General Introduction

Gastrointestinal nematodosis (GIN) is the principal disease of sheep in Australia and world wide. In the most recent published estimate of its cost to the Australian sheep industry GIN was estimated at $222 million dollars per year and it caused the greatest economic loss of all sheep diseases (McLeod 1995). Chemical anthelmintic treatments have been the principal method of worm control over the last 40 years, however, resistance has developed to all major classes of anthelmintic compounds, with the possible exception of Napthalophos, an organophosphate, and is rapidly worsening. The development of new anthelmintic compounds is constrained by the relatively small size of the worldwide market for sheep anthelmintics and the lack of significant anthelmintic resistance problems in the much bigger cattle market. Hence, alternative control methods are required to form part of an integrated approach to gastrointestinal nematode control. Grazing management is an obvious alternative to anthelmintic treatment and has been used with mixed results to date. Successful grazing management strategies include dilution strategies such as mixed grazing of susceptible sheep classes with non-susceptible animals (eg: cattle or older dry sheep) and preventative strategies such as grazing cattle alternately with sheep to 'clean' the pasture of infective larval nematodes. Our understanding of the ecology of the free-living stages of parasitic nematodes also suggests that rotational grazing systems could assist in the control of GIN in sheep by interrupting the nematode lifecycle (Donald 1967). However, up to the late 1980s, there was little success in developing and implementing practical rotational grazing systems that reduced GIN. Early studies on rotational grazing in cool temperate environments involved grazing periods of 7 days and rest periods between grazing events ranging from 3 to 7 weeks (Morgan 1933; Morgan and Oldham 1934; Roe *et al.* 1959; Gibson and Everett 1968). These rotations, however, were ideal for the proliferation of parasitic nematodes allowing both autoinfection from the current grazing period in summer months, and re-grazing at the peak of L_3 availability. *Haemonchus contortus* will develop from egg to L_3 in 3-5 days at 25-26°C but will take 15-30 days at 10-11°C (Rose 1963). Season therefore determines the length of safe grazing periods that prevent autoinfection. The time of peak L_3 on pasture in the Sydney Basin, NSW is generally around 35 days after deposition with smaller peaks at days 14 and 28 (Donald 1967). This author concluded that the spelling period for a paddock should be no less than 8 weeks to enable a significant reduction in pasture infectivity. This may also vary with season as L_3 on pasture survive longer in cooler conditions than warm or hot conditions (Dinaburg 1944a; Thomas and Boag 1972; Southcott *et al.* 1976; Besier and Dunsmore 1993a).

Donald (1967) thought that such long rest periods were inefficient in terms of optimal pasture utilisation. This is supported to some extent by Robertson and Fraser (1933) using much longer rest periods than those suggested by Donald. Their study in north Scotland achieved control of GIN through what they termed 'progressional grazing' and was especially effective in reducing the incidence of *H. contortus*. The rotation employed for 'progressional grazing' was 10 days of grazing followed by 100 days of rest. The authors concluded that 10 days was a sufficiently safe period of time for grazing before eggs reached the infective stage. However, over-mature grass undermined the success of 'progressional grazing' with sheep failing to maintain body weight despite lower parasite burdens.

Some 60 years after Robertson and Fraser (1933) an effective rapid rotational grazing system was devised by Barger *et al.* (1994) for small ruminants in the humid tropics based on the findings of Banks *et al.*(1990) in Fiji on the rates of larval survival and mortality in hot, humid environments. The system comprised a grazing period of 3.5 days and a rest period of 31.5 days and has been used with success throughout the tropics in both sheep and goats (Barger *et al.* 1994; Chandrawathani *et al.* 1995; Sani *et al.* 1996; Gray *et al.* 2000). However, Banks *et al.* (1990) and Barger *et al.* (1994) suggest that rapid rotational grazing would not be economically viable in cooler climates, presumably because the rigid application of timing of graze and rest periods would be unsuitable given the seasonal variability of temperature and rainfall which are the major drivers of development and survival of the free-living stages of parasitic nematodes in these environments.

In the early 1990s in the temperate regions of Australia, intensive rotational grazing systems such as 'cell grazing' and 'holistic grazing' were introduced on the basis of improved pasture and animal performance. These systems claimed to increase pasture biodiversity, ground cover, and the ratio of palatable to non-palatable plant species, and improve soil structure (Earl and Jones 1996; McCosker 2000; Sparke 2000). They involve the use of large groups of animals at high stock densities moving through a series of 20 to 40 paddocks at a rate dependant on the amount of feed on offer and pasture growth rate (not based on rigid time periods). The grazing period generally ranges from 1-3 days with rest periods of 40-90 days, resulting in paddocks being rested for 90-95% of the year (Earl and Jones 1996). This type of grazing management has become increasingly used throughout Australia with its highest prevalence being in the New England region on the Northern Tablelands of NSW (Reeve and Thompson 2005). The consequences of such intensive grazing systems on GIN in sheep in cool temperate climates have

not been documented despite considerable anecdotal evidence of marked reductions in faecal worm egg counts (WEC) and the number of anthelmintic treatments required.

The Cicerone project is a farmer-led project comparing three sheep management systems in the New England region of Northern NSW. The 3 management systems are Typical (TYP), High Input (HI), and Intensive Rotational Grazing (IRG) and are detailed later in the thesis. There was evidence that sheep on the IRG management system, had lower faecal worm egg counts than those run with slower rotational grazing management on the TYP and HI systems. A retrospective analysis of the routine faecal worm egg counts under the three management systems strongly supported the proposition that intensive rotational grazing reduced GIN (Healey *et al.* 2004). This doctoral study was designed to confirm this finding in a controlled, balanced study and to:

- determine on which classes of stock it was operative
- determine which times of the year it is operative
- determine which parasite species are affected by it
- uncover the underlying mechanisms by which its was working

The study also aimed to investigate what differences, if any there were in GIN between the TYP and HI management systems on the Cicerone project.

This thesis details the epidemiology of GIN on the Cicerone Project farmlets dissecting the host, environment and pathogen effects on the disease under the 3 different management systems. A two-year longitudinal study (Experiment 1) aims to confirm the effect of the three management systems on GIN and animal production and provide insight into possible mechanisms. A fixed larval challenge study (Experiment 2) investigates host effects on GIN while the pasture larval contamination is investigated in a tracer sheep experiment (Experiment 3). The development and survival of free-living stages of *Haemonchus contortus* on the Cicerone project is investigated in a plot study (Experiment 4). Finally, the cost of GIN on animal performance is investigated by comparison of 'worm-free' sheep with those managed for worm control within each management system in Experiment 5. Grazing systems in general are not designed for worm control, so an holistic approach has been adopted with animal performance measured in this doctoral study whilst a fellow doctoral student, Libuseng Shakhane, has investigated the pasture aspects.

The general hypothesis under test in this thesis is that intensive rotational grazing reduces faecal worm egg count in sheep through interruption of the nematode lifecycle in its free-living stages.

Subsidiary hypotheses are that the reductions in WEC associated with IRG will be greatest for *Haemonchus contortus* due to the greater susceptibility of its free-living stages to desiccation and cold, and that WEC will be higher in the high input grazing system than the typical grazing system.

CHAPTER 1: Review of the literature

1.1 Introduction

Gastrointestinal nematode infections are endemic in livestock across much of Australia and throughout the world. The nature of the disease varies with location, temperature and rainfall distribution. The adverse impact on animal production is greatest in warmer wetter regions of the world, where temperature and rainfall are not limiting to the development of the free-living stages of the parasites. In cooler climates there are seasonal fluctuations in severity. This review will briefly cover the pathogenicity of gastrointestinal nematode disease and the host responses to it. It will cover in more detail the ecology of the free-living stages of the parasites, the use of grazing management for control of the disease, other control measures available and their integration.

1.2 Australian sheep production, rainfall zones and the distribution of the major gastrointestinal nematodes of sheep

The Australian sheep flock comprises 84.6% merino, 11.3% crossbred and 4% other breeds and is spread across the western region of Western Australia, the south-east regions of South Australia and Queensland and throughout New South Wales and Victoria (AWI 2005, Figure 1-1). There is also sheep production in the high rainfall regions of Tasmania. New South Wales has the highest proportion of the total sheep flock (34.7%), followed by Western Australia (23.6%) and Victoria (20.4%, Figure 1-2). Overall, the wheat-sheep zone contains 55% of the Australian sheep flock, 33% in the high rainfall zone and 12% in the pastoral zone (AWI). Production of sheep occurs across a wide range of climates, from the high rainfall zones to semi-arid regions of Australia. There are regional differences in the abundance and importance of the parasitic nematode species, largely dependant on seasonal rainfall patterns. The summer rainfall zone, which includes the Northern Tablelands of NSW and the sheep production regions of Queensland, is dominated by *Haemonchus contortus*, which can cause widespread disease and animal deaths in spring and autumn. *Trichostrongylus colubriformis* and *Teladorsagia (Ostertagia) circumcincta* are also of importance in winter and spring but are not as damaging as *H. contortus* outbreaks. A band of non-seasonal rainfall stretches across the middle of Australia and includes the Central Tablelands of NSW. The winter rainfall zone encompasses parts of

southern NSW, Victoria, South Australia, Western Australia and Tasmania (Figure 1-3). The most prevalent parasites in these zones are *Trichostrongylus* spp. and *Teladorsagia circumcincta*; *H. contortus* is present in smaller numbers with sporadic outbreaks occurring in the south of Western Australia, south-east of South Australia, Central Tablelands of NSW and the wetter parts of northern Victoria.

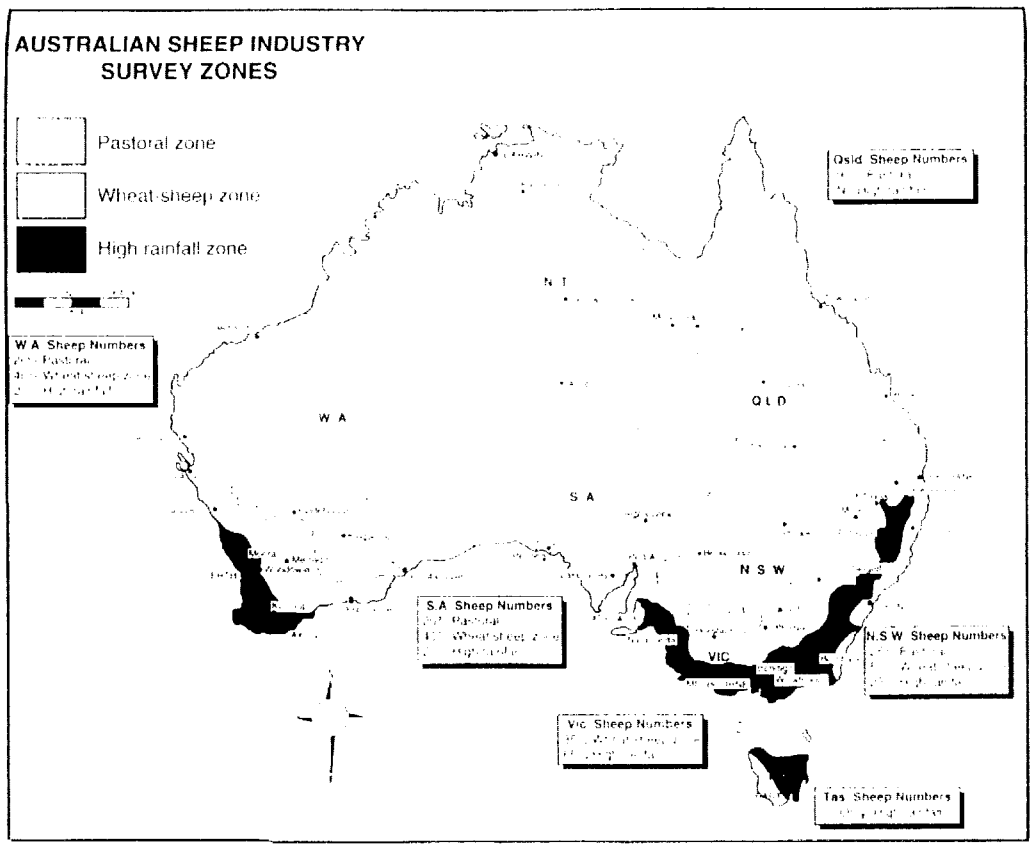


Figure 1-1: Distributions of sheep enterprises across Australia (UNE lecture notes ANPR211, 2005).

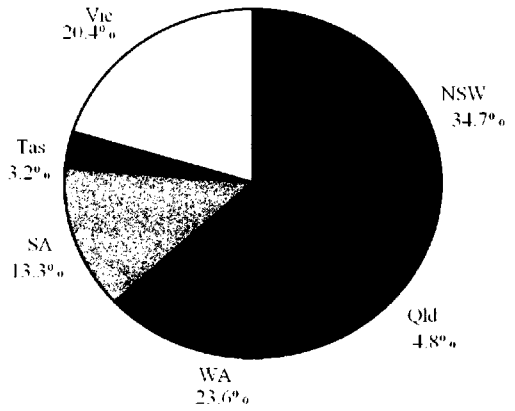


Figure 1-2: Proportion of sheep production per Australian state (Australian Wool Innovation website, accessed in June 2006, www.wool.com.au).

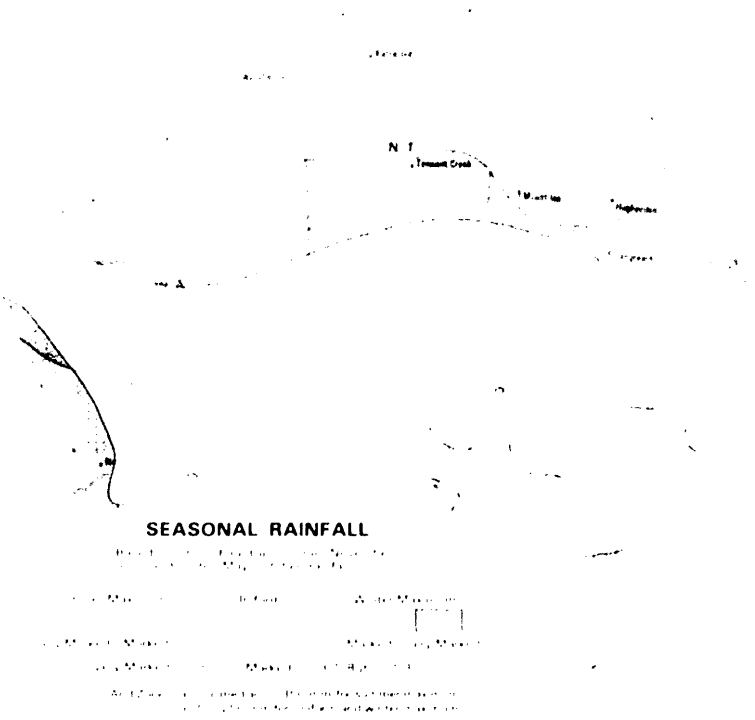


Figure 1-3: Rainfall distribution across the continent of Australia showing the different rainfall zones (Crowther 1995).

1.3 Life cycles of the major gastrointestinal nematodes of sheep

The basic life cycles of the three major nematode parasites for sheep in Australia (*Haemonchus contortus*, *Trichostrongylus* spp. and *Teladorsagia circumcincta*) are identical. The lifecycle is direct without the involvement of an intermediate host, and comprises phases in the environment (egg, L_1 L_2 and L_3) and in the host (L_3, L_4, and adult) (Cole 1986). The main differentiating factors between the species are the temperatures and precipitation required for development of the free-living stages of the lifecycle. Eggs passed in the faeces of a sheep hatch releasing first stage larvae (L_1), which feed in the faecal pellet before moulting into second stage larvae (L_2), which also feed on bacteria in their environment. The L_2 go through an incomplete moult leaving a sheath enclosing the third stage larvae (L_3). The L_3 cannot ingest food and must survive on energy reserves, ceasing to be free-living and becoming obligatory parasites. The L_3 is infective to sheep and exits the faecal pellet moving onto pasture or into the upper layers of the soil profile and mulch layer. If the L_3 are ingested by the host sheep they exsheathe, usually in the organ prior to their infection site where they burrow into the mucosa. They then develop into fourth stage larvae (L_4) and either become arrested at this stage in what is termed hypobiosis or go on

immediately to develop into adult nematodes. It generally takes 21 days for the ingested larvae to develop into mature egg laying adults in the absence of hypobiosis (Donald *et al.* 1978b).

1.4 Clinical disease and pathological changes

Clinical signs of GIN vary with the species of the parasitic infection and include general ill thrift, diarrhoea , anorexia (*Trichostrongylus* spp.), anaemia (*H. contortus*), reduced growth rate and bodyweight, reduced wool quality and reproduction rate. Depression in growth rates in younger sheep and a reduction in body weight in adults, is common with *T. colubriformis* or *T. circumcincta* infections. In a study undertaken in central Victoria, Thompson *et al.* (1978) reported that lambs with a natural infection drenched as recommended showed a 21—23% reduction in liveweight compared to lambs drenched weekly. The location of the study suggests that *T. colubriformis* and *T. circumcincta* would have been the predominant nematodes. Details of the recommended drenches were not given. Brunsdon and Vlassoff (1982) showed a significant difference in liveweight gain between lambs grazed on pastures with differing infectivity. Lambs that were grazed on the most heavily infected pasture (100 larvae/kg herbage) were 5.8 kg lighter after the five-month experimental period than those grazed on virtually clean pasture (nil larvae/kg herbage). Albers *et al.* (1988) demonstrated a significant difference between lambs infected with *H. contortus* and those not infected; the average depression of liveweight gain was 1.29 kg or 38% of the total gain in uninfected animals over a four-month period. Alterations in body composition also occur with reduced fat deposition, protein synthesis, and skeletal calcium and phosphorus (Sykes and Coop 1977; Jones and Symons 1982).

Pathological changes in the gut during nematode infection also vary with location of infection and nematode species. Abomasal changes occur during infection with *T. circumcincta*, with major damage occurring when the fourth stage larvae emerge from the gastric glands. Parietal cells lose function and acid secretion is impaired leading to elevated pH levels (Ritchie et al 1966 cited in Holmes 1985). Lesions caused by *H. contortus* cause similar damage, although the hematophagia of the adults and L_4 cause more damage to the host with the mucosal surface being covered in coagulated blood (Soulsby 1965). Heavy infections of *T. colubriformis* in the small intestine cause extensive villous atrophy, mucosal thickening, stunting of microvilli and erosion of the epithelium (Coop and Angus 1975).

1.4.1 *Haemonchus contortus*

Haemonchus contortus is commonly known as 'Barber's Pole' worm, named for the barber's pole-like appearance of the white oviduct of the adult female entwined with the red, blood-filled, gastrointestinal tract. The site of infection for this nematode is the abomasum, where it burrows deep into the abomasal wall and feeds on the host's blood. Both males and females are hematophagous, with a lancet within the buccal cavity used to pierce small blood vessels during feeding. The male adult *H. contortus* is 19--22mm in length, with the females being 25--34mm in length (Dunn 1978). The female *H. contortus* is extremely fecund, producing an average 6500 eggs per day with a range of 5000--10 000 (Coyne *et al.* 1991), and generally begins shedding eggs around 21 days after entering the gut as a third stage larva..

There are three recognised forms of haemonchosis: hyperacute, acute and chronic. Hyperacute haemonchosis occurs following ingestion of large numbers of *H. contortus* L_3 (up to 50 000). The host experiences severe blood loss and bleeds to death within 7 days; losses of up to 500ml of blood per day have been reported (Dunn 1978). Acute haemonchosis occurs when the adult population within the sheep reaches moderate levels (up to 10 000). Liveweight and weight gain are reduced, and other clinical signs include: anaemia; hypoalbuminemia; facial, sub-mandibular and abomasal oedema; loss of wool; and dark faeces. Mortality in the acute form of the disease is high. Because of the high fecundity of *H. contortus*, heavy infections can develop rapidly under favourable weather conditions and fat, healthy-looking sheep can be killed within a short space of time, often with low faecal worm egg counts (Cole 1986). The chronic form of haemonchosis occurs with low levels of adult worm/nematode infestation (less than 1000) and leads to low blood iron concentrations and reduced protein levels through slow leaching of blood and damage to body tissue. Where nutrition is inadequate death may occur. There is a general look of malnutrition in chronically affected animals, with loss of body weight, break in the wool fibre causing peeling of wool, lethargy and weakness (Dunn 1978).

1.4.2 *Trichostrongylus* spp.

The main species of Trichostrongylus responsible for disease in sheep is *Trichostrongylus colubriformis*; the secondary species is *Trichostrongylus vitrinis*. *Trichostrongylus rugatus* is present in Western Victoria and south-east South Australia. A fourth species, *Trichostrongylus axei*, is a cattle nematode but it also has a widespread occurrence in sheep (Cole 1986). It is difficult to determine the difference between these species with basic larval differentiation

methods, so they will be dealt with together as *Trichostrongylus* spp., unless species is indicated. The *Trichostrongylus* spp. are commonly referred to as 'Black Scour worm'. The adult male nematodes are smaller than those of *H. contortus* at 5.5--7.0mm long, with the females ranging from 6.0--8.0mm long (Dunn 1978). The adult *Trichostrongylus* spp. resides in mucus-covered tunnels in the surface epithelium of the proximal third of the small intestine (Wagland *et al.* 1996). The eggs are of very similar size and appearance to those of *H. contortus*, *Teladorsagia circumcincta* and *Oesophagostomum* spp., and cannot be easily differentiated morphologically. *Trichostrongylus* spp. are not as fecund as *H. contortus*, mainly due to the smaller size of the adults. A study by Coyne *et al.* (1991) reported an average of 262 eggs per day with a range of 7--933 eggs per day. The disease, as its common name suggests, causes dark diarrhoea and sheep generally lose body condition. A marked reduction of feed intake is a major aspect of this disease. The damage caused by the nematode to the small intestine and the host response to it also causes protein loss into the gut lumen. Much of this is reabsorbed lower in the gut, however, there is usually a net loss of nitrogen from sheep during infection (Roseby 1977). This disease has both acute and chronic forms. The acute form occurs with ingestion of large numbers of larvae and results in anorexia, dark diarrhoea, facial oedema and death after 13 days (Dunn 1978). The chronic form of the disease occurs with moderate ingestion of larvae and causes anorexia, soft faeces, some facial oedema and anaemia. Some animals spontaneously recover from chronic Trichostrongylosis, whilst others die from its effects.

1.4.3 *Teladosagia circumcincta* (formerly *Ostertagia circumcincta*)

The parasitic nematode *Teladosagia circumcincta* was previously named *Ostertagia circumcincta* and is referred to as such in much of the literature. The common name is 'Small Brown Stomach worm' and it is also still commonly called 'Ostertagia'. The adult nematodes live in the abomasum and feed on the epithelium and mucosa (Schallig 2000); the adult males are 7--9mm long with the females being 8--12mm long (Dunn 1978). *Teladosagia circumcincta* have very similar eggs to the other strongyles described previously and the differences cannot be detected reliably under the microscope. The pathogenic effects of *Teladosagia circumcincta* are almost indistinguishable from those of *Trichostrongylus* spp. The acute disease is most common in lambs in summer, causing diarrhoea, dehydration and anaemia with rapid weight loss. In winter and spring a sub-acute form of the disease can occur, resulting from the mass emergence of hypobiotic larvae, which have over-wintered in the abomasal mucosa. This generally occurs in peri-parturient ewes. Chronic ostertagiasis is a wasting condition of ewes which is

indistinguishable from malnutrition. Anaemia, body weight loss and emaciation are all found with the chronic form (Dunn 1978; Cole 1986).

1.4.4 Minor species

There are a number of nematode parasites present in sheep that do not commonly cause disease to the extent of the nematodes outlined above. The most common of these are *Nematodirus spathiger*, *Nematodaris filicollis* *Oesophagostomum venulosum*, *Oesophagostomum columbianum*, *Cooperia curticei*, *Chabertia ovina* and *Trichuris globulosa*. *Nematodirus* spp. (thin-necked intestinal worm) produce only a small numbers of eggs per day (40 eggs/day) (Coyne *et al.* 1991), and these are easily distinguished from other strongyle eggs by their large size and by the 7--8 granular cells visible within the egg (Soulsby 1965). This nematode only causes disease in lambs and problems are typically seen after drought conditions (Cole 1986). Development to L_3 occurs within the egg, which is highly resistant to environmental influences. The L_3 can over-winter within the egg, which is very tolerant of freezing and can survive for up to 2 years. Eggs are also very drought tolerant, ensuring transmission over a number of years (Dunn 1978). *Oesophagostomum. columbianum* is also commonly identified in faecal cultures and was once a major parasite of summer rainfall zones, but it has gradually disappeared and is of little importance currently (Cole 1986). It is reported to have a very high fecundity, producing an average of 11 000 eggs per day (Dunn 1978; Coyne *et al.* 1991). *Cooperia curticei* is also commonly seen in faecal cultures, but mainly those of young sheep. It can cause ill thrift in lambs and weaners, but is uncommon and has no characteristic clinical signs (Cole 1986).

1.5 Pathogenesis of ovine gastrointestinal nematodiasis

Gastrointestinal nematode infection is generally associated with a loss of animal production through liveweight losses, poor growth rate, lost wool production and animal deaths (Steel 1974). There are three main mechanisms underlying production losses: appetite depression, changes in gastrointestinal function, and alterations in protein metabolism (Fox 1997). The manifestation of the disease is dependent upon the species present in the host (outlined in section 1.4). The effects of gastrointestinal nematode infection are less pronounced in older sheep than in young weaner sheep, mainly because of acquired immunity (Donald 1979). There is also a difference in susceptibility between sexes, with rams being generally more susceptible to infection than ewes (Barger 1993).

1.5.1 Effect on feed intake

A reduction in voluntary feed intake is a common feature of parasitic nematode infections; it varies considerably in severity and is a major contributor to poor animal performance. Roseby (1973) found a reduction in feed intake of 20--30% over 40 days in weaners infected with a single dose of 30 000 *T. colubriformis* infective larvae. Dynes *et al.* (1990) reported a slightly higher depression in feed intake, 40%, in weaner lambs given 4000 *T. colubriformis* L_3 daily for 12 weeks. This suggests that the constant infection caused a greater depression in feed intake than a single dose of larvae. Sykes and Coop (1977) found an intake depression of 20% in lambs infected daily for 14 weeks with 4000 *T. circumcincta*. Coop *et al.* (1982) reported a smaller reduction in feed intake of around 12% after daily doses of 5000 *T. circumcincta*. They also found that feed intake reduced further with increasing larval intake even though there were no clinical signs of disease. From these studies, it seems that *T. colubriformis* has a greater impact on appetite than *T. circumcincta*. In sheep infected with *T. colubriformis*, reduction in voluntary feed intake is not apparent until 3--4 weeks after infection, coinciding with the establishment of mature nematodes (Kyriazakis *et al.* 1998). Lambs infected with *T. colubriformis* have also demonstrated a slowing down of the rate of digesta flow (Roseby 1977) and, as feed intake in sheep is related to the distension of the rumen, reduced digesta flow may be a contributing factor to inappetence. Kyriazakis *et al.* (1998) state that reduction of feed intake of sheep with nematode parasitism is not dose dependant, however, there is a level below which anorexia is not observed and the higher the level of infection the more severe the anorexia. In contrast to Kyriazakis *et al.* an experiment by Symons *et al.* (unpublished cited by Steel 1978) shows a dose dependent reduction in feed intake in lmabs over four weeks for both *T. colubriformis* and *T. circumcinta*. Kyriazakis *et al.* (1998) put forward two hypotheses for the occurrence of anorexia: 1) Food intake decreases for the purpose of promoting an effective immune response, and 2) Anorexia allows the host to become more selective in its diet (possible reducing further larval intake or changing plant selection).

1.5.2 Effect on feed utilisation and metabolism

Feed utilisation is also affected during gastrointestinal nematode infection. Studies on digestion showed a reduction in the digestibility of dry matter, organic matter, crude fibre and crude protein (Parkins and Holmes 1989). There are a number of reviews on the metabolic consequences of intestinal parasitism (Sykes 1983; Holmes 1985; Parkins and Holmes 1989; MacRae 1993). Symons (1981) (cited in Holmes 1985) concluded that net movement of amino

acid nitrogen, from muscle and skin to the liver and intestines, decreases its availability for growth, milk and wool. The movement of amino acids was due to inappetence, enteric losses of protein and increased rates of intestinal tissue protein metabolism. Roseby and Leng (1974) found higher levels of urea in the urine of sheep given a single dose of 30 000 *T. colubriformis* infective larvae. They concluded that the increased urea was due to an increased rate of urea synthesis and not a change in urea losses. Kimambo *et al.* (1988) showed a higher flow rate of nitrogen in the ileum of sheep dosed daily with 2500 *T. colubriformis*; they attributed this to enhanced endogenous secretions into the small intestine rather than reduced nitrogen absorption. However, Sykes and Coop (1977) found nitrogen digestibility to be reduced from 60% to 44% after 2--3 weeks of daily infection with 4000 *T. circumcincta.* Bown *et al.* (1991b) calculated the total plasma loss into the small intestine was increased two- to three-fold in sheep given 3000 *T. colubriformis* and 3000 *T. circumcincta* daily for 18 weeks, however, most of the plasma protein was reabsorbed before the end of the ileum. Poppi *et al.* (1986) had similar results, which indicated that the nutritional penalty associated with the development of resistance to infection is greater than that caused by the primary infection.

Barger (1973) found that absorption of phosphorus was similar in non-infected and infected sheep (initial dose of 15 000 *T. colubriformis* larvae and a second dose of 1 500 000 L_3 6 weeks later), however, calcium absorption was one tenth of that of non-infected sheep. Poppi *et al.* (1985) found the opposite, with calcium absorption and retention not affected by a daily infection of 2500 *T. colubriformis* larvae. Phosphorus metabolism, however, was markedly affected, with reduced blood concentrations, reduced absorption and a higher rate of flow of P in the small intestine. This disparity could be due to differences in the larval dosing strategies of the two trials, with Barger (1973) administering 2 very large doses 6 weeks apart and Poppi *et al.* (1985) giving daily doses of 2500. A later study by Bown *et al.* (1989) found that lambs given concurrent doses of 3000 *T. colubriformis* and 3000 *T. circumcincta* infective larvae daily for 18 weeks had increased losses of calcium and phosphorus and reduced absorption of both minerals. They also found that magnesium metabolism was unaffected by parasitism. It appears that the effect of parasitism on calcium and phosphorus absorption is dependant not only on which species are present, but also the rate of larval intake.

1.5.3 Effect of nematodes on wool and milk production

A comprehensive review by Donald (1979) provides strong evidence that gastrointestinal nematode infections cause a reduction in wool production; this is mainly associated with *Trichostrongylus* spp. and *Teladosagia circumcincta* infections. A number of experiments involving artificial and natural infections in sheep have demonstrated a depression in wool production ranging from 18% to 59% (Carter *et al.* 1946; Barger and Southcott 1975; Thompson *et al.* 1978; Leyva *et al.* 1982). Lambs concurrently infected with *T. colubriformis* and *T. circumcincta* (900 or 3000 *T. colubriformis*/week and 38 000 *T. circumcincta*/week, individually or both parasites concurrently over 16 weeks) produced 66% less wool than non-infected lambs (Steel *et al.* 1982). Another experiment by Barger (1973) on mineral absorption and feed digestibility found that infected sheep (single dose of 15 000 *T. colubriformis* and 1 500 000 larvae 6 weeks later) produced 0.36g of wool/g of nitrogen absorbed compared to 0.46g wool/g of nitrogen absorbed for non-infected sheep. The experiments outlined in Donald (1979), and those experiments mentioned above, involved artificial infection with *T. colubriformis* and *T. circumcincta* or natural infections in areas of winter dominant rainfall predominated by these species. Johnstone *et al.* (1979) showed that *H. contortus* also had a significant effect on wool growth with sheep given a high level of worm control (anthelmintic treatment every 4 weeks, with another treatment between those if high *H. contortus* infections were expected) producing 34.2% more wool than those given salvage treatments (treated to avert death). A study by Albers *et al.* (1988) found sheep infected with *H. contortus* had both reduced fibre quantity and lower fibre diameter. The effect on wool production in this study showed a markedly different pattern to the liveweight losses, with a 25-day delay in the effect on wool growth after the first observations of liveweight changes. Leyva *et al.* (1982) demonstrated a reduction in wool growth of 20% in sheep infected with *T. circumcincta*; the point of break in the staple was also moved in the infected sheep from parturition (non-infected) to the period of infection.

Studies in the effect of parasitism on milk production in sheep demonstrated a reduction in milk production by 17% with *T. circumcincta* infections and 23% reduced milk production with *H. contortus* infections (Leyva *et al.* 1982; Thomas and Ali 1983).

There are no studies that this author could locate on the direct effects of GIN on reproduction, however, the major physiological impact that GIN has on metabolism, nutrient intake and absorption would translate into a reduction in the reproductive rate of ewes and possibly rams. Fat score and liveweight both directly relate to the reproductive performance of ewes (Kenyon *et*

al. 2004). As such it is not unreasonable to link GIN with lower reproductive rates as it significantly reduces feed intake and bodyweights as discussed above.

1.6 Epidemiology of ovine gastrointestinal nematodiasis

Epidemiology is the study of disease in populations and the factors that determine its occurrence and severity (Thrusfield 1995) and traditionally deals with the host, pathogen and environmental influences and their interactions on the occurrence of disease. The epidemiology of gastrointestinal nematode infection involves an understanding of the development and survival of the free-living stages, the effect of the host and host nutrition, and parasite factors, such as egg output and population dynamics. The fact that each infective larvae on pasture resulted from an egg and every nematode in the host was acquired as an infective larvae from pasture leads to the obvious assumption that factors influencing the abundance of infective larvae on pastures are of great epidemiological importance and play a major role in control measures (Donald 1973).

The free-living stages of the nematode lifecycle are the most susceptible to adverse environmental influences, with temperature and moisture being the major limiting factors for development and survival. An understanding of the time taken for development to L_3, time of peak L_3 and L_3 survival is also important when considering control strategies (Gordon 1948; Donald 1973; Barger 1999). These features of the free-living stages are solely determined by climatic events. Dry, hot weather and dry, cold weather are not conducive to the development of nematode eggs and larvae although there is some development of species at low temperatures where moisture is not limiting (section 1.5.2.1). Warm, wet weather provides optimum conditions for the development of the major nematode species.

The species of importance varies with climate, with rainfall and temperature patterns being the determining factors as to whether *H. contortus* or *T. colubriformis* and *T. circumcincta* are dominant. Generally, warm wet weather leads to the dominance of *H.contortus*, such as in tropical zones and summer rainfall zones. *Teladosagia circumcincta* and *Trichostrongylus colubriformis* predominate where rainfall is either non-seasonal or winter dominant and where temperatures during the main periods suitable for larval development are generally cool.

1.6.1 Epidemiology of gastrointestinal nematode infections in sheep on the Northern Tablelands of NSW

The climate on the Northern Tablelands of NSW is cool temperate with warm, wet, summers and cold, dry, winters with many frosts and the occasional snowfall. Rainfall is summer dominant with an average annual total of 790mm. Nematode infestations generally rise in spring, reaching a peak in mid- to late summer (January/February) through to autumn (March/April) (Gordon 1948). There are often peaks of the disease in late November, which coincide with frequent thunderstorms. The climate on the Northern Tablelands is most favourable for *H. contortus*, the incidence of which explodes in warm wet weather causing numerous cases of acute haemonchosis occasioning death in all classes of sheep, including fat, healthy sheep. Chronic haemonchosis is also common in older ewes that are not in late pregnancy or lactating. The phenomenon of self-cure was noted in earlier epidemiological studies on the Northern Tablelands (Gordon 1948). Sheep were observed to expel large infections of *H. contortus* after periods of rainfall which bring on young lush green grass. Gordon (1948) states that 'self-cure plays a big part in the epidemiological fluctuations in the population of strongylid nematodes and the discovery of its cause is one of the urgent problems of parasitological research', however, it has not become a major factor in control strategies most probably due to our inability to control its occurrence and the widespread use of chemical anthelmintics.

Typical patterns of infection and levels of infective larvae on pasture of the 3 major worm species on the Northern Tablelands is shown in Figure 1-4 (left). *Haemonchus contortus* rises slowly in spring to reach large peak infections in summer/autumn. A second peak occurs in winter and is attributed to inhibited larvae which have no effect on faecal worm egg count. Infective larvae are in very low numbers in late autumn to early spring when conditions are unfavourable for survival of the free-living stages of this species. *Teladosagia circumcincta* (referred to as Ostertagia in Figure 1-4) peaks slightly earlier than *H. contortus* in both larval numbers on pasture and infection. This is due to its ability to survive and develop into infective larvae at lower temperatures. Infections of this species drop off in the heat of summer, peaking again in spring. *Trichostrongylus* spp. infection tend to rise in late spring and decline during the hotter months. Pasture infectivity of *Trichostrongylus* spp. rises earlier than *H. contortus*, again due to the ability of the eggs and larvae of *Trichostrongylus* spp. to survive at lower temperatures over winter. To demonstrate the influence of rainfall distribution on nematode infestation and dominant nematode species, the right hand diagram in Figure 1-4 shows the pattern of nematode

infestation in the winter rainfall region of Western Victoria. Whilst temperatures are similar throughout the year, rainfall is greatest in winter, with hot dry summers. This climate is not conducive to the survival of *H. contortus*, which is rarely seen in this region. The dominant species are *T. circumcincta* and *Trichostrongylus* spp. with peak infections in late autumn/winter coinciding with high rainfall. Infections are low in the hot dry summer months.

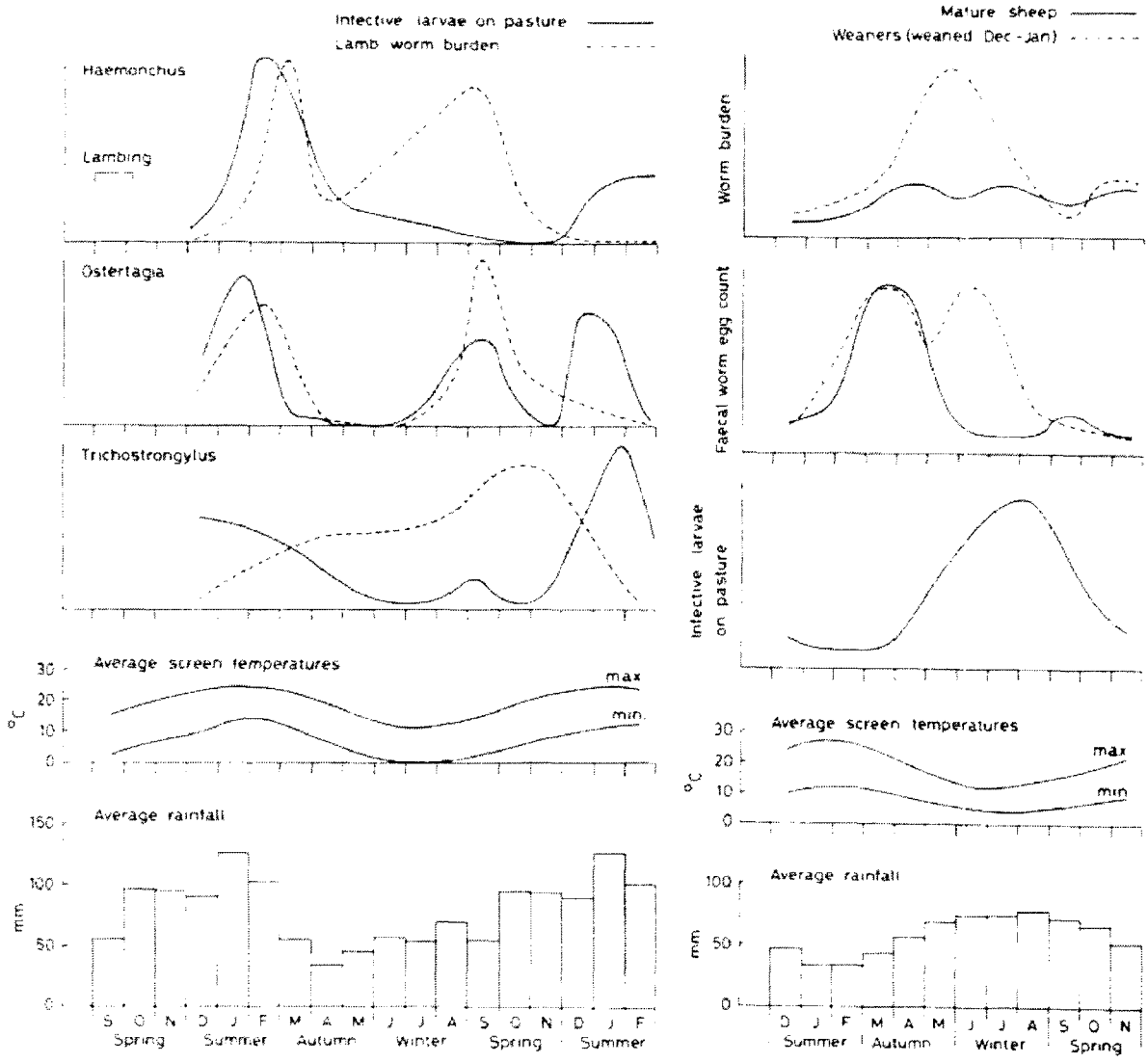


Figure 1-4: Seasonal patterns of nematode infection in summer and winter rainfall areas: (left) *Haemonchus contortus* in spring-born lambs grazing contaminated pastures from birth to 18 months of age. Average rainfall and screen temperatures at Armidale, NSW; (right) *Teladorsagia* spp. (*Ostertagia*) and *Trichostrongylus* spp. infections in the Western District of Victoria. Average rainfall and screen temperatures at Hamilton, Victoria (Donald *et al.* 1978b).

1.6.2 Environmental influences on epidemiology: Ecology of the free-living stages of parasitic Trichostrongylid nematodes

Meteorological factors are of great importance for the development and transmission of the infective stages of parasitic nematodes outside the host. Precipitation, temperature, humidity, wind and sunlight are local factors which have a large impact on the development and survival of the free-living stages of gastrointestinal nematodes. Generally there are 4 egg and 3 larval stages identified during observation of the free-living stages of trichostrongylid nematodes. For the egg these are: E_1, early blastomere to morula; E_2, differentiating gastrula; E_3, tadpole or early vermiform; E_4, late vermiform to pre-hatch; for the larva: L_1, first-stage larva; L_2, second-stage larva; L_3, third-stage larva (Silverman and Campbell 1959). Temperature and moisture are the main determinants for the development of eggs to L_3 and L_3 survival on pasture. When moisture is not limiting, temperature has the greatest effect on development and survival. The stage that is most resistant to the effects of temperature and moisture is L_3; the embryonated egg (E_4) is the next most resistant (Ciordia and Brizzell 1963; Anderson *et al.* 1965; Anderson *et al.* 1966; Anderson and Levine 1968). Anderson *et al.* (1966) found that, in general, a higher percentage of L_2 than L_1 remained alive at a large range of temperatures; this was more obvious at temperatures above 35°C and below 4°C.

1.6.2.1 Development of egg to third stage larvae

Levine and Todd (1975) state that temperature and soil moisture are the most important factors influencing development and survival of the eggs and larvae of parasitic nematodes on pasture. There have been comprehensive studies on the effect of temperature on development, both in the laboratory and in the field. Studies on the effects of moisture have been more limited. The recovery rate of L_3 from pasture as a percentage of eggs deposited ranges from 0.03% to 0.9% (Niezen *et al.* 1998b).

1.6.2.1.1 Effects of temperature

1.6.2.1.1.1 Haemonchus contortus

The earliest laboratory studies on *H. contortus* development by Ransom (1906) were quite comprehensive in determining the lifecycle of the nematode and the susceptibilities and resistant qualities of the eggs, and the larval stages, L_1, L_2 and L_3. He observed that eggs and newly hatched embryos (presumably L_1 and L_2) were quite vulnerable to freezing and drying and that a 'large number' of eggs passed in faeces are unlikely to develop. Eggs kept at a temperature

below 40°F (4.4°C) could remain dormant and may retain their viability for 2 to 3 months. Veglia (1915) noted that development stopped altogether at 5°C, a similar temperature to that observed by Ransom (1906), and Coyne and Smith (1992a) who found no development of *H. contortus* eggs and larvae at 5°C at constant 100% humidity. Berberian and Mizelle (1957), however, found that the minimum lethal temperature for *H. contortus* eggs was 5.6°C, which conflicts with Ransom's finding that the eggs become dormant but remain viable. Berberian and Mizelle also found some hatching of embryos at 8.89°C but no L_3 emerging; the minimum temperature at which they recovered L_3 was 12.2°C. Silverman and Campbell (1959), reported development and survival of *H. contortus* eggs below 13°C. They also found some larval development at temperatures as low as 7.2°C, but the bulk of the population were inhibited between 7--11°C, with the proportion of eggs developing increasing as temperature increased. Work outdoors by Dinaburg (1944a) concluded that the mean maximum temperature below which there is no development of eggs or larvae was 65°F (18.3°C), which is substantially higher than that reported by others. There may have been some confounding with moisture inhibition in Dinaburg's experiment while all the other above-mentioned experiments were performed in the laboratory (Ransom 1906; Veglia 1915; Berbarian and Mizelle 1957; Silverman and Campbell 1959). Crofton and Whitlock (1965 cited in: Crofton and Whitlock 1965b) reported real differences between minimum hatching temperature for *H. contortus* isolates from Great Britain (9°C) and those from the United States (15°C). This may account for some of the discrepancies between minimum temperatures reported for development of egg to L_1 between countries. Gibson and Everett (1976) stated that it was generally agreed that 10--11°C was effectively the lower limit for *H. contortus* larval development on pasture, and given the results of the laboratory experiments already mentioned this is not an unreasonable assumption (range of temperatures for minimal development being 7.2°C to 12.2°C).

Jasmer *et al.* (1986) tested the influence of cold temperatures on eggs of *H. contortus* collected in the Washington area (USA) and found slow development of *H. contortus* eggs at 10°C, with few eggs hatching after exposure to -18°C for 96 hours. This led them to the conclusion that this isolate would not contribute a lot to the over-wintering of larvae on pastures. Monnig (1930) states 'there is an optimal temperature for every living organism; lower or higher temperatures cause slower or more rapid development and frequently deterioration and death.' This statement holds true with variation in optimal temperatures for development and survival between the major nematode species. Ransom (1906) found the range of hatching times varied from two to

seven days at temperatures of 16--20°C. Later work by Veglia (1915) found that *H. contortus* eggs hatched within 14--24 hours at temperatures ranging from 26--35°C, whilst in uncontrolled temperatures of 15--18°C 50% of eggs hatched after 4 days. Kauzal's (1941) range for optimal development (25--30°C) was similar to Veglia (1915), reaching the L_3 stage by 4--6 days. In the laboratory, Coyne and Smith (1992a) also report optimum temperatures for L_3 development ranges between 20--30°C which is in concurrence with previous studies (Veglia 1915; Silverman and Campbell 1959; Gibson and Everett 1976). Berbarian and Mizelle (1957) found a much higher optimum temperature for development of *H. contortus* to L_3 of 33.3°C taking 60 hours to reach the infective stage. Thus, the higher the temperature, the faster the rate of development from egg to L_3; this is also supported by Rose (1963) (Table 1-1). The definition of optimal temperature for development seems unclear. Optimal temperature for some researchers means the temperature at which development is at its fastest rate, regardless of the concurrent death rate. Others seem to report the optimum temperature as that temperature at which the maximum rate of development is balanced with an acceptable death rate. The latter explanation seems to hold true for Besier and Dunsmore (1993b), who reported that optimal conditions for larval development on pasture over the few days following egg deposition were mean daily temperatures of 13.5--16.6°C, mean maximum temperatures of 18--22°C and visibly green pasture. These optimal temperatures are much lower than those reported by others mentioned above, however, they paint a truer picture than laboratory experiments provide. Optimal temperatures for development observed in laboratory conditions do not necessarily transfer to the field, where temperatures fluctuate daily and adequate moisture is not guaranteed. An optimum temperature of 25°C in the laboratory resulting in hatch within a week may not be a realistic outcome when transferred to the field. During the hottest part of the day, temperatures may reach 40°C leading to increased development but also increased mortality. Thus, optimum temperatures for development of the free-living stages in the field would be lower than any recorded in laboratory experiments, as demonstrated by Besier and Dunsmore (1993b).

Table 1-1-1: The effect of temperature on the rate of development of the free-living stages of Haemonchus contortus in moist faeces (Rose 1963):

	Time taken for development to third-stage larvae	
Temp (°C)	Minimum days	Maximum days
25-26	3	5
20-21	5	15
15-16	7	21
10-11	15	30

The magnitude of larval development and survival differs between field and laboratory studies. Rose (1963) found 100% development and hatching in laboratory conditions and one to seven percent recovery out-of-doors. He put this discrepancy down to the effects of continuous freezing and desiccation rather than egg infertility. Todd *et al.* (1970) reported recoveries in laboratory conditions of 50% and an average of 0.03% in the field situation. Other authors have reported a similar phenomenon in their studies (Kates 1950; Niezen *et al.* 1998b). The discrepancies between development of eggs to L_3 in the laboratory and in the field could be due to a number of factors, including: desiccation, unfavourable temperature, diurnal temperature fluctuations, moisture limitations, and predation by nematophagus fungi, dung beetles, other soil nematodes and possibly soil bacteria. This is another area in which laboratory experiments fail to simulate the reality of field situations.

1.6.2.1.1.2 Trichostrongylus colubriformis

Gibson and Everett (1967), in the United Kingdom reported the minimum range for development of *T. colubriformis* from eggs to L_3 was 10--15°C. In spring time (April/May) infective larvae did not appear on pasture until 14 weeks after deposition; later in the year, when conditions were more favourable, larvae on herbage reached a peak at 6 weeks. Larvae persisted on the herbage in large numbers for as long as 20 weeks. Boag and Thomas (1970), also in the U.K., found similar results to those above, with *T. colubriformis* larvae first recovered after 2 weeks in summer with a peak in infection after 6 weeks. Levine and Anderson (1973) implemented plot trials in 1965--66 to determine the development and survival of *T. colubriformis* on pasture and found that there was no embryonation when the mean weekly maximum temperature was 7°C, and unembryonated eggs died. They reported that optimal embryonation occurred at 36.8°C, which is considerably warmer than that reported by Ciordia and Brizzell (1963) in their laboratory study (25°C) and Monnig (1930) who found 26°C to be optimal for development for *Trichostrongylus* spp. Ciordia and Brizzell (1963) reported faster embryonation at 35°C, but found that this temperature was not favourable for complete development of the free-living stages, as first stage larvae were quite susceptible at this temperature. When temperatures rose above 40°C, Ciordia and Brizzell (1963) found eggs were killed within a few hours and no first or second stage larvae were isolated. Monnig (1930) had similar results with *Trichostrongylus* spp., finding that eggs developed rapidly at 37°C, but rarely hatched or the larvae died soon after. Levine and Anderson (1973) concluded that good pasture transmission conditions should occur for *T. colubriformis* when the total monthly rainfall is greater than 25 mm and the mean monthly temperature at soil surface beneath 7--10 cm of herbage is above 16°C. Anderson *et al.* (1966)

demonstrated, in the laboratory, that in the faecal pellet, where moisture was not limiting, the optimum temperature for survival of all egg and larval stages of *T. colubriformis* was 4°C. Embryonated eggs survived in large numbers (80%) at 4°C for 16 days, whilst 14% survived for 64 days at the same temperature. Embryonated eggs had a much greater survival rate at all temperatures than unembryonated eggs (Figure 1-5). As there is no development of eggs or larvae at this temperature, it may be inferred that embryonated eggs can remain dormant during colder months and subsequently develop when temperatures rise.

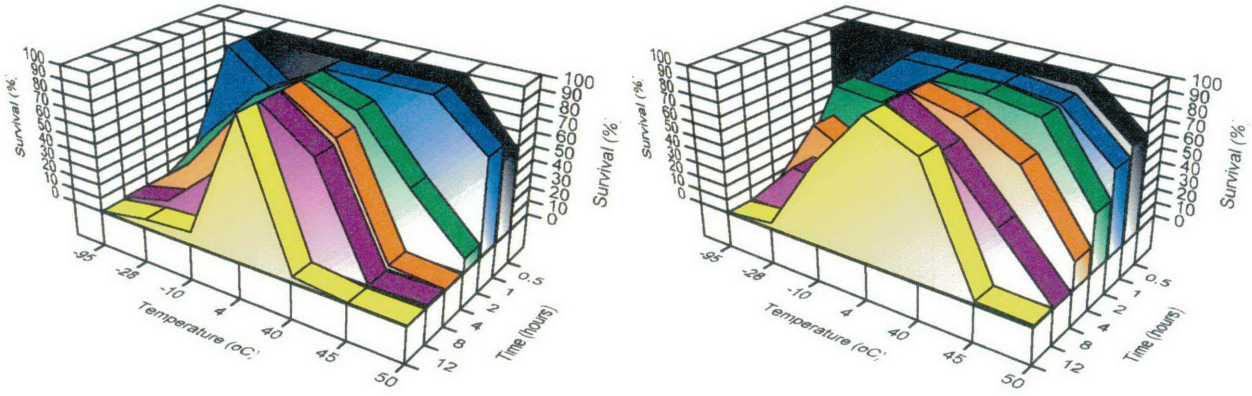


Figure 1-5: Survival of *Trichostrongylus colubriformis* unembryonated (left) and embryonated (right) eggs at varying temperatures tested over six time periods (Data from Anderson *et al.* 1966).

In the tropical environment of Fiji, Banks *et al.* (1994) recovered *T. colubriformis* throughout the year, including during the dry season on Viti Levu's western region; *H. contortus* failed to develop in the western region. During those dry months the optimum time for recovery of *T. colubriformis* was delayed, suggesting that the nematodes were affected by the lack of moisture. On the wetter eastern side of the island, larvae from both species were found year round. Larval counts were highest seven days after contamination, with proportionately higher numbers of *T. colubriformis* than *H. contortus* recovered. Survival of *T.colubriformis* was up to 9 weeks in mid-summer and up to 13 weeks as temperatures became more moderate (~23--25°C).

1.6.2.1.1.3 Teladorsagia circumcincta

Dinaburg (1945) studied the effect of low outdoor temperatures on the free-living stages of *Teladosagia circumcincta*, *Trichostrongylus colubriformis*, *Cooperia curticei* and *Oesophagostomum columbianum*. He found that the pre-infective stages of *T. circumcincta* were the most resilient to cold conditions, with survival below mean minimum temperatures of -9.4°C and development of the eggs to L_3 in a daily temperature range of minimum -1°C to maximum 15°C. At these same temperatures, the pre-infective stages of the other species were either just

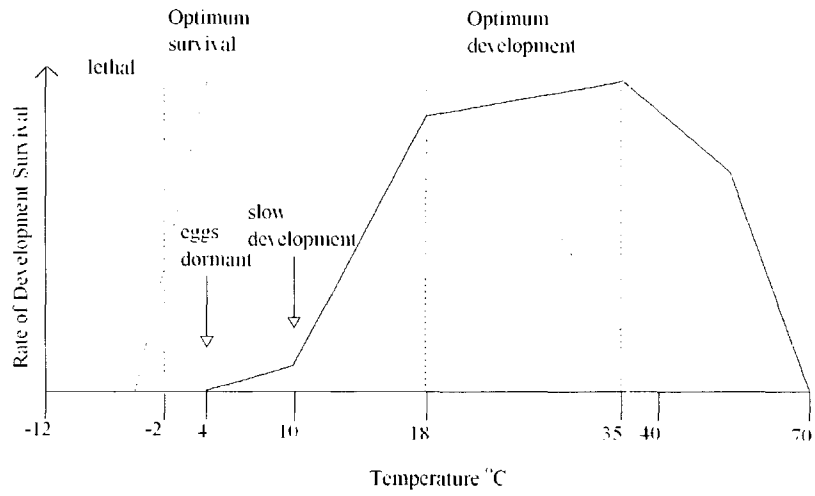
surviving (*T. colubriformis*) or dying (*C. curticei* and *O. columbianum*). Dinaburg's study, however, is confounded by moisture, as the pots containing the faecal matter were covered to protect them from rain, sunlight and snow and there is no indication that the pots were watered. Thus the failure of eggs to hatch and develop could also be attributed to lack of moisture. Crofton and Whitlock (1965b) reported hatching of *T. circumcincta* eggs at temperatures as low as 4°C and Gibson and Everett (1972) found development at all times of the year with mean maximum soil temperatures ranging from 2°C --23°C. The eggs of this nematode have been demonstrated by Kates (1950) to over-winter in below freezing temperatures. However, as with all outdoor studies, mention should be given to the insulating properties of snow found by other researchers (Gibson and Everett 1967). Jasmer *et al.* (1986) discovered a vast difference in the ability of *H. contortus* and *T. circumcincta* eggs to withstand cold temperatures. After exposure to -18°C, the survival of the eggs of *T. circumcincta* was >87% but <4% for the eggs of *H. contortus*. There was a significantly greater ($P<0.05$) amount of lipid stored in the *T. circumcincta* eggs, suggesting a cryoprotectant effect or greater energy stores promoting better survival of the eggs. Furman (1944) conducted studies into the effect of temperature and humidity on *T. circumcincta* eggs in the laboratory and found that temperatures above 37°C killed the eggs of this nematode within a few days. He reported an optimal temperature for development of *T. circumcincta* eggs of 27°C but survival was limited at this temperature. At the lower end of the scale the eggs survived for 1--2 months at 5°C in water and survived for the longest period (271 days) at temperatures just above freezing. Pandey (1989) reported a lower optimal temperature for development of *T. circumcincta* eggs of 16°C in water with eggs developing at 4, 16, 25 and 35 °C. When incubated in faecal material the eggs developed to L_3 at all temperatures except 4°C which contradicts other reports that this worm species develops at temperatures slightly colder than this (Gibson and Everett 1972). It is possible that there were variations between the strains used for these experiments with Pandey located in Morocco and Gibson and Everett in England.

In Armidale, Southcott *et al.* (1976) found larvae to be available within 2 to 12 weeks of faecal deposition, with infection peaks of *T. circumcincta* following autumn infections, with the peaks occurring in late winter and spring. The climate was milder than the previously mentioned studies, so the L_3 over-wintered easily and no lower temperature limitations on development to L_3 were observed. Similar results were obtained by Callinan (1978) in Western Victoria.

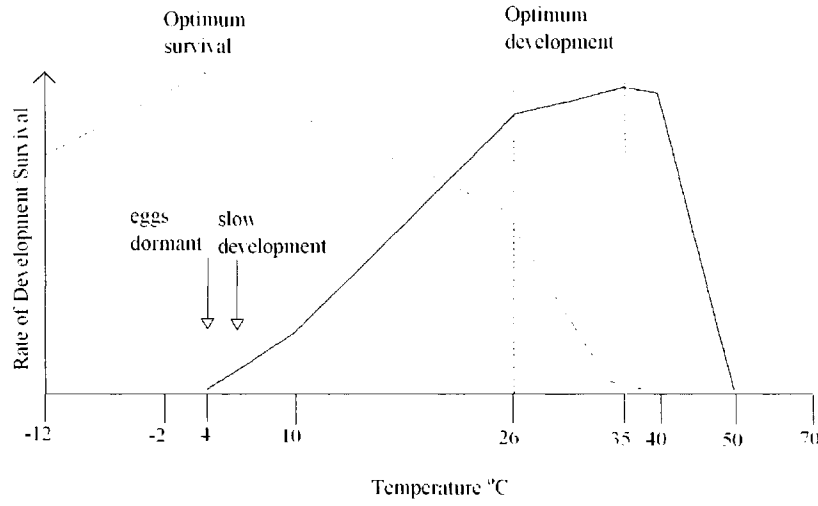
All three major nematode species have similar optimal temperatures for development in the laboratory: *H. contortus* 20--30°C, *T. colubriformis* 25--28°C and *T. circumcincta* 27°C (Figure 1-6). There was a larger range reported for *H. contortus*, from 25 to 35°C, and very few reports of an optimal temperature for *T. circumcincta*. The main differences between the effect of temperature on the eggs of these species is their ability to survive cold and below freezing temperatures. *Trichostrongylus colubriformis* and *Teladosagia circumcincta* were able to develop and survive at much lower temperatures (4--5°C) than *H. contortus* (~10°C), which was also severely affected by freezing and thawing. *Teladosagia circumcincta* was the most resilient of the three species against freezing and has been shown to develop at temperatures as low as 2°C, with eggs hatching at temperatures as low as 4°C. In general, development rate increases with increasing temperature, whilst survival time decreases (Figure 1-6).

The effect of temperature on the development of egg to L_3 has been extensively studied in the laboratory situation and in a more complicated manner in the field. Field experiments provide crucial information about development and survival in a real situation, however, in these situations moisture and temperature cannot be separated. Genetic comparisons of isolates of different origins may be of use to aid identification of isolates and comparison of results across continents.

***Haemonchus contortus* eggs**



***Trichostrongylus colubriformis* eggs**



***Teladorsagia circumcincta* eggs**

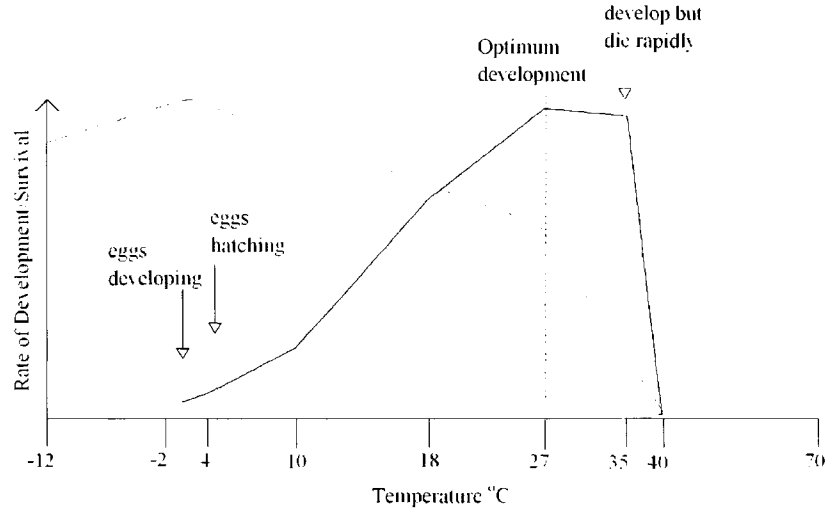


Figure 1-6: Representation of the rate of development (⟵) and survival (- -) of the eggs of three major nematode species at varying temperatures. The values for these figures are taken from various authors cited in section 1.5.2.1.1.

1.6.2.1.2 Effects of moisture

Gibson and Everett (1967) infer from their data that moisture is not as important a limiting factor as temperature. However, when conditions were dry, eggs perished rapidly, indicating again the interaction between temperature and moisture for the survival of infective larvae and the ambiguity of Gibson and Everett's inference. Monnig (1930) found embryonated *Trichostrongylus* spp. eggs to have resistance far exceeding other pre-infective stages and much greater resistance to drying than the infective larvae. Some embryonated eggs developed, when rehydrated, up to 15 months after drying. The pre-infective stages of *H. contortus* and *O. columbianum* were also tested and found not to recover from just one drying event. This is supported by an earlier study by Ransom (1906), who found that eggs and pre-infective stages of *H. contortus* were rapidly killed by drying. The effect of desiccation on *T. colubriformis* eggs and larvae was also investigated by Anderson and Levine (1968). They reported that eggs in the advanced stage of embryonation (E_4) were approximately as resistant to desiccation as third-stage larvae; this differs slightly from Monnig's findings. Unembryonated eggs were only slightly resistant to desiccation and first and second stage larvae removed from the pellet were highly susceptible to drying. They speculate that there is enough moisture within the pellet to facilitate development to the resistant embryonated egg (E_4) stage and that additional external moisture is required for further development to L_3.

Waller and Donald (1970) studied the effect of humidity on *T. colubriformis* and *H. contortus* in the laboratory, finding a marked difference between the two species. There was no development of *H. contortus* eggs in 4 of the 5 humidity treatments. The only treatment at which *H. contortus* would develop was 93% relative humidity (RH). The best response was from the E_4 stage, with some E_{2-3} developing and no E_1 developing in any treatment. The eggs of *T. colubriformis* developed in all humidity treatments, with the E_4 stage being most resistant to desiccation. The more advanced the eggs were at the time of desiccation the greater the chance of survival. Waller and Donald (1970) concluded that the only free-living stage of *H. contortus* resistant to desiccation is the L_3, as all the egg stages were highly susceptible to desiccation. This contradicts the results obtained by Silverman and Campbell (1959), who showed that *H. contortus* eggs resembled those of *T. colubriformis* in their ability to survive for long periods in the pre-hatch stage. Waller and Donald (1970) dismissed Silverman and Campbell's results as having little or no significance in the field, since the results obtained by Silverman and Campbell (1959) occurred under very exceptional circumstances found in their laboratory. Hsu and Levine (1977)

conducted laboratory tests on the effect of humidity on *H. contortus* and *T. colubriformis* eggs, producing results supporting those of Waller and Donald (1970), with *T. colubriformis* developing at lower relative humidity than *H. contortus* which would not develop below 85% RH. The egg shell of *H. contortus* was more permeable to water than *T. colubriformis*, making more susceptible to drying.

Rossanigo and Gruner (1995) studied the effects of faecal moisture content and temperature on eight species of gastrointestinal nematodes. An optimal value of between 60--70% faecal moisture content for development to L_3 held true for all species studied, including *H. contortus*, *T. colubriformis* and *T. circumcincta*. The development rate decreased rapidly when faecal moisture either increased or decreased from this value. This could possibly be due to lack of adequate moisture below 60--70% for egg development and lack of available oxygen for L_1 and L_2 development when the moisture content was above 60--70%. For *T. circumcincta*, the best rate of development was at 23°C and 60% faecal moisture content and at 18°C at a higher humidity. *Trichostrongylus colubriformis* eggs developed at an optimal temperature of 28°C when the faecal moisture content was 55--60%. This is 3°C higher than Ciordia and Brizzell (1963) reported, and approximately 9°C lower than the temperature reported by Levine and Anderson (1973). The faecal moisture content for optimal development for *H. contortus* was 70%. The moisture content reported in Rossanigo and Gruner (1995) of faeces at the time of emission from the sheep was 55--65%, suggesting that moisture may not be limiting for initial development of *T. circumcincta* and *T. colubriformis* but may be limiting to *H. contortus*. *Haemonchus contortus* and *T. colubriformis* were both limited by moisture below 60% faecal moisture content, whereas *T. circumcincta* were able to develop at much lower moisture contents with substantial development (10--20 L_3/100 eggs) occurring at 40--50% faecal moisture content (Rossanigo and Gruner 1995). It is difficult to reconcile the results where moisture is measured by faecal moisture or by humidity, as there is no indication of what the humidity is at different faecal moisture levels. Faecal moisture is a natural progression from fundamental humidity trials and may be a better guide to nematode egg development as it maintains the faecal pellet environment to which the eggs would be subjected.

Egg size has also been shown to affect the ability of an egg to survive desiccation. *Trichostrongylus colubriformis* eggs with a lower surface area to volume ratio had/have a higher probability of developing to the pre-hatch stage and surviving (Waller and Donald 1970). Waller

and Donald (1970) also reported more eggs hatched with increasing relative humidity and the mean egg volume increased along with humidity. There was also a shorter time required for smaller eggs to develop and hatch. This phenomenon was also demonstrated in *H. contortus* by Crofton and Whitlock (1965a).

Faecal pats have been shown to be a reservoir for infective larvae of cattle parasites providing a source of infection during drought and after it breaks (Barger *et al.* 1984). The larvae seem to survive in a dormant, desiccated state until re-hydration leads to reanimation. It is possible that this occurs with sheep parasites, however, the large bulk of cattle faecal pats would provide a better environment for survival over longer periods of drought.

The requirement for moisture for development from egg to L_3 differs remarkably between the 3 major parasites, with *H. contortus* being most susceptible to desiccation. *Trichostrongylus colubriformis* and *Teladosagia. circumcincta* are much more resistant to drying, with the latter being the most able to develop at lower moisture levels. All, however, were found to share the optimal level of moisture for development. Both the embryonated egg and L_3 stages of *T. circumcincta* and *Trichostrongylus* spp. are resistant to drying, whereas only the L_3 stage of *H. contortus* was resistant, with the eggs being highly susceptible to drying. More studies need to be conducted to measure the interaction between moisture/humidity and temperature on the development of *H. contortus* eggs to L_3, as many studies focus on one or the other with temperature studies often being confounded by moisture limitations.

1.6.2.2 Survival of third stage larvae on pasture

Survival of L_3 on pasture varies greatly, depending on the environment and species of nematode. Third-stage larvae can survive on pasture for many months with temperature and moisture again playing significant roles in the survival of the L_3 stage in the environment (Southcott *et al.* 1976). Herbage mass, pasture species, soil moisture, evaporation, and predators also affect the survival of L_3 on pasture. The optimum temperature for development to L_3 is generally higher than that for L_3 survival (Boag and Thomas 1985). Third-stage larvae are the most resistant to temperature extremes and desiccation of the free-living stages of nematode parasites. The larvae are thought to move from the faecal pellet and migrate up the pasture sward presenting themselves for ingestion. Being unable to consume food because they are enclosed in a sheath left over from the L_2 stage means that L_3 need to find a host or perish.

1.6.2.2.1 Effects of temperature and moisture on L_3 survival

1.6.2.2.1.1 Haemonchus contortus

As mentioned in 1.5.2.1.1, Ransom (1906) published the earliest observations on *H. contortus*, and noted that L_3 in the field could withstand continuous freezing and thawing with higher food reserves remaining in the gut cells than L_3 that had been kept in the constant laboratory conditions. He concluded that the cold conditions of winter cannot be relied upon to clear the pasture of infective larvae. Ransom inferred from the data that high humidity and moisture promotes the movement of L_3 out of the soil and up the grass blade, with movements ceasing as conditions become dry. Waller *et al.* (2004), in a Swedish study, reported a virtual absence of *H. contortus* in tracer sheep which were turned-out to graze in the early spring. They found high numbers of *H. contortus* in arrested development in tracers heading into the winter months, suggesting that *H. contortus* over-winters in the host rather than on pasture in Sweden. Donald (1968), near Sydney, NSW, did not find *H. contortus* L_3 until the end of winter and early spring, when minimum temperatures began to rise. A field study by Knapp (1964) in Urbana (USA) showed *H. contortus* larvae over-wintered and infected lambs the following spring. The pasture plots used in this study were infected with three-week old *H. contortus* L_3 at a rate of 10 000 L_3/foot. The minimum monthly temperatures were below freezing for 3 of the experimental months and temperatures ranged, in the coldest month, from -14.4 to 3.3°C (6 to 38°F). It is possible that snow cover acts as insulation with actual soil temperatures much warmer than the recorded air temperature (see section 1.5.2.2.2) (Griffith 1937 cited in Gibson and Everett 1967). Another study conducted in Urbana, by Levine *et al.* (1974) concluded that *H. contortus* and *T. colubriformis* larvae are affected in different ways by pasture conditions. The cold conditions of winter, with mean maximal temperatures below zero, killed *H. contortus* L_3 but not *T. colubriformis* L_3. In fact, *T. colubriformis* L_3 had greater survival overall, with the peak number of days they were recovered from vegetation being 80 days in January (mid-winter). There were two peaks in number of days *H. contortus* survived; these were in spring and autumn, with a dip in survival during the summer months (Figure 1-7). Jasmer *et al.* (1987) found the L_3 of their Washington isolates of *H. contortus* highly susceptible to freezing and freeze/thaw situations, supporting the results of Levine *et al.* (1974).

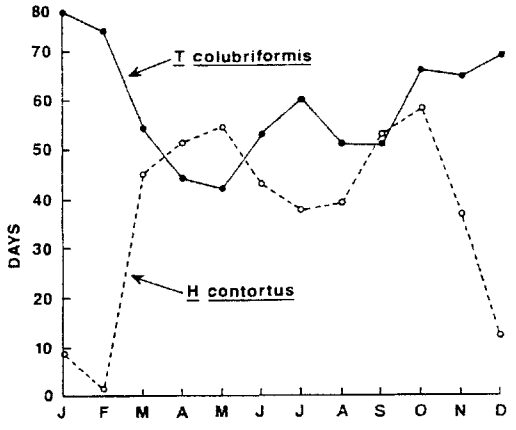


Figure 1-7: Number of days *Haemonchus contortus* and *Trichostrongylus colubriformis* infective larvae were recovered from vegetation after L3 had been placed on pasture, monthly for 1967--1969 (*H. contortus*) and 1965--1966 (*T. colubriformis*) (Levine *et al.* 1974).

Monnig (1930) reports the longest survival period for *H. contortus* to be 4.5 months under unfavourable conditions (dry slide in lab) and 9.5 months in more favourable conditions in irrigated sheltered pots of clay soil. In 'natural and favourable' conditions, larvae were reported to live up to 3 months in the soil but were killed by dryness and heat. Dinaburg (1944b) carried out plot trials on outdoor grass plots in Beltsville, Maryland, USA and found that larvae did not survive for longer than about 2.5 months regardless of the season. However, season did seem to affect survival with one percent of L_3 from the inoculum recovered after 14--28 days in summer, 27--41 days in winter, 42--56 days in autumn and 56--70 days in spring. With larvae being susceptible to desiccation and abnormally high or low temperatures, the ranking of season from shortest to longest L_3 survival times were summer, winter, autumn, spring for this study. Many of the early studies on *H. contortus* ecology presume that infective larvae die out by around 3 months and that most are dead before 6 months after deposition (Kates 1950; Donald *et al.* 1978a). Over-wintering of *H. contortus* was observed by Rose (1963) in south-east England, Thomas and Boag (1972) near London and Southcott *et al.* (1976) found *H. contortus* and other nematode species were picked up by tracer sheep 12 months after egg deposition near Armidale, NSW. Larval survival in the tropics presents a different picture. In the rainy season, Aumont and Gruner (1989) reported that L_3 appeared on pasture seven days after contamination with a peak on L_3 between day 14 and 21 after contamination. Maximum survival of L_3 ranged between 49 and 56 days. In the dry season L_3 were only found on two occasions and never in the cultured faeces. Banks *et al.* (1990) conducted ecological studies in Fiji, reporting maximum larval counts one week after contamination with conditions for hatching being ideal (high daily rainfall, mean

monthly maximum 25-30°C). Larval counts were found to be below detectable levels only 9 weeks after contamination, and the majority of the larvae dead at 4--5 weeks. So, whilst conditions in a tropical environment are often favourable for rapid development to L_3, their survival is much shorter than in temperate environments.

Dinaburg (1944a) reported that, in the high temperature range (maximum daily temperatures of 86 to 93°F (30 to 34°C), exposing L_3 to full sunlight resulted in lower recovery of larvae than exposure to full or partial shade,. This most likely occurs because the larvae use up their metabolic reserves at the higher temperatures. Recoveries were greater in full shade than partial shade, regardless of rainfall, and this could be due to the indirect effect of increasing desiccation with increasing sunlight and temperatures. His observations on the development of larvae shows the number of larvae recovered clearly increases as rainfall increases within mid-range temperatures (66--84°F or 19--29°C). There is little development in the low temperature range for all rainfall (0--7.5mm) and moderate recovery in high temperatures but only in full shade. Dinaburg concluded that '... in designating large periods of the year as unfavourable for the survival and development of the eggs of *H. contortus*, temperature is a more reliable weather factor than rainfall.'

Kates (1950), who worked in Beltsville, Maryland, also reported the destructive effect of high temperatures and dry conditions on L_3. The best recoveries were in August, when there was moderate rainfall and moderate weekly maximum, minimum and average temperatures. The L_3 that developed from eggs deposited during August survived 3.5 months after deposition, whereas larvae in less favourable conditions were not recovered after 1 month. Cool-dry conditions and warm-dry conditions were both destructive to *H. contortus* L_3. Kates (1950) concluded that the free-living stages of *H. contortus* are the least resistant to weather of the 10 parasitic nematode species he studied, with development and survival only occurring when warm and moist weather is continuous. Development and survival are confounded in Kates' experiments as he was estimating L_3 survival based on egg deposition.

Besier and Dunsmore (1993a), working in the south of Western Australia, reported that infective larvae of *H. contortus* were recovered for longer periods as temperature decreased and rainfall increased. They reported a linear relationship between survival period and temperature, where survival increased as temperature decreased. Conversely larval movement on the sward is

positively correlated with temperature (Krecek *et al.* 1992). Shorb (1944) demonstrated that watering of plots daily and weekly with varying amounts of water yielded 24 times as many L_3 as plots without water. The increase in larval survival appeared to be more a result of constant water than the amount given, although there were three times as many larvae on the plots given a quart (946ml) daily than those given 0.33 quart (312ml) daily, and five times as many larvae when given a quart (946ml) weekly than plots given 0.33qt (312ml) weekly. Studies in Ibdan, Western Nigeria, showed that, when temperature is constantly favourable (minimum 24.8 to maximum 30.0°C), the most important epidemiological factor was rainfall. When rainfall was at least 3mm per day, the development and survival of larvae on pasture increased from zero to 60 days (Okon and Enyenihi 1977). Besier and Dunsmore (1993a) demonstrated a positive linear relationship between larval survival and mean rainfall frequencies and a negative linear relationship between larval survival and air temperature and mean evaporation. However, no significant relationship was observed between mean rainfall, dew point and ground level temperatures and larval survival but there was a significant positive correlation between green pasture material and larval survival. The green material content of the pastures was positively correlated with rainfall.

Levine and Todd (1975) stated that soil moisture and temperature were the two most important determining factors in L_3 survival on pasture and that meteorological conditions recorded at a weather station do not reflect conditions at ground level. However, Krecek *et al.* (1992) showed a close correlation between soil temperatures and air temperatures, concluding that air temperature is a good predictor of L_3 movement on pasture displaying a positive linear relationship between L_3 recovered on pasture and temperature.

Barger *et al.* (1972) developed a model for simulation of pasture larval populations of *H. contortus* based on data from a number of sources (Dinaburg 1944a; Roe *et al.* 1959; Silangwa and Todd 1964; Donald 1968; Swan 1970). The assumptions of the model are based on the work of Donald (1968), Swan (1970), and Dinaburg (1944a) and include a maximum egg hatch time of five days, minimum daily temperature for hatching of 10°C with maximum daily temperature 18°C or above and percentage of eggs hatching varying with temperature. After hatching, the L_3 stage must be reached within 21 days or death will occur. The cumulative precipitation:evaporation ratio must exceed one for up to three weeks after hatching. The death rate of L_3 is considered a function of temperature and humidity and is assumed to be exponential.

This model has good agreement with actual observations taken by the experimenters ($R^2 = 0.86$). A representation of the model is shown in Figure 1-8. At higher temperatures the rate of larval die off is faster with the half-life of L_3, being 24 days at 30°C, 35 days at 22°C and 49 days at 15°C.

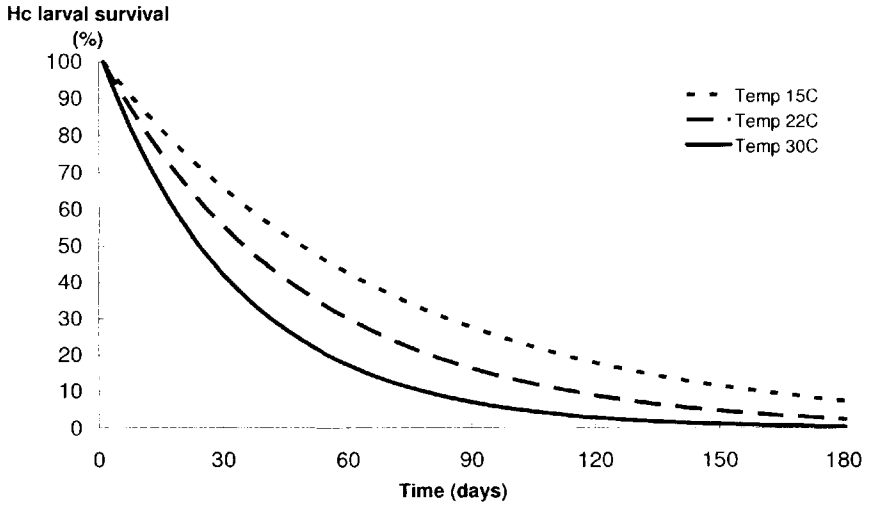


Figure 1-8: An application of the model of Barger *et al.* (1972) showing the effect of temperature on survival of *Haemonchus contortus* larvae on pasture as a percentage of larvae surviving from an original population. (Created by L. Kahn using data from Barger *et al.* 1972).

From Barger's model and other experiments reviewed in this section, it is clear that *H. contortus* develops faster in warm moist conditions. Its lower limit for development is generally 10°C and the species does not respond well to freeze/thaw situations. However, *H. contortus* survives well as L_3 in cooler temperatures and has been shown to survive freezing temperatures to over-winter on pasture in some climates. These factors combined mean that *H. contortus* outbreaks are generally restricted by climatic conditions. Warm, wet summers and mild winters are conducive to endemic haemonchosis.

There are many conflicting observations on the effect of temperature on the free-living stages of *H. contortus* and often experiments on L_3 survival are confounded by moisture and by egg and pre-infective larval development. Eggs deposited on pasture are often used to measure L_3 survival rather than the third stage larvae themselves. The use of deposited eggs to determine the survival of infective larvae on pasture gives an indication of the real dynamic process that is occurring in faecal pellets and on pasture. Donald (1973) writes that the use of cultured infective larvae for determining larval survival gives an incomplete or misleading understanding of the

likely course of infective larval availability on pasture. However, the requirements of temperature and moisture for development of the pre-infective stages and the survival of infective larvae are quite different; thus studies involving L_3 deposition should not be discounted.

Conflicting results involving temperature generally occur between different climatic regions and continents, which could indicate a difference in nematode isolates and/or the localised effects of climate on the survival of L_3 on pasture. It is difficult to extrapolate the findings of researchers in Urbana, where snow is frequent in winter, and apply them to control programmes in Africa, Fiji, New Zealand or Australia. Ecological studies should be referred to in the context of the climatic conditions in which they were conducted. Each region should acquire their own ecological knowledge for the development of control measures.

1.6.2.2.1.2 Trichostrongylus *spp.*

Monnig (1930) exposed larvae of *Trichostrongylus* spp to light, high temperatures, desiccation and freezing; measured longevity in the soil; conducted larval migration studies; and also did some outdoor pot studies. He came to the following conclusions concerning L_3 survival: L_3 of some *Trichostrongylus* spp. (chiefly *T. instabilis* with some *T. rugitus*, *T. falculatus*, *T. colubriformis* or *T. axei*) prefer mild diffused light; they are able to infect lambs after exposure to freezing for 10 days; and they revived after being on dry slides for up to 8.5 months and were able to infect lambs. In sheltered pots of clay soil watered from the bottom, L_3 lived for up to 8.5 months but in natural conditions will survive for only 3 months as they are rapidly killed by dryness and heat. Through his experiments in Beltsville, USA, Kates (1950) found *Trichostrongylus* spp. L_3 (mainly *T. colubriformis* with small numbers of *T. axei* and *T. vitrinis*) to be similar to *H. contortus* in their ability to over-winter, but to be more resistant to warm-dry and cool-dry weather. The survival patterns in spring and summer were similar to *H. contortus* but with a higher proportion of *Trichostrongylus* spp. surviving. Anderson *et al.* (1966) demonstrated, in the laboratory, that the optimum temperature for survival of *T. colubriformis* L_3 is 4°C, whereas the optimal temperature for development has been reported as 25--36.8°C (Ciordia and Brizzell 1963; Levine and Anderson 1973). Ciordia and Brizzell (1963) reported high mortality rates of larvae above 35°C in laboratory studies. Anderson (1966) found temperatures of 45 °C and 50°C were rapidly fatal to *T. colubriformis* infective larvae. There were small numbers of L_3 alive after 65 days (1%) at below freezing temperatures (-10, -28 and -95°C). This suggests that, in most cases, a very small number of *T. colubriformis* L_3 will survive

below freezing winter conditions. In laboratory studies, Anderson and Levine (1968) found that desiccation of *T. colubriformis* L_3 was beneficial to survival of below freezing temperatures. Half of the L_3 that had been desiccated prior to freezing survived the low temperatures for 128 days compared to zero survival for the non-desiccated larvae. This success did not translate to larvae in the faecal pellet, with only 7% surviving -95°C, 5% surviving -20°C, and 12% surviving -10°C, if desiccated in the faecal pellet. Desiccation of larvae prior to freezing possibly increases survival through lowering the amount of water available to form cell damaging crystal formations.

A comprehensive study on the ecology of the free-living stages of *T. colubriformis* by Gibson and Everett (1967) in southern England reported no loss of larvae, whilst their plots were covered with snow. An insulating effect of the snow was demonstrated with air temperatures frequently as low as -8°C, while the temperature under the snow was constant, rarely falling below 0°C. *Trichostrongylus colubriformis* larvae have been found to survive in Canada at below freezing conditions, when air temperatures were as low as -26°C (Griffith 1937 cited in Gibson and Everett 1967). Another Canadian researcher found ground temperatures to which the larvae would have been exposed to under the snow to be really more like -4 to 0°C (Swales 1940 cited in Gibson and Everett 1967). Anderson *et al.* (1970) found similar results to Gibson and Everett (1967) in Urbana, USA, with larvae placed in experimental plots in late autumn, winter and early spring surviving for longer periods than those placed in plots in summer. It was inferred that the effects of temperature impacted on the survival of *Trichostrongylus* spp.; the cooler the temperature, the better the survival of the L_3.

A later study testing the importance of over-wintering by Thomas and Boag (1972) found over-wintering of the L_3 of a number of parasitic nematode species (including: *Haemonchus contortus, Trichostrongylus* spp., *Teladosagia circumcincta*) on pasture produced a wave of infection in spring, independent of the peri-parturient rise in faecal egg counts. It was reported that dosing ewes prior to the 'spring rise' reduces infection on clean pasture but not on pasture that had been contaminated the previous summer/autumn. *Teladosagia circumcincta*, followed by *Trichostrongylus* spp., showed the greatest ability to over-winter, indicating over-wintering as L_3 on pasture is an important survival mechanism for these two nematode species. In Armidale, Southcott *et al.* (1976) reported over-wintering of *Trichostrongylus* spp. and infection peaks in

summer, late winter and spring, although temperatures in that region do fall as far below zero during winter as those in the United States and Europe.

1.6.2.2.1.3 Teladosagia (Ostertagia) circumcincta

The literature on *Teladosagia circumcincta* indicates that it develops and survives at lower mean temperatures than *Trichostrongylus* spp. and *Haemonchus contortus*. In laboratory experiments, infective larvae survived over 271 days at 5°C; mortality increased at 27°C, 37°C and 45°C. Although the infective larvae were highly resistant to very hot and very cold temperatures, desiccation was quite destructive (Furman 1944). Dinaburg (1945) observed that the infective larvae of *T. circumcincta* were very resistant to sustained below freezing temperatures. Field studies by Kates (1950) confirm these reports with optimum development and survival of *T. circumcincta* occurring in the cool seasons, with the infective larvae and eggs being resistant to winter temperatures including extended periods of below freezing temperatures. Kates (1950) also observed that summer conditions with high temperatures and high evaporation causing desiccation adversely affected *T. circumcincta* infective larvae. Large numbers of free-living *T. circumcincta* over-wintered from eggs deposited the previous autumn. In summer in Northumberland, England, *T. circumcincta* larvae were found after 3 weeks and peaked at 6 weeks after deposition of eggs. In April, whilst *T. colubriformis* larvae disappeared altogether, the *T. circumcincta* larvae increased and disappeared only in May. Small numbers of *T. circumcincta* were recovered in the winter months, with that species being more numerous and persistent in the following spring and summer months. Gibson and Everett (1972) in the U.K. also observed *T. circumcincta* larvae surviving low winter temperatures. In the summer months the larvae peaked between 4--6 weeks and fell sharply to remain at lower levels throughout summer. It is difficult to gauge which starting point the authors are referring to when they say larvae survived until July/August the following year, but from the graphs it seems that larvae persisted on pasture for approximately 12 months. The over-wintering ability of *T. circumcincta* was also demonstrated by Thomas and Boag (1972), when they administered an anthelmintic treatment to ewes prior to lambing and placed them on a pasture that had been contaminated the previous summer/autumn. The predominant species found in the lambs was *T. circumcincta*. Another experiment by Boag and Thomas (1985) on the effect of temperature on survival of nematode parasites showed that *T. circumcincta* survived longer than any other species at both low and high temperatures (over 800 days at 5°C, 154 days at 30°C, Figure 1-9). These results are similar to those reported for the bovine nematode *Ostertagia ostertagi* by Pandey (1972), who found the optimal temperature for survival of *O. ostertagi* was 4°C, with the larvae

surviving for shorter periods at higher temperatures. Subjecting *T. circumcincta* to a single freeze/thaw event decreased viability to 32% (-15°C/3°C) and 48% (-10°C/3°C) compared to *H. contortus* where no larvae survived. Jasmer *et al.* (1987) studied the effect of cold temperatures on *T. circumcincta* L_3 in the laboratory, finding 85% survived a single exposure to -18°C for 5 hours, compared to <5% *H. contortus* larvae surviving the same conditions; these are much higher survival rates than those observed by Pandey (1972).

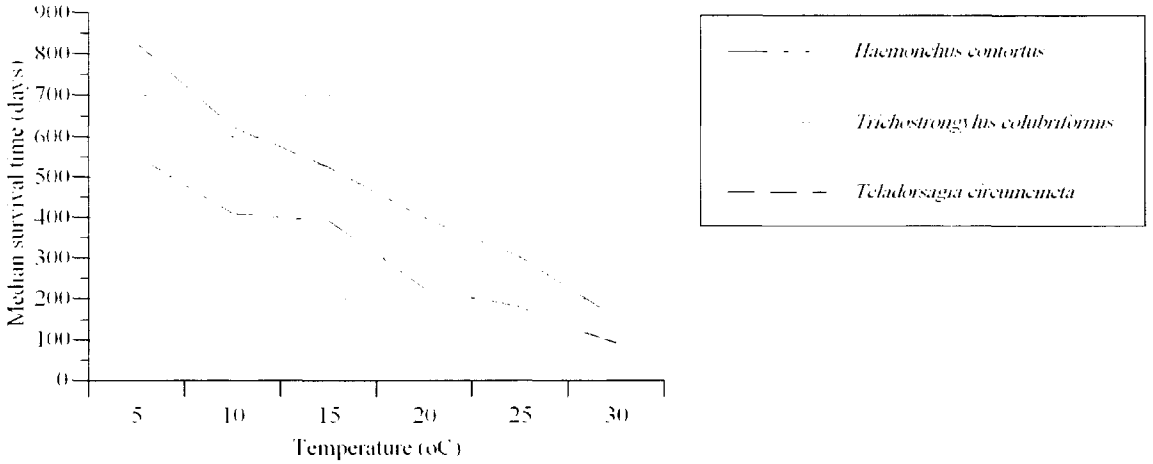


Figure 1-9: Graphic representation of the data collected by Boag and Thomas (1985) on the survival time of the third stage larvae of 3 major nematode species.

Teladosagia circumcincta L_3 seem to be the most resilient to high and low temperatures of the 3 major parasitic nematode species but are susceptible to desiccation. *Trichostrongylus* spp. have similar properties in the mid range of temperatures (10--20°C) to *Teladosagia circumcincta* but are more resilient to drying. *Haemonchus contortus* has the worst resilience to low temperatures with no firm data on the effect of moisture on the third stage larvae of this species. *Haemonchus contortus* does not differ in its resilience at higher temperatures from the other nematode species (above). All nematode species have a small number of L_3 surviving for more than 12 months. All seem to have a similar temperature of 4--5°C for optimal survival. This temperature is identical to that for survival of their eggs. This is most likely due to a slowing of the metabolic rate at this temperature so there is not the damage that is usually associated with freezing.

1.6.2.2.2 Migration of third stage larvae

The ability of larvae to migrate from the faecal pellet and to climb vertically up the pasture sward is an important factor in their presentation to the host animal. Ransom (1906) noted the facility of *H. contortus* L_3 to ascend perpendicular surfaces when the air was saturated with

moisture; this is now referred to as vertical migration and is not unique to *H.contortus*. Rogers (1940) carried out a number of experiments in a laboratory situation on grassed pots. He observed that *Trichostrongylus* spp., *Haemonchus contortus*, *Teladosagia circumcincta* and *Chabertia oxina* all had the ability to migrate vertically, and found that moisture favoured vertical migration, with increasing quantities of moisture up to 85% saturation in the soil assisting larvae to move up the grass sward. However, more than 0.12ml of water per square centimetre on the soil surface impeded movement and 0.2ml/square centimetre prevented migration up the grass. Light and temperature were also found to affect migration. The greatest numbers of larvae migrated when the light intensity was 62 f.c. with the range tested being 0 to 1240 footcandles (f.c). There were two peaks of migration for temperature, a small one at 5°C and a larger one at 45°C; the range of temperatures tested was also between those 2 figures. The greatest number of larvae were recovered from grass in the early morning. An earlier study by Monnig (1930) observed that larvae cannot migrate upwards out of water due to surface tension, thus, although moisture can assist the movement of larvae, it can also impede it if volume is too high. Rees (1950) also found that the most important factors in larval migration were temperature, humidity and light, with the greatest number of larvae recovered in the early morning and evening. When passing from winter to summer, the time of the morning peak in larval availability became progressively earlier and the time of the evening peak progressively later, suggesting an effect of temperature and light on larval activity. It was found that low and high temperatures inhibited vertical migration on grass and that high humidity did not prevent migration. These results differ slightly to those of Rogers (1940) who observed an evening peak. Silverman and Campbell (1959) observed that both *H. contortus* L_1 and L_2 migrated from faecal pellets. The number of L_1 migrating was very small, however, the numbers of L_2 migrating out of the pellet were substantial and, at times, exceeded the numbers of L_3 recovered. Rose (1963) reported larvae migrated from faeces to herbage within a short time of becoming infective, observing that the majority of larvae did not migrate further than 2 inches (5cm) from the outer edge of the faecal pile, a minority migrating between 2 and 6 inches (5 to 15cm). Vertical migration of larvae to both the 'lower' (<5cm) and 'upper' herbage (>5cm) was observed, with the greater proportion of larvae being on the former. Large numbers of larvae were recovered from the soil on two occasions, with very few recovered from the soil at the majority of sampling times. A further study by Silangwa and Todd (1964) found that, although larvae have the ability to move up the grass blade, they are generally found at the base of the vegetation. The morphology of the grass can also have an effect on the ability of larvae to migrate, with smooth

grasses affording an easier migration than hairy grasses (Niezen *et al.* 1998a). Silangwa and Todd (1964) reported that migration was favoured by higher relative humidity and moderate temperatures. The vertical migration of nematode larvae has been well established. Lateral migration of *H. contortus* was investigated by Skinner and Todd (1980), who found a few larvae had migrated up to 90cm from the faeces after 24 hours, although generally 90% of the larvae were found within 10cm of the faeces. The number of larvae diminished logarithmically with distance from the faeces. They also noted that more lateral migration occurred in cool, wet weather than in cool, dry weather and was positively correlated with larval survival. There is also a large amount of migration of infective larvae into the soil matrix. Callinan and Westcott (1986) found eight times as many larvae in the soil than on herbage and no positive migration from soil to herbage was observed during their experiments. After recovering 20% of the original infection over 4 days, only 2% of the larvae were on the herbage, 1.3% were found below 2cm of soil, with the majority, 71%, found in the top 2cm of soil.

1.6.2.2.3 Effects of pasture height and pasture type on L_3 survival

Rose (1964) concluded that pasture height and soil moisture were the most important factors in L_3 survival of *H. contortus*. Knapp (1964) found that pasture species also had a marked effect on the ability of *H. contortus* to over-winter, with clover species producing significantly higher infections in tracer lambs than grass species. The clover species had heavier mats and a greater abundance of foliage than the grasses, indicating that plant structure and density may be important factors in the survival of *H. contortus* larvae during cold temperatures. Grasses may leave larvae more exposed to fluctuations in temperature. Moss and Vlassoff (1993) found a ryegrass (*Lolium perenne*) based pasture to have the best survival rates for predominantly *Trichostrongylus* spp. and *Teladorsagia* spp., however, that pasture type had a significantly higher proportion of white clover (*Trifolium repens*) (19% rye grass with 61.6% white clover) than the other pasture types (treatment species varied from 53%--91%), which clouds the results of this study. The authors all but dismiss the effects of clover on the premise that the ryegrass is more conducive to larval movement than the other grass species used. The other grass was Prairie grass (*Bromus unioloides*), which has small hairs covering its leaves. However, given the low proportion of ryegrass as a treatment species compared to the other species trialled, it is clearly misguided of the authors to attribute so much to the presence of rye grass affecting the survival of L_3, especially in the presence of evidence from Knapp (1964) that clover has such a positive effect on larval survival. Niezen *et al.* (1998a) found an effect of herbage type on

development of *Ostertagia* spp. and *Trichostrongylus* spp. but no effect on larval survival. Topographical aspect is another variable which affects the survival of infective nematode larvae. In New Zealand, Neizen (1998b) found significantly greater numbers of infective larvae on herbage on the north than south-facing aspect, with the difference in larval numbers attributed to the faster faecal decay on the southern aspect due to more moist conditions. Besier and Dunsmore (1993a) found the factor most correlated with *H. contortus* larval survival was the visual assessment of pasture growth, with the percentage of green herbage also correlated with greater larval survival. Shorb (1944) demonstrated the importance of pasture height, when he found low recoveries of L_3 (21 larvae) from plots with no ground cover compared to plots with 4 inch (10cm) tall grass which recovered 5000 L_3. Shorb concluded that adequate grass cover favours the survival of L_3. However, that study involved the deposition of eggs onto grass plots, thus L_3 survival in this case was confounded by larval development.

1.6.3 Pathogen influences on epidemiology

Pathogen influences on epidemiology vary with species and include regulation of the parasite population (in the host and in the environment) through egg output and differences in free-living ecology (as demonstrated above). There are major species differences in the population dynamics of gastrointestinal nematodes within the host. Populations of adult *H. contortus* nematodes have been shown to have a rapid turn over rate, with senescing adults being constantly replaced by incoming infective larvae. It is thought that the adult nematode population reaches a threshold at which incoming larvae are inhibited from further development causing arrested development (Courtney *et al.* 1983). Barger *et al.* (1985) noted that the pattern of worm counts started with an accumulation of adult nematodes for 6--9 weeks at a rate that was positively correlated with the level of larval intake. This peak at 6--9 weeks was followed by a loss of nematodes. There was also an accumulation of early L_4 in the first 9 weeks, followed by a decline in adults, early L_4, and late L_4, possibly the result of resistance by the host. They concluded that the establishment rate of incoming larvae declined rapidly after several weeks of infection and the rate of decline was not related to rate of larval intake. However, loss of established adult worms was more influenced by current rates of larval intake than they were by adult worm numbers. Thus, for a given age of sheep and set of conditions, intense infections resulting in high levels of pasture contamination will be short-lived, whilst low-level infections of *H. contortus* will be more persistent. Another aspect of *H. contortus* is the ability of the inhibited larvae to survive over winter within the host body; this is called hypobiosis. What

regulates this phenomenon is not clear, Waller and Thomas (1975) do not believe that it is mediated by host resistance or by seasonal changes in temperature. However, they did not rule out changes in photoperiod as a possible contributor and considered hypobiosis to be an adaptation of the nematode to overcome unfavourable external conditions. The phenomenon of hypobiosis also occurs with *T. circumcincta* larvae, Waller *et al.* (2004) found that it occurred to a lesser extent in this species than with *H. contortus*.

Populations of *T. colubriformis* are not regulated by rapid turnover of adults. Instead the adult nematode population tends to accumulate over time and is not generally associated with the level of larval intake (Courtney *et al.* 1983). A series of experiments were conducted by CSIRO livestock researchers to determine the adult establishment rate of *T. colubriformis*, which led to the development of a model to simulate experimental infections (Barnes and Dobson 1990; Dobson *et al.* 1990a; Dobson *et al.* 1990b; Dobson *et al.* 1990c). Populations of established adults increased until the development of primary host immunity, which caused a reduction in the proportion of incoming larvae that became established. Arrested development (hypobiosis), reduced fecundity and loss of the adult population followed.

These differences in population dynamics of these species translate into differences in epidemiology of their diseases. Both species at high doses trigger an immune response in the host, but this response took a number of weeks to take effect, changing egg output and nematode populations. Other influences of the pathogen include fecundity of adults. Species differences were mentioned in the life cycle section of this review but are again briefly described here. *Haemonchus contortus* has a daily egg output that far outstrips other major nematode species (*H. contortus* 6582 eggs/day, *T. colubriformis* 262 eggs/day). Egg production is largely a function of parasite size but the susceptibility of the free-living stages of *H. contortus* to desiccation and low temperatures is an additional reason for this parasite to produce such a high number of eggs (see section 1.4.2). *Haemonchus contortus* infections tend to have periods of rapid accumulation triggering acute disease, whereas *Trichostrongylus colubriformis* and *Teladosagia circumcincta* infestations tend to be characterised by slow accumulation and persistence of populations.

1.6.4 Host influences on epidemiology

The host has the ability to affect the epidemiology of gastrointestinal nematode infections through resistance and resilience to infection and rejection of the nematode population or

restricting nematode egg output through immunological response mechanisms. As mentioned in the pathogenesis of gastrointestinal nematodes (section 1.4), the age and sex of the hosts have a major impact on the level of disease. Older sheep, which have developed immune systems, are better able to resist parasitic nematode infection than immunologically naïve lambs. Also, female sheep are more immune than entire males and possibly more immune than emasculate males, however, there are no conclusive studies on the latter. Reproductive status also has a major impact on epidemiology, with the peri-parturient relaxation of immunity in ewes being a major contributor to lamb infection levels in some situations.

1.6.4.1 Immune response

The immune response to helminth infection is complex, with the antibody-mediated immune response, predominantly IgE, being the most important. It is directed primarily against extracellular or exogenous invaders and thus is of primary importance when considering the immune response to helminth infections (Tizard 1996). Specific antibody is produced in response to antigens which stimulate an immune response. Antibodies come in a number of isotypes. Immunoglobulin G (IgG) is found in high concentrations in the blood. Structurally it is quite small (180 000 Da) and is rapidly transported throughout the body to a site of antigen detection. There are two sub-classes of IgG, namely IgG1 and IgG2. They have similar binding properties but have some differences in biological activity (Aalund 1972). Immunoglobulin A (IgA) is found in low concentrations in the blood and prevents foreign antigens from adhering to body surfaces. It is thought that IgA and IgG antibodies contribute to resistance by neutralising vital metabolic enzymes of *H. contortus*, interfering with the nematodes' ability to feed (Schallig 2000). Sheep bred for resistance to the disease have been shown to have significantly higher levels of IgA, IgE and IgG than non-resistant sheep (Gill *et al.*; Bisset *et al.* 1996; Shaw *et al.* 1999).

Immunoglobulin E (IgE) is commonly involved with Type 1 hypersensitivity reactions and is largely responsible for immunity to parasitic nematodes. It has a very low concentration in the blood of infected animals and is found primarily in tissue bound to mast cells and eosinophils (Aalund 1972). Nematode antigens promote the activity of T helper 2 cells (Th2), which in turn promote the elevation of IgE antibody levels (Finkelman *et al.* 1991; Tizard 1996). The activated Th2 cells produce increased levels of interleukin-5, which triggers an increase in eosinophil numbers, which can rise by 10- to 30-fold. The eosinophils, along with macrophages and platelets, bind to the IgE coated nematodes and are activated, leading to the destruction of the

parasite. Gut and vascular permeability are also increased, allowing an efflux of fluid into the lumen, which may cause the dislodgement and expulsion of the nematode population. This IgE dependent eosinophil-mediated response is thought to be the most significant mechanism of host resistance to helminth parasites and is involved in the 'self-cure' reaction (Figure 1-9). However, other immunoglobulin responses also play a protective role, including, IgA, IgG and IgM (Tizard 1996). Other immune responses, such as the cell-mediated response involving the T cell system, are also involved in resistance to helminth infections. Lymphocytes are thought to have an important role in the innate immune response against nematode infections, as sheep that are totally naïve to *H. contortus* showed lymphocyte proliferation when exposed to soluble L_3 antigens (Torgerson and Lloyd 1993).

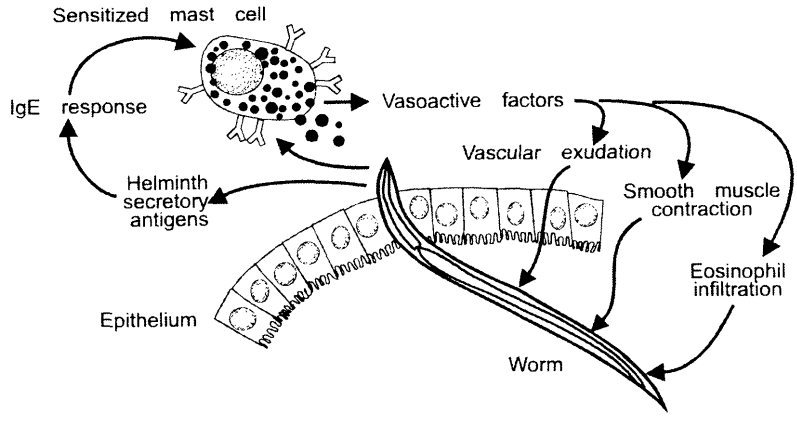


Figure 1-10: Self-cure reaction to *Haemonchus contortus* infection involving IgE mediated response (Tizard 1996).

1.6.4.1.1 Peri-parturient rise in faecal worm egg counts

The phenomenon of the peri-parturient rise in faecal egg counts coupled with a relaxation of immunity in the ewe is well documented. The relaxation in immunity is thought to be more closely related to lactation than the actual parturition, as ewes that do not raise a lamb do not express the rise in faecal egg counts (Connan 1967). However, Gibbs and Barger (1986) strongly disagree with this statement, suggesting that the rise in faecal egg count occurs before lactation and is more closely associated with lambing. The same study found that lactating ewes had acquired greater burdens of *T. circumcincta* and *T. colubriformis* than dry ewes but were equally resistant to infections of *H. contortus*. There are a number of causes thought to contribute to the peri-parturient relaxation of immunity in sheep; these include hormonal suppression of the immune system, immune regulatory proteins that circulate during lactation (Lloyd 1983), and stimulation of quiescent larvae through hormonal changes (Salisbury and Arundel 1970).

1.6.4.1.2 Self-cure phenomenon

Self-cure was a phrase coined by Stoll (1929) to describe a sudden and massive expulsion of worms from the abdomen (referring to *H. contortus*), after which eliminated nematodes may be found in the faeces. It is often associated with *H. contortus* infection (Gordon 1948; Olsen and Krakowka 1979) but others report self-cure in *T. colubriformis* infections (Stewart 1953; 1955). There is some conflict over the actual mechanisms of self-cure, with two schools of thought, one promoting the influence of new pasture growth, the other promoting an immune response. Gordon (1948) hypothesised that self-cure is not a mechanism of resistance, improvement in nutrition, or worms 'dying of old age', rather it may be caused by a possible 'anti-helmintic' compound present in rapidly growing grazing plants, as self-cure often occurs after rainfall and the onset of new green growth of pasture. Stewart (1955) described it as the result of a hypersensitive immune reaction caused by the intake of infective larvae by a sheep previously infested with worms. Intake of *H. contortus* larvae provoked a self-cure of *H. contortus* infections as well as *Trichostrongylus colubriformis*, *T. axei* and *Teladosagia circumcincta* infections and intake of larvae of the latter two species also caused self-cure in *T. colubriformis* and *H. contortus*. Stewart (1955) postulates that this means the reaction involved in self-cure is localised to the organ of infestation, as antigens can pass down the alimentary tract from the abomasum (*H. contortus, T. circumcincta* and *T. axei*) to the duodenum (*T. colubriformis*) causing expulsion of any species present, but that antigens cannot pass from the small intestine back up to the abomasum. He makes this conclusion without reporting evidence that *T. colubriformis* does not cause self-cure in the abomasum. Gibson (1965) reports in his review of helminthiasis in sheep that *T. colubriformis* ingestion only causes self-cure against *T. colubriformis* worms; he is at that point quoting Stewart (1953). Allonby and Urquhart (1973) support Gordon's theory that self-cure is not immune-mediated, as they report that both adult and young sheep express the phenomenon. Also sheep with low burdens and high burdens can both undergo self-cure without subsequent display of resistance and climatic conditions alone can trigger self-cure independent of larval ingestion. They report that, during their study, self-cure was always associated with a period of significant rainfall and all sheep in the study reacted similarly, whether they grazed clean pasture or contaminated pasture and had a prior history of infection or not. This is contrary to results reported by Stewart (1955) where only sheep given a prior infection showed a self-cure response. Allonby and Urquhart (1973) put the self-cure phenomenon down to new pasture growth, through anthelmintic properties, change in abomasal pH, or new pasture replicating the hypersensitivity reaction described by Stewart (1955). The

two camps of thought on self-cure seem to be divided into theories developed using sheep indoors (immunology theories) or sheep outdoors in a 'natural' environment (pasture influences). There does not seem to be any crossover in theories by these camps, but the results of either cannot be ignored. It may be possible that the immune response described by the immunologists during indoor experiments is accelerated by significant rainfall events in outdoor experiments and interactions with pasture plants and their stages of growth. Another, more likely, explanation is that the two camps of thought are using the same term to describe two different phenomena.

1.6.4.2 Resistance and resilience to gastrointestinal nematode infection

Two host traits can limit the effect of gastrointestinal nematode infections in sheep: resistance and resilience. Resistance is 'the ability of the host to reduce the establishment, survival or reproductive rates of a pathogen' and resilience is 'the ability of a host to limit the adverse effects of infection with a pathogen by mechanisms other than resistance' (Walkden-Brown and Kahn 2002). Sheep that are resistant to gastrointestinal nematode infections are not necessarily resilient. Thus, genetically resistant sheep that display lower faecal worm egg counts and worm counts do not necessarily display higher levels of production than non-resistant sheep, even in a parasitised state (Eady and Smith 2001). Resilient sheep are able to maintain production of wool and maintenance of body weight throughout an infection. The ability of a host to resist nematode infection is determined by age of the host, breed, sex and reproductive status (Barger 1993).

1.6.4.2.1 Genetic resistance nematode infection

As with all biological populations, plant or animal, some sheep are genetically predisposed to better resist nematode parasitism. There are breed differences in genetic resistance with some breeds, such as the Rambouillet, Scottish Black Face, Perendale, Romney and crosses (Europe), the Red Massai and Dorper (Africa), and the St Croix, Florida Native and Barbados Blackbelly (Tropical breeds), being more resistant to nematode parasitism than other breeds (Barger 1989; Baker *et al.* 2003). There is also heritable variation within breeds to single infections of *H. contortus* and *T. colubriformis* as well as to mixed infections. The heritability of resistance appears to be moderate (h^2 = 0.23) based on one faecal egg count test, increasing when based on the mean of two faecal egg counts (h^2 = 0.35) (Morris *et al.* 1995). Barger (1989) reports the heritability of resistance at a higher range of 0.3 to 0.5, stating that heritability is similar to production characteristics, such as fleece weights or body weight, which have been successfully selected for by breeding. Sheep bred for resistance to parasites have fewer faecal egg counts and fewer worms, but this does not necessarily transfer to greater production. Eady and Smith (2001)

reported that there was no difference in wool production between lines selected (IRH) and non-selected for resistance, when the sheep were infected with *H. contortus*. There was also no difference in liveweight when the sheep were supplied with adequate nutrition. However, liveweight was lower in non-selected sheep when nutrition was inadequate. There was also a difference in 'mortality' rates, which were determined by PCV<14%. The IRH sheep had 0% 'mortality' overall whilst the non-selected sheep had a 'mortality' of 25% on the low quality rations. This study suggests that the immune response mounted by the IRH line comes at a cost to production, as the sheep have fewer worms without displaying greater production. Walkden-Brown and Eady (2003) reviewed the influences of nutrition on the expression of genotypic resistance, postulating that an interaction between nutrition and resistance genotype, such that genotypes show marked differences in resistance under poor nutritional conditions, small differences under moderate nutrition and virtually no differences under good nutritional conditions. New Zealand researchers report a slightly negative correlation between selection for resistance and growth rate of challenged lambs (McEwan *et al.* 1995). Morris *et al.* (1997) showed resistant selection lines produced lower yearling and ewe fleece weights than their susceptible counterparts, when grazed together. Bisset *et al.* (2001) suggest that genetic gains may still be possible in both resistance and production traits, with an appropriate selection index. The resistant sheep may also be useful for maintaining low levels of pasture infection, which could be used for weaner paddock preparation.

1.6.4.2.2 Resilience to nematode infection

Assessing resilience is not as straight forward as testing for resistance (use of faecal egg count). Albers *et al.* (1987) used measurement of growth rate in lambs subjected to a roundworm challenge, relative to their growth rate when not subjected to challenge. This method was deemed impractical in a real production system due to the associated rate of mortality in non-resilient lambs. New Zealand researchers (Bisset and Morris 1996) used a drench on demand method to identify resilient sheep. The criterion for drenching involved observation of body weight change or a visual assessment of body condition. This method, although useful for *T. circumcincta* and *T. colubriformis*, may not be as useful for determining resilience to infection with *H. contortus*, which does not cause as severe or as rapid inappetence or body weight loss as the first two parasites. Indeed, Roberts and Swan (1982) found that body weight was not predictable from either worm count or haemoglobin levels in merino sheep infected with *H. contortus*.

Host resilience to parasite infection is largely influenced by the nutritional status of the host, with those on a higher plane of nutrition being more resilient to infection. A review by Walkden-Brown and Kahn (2002) describes the modulation of resilience and resistance to nematode parasites through nutritional manipulation. Kahn *et al.* (2000a) demonstrated that lambs infected with *T. colubriformis* fed a high protein diet (165kg CP/kg DM) had 25% improvement in both liveweight gain and feed conversion compared to those on a low protein diet (96kg CP/kd DM). Breeding for resilience has been an objective for New Zealand researchers aiming to reduce the number of drenches given in a year whilst boosting production (Bisset and Morris 1996; Bisset *et al.* 1997; Morris *et al.* 2001). Morris *et al.* (2001) tested lambs from the Elite Resilient-line reporting they had 27% higher post-weaning weights relative to the control line. They also displayed a 0.48 unit reduction in dags and 45% reduction in total drench requirement, yet the faecal egg counts were not significantly different between the lines. This study found the heritability of resilience traits to range from 0.10 to 0.19, however a considerably higher heritability was expressed in a flock that was exposed to a higher level of cumulative challenge (h^2 = 0,24—0.53). Bisset *et al.* (1996) also reported no correlation between resilience and faecal egg counts.

1.7 Control of ovine gastrointestinal nematodiasis

1.7.1 Approaches targeting the parasitic nematode in the host

1.7.1.1 Chemotherapy

The use of anthelmintics has long been the main method of nematode control, based on the removal of the nematode population from the host animal. Anthelmintic control is largely based on a limited number of groups of compounds, divided into broad-spectrum and narrow-spectrum classes. The broad-spectrum compounds include the benzimidazole (including thiabendazole) (BZ), levamisole (LV) and the macrocyclic lactone (ML) groups (includes avermectins, abamectin, ivermectin, milbemycin and moxidectin). The use of anthelmintic compounds will continue to have a large place in the future of worm control. However, there is widespread resistance to all the above mentioned broad spectrum anthelmintic groups (Besier and Love 2003), which necessitates the formulation of more sustainable worm control programmes so that the effective 'life' of anthelmintic products can be extended or their use avoided altogether.

1.7.1.1.1 Anthelmintic resistance

Anthelmintic resistance is widespread throughout Australia, the European Union, Africa, South-East Asia, North America and South America (Anonymous 1989; Waller 1997; Besier and Love 2003). The first report of anthelmintic resistance was in 1968 in the New England area of northern NSW, with thiabendazole failing to control *H. contortus* on 3 properties (Anonymous 1989). In the mid-1970s producers began to use the levamisole/morantel group of chemicals in the face of wide-spread thiabendazole resistance. This led to the development of resistant strains of *Teladosagia circumcincta* and *Trichostrongylus* spp., with *H. contortus* still remaining largely susceptible. Levamisole is now regarded as a narrow-spectrum drug for use in control of *H. contortus*. Closantel was released in the early 1980s for the control of *H. contortus* but by the mid-1990s was all but ineffective for *H. contortus* control (Love *et al.* 1998). Ivermectins and moxidectins are also increasingly ineffective (Besier and Love 2003). According to Dobson *et al.* (1996), the rate at which anthelmintic resistance develops in a population depends on: 1) genetic factors, including mutation rate and dominance of the trait; 2) reproductive factors, including generations per year and fluctuations in population size; 3) behavioural/ecological factors such as migration of the nematodes and ability to avoid the anthelmintic; and 4) operational factors, such as the proportion of the population exposed to the anthelmintic and the persistence of the chemical control agent. Anthelmintic resistance has been recognised as a pre-adaptive phenomenon, that is, the genes that confer resistance already exist within the population (Jackson and Coop 2000). It is inevitable that anthelmintic resistance will occur, with the rate at which it accelerates being determined by the factors above. Lack of knowledge about anthelmintic resistance has led to the acceleration of its development under strategic control programmes. Wormkill in 1984 was the first strategic control programme in Australia and it was developed for the summer rainfall region of the New England. The programme aimed to reduce the number of drenches being used, focusing on the use of Closantel to control *H. contortus* at strategic times of the year. Wormkill is, generally, no longer in use in the New England with the loss of Closantel as an effective narrow-spectrum drug across much of the region, however, there are pockets of properties which seem not to have developed resistance to Closantel and are related through their geographical location (Dr E. Hall pers comm. 2004).

Strategic control programmes in winter rainfall zones generally comprised two summer treatments, the first given when pastures are drying off, the second during mid-late summer. The objective was to limit pasture contamination in summer and autumn, thereby reducing the

number of larvae available in winter and spring. This theory forms the cornerstone of Drenchplan, Worm plan, Wormcheck and CRACK programmes (NSW, VIC, SA, WA) (Anonymous 1989). These programmes contain recommendations to treat weaners in April or 4--6 weeks after autumn rains and again in July.

Strategic control programmes in both rainfall regions accelerated the development of anthelmintic resistance, as treatments were given when the free-living nematode population was at its lowest and the large part of the nematode population in the host. This means that virtually the whole parasite population is exposed to the chemicals with only the resistant worms making a contribution to the next generation (Besier and Love 2003). The process may be slower in set-stocked sheep where the host is exposed to larvae from nematodes that were not subjected to the chemical. The same principles stated above for development of resistance would apply to the drench and move strategy (section 1.6.3). This concept of 'unexposed' larvae is referred to as *refugia* and describes that portion of the population that is not exposed to the anthelmintic treatment (either as larvae on pasture, or in untreated sheep) (reviewed by Besier and Love 2003). The progeny of worms in the host that survive a given anthelmintic treatment will be among the free-living worms *in refugia*. Michel (1985), Martin (1981) and Van Wyk (2001) all outline the importance of *refugia* in the development of anthelmintic resistance, with the size of the population of worms *in refugia* directly affecting the degree of selection for resistance. Small levels of *refugia* leads to higher selection pressure due a small gene pool, whereas larger numbers of nematodes *in refugia* leads to low selection pressure due to a larger available gene pool.

One problem with determining the level of anthelmintic resistance in a population is the accuracy and reliability of tests for its presence. The faecal egg count reduction test (FECRT) has been widely adopted as a reliable method of detection, however a recent study by Kemper and Walkden-Brown (2004) found the Australian standard method for FECRT can underestimate the level of anthelmintic resistance and also return nonsensical results. A similar, more reliable method includes the use of a control group of sheep to track the change in faecal egg counts in the host population. The FECRT is also time consuming and labour intensive, especially if all anthelmintic compound groups are to be tested. *In vitro* drench resistance tests are also available in the form of a larval development assay (LDA) which do not generally have the sensitivity of FECRT (Kemper and Walkden-Brown 2004).

1.7.1.1.2 Anthelmintic plants

The anthelmintic properties of plants has long been recognised and used for worm control, especially prior to the development of synthetic anthelmintics or where synthetic anthelmintics may not be accessible or affordable. A FECRT of 70% is a figure used as a cut off point by Githiori *et al.* (2003) to class a compound as biologically active. They tested a number of remedies used by Kenyan pastoralists and smallholder farmers. Githiori *et al.* tested for gastrointestinal nematodes in mice infected with *Heligmosomoides polygyrus*, finding that none of the remedies fulfilled the 70% efficacy benchmark. Although the parasite used, *H. polygyrus*, is in the same family as ruminant nematodes, it may not be suitable to test remedies for ruminant parasites in a monogastric animal. The diversity between even ruminant nematode parasites in their reaction to different compounds leads to the suggestion that these compounds be further tested on ruminant parasites in their natural hosts. A comprehensive review of alternative de-wormers by (Anonymous, 2004) included: wormwood, garlic, wild ginger, conifers, crucifers, cucurbits, fern, Umbelliferae, Tansy, *Chenopodium* sp. and *Carica papaya*. Some of these plants have undergone scientific testing with varying results and all of these plants require extraction or preparation for oral dosing. Some research has gone into the use of pasture species as a means of reducing worm burdens, also with varying results. The reason for varied results in trials using plants with anthelmintic properties could lie with differences in preparation, dose rates and possibly seasonal or regional differences in the concentration of the anthelmintic properties of the plants.

Condensed tannins, found commonly in leguminous plants, are thought to have direct and/or indirect effects on the resistance and resilience of stock to gastrointestinal nematode infection (Kahn and Diaz-Hernandez 2000). The effects of plants containing condensed tannins differ between plant species. Some species cause a direct interaction between the nematodes and tannins, causing reduced worm viability. The more indirect effects include enhancement of mineral and protein metabolism increasing resilience to nematode infestations (Kahn and Diaz-Hernandez 2000).

1.7.2 Approaches targeting the host

1.7.2.1 Nutrition – parasite interaction

As mentioned earlier, nutrition has a profound effect on the ability of a host to mount an immune response to infection. This is especially so for gastrointestinal nematode infections. An early

study by Clunies Ross and Gordon (1933) demonstrated, with an experiment containing only five sheep, that sheep fed a low-protein diet (3% protein) had lower resistance to *H. contortus* infection than sheep fed a 'normal' diet (7.5% protein). A study by van Houtert *et al.* (1995) investigated the effects on *T. colubriformis* infection of supplementation of sheep with different levels of bypass protein. When protein supplementation was high, the liveweight gain was only reduced by 11% compared with an 18% reduction in moderately supplemented sheep and 43% liveweight losses in sheep not given protein supplementation. There was no effect on feed intake in animals on the high protein diet, whilst those on the nil supplement diet ate 7% less than normal. There was no reduction in wool production in the high protein supplement group, whilst a reduction of 20% in wool production was seen in the low and nil protein supplement groups. The study by van Houtert *et al.* (1995) also showed that the high protein supplementation boosted the immune response of the sheep in that group, with higher levels of eosinophils. There was, however, no increase in the circulating antibody levels. Bown *et al.* (1991a) reported that the effects of *T. colubriformis* on young sheep were markedly reduced by post-ruminal infusion with protein in the form of casein, but much less by post-ruminal infusions of an isoenergetic amount of glucose or mineral solution. These two studies show that protein supplementation plays an important role in boosting the immune response to nematode infections and reduces the susceptibility of a sheep to infection. However, Kahn *et al.* (2000b) found energy supplements had a greater effect on resistance to *T. colubriformis* infections than protein supplements (1000 *T. colubriformis* 3 days a week for 10 weeks). There have been many comprehensive reviews of the literature describing the effects of nutrition and especially the role of protein on the immune response of sheep to nematode parasitism (Steel 1978; Topps 1983; Coop and Holmes 1996; Coop and Kyriazakis 1999; Walkden-Brown and Kahn 2002; Steel 2003). Protein supplementation is generally an expensive exercise and may not yield returns equal to the expenditure. However, Knox (2003) reviewed the use of non-protein nitrogen, such as urea and urea-molasses blocks, for enhancing resistance and resilience in sheep and concluded that they were often successful and economically viable. Numerous studies have demonstrated higher feed intake, weight gain and wool production in infected sheep given urea supplements on high roughage diets (Wallace *et al.* 1998; Knox and Steel 1999; Stear *et al.* 2000).

A study by Niezen *et al.* (2002) investigated the effect of pasture species on lamb performance and parasitism and found that lambs grazed on ryegrass (*Lolium perenne*) with white clover (*Trifolium repens*, 7% in year 1 and 30% in year 2) had lower faecal worm egg counts and higher

antibody titres than lambs grazed on Yorkshire fog (*Holcus lanatus*) with low levels of white clover (~1% both years). The lower faecal worm egg counts were attributed to the higher white clover content of the ryegrass pasture, and resultant increase in animal performance (R^2 = 85.4, P<0.0001).

1.7.2.2 Vaccines

The idea of developing a vaccine for the control of gastrointestinal nematode infections is not new, but to date there has been little success in developing one for commercial use. The main difficulty is that the organism to be vaccinated against is a multicellular, complex organism. The immune system has far greater success in removing simple organisms, such as bacteria, than it does complex organisms. Thus developing a vaccine to elicit a specific immune response to a multicellular organism is no mean feat. Mulligan *et al.*(1961) found minimal immunity developed in lambs treated with either irradiated or non-irradiated larvae, so the results were inconclusive. Other more recent studies have also found vaccination to be unreliable and generally ineffective, with the immune response being more influenced by nutrition than by vaccination (Wagland *et al.* 1984; McClure *et al.* 1998).

1.7.3 Approaches targeting the parasitic nematode in the environment

1.7.3.1 Grazing management for control of nematode infections

Studies into the ecology of the free-living stages of gastrointestinal nematodes led to numerous theories on the use of grazing management for the control of nematode infections. The earliest control strategies involved the use of rotational grazing, where grazing period and rest periods for paddocks were devised. With the failure of testing of rotational grazing came other forms of grazing management, such as mixed species and mixed class grazing, diluting strategies, and preventative strategies, such as cropping and preparation of weaner paddocks (Michel 1985; Barger 1997). These strategies have been used to varying effects.

1.7.3.1.1 Rotational grazing versus continuous/set-stock grazing

1.7.3.1.1.1 Temperate and cool climates

Over the last century, rotational grazing and set stocking have been discussed in detail in relation to control of gastrointestinal nematode infection (Morgan 1933; Morgan and Oldham 1934; Gordon 1948; Gibson and Everett 1968; Barger *et al.* 1994; Barger 1997; Barger 1999). Systems of rotational grazing were suggested according to the understanding of the free-living stages and

tested against set stocking for animal performance and for nematode control. In their most basic form, rotational grazing strategies involve allowing sheep to graze for a short period of time with removal before the eggs laid down in the their faeces develop into infective larvae. The other significant theory of rotational grazing involves the paddock being spelled for sufficient time to allow for the majority of the infective larvae to die off. Morgan (1933) provides a description of the 'new system of grassland management' in England which involved the application of nitrogenous manures and a rotation of 7 cropping paddocks each grazed for a 'week or so'. The goats on the 'new system of grassland management' were compared to goats set-stocked in a paddock of an area equal to that of the 7 paddocks of the 'new system' combined. Morgan (1933) suggested that the 'new system of grassland management' would allow for an increase in stocking rate, particularly on pasture that was previously 'not well managed'. This rotation of one-week graze and 7-weeks rest left grass grazed very short. The animals used in this experiment were goats, which are traditionally more susceptible to gastrointestinal nematode infection. All of the goats run on the 'new system' died by the end of the experiment with egg counts of over 7000 eggs per gram of faeces. There are many confounding factors in this experiment with goats being fed at different times, grass being mowed in one treatment and not the other, and then the 'new system' animals allowed to graze over all the plots due to a lack of pasture. The set-stocked, controls had a greater area, thus lower stocking rate and, as noted by Morgan, a substantial amount of rank feed in the paddock at the end of the trial. Only 1 goat is reported to have died in the control paddock. The same experiment was carried out again by Morgan and Oldham (1934) with one-year old sheep. The control paddock was further increased to twice the size of the experimental plots. Again all the experimental animals died with no firm result from the study. However, the results obtained by Morgan in both 1933 and 1934 suggest that the grazing management described (approximately 7 days graze and 7 weeks rest) was not beneficial for the control of gastrointestinal nematodes in either sheep or goats in England.

Robertson and Fraser (1933) conducted a study in Scotland on what they termed 'progressional' grazing, claiming that it provided complete control over *H. contortus*. The 'progressional' grazing allowed 3 Blackface ewes and their lambs to graze on $1/10^{th}$ of 1.5 acres being moved every 10 days to the next 'clean' section of paddock; thus the sheep came back to the original plot by 100 days. The experimenters report that the original infection in the ewes was a moderate to heavy infestation of *H. contortus* and *T. circumcincta*; in the lambs the infection was almost entirely *T. circumcincta*. The average number of *T. circumcincta* recovered from the 'non-

progressional' group was 80% more than that recovered from the 'progressional' group. The average number of *H. contortus* recovered from the 'progressional' group was reduced by 99% compared with the 'non-progressional' group. This experiment was carried out in Scotland, where it can be assumed that temperatures may only be conducive to development of the free-living stages of *H. contortus* in summer and this coupled with the long rest periods of 100 days, significantly reduced *H. contortus* and *T. circumcincta* infections. The cooler climate would have slowed development from egg to L_3 providing a longer grazing period (10 days) before nematode eggs could develop into infective larvae; thus the sheep were possibly removed before autoinfection. These authors have demonstrated an early version of intensive rotational grazing having beneficial effects on worm burdens in a cool temperate environment. The reasons behind the lack of adoption of this technology could lie with the experimenters reporting reduced feed value and lower lamb growth rates in the 'progressional' system due an over-maturation of grass on those plots.

Roe *et al.* (1959) reported no significant difference in parasitic infestation between sheep on continuous and rotational grazing treatments carried out near Armidale, NSW. There were three treatment groups of continuously stocked sheep with varying stocking rates of one sheep per 1.25 acre, 1 acre or 0.75acre. The results also suggest that there is no effect of stocking rate in this range on gastrointestinal nematode infections. The rotational grazing treatment consisted of a stocking rate of one sheep per acre rotated weekly through four sub-paddocks. The predominant infection was *H. contortus* with some *T. colubriformis* and *T. circumcincta*. This rotation would not be sufficient rest to allow for larval die off, given that most of the L_3 that were going to hatch would have done so by 3 weeks. The sheep would be coming back onto paddocks heavy with the L_3 they had deposited 3 and 6 weeks prior, especially in summer and autumn. The grazing period of 7 days may also mean autoinfection in warmer months but less so in winter. Given these simple ecological facts, it is little wonder that there was no difference between continuous and rotational grazing for nematode infestation. Gibson and Everett (1968) confirmed the results of Roe *et al.* (1959) with a study in England. Their rotation consisted of one-week graze rotated through 6 paddocks (five-week rest). The sheep used in the experiment were Suffolk-cross ewes and their lambs, and Dorset Horn lambs were run with the experimental sheep to provide an artificial infection of *T. colubriformis*, which would usually have been put down by the ewes. The artificially infected Dorset Horn lambs remained with the Suffolk lambs after weaning. As they were to be a replacement for the ewe contamination, it is unclear why the

Dorset Horns remained for 2 months after the Suffolk ewes were removed. The rotation in this experiment proved to be insufficient for the control of parasitic infections. The common failings of these experiments seem to be in the length of the rotation and inflexibility of rotations with the changes in season. One-week grazing may be too long in summer but fine in winter. The rest periods are too short given evidence from Donald (1967) that infective *H. contortus* and *Trichostrongylus* spp. larvae can persist on pasture in sufficient numbers until at least 9 weeks after egg deposition; this was the duration of that/the above? experiment. This work was carried out from summer to early autumn; thus the results suggest that pasture should be rested for at least 8 weeks in summer. These results also point to a peak of L_3 availability 5 weeks after infection, with substantially high numbers two and three weeks after infection. In fact, Donald (1967) states that there is no sound evidence that pastures should be spelled for periods shorter than 2 months.

This further outlines the inadequacies of the rotations implemented in studies by Morgan (1933), Morgan and Oldham (1934), Roe *et al.*(1959) and Gibson and Everett (1968), which had rest periods ranging from three weeks to seven weeks. However, some of these researchers were working in a time when ecological information on the free-living stages of gastrointestinal nematodes was limited. It also highlights the reasons for the success of the 'progressional' grazing used by Robertson and Fraser (1933) in reducing worm burdens with 10 days graze and 50--100 days rest. However, the grazing periods used in the British studies mentioned above seem reasonable, as a study in the British Isles concluded that *H. contortus* eggs require upwards of 2 weeks to develop in summer and considerably longer at other times of the year (Silverman and Campbell 1959). The study by Roe *et al.* (1959) was carried out in Northern NSW, Australia, where larval development can occur within 5--7 days in summer (Monnig 1930; Rose 1964). That particular system of rotation, one-weekgraze, three-weeks rest, provided perfect conditions for the perpetuation of the lifecycle of the parasitic nematode. In Australia, Southcott *et al.* (1976) found pasture to be potentially infective up to 12 months after sheep were removed. This persistence of larvae on pasture is of great importance when considering rotation periods for cool temperate grazing systems.

1.7.3.1.1.2 Tropical climates

Helminthosis in a tropical environment differs in severity and ecology from the disease in cool temperate environments. Banks *et al.* (1990) and Aumont and Gruner (1989) both report more rapid development from egg to L_3 and shorter survival times of infective larvae on pasture in the

tropics. From their results in Guadeloupe (latitude 16°15'N), Aumont and Gruner (1989) suggested that certain grazing management practices may help in reducing the risk of parasitic infection, the criteria being that the graze period be no more than 7 days and grass regrowth time should be more than 28--35 days. The ecological studies by Banks *et al.* (1990) in Fiji found that, under ideal conditions, the maximum larval count was reached at one week post-contamination, with the majority of the larvae dead at 4--5 weeks. A trial of a rotational grazing system based on these findings was conducted using goats in Tonga, an environment with slightly milder temperatures than Fiji (Barger *et al.* 1994). Barger *et al.* (1994) devised a ten paddock rotation with the graze period set at 3.5 days and a rest of 31.5 days (~4.5 weeks), a control group of set-stocked does were run adjacent to the rotation paddocks. All goats were housed at night to prevent theft or dog attack. The set-stocked goats had significantly higher faecal worm egg counts and required three more drenches than the rotationally grazed goats. The rotationally grazed goats were drenched only once strategically, at kidding (Figure 1-11).

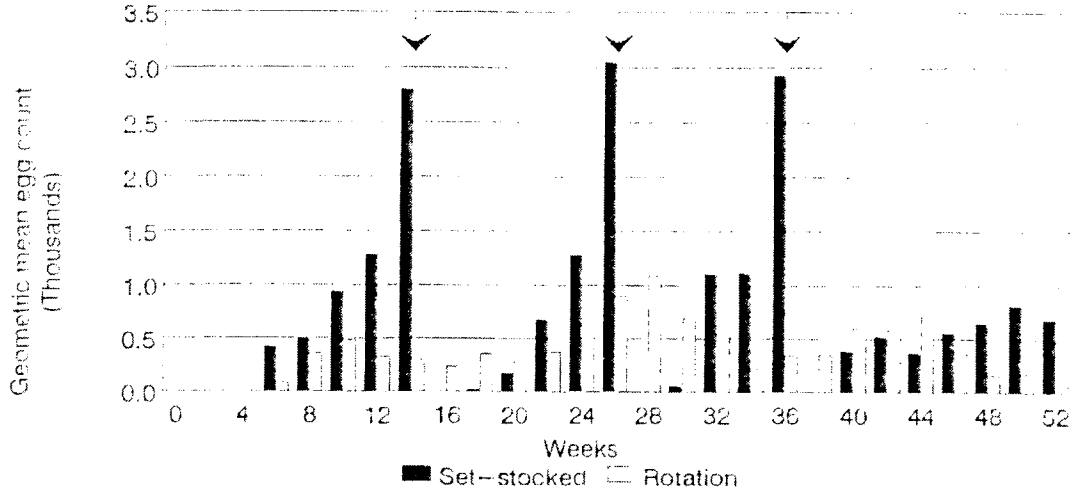


Figure 1-11: Geometric mean faecal egg counts of set-stocked or rotationally grazed goats in Tonga from October 1991 to October 1992. Anthelmintic treatments for set-stocked goats shown by arrows. (Barger *et al.* 1994).

Chandrawathani *et al.* (1995) obtained almost identical results with a rapid rotational grazing system in Malaysia, the only exception being that the animals used were sheep. The sheep in the rapid rotational grazing system had significantly lower faecal worm egg counts than the set-stocked sheep (350 and 3237 eggs per gram, respectively). Another study in Malaysia reports on grazing management for worm control in a rubber plantation (Sani *et al.* 1996). This study again used sheep in a rotation of 3--4 days with a rest period of 35 days. The hedgerow of rubber trees was used as a boundary between paddocks. The sheep on the rotationally grazed paddocks again

had lower faecal worm egg counts than those on a set-stocked paddock. A study in the Philippines using goats also followed the rotation described by Barger *et al.*(1994), with the rotationally grazed goats having significantly lower faecal worm egg counts than the set-stocked goats and receiving no drenches compared with 3 drenches given to the set-stocked animals (Gray *et al.* 2000). The overwhelming message from these experiments is that sound knowledge of the parasitic nematode lifecycle in a given region has led to the formulation of an effective form of rotational grazing for the control of gastrointestinal nematodes in sheep and goats. Apart from work done by Robertson and Fraser (1933) in Scotland, the type of rapid rotational grazing used in the tropics specifically for the control of nematode parasites has not been applied to a cool temperate environment; it has been implemented for pasture management (Earl and Jones 1996; McCosker 2000; Sparke 2000).

An early study on rotational grazing for worm control in Fiji was conducted by Singh *et al.* (1972). They tested 4 treatments for worm control: set-stocked/not drenched, set-stocked/drenched fortnightly, rotationally grazed/not drenched and rotationally grazed/drenched fortnightly. The sheep given fortnightly anthelmintic treatment (the chemical compound used was not mentioned) were virtually free of worms for the period of the trial whereas non-treated sheep, both set-stocked and rotationally grazed, had very large worm infections (*H. contortus*, *Cooperia* spp., *Brunostomum* spp., *Oesophagostamum* spp. and *Trichostrongylus* spp.), and body weight loss and some died. The rotation used considered only the length of the rest period (30 days) with the average grazing period not reported. However, they employed 5 paddocks of 3.5 to 9 acres in size, stating that graze period varied with paddock size. Barger *et al.* (1994) required 10 paddocks for their rotation of 3.5-days graze and 31.5-days rest, thus the average graze period in this study must have been around 7 days. The authors state that this form of rotational grazing was ineffective for worm control in Fiji. This is hardly surprising given that they only considered L_3 survival in devising their rotation and did not include the time for development of egg to L_3. The downfall of this rotation was that sheep were continually exposed to autoinfection through overly long grazing periods. Again, the stark contrast between the success of the two rotational grazing methods (Singh *et al.* 1972; Barger *et al.* 1994) used in the tropics indicates the need for sound ecological knowledge for the development of grazing control programmes.

1.7.3.1.2 Other strategies of grazing management for control of gastrointestinal nematodes

Epidemiological studies have identified peaks and troughs in helminthosis throughout the year. These fluctuations are unique to each region, but the principles behind them are identical. The main driver of these peaks and troughs is L_3 availability to the grazing sheep, which is, in turn, dependant upon temperature and moisture (see section 1.6.2). The aim of preventative control strategies is to break the parasitic lifecycle in its free-living stages, providing 'clean' pasture, which is especially important for young immunologically naïve stock. Southcott and Barger (1975) demonstrated that sheep pasture can be effectively decontaminated through the grazing of cattle for 6, 12 and 24 weeks near Armidale, NSW. They also demonstrated an effective 'cleaning' of cattle pasture by grazing sheep for 24 weeks, although there was a minimal success with grazing sheep for 12 weeks and no effect when grazing for 6 weeks. In this study, they found cross-species infections with tracer calves hosting the sheep nematodes *H. contortus* and *T. colubriformis*, and to a lesser extent tracer lambs carrying the cattle parasite *Cooperia oncophora*. O'Callaghan *et al.* (1992) also report some cross-infection with a natural infection of *O. ostertagi* occurring in lambs on pasture previously grazed by cattle, but state that this is highly uncommon. A follow-up study by Barger and Southcott (1978) further demonstrated the exploitation of host specificity of nematode parasites by grazing cattle alternately with Merino weaners. The weaners in the control treatment group (SC) were put out on the same paddock each year at weaning, while the second treatment (SC-6) involved sheep and cattle grazing alternately for six-month periods and the third treatment (SC-12) involved sheep and cattle grazing alternately for 12-month periods. The weaners in the SC-6 treatment had significantly lower faecal egg counts (Figure 2-5) and lower burdens of *H. contortus*, *T. axei*, *T. colubriformis* and *Nematodirus* spp. than the controls but had higher levels of *Cooperia oncophora* and similar levels of *T. circumcincta*. The SC-6 also had higher liveweight gain, heavier fleeces and had a lower mortality rate than SC, with the SC-12 group generally being intermediate. In the third year there was no difference between the SC-6 and SC-12 and both were superior to SC in worm control. This study demonstrates that alternate grazing with cattle is an effective tool for lowering nematode infections in weaner sheep and that even short periods of grazing with cattle prior to weaners (e.g. 6 months) will significantly reduce worm burdens. However, given that anthelmintic treatment was administered at each pasture change, the possibility of the development of anthelmintic resistance is high due to sheep entering clean pastures straight after anthelmintic treatment.

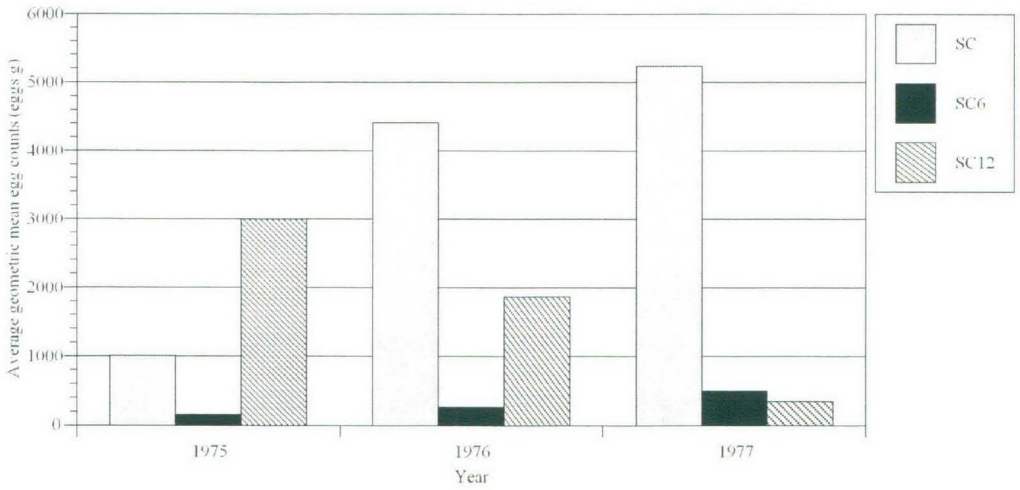


Figure 1-12: Average geometric mean egg counts per gram of faeces for sheep in each year of the experiment (Barger and Southcott 1978). The control group of sheep were weaned onto the same paddock each year with no cattle grazing in the interim; SC 6 treatment involved alternately grazing paddocks with cattle and sheep for 6 months and SC 12 involved alternate grazing for 12 months of cattle and sheep.

A study by Donald *et al.* (1987) combined the use of anthelmintic treatment and grazing management through cattle-sheep alternation. They demonstrated that paddock moves in the cattle-sheep rotations did not need to be accompanied by anthelmintic treatment to achieve effective worm control. Their study was conducted near Canberra, ACT, where rainfall is uniform throughout seasons and *T. colubriformis* and *T. circumcincta* are the dominant nematode species. *Haemonchus contortus* occurs in some years. There were two grazing treatments: i) sheep and cattle were alternated between 2 paddocks in December, February and July (S-C) and were given 4 levels of anthelmintic treatment; ii) sheep set-stocked on one paddock (S-S) given 4 levels of anthelmintic treatment. Each of the S-S and S-C groups were given two-week anthelmintic treatments (rotations of double doses of levamisole or thiabendazole) to provide a 'worm-free' comparison. The sheep on the S-C system had significantly higher liveweight gains and wool growth than set-stocked sheep (except for S-S given two-weekly treatments). This includes the sheep in the lowest level of anthelmintic treatment, which was one treatment in December at weaning. The greater production of sheep in the S-C groups was attributed to greater worm control achieved through the alternation of cattle with sheep, reducing availability of infective larvae on pasture.

Breaking the worm lifecycle has been more commonly achieved by using controlled release anthelmintic devices or timed conventional anthelmintic treatments. Anthelmintic treatment (either controlled release or conventional) is generally given at weaning, with a move to a clean

paddock to counter the effect of burdens picked up from lambing paddocks. Preparation of weaner paddocks is essential to this method, by providing low worm pastures for young susceptible sheep (Barger 1997). A method of weaner paddock preparation was devised and tested by Niven *et al.* (2002) for use in the winter rainfall regions of southern Australia. It has been termed 'smart grazing' and combines the two summer drench programme with strategic grazing methods. Older ewes or wethers are given their first early summer anthelmintic treatment (late October) and put into the chosen weaner paddock at 2--3 times the normal stocking rate for a period of 1 month. After the second summer drench (early February), the older ewes or wethers return to the weaner paddock and graze for another month. The weaner paddock is left empty until the autumn break (rainfall) arrives and the pasture grows. The weaners are then given an anthelmintic treatment and introduced onto the prepared weaner paddock (15 weaners/ha) where they will remain until the spring. This method was compared to the 'set-stocked' method commonly used, where older wethers are given both the summer anthelmintic treatments in October and February but are allowed to remain on the paddock for the entire period from October to weaning (April). Pastures prepared using the 'smart grazing' strategy had significantly lower larval contamination and reduced total worm counts in weaners by 50% to 95% compared with the 'set-stocked' preparation. Worm egg counts were lower (reduced by 50%), fleece weights were heavier and liveweights were greater in weaners on the 'smart grazing' paddocks. The researchers attribute the improved production to both better pasture growth in early winter and reduced parasitism. This type of grazing management, where sheep are put onto a 'clean' paddock straight after anthelmintic treatment, will lead to an acceleration of anthelmintic resistance with low levels of *refugia* on pasture (see section 1.7.1.1.1 on anthelmintic resistance and *refugia*).

'Smart grazing' could fall into the category of evasive grazing strategies which rely on the removal of infection, either by drenching and moving to a clean paddock or by rapid rotational grazing as used in the tropics, in which sheep and goats avoid infection through exploitation of the rapid development and death of infective larvae (See section 1.6.2.2). Githigia *et al.* (2001), in Denmark, found that lambs given an anthelmintic treatment and moved to a clean pasture had zero egg counts at 4 weeks after the drench and lower worm counts at the end of three months than lambs moved to a clean paddock without an anthelmintic. Eysker *et al.* (2005) explored the possibilities and limitations of evasive grazing, using a complex experimental design, with sheep being moved to a new paddock at 4 weeks, then every 3 weeks, then 2 weeks, based on peak

infectivity of pasture. Sheep were moved to clean pasture without an anthelmintic treatment. The results suggest that there was limited success with this method of evasive grazing. Better control was observed when the move to a clean paddock was reduced to 2 weeks. The provision of 'clean' pasture was achieved by mowing prior to grazing. This may remove a large proportion of the infection but it seems to have left some larvae capable of infection behind. Drench and move strategies are commonly used in Australia and have led to a rapid increase in parasite resistance to anthelmintic chemicals (Waller 1997; van Wyk 2001). Whilst it may still be a valid worm control option for young lambs, its continued general use is predicated on the idea that more anthelmintics will be developed as the current ones become obsolete due to anthelmintic resistance.

Diluting strategies involve the grazing of young susceptible stock with a greater number of non-susceptible animals (Barger 1997). The principle of dilution is that greater numbers of non-susceptible stock will produce larger quantities of faeces of lower infectivity, diluting the lower volume of higher infection faeces. Older ewes, wethers or cattle are commonly used for dilution strategies. Arundel and Hamilton (1975) grazed steers with lambs and reported a decrease in sheep parasites (*T. circumcincta* and *Nematodirus* spp.). They reported a concurrent increase in infestations of some cattle parasites, *T. axei* (also a sheep parasite) and *C. oncophora*. Jordan *et al.* (1988) also reported the benefits of mixed grazing of cattle with sheep for sheep production. The mixed grazing, however, led to a decrease in cattle performance due to increased parasitism in the calves. They demonstrated that lambs grazed with both cattle and sheep had fewer worms than those grazed with sheep alone, however, calves grazed with sheep and cows had higher worm infestations and lower body weights than those grazed with cows alone. Mixed grazing may be beneficial in sheep producing operations but may be a disadvantage in cattle production.

Grazing management is a powerful tool for controlling gastrointestinal nematodiasis and various forms of grazing management have been use to great success (mixed grazing in temperate climates and rapid rotational grazing in the tropics). However, extension of successful treatments to sheep producers has generally been limited with the focus on grazing management strategies that promote rapid development of anthelmintic resistance (drench and move strategies and 'smart grazing'). In temperate climates, mixed grazing with cattle or non-susceptible sheep with weaner lambs has not been as widely used as it should be, given the promising results of studies in that field. Application of more intelligent rotational grazing based on local ecological

knowledge would also be of benefit to sheep producers. However, the impact of rapid rotational grazing on selection pressure will need to be monitored with the possibility of selecting for infective larvae with a tendency for longevity.

1.7.3.2 Non-chemical approaches

With widespread anthelmintic resistance and an increase in the prevalence of organic farming, alternatives to chemotherapy are being increasingly explored. Apart from grazing management, biological control has emerged as a forerunner in the non-chemical approach to worm control. Nematophagus fungi and dung beetles have been used with some success, while anthelmintic plants and vaccines are still under investigation. The main problems facing non-chemical control is application and integration into industry practices.

1.7.3.2.1 Biological control

Perhaps the most promising of the biological control agents for worm control is the nematophagus fungus *Duddingtonia flagrans*, which usually invades the faeces on the pasture. This fungus is capable of surviving the passage through the ruminant gastrointestinal tract, as it has robust chlamydospores which have been shown to germinate and spread in fresh dung, capturing large numbers of infective larvae before they migrate onto pasture (Larsen *et al.* 1992; Waller and Larsen 1996; Sanyal 2000). A recent study on *D. flagrans* in New Zealand reported an average efficacy of larval killing of 78%, with group means ranging from 40--93% at two dose rates of 250 000 or 500 000 spores/kg liveweight. The treatment was successful in both sheep and goats, being effective against *H. contortus*, *T. colubriformis* and *T. circumcincta* larvae (Waghorn *et al.* 2003). Waller and Larsen (1996) note a number of 'hurdles' to be negotiated if predacious fungi are to be used as a widespread biological control. These include:

1. Spectrum of activity -- whilst there are no fungal preferences for species of parasitic nematodes, some nematode species are slower to develop to larval stages and may miss the activity of the fungus in the faeces.
2. New fungal species -- there may be more superior fungal agents than those currently used. These need to be tested for their ability as a biological control agents.
3. Ecology of the fungus versus nematode -- the ecology of the fungus needs to be similar to that of the nematodes to optimise the activity of the fungus when seasonal larval peaks occur.
4. Environmental impact assessment needs to be made for fungal registration purposes.
5. Human health and safety is important, if the fungus is to be produced in large quantities and have a widespread industry use.

6. Product development -- local isolates may be easier to market than a blanket isolate for each continent.

One issue that is not covered by these points is that of providing a reliable means of ensuring the continuous presence of spores in the manure for long periods. Quarantine issues with a live organism in a feed supplement may impede the use of a common fungus across continents. One other point worth adding to this list is the method of delivery. The three main methods of delivery that have been explored are feed supplements (incorporated into feed pellets or top dressing), feed blocks (voluntary consumption) and controlled release devices which would provide each animal with a predetermined dose. Chandrawathani *et al.* (2004) combined the use of a daily supplement of *D. flagrans* with intensive rotational grazing and this has been shown to reduce faecal worm egg counts in Malaysia (Sani *et al.* 1996). They found significantly lower faecal worm egg counts and higher growth rates in lambs rotationally grazed with fungal supplements versus those rotationally grazed without fungal supplements. The fungal supplements were given with concentrates and hay when the sheep were housed each night. The control group received the same supplements without the fungal additive. This integration of tools for worm control may be a successful alternative to chemical therapy.

Another possibility for biological control lies with the dung beetle. Fincher (1973) demonstrated the potential of dung beetles as a biological control agent for nematode parasitism with a 14.7 fold reduction in *O. ostertagi* larvae on plots with increased dung beetle populations compared to plots without beetles. The plot with a 'natural' dung beetle population reduced the number of *O. ostertagi* by 3.7 times. Cattle dung on the plot with the higher population of dung beetles disappeared rapidly, with 80% of faeces buried within 24 hours and 100% buried within 72 days. It is hypothesised that this burial of the cattle dung reduces the availability of *O. ostertagi* larvae to its host. Fincher (1973) also reported a reduction in infestation in calves grazed on pasture with the increased beetle population. He repeated this success in a later study with varying dung beetle population densities (Fincher 1975). The higher the population density of the dung beetle the greater the success in reducing worm infestations in calves. This density dependence may limit the use of dung beetles as a biological control agent through the need to maintain beetle populations on farm. Whilst dung beetles have been shown to successfully reduce worm infestations in cattle, a study in New Zealand has shown that they increased the recovery rate of *T. circumcincta* L_3 on pastures grazed by sheep (Waghorn *et al.* 2002). Waghorn *et al.*'s theory

was that the burying of the sheep faeces led to faster transmission of L_3 through migration from the soil to the pasture. It is possible that the beetles involved did not bury the faeces as deeply as those in Fincher (1973, 1975) It remains unclear as to whether dung beetles will have a positive or negative impact on gastrointestinal nematode infections in sheep.

1.8 Conclusions

Over the last 100 years, there has been considerable research into every aspect of gastrointestinal nematode infections, however, the interactions between host, environment and pathogen are complex and not yet fully understood. The focus of most modern parasitological work in this area is on control of the disease, since eradication has long been dismissed as an option.

As Michel (1985) stated '... the imposition of control measures can exert a powerful selection in favour of worms which, for one reason or another, are unsusceptible to them. To this extent, all control procedures must be expected to have a limited working life.' This has led to parasitologists considering more integrated approaches; where worms are not susceptible to one control treatment they may be susceptible to two or three control treatments used in conjunction. Integrated parasite management (IPM) is the combination of multiple tools, such as breeding for genetic resistance, biological control mechanisms, grazing management, nutritional manipulation, and, possibly in the future, vaccination. Integrated pest management seems to be a natural progression after decades of research into various aspects of worm control. It allows more flexibility than the implementation of just one control tool which, according to Michel (1985), is doomed to eventually become redundant.

There is a need for more investigation into the use of rotational grazing for worm control, with the success of rapid rotational grazing in the tropics possibly being applied in more temperate environments. Rotations that were previously tested in temperate climates had obvious flaws when ecological factors are considered. A more careful consideration of the ecology of the free-living stages of gastrointestinal nematodes in a localised situation could lead to the development of more successful rotations. Banks *et al.* (1990) and Barger *et al.* (1994) concluded that the rapid rotational grazing they employed in the tropics would not work in more temperate climates and they were most probably right. The rigid time periods imposed on the rapid rotations work well in the tropics, where temperatures are always warm and rainfall relatively constant, resulting in constant development of eggs into infective larvae. They would be unsuitable in

temperate climates due to the large variability in rainfall and temperature throughout the year, with large fluctuations in development time and survival of the free-living stages.

Intensive rotational grazing systems, such as 'Cell grazing' and 'Holistic grazing', are being implemented across Australia, providing anecdotal evidence from producers of reduced anthelmintic use and reduced nematode burdens. These rotational grazing systems have flexible grazing and rest periods and, with movements based on feed on offer, rotations speed up or slow down according to pasture growth and recovery rates. If intensive rotational grazing is successful in a cool temperate environment, its success may be enhanced by other factors, such as the introduction of nematophagus fungi to help control infections during periods where rotation may need to be more rapid than desirable for worm control, such as when pasture growth rates are high. The suggestion that the use of IPM could potentially eliminate the need for anthelmintic use is not without merit. This is particularly applicable/attractive to sheep producers in the current market place and to organic producers.

A unique opportunity arose through the Cicerone Project Inc. to test the impact of intensive rotational grazing on gastrointestinal nematode infection against traditional and high input grazing management systems with slower paddock rotations. It was hoped that the impact of intensive rotational grazing on GIN would be confirmed and quantified followed by detailed investigation into the mechanisms underlying the observed effect, both host and environmental. The backbone longitudinal study (Experiment 1) tracked the incidence of worm egg counts over 2 years, along with body weights, fat scores and bi-monthly blood samples in all classes of sheep on the three Cicerone Project farmlets. The second experiment teased out the host effects on worm infection using a fixed larval challenge. A third experiment investigated the level of pasture contamination with infective larvae using tracer sheep and thus tested the environmental effects on the lifecycle induced by the different management systems. A further investigation of environmental factors was carried out on the intensive rotational grazing system only, looking at larval development and survival (Experiment 5). All these studies were repeated in each season of the year. The final study (Experiment 6) was conducted to determine whether there was a difference in production between sheep that were given worm control in a conventional way (i.e. anthelmintics and/or grazing management) and those that were 'worm free', and whether the production differences were the same between management systems.

It is hoped that the results of these experiments will lead to a better understanding of rotational grazing as a tool for worm control in cool temperate climates.

CHAPTER 2: General Materials and Methods

2.1 The Cicerone Project Inc.

All of the work presented in this thesis was carried out on The Cicerone Project Inc. Farmlets. The Cicerone Project Inc. is a producer led group that aims to increase the profitability and sustainability of grazing based agriculture in the New England region of Northern NSW with the farmlets commencing in 2000. The project is funded by Australian Wool Innovation Limited and member subscription. The majority of its board members are sheep producers with representation also from CSIRO, TAFE, the University of New England and NSW Department of Primary Industries. The Cicerone Project farm is located on land leased from the CSIRO Livestock Industries, F.D. McMaster Laboratory property "Chiswick" approximately 20km south of Armidale, NSW (30°31'S, 151°39'E), the elevation is 900 metres above sea level. Climatic data for Armidale during the experimental period are presented in Figure 2-1. Armidale has a cool temperate climate with the majority of rain falling in the summer months. Winters are generally dry, cold and frosty with occasional light snowfall. Summer days are warm but nights are generally cool. The average annual rainfall is 790mm. The topography around Armidale is undulating, but the Cicerone Project site was a flat, open plain. The majority of soil on the Cicerone Project is Brown Chromosomal soil (previously named Podsolic) with some Brown Dermosol (Previously named Basalt, McLeod 2002). The main native pasture species are mostly perennial, with very little or no legume content; Wallaby Grass (*Danthonia* spp.), Weeping Rice Grass (*Microlaena stipoides*), Tussocky Poa (*Poa seiberana*) and Wheat Grass (*Agropyron scabrum*). Queensland blue grass (*Dicanthium sericeum*), Red grass (*Bothriochloa macra*), Lovegrass (*Eragrostis* spp.), Parramatta Grass (*Sporobolus elongatus*) and Spear grass (*Aristida* spp.) are also present (Robinson 1983).

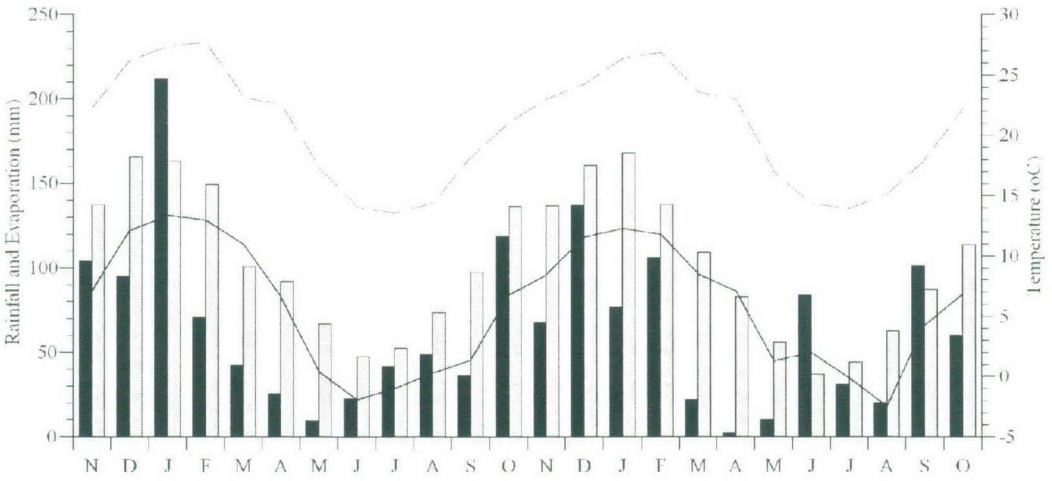


Figure 2-1:Rainfall (black), evaporation (grey), minimum (solid line) and maximum (dashed line) temperatures for the experimental period November 2003 to October 2005 (Burr 2006).

The farm is divided into three farmlets of approximately 50ha in area, each testing a different management system, as detailed in Section 2.2. The sub-division of land was a complex process of apportioning hydrology, slope, soil type and fertilizer history equally between the three Farmlets, hence the 3 farmlets represent interspersed allocations of paddocks rather than geographically discrete sections of the project (Figure 2-2, unpublished Munroe and Scott 2005). The farmlets are stocked with fine wool merinos which were allocated to the different farmlets in 2000 on the basis of liveweight and place of origin.

Scott *et al.* (2004) presented evidence of the equivalence of the farmlets at the starting point of the Cicerone Project Inc. experiment in carrying capacity, herbage mass, fertiliser responsive pasture species, sheep liveweights, fibre diameter and faecal worm egg counts. Fencing of paddocks was completed by July 2000 and the first pasture sown and fertilised in Autumn 2000. A flexible ratio of 60:40 DSE, sheep:cattle was set as the district average, however this ratio has fluctuated dramatically with the change in seasons over the course of the project with sheep being the predominant commercial species.

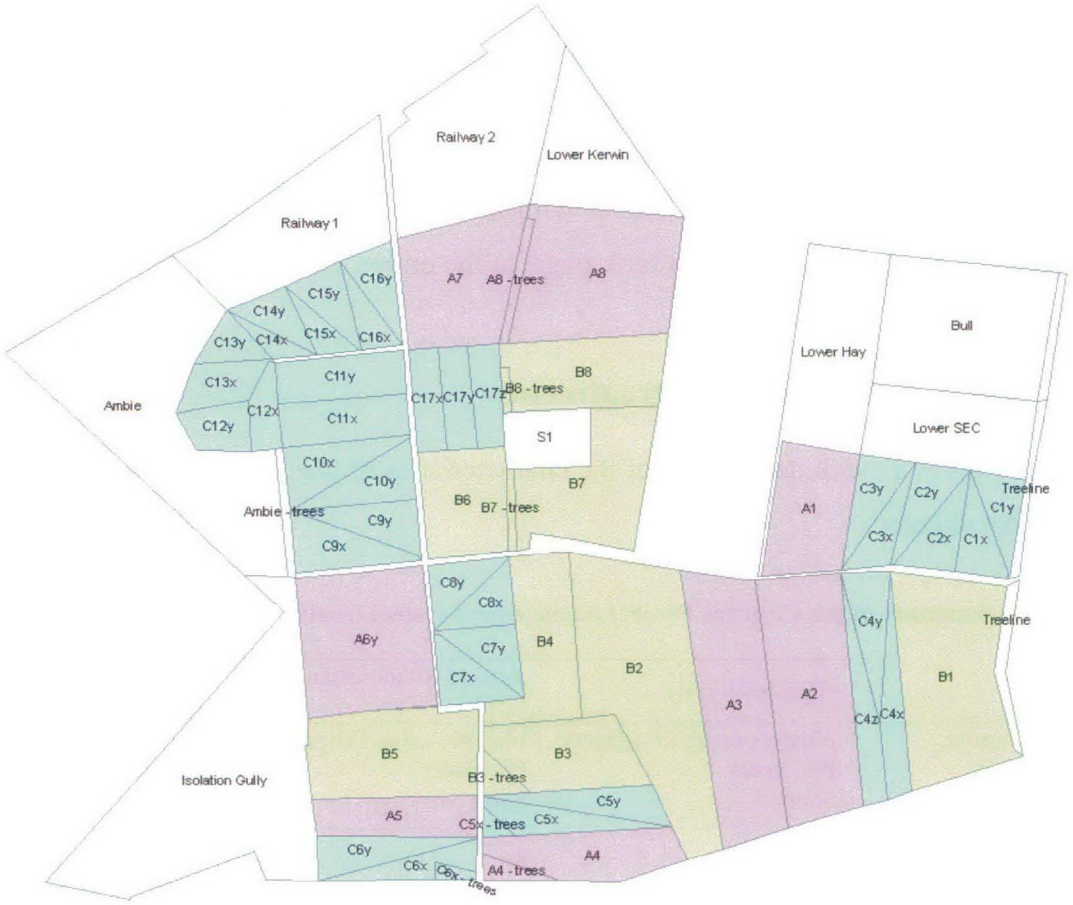


Figure 2-2: Cicerone Project map of farmlets showing paddock distribution across the farm. Farmlet A – High Input (pink), Farmlet B - Typical (green) and Farmlet C – Intensive Rotational Grazing (blue), periphery paddocks (white).

2.1.1 Terms used throughout the thesis

The years of Experiment 1 (Chapter 3) will be referred to as 'Year 1' (November 2004 to October 2004) and 'Year 2' (November 2004 to October 2005). The term 'ewes' will refer to female animals over 2 years of age, 'hoggets' will refer to animals between 12 and 24 months of age and the term 'lambs' will refer to animals less than 12 months of age. Each animal remained within their class (ewe, hogget and lamb) for Year 1, animals in Year 2 were different for the hogget and lamb classes as hoggets became ewes, lambs became hoggets and new lambs were selected.

2.1.2 Animals and their husbandry

All experimental sheep were superfine wool merino ewes. Mating occurred over a 5-week period in April/May each year with ewes and hoggets from all management treatments moved to a common periphery paddock for joining with common sires. Lambing started in mid-September and was finished by the end of October with lamb marking in mid-November and weaning in late January. Shearing of all sheep took place at the end of July each year in an off-site shearing shed.

2.2 Farmlet management treatments

Table 2-1 gives a quick description of the characteristics of the 3 farmlets which are described in further detail below.

Table 2-1: Summary of the Cicerone Project management system treatments.

	High Input (HI)	Typical Management (TYP)	Intensive Rotational Grazing (IRG)
Grazing/Pasture Management	Flexible using Prograze® Principles	Flexible using Prograze® Principles	Intensive Rotational Grazing
Rest periods	Short	Short	Long
Graze periods	Long	Long	Short
Number of paddocks	8	8	36-64
Sown pastures	50-80%	30-50%	30-50%
Fertiliser inputs	High	Moderate	Moderate
Stocking rate	High	Moderate	Moderate
Number of sheep (2003 to 2005)	581	444	395

2.2.1 Farmlet A; High Input (HI)

This treatment comprised 8 paddocks with flexible grazing applied using Prograze® principles of stock movement based on estimated pasture availability (Noad 2003). The initial aim was to achieve a minimum stocking rate of 15 dry sheep equivalents (DSE) per hectare within 5 years but the average during the experimental period was 13.4 DSE/ha based solely on sheep numbers as cattle were only used for relatively short periods. In year 1 sheep numbers totalled 520 (238 ewes, 31 hoggets, 92 lambs and 159 wethers) while in year 2 they totalled 581 (148 ewes, 94 hoggets, 132 lambs and 207 wethers). Cattle were used on 2 occasions, from October 2003 to May 2004 (15 steers) and from September 2004 to March 2005 (19 steers). An aim of 100% of pastures sown to deep-rooted grasses and persistent legumes was set resulting in a high proportion of area been re-sown with consequent reduced options for stock movements. The pasture was dominated by deep-rooted fertiliser responsive perennials which declined from 2003 to 2005 (60% to 40%) with low levels of native species (~5%) (Mpiti-Shakhane 2006). The

target soil phosphorus (bicarbonate extract) and sulphur levels (KCl_{40}) were 60ppm and 10ppm, respectively and were maintained by appropriate fertilizer application. Supplementary feeding was generally at a higher rate than for the other management systems as shown in Table 2-2.

2.2.2 Farmlet B; Typical management (TYP)

This treatment also comprised 8 paddocks with flexible grazing applied using Prograze® principles. The target stocking rate was 7.5 DSE/ha and only 1 paddock was re-sown to new pastures in 2004. The average stocking rate over the experimental period was 9.2 DSE/ha. In year 1 sheep numbers totalled 338 (138 ewes, 39 hoggets, 59 lambs and 102 wethers) and in year 2 they totalled 444 (166 ewes, 59 hoggets, 93 lambs and 126 wethers). Cattle were used on 2 occasions, from October 2003 to May 2004 (18 steers) and from September 2004 to March 2005 (26 steers). Pastures were dominated by native grasses (~62% in 2005) with a very low proportion of deep-rooted fertiliser-responsive perennials (~5% in 2005) (Mpiti-Shakhane 2006). The target soil phosphorus and sulphur levels were 20ppm and 6.5ppm, respectively. Supplementary feeding was at a lower rate than for the HI system and similar to the IRG system (Table 2-2).

2.2.3 Farmlet C; Intensive rotational grazing (IRG)

The initial aim was to achieve high pasture utilisation with short grazing periods followed by long rest periods. The original 16 paddocks were divided into 33 paddocks to ensure appropriate grazing pressure, pasture utilisation and rest periods. At times of feed shortage, these 33 paddocks were further split into a total of 66 paddocks with temporary electric fences. During lambing ewes were set stocked to minimise miss-mothering of lambs. The duration of this set stocking was generally 6-8 weeks. The target stocking rate was 15 DSE/ha but during the experimental period the average rate was 8.8 DSE/ha. In year 1 sheep numbers totalled 294 (125 ewes, 30 ewe hoggets, 54 ewe lambs and 85 wethers) while in year 2 they totalled 395 (150 ewes, 53 hoggets, 72 lambs and 120 wethers). Cattle were used on 2 occasions, from October 2003 to May 2004 (15 steers) and from September 2004 to March 2005 (29 steers). Only 1 paddock was re-sown in 2004. Under this management system deep-rooted fertiliser responsive perennial species comprised 18% of pasture in 2005 with native grasses increasing in proportion from 38% in 2000 to 50% in 2005 (Mpiti-Shakhane 2006). The targets for soil phosphorus and sulphur were as the conventional system, and supplementary feeding levels were also similar (Table 2-2).

Table 2-2: Total days of supplementary feed per sheep class under each management system over the experimental period from November 2003 to October 2005. The main type of supplement given was lupins and with some maize given on the HI management system.

Management system	Class	Total Days fed		Average rate of Feed (kg/sheep/week)	
		Year 1	Year 2	Year 1	Year 2
High input (HI)	Ewes	118	97	1.3	2.5
	Hoggets	133	0	1.0	0.0
	Lambs	0	271	1.0	3.5
Conventional (TYP)	Ewes	111	77	0.7	2.0
	Hoggets	133	0	1.0	0.0
	Lambs	0	272	0.0	1.7
Intensive rotational grazing (IRG)	Ewes	118	91	0.7	1.2
	Hoggets	133	0	1.0	0.0
	Lambs	0	272	0.0	1.7

Pasture dry matter availability under each of the management systems throughout the experimental period is shown in Figure 2-3. The HI treatment had lower total dry matter with a higher proportion of green dry matter than the TYP and IRG treatments, which were similar.

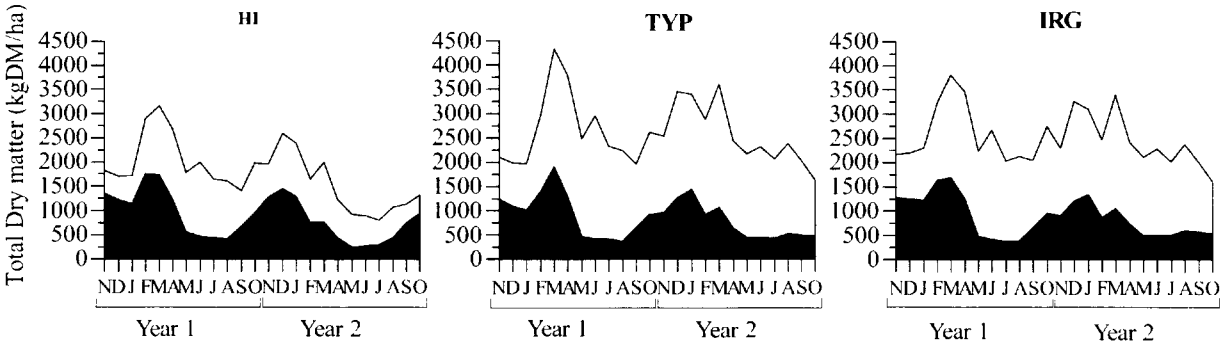


Figure 2-3: Total pasture dry matter divided into green dry matter (black) and dead dry matter (white).

2.2.3.1 Anthelmintic treatment on the Cicerone Farmlets

Anthelmintic treatments comprised two fixed treatments across all management treatments and tactical treatments applied within management groups on the basis of monitored faecal worm egg count (WEC), larval differentiation and advice from a consulting parasitologist. The fixed treatments comprised a quarantine treatment given to all sheep as they moved off the property

for shearing in July and a pre-mating treatment for all ewes in March-April prior to grouping in a clean periphery paddock external to the management treatment areas. All anthelmintic treatments for the duration of the experimental period are detailed in Table 2-3. The anthelmintics used were moxidectin (Cydectin®, Fort Dodge Australia Pty Ltd), albendazole oxide) + levamisole (Combi®, Novartis), levamisole (Levamisole Gold®, Virbac) and naphthalophos (Rametin®, Bayer Healthcare).

Table 2-2-3: Anthelmintic treatments during the experimental period by sheep class and management treatment; moxidectin (MOX), albendazole (ABZ), levamisole (LEV), napthalophos (NAP). Where LEV was given without other anthelmintics it was administered at double the recommended dose rate under veterinary advice (Dr E. Hall, personal communication). Asterisks denote fixed treatments imposed for reasons other than perceived risk of GIN.

Class	Date of treatment	Anthelmintic treatment		
		High Input (HI)	Conventional Management (TYP)	Intensive Rotational Grazing (IRG)
Ewes	23/12/2003	MOX + ABZ + LEV	MOX + ABZ + LEV	MOX + ABZ + LEV
	*19/04/2004	LEV double dose	LEV double dose	LEV double dose
	*30/07/2004	MOX + ABZ + LEV	MOX + ABZ + LEV	MOX + ABZ + LEV
	17/11/2004	LEV double dose	LEV double dose	
	14/12/2004	MOX + ABZ + LEV	MOX + ABZ + LEV	
	24/01/2005	LEV double dose	LEV double dose	
	24/02/2005	NAP/ABZ	NAP/ABZ	NAP/ABZ
	*23/04/2005	NAP/ABZ	NAP/ABZ	
	*23/07/2005	NAP/ABZ/LEV	NAP/ABZ/LEV	NAP/ABZ/LEV
Hoggets	23/12/2003	MOX + ABZ + LEV	MOX + ABZ + LEV	MOX + ABZ + LEV
	*19/04/2004	LEV double dose	LEV double dose	LEV double dose
	*30/07/2004	MOX + ABZ + LEV	MOX + ABZ + LEV	MOX + ABZ + LEV
	28/9/2004	LEV double dose		
	15/11/2004		LEV double dose	
	5/01/2005		MOX	
	24/01/2005	LEV double dose	LEV double dose	
	24/02/2005	NAP/ABZ	NAP/ABZ	NAP/ABZ
	*23/04/2005	NAP/ABZ	NAP/ABZ	
	*23/07/2005	NAP/ABZ/LEV	NAP/ABZ/LEV	NAP/ABZ/LEV
Lambs	23/12/2003	MOX	MOX	MOX
	10/03/2004	LEV double dose	LEV double dose	
	8/05/2004	LEV double dose	LEV double dose	LEV double dose
	2/07/2004	LEV double dose	LEV double dose	
	*30/07/2004	MOX + ABZ + LEV	MOX + ABZ + LEV	MOX + ABZ + LEV
	28/09/2004	LEV double dose		
	14/12/2004	MOX	MOX	
	24/01/2005	LEV double dose	LEV double dose	
	24/02/2005	NAP/ABZ	NAP/ABZ	NAP/ABZ

	23/04/2005	NAP/ABZ	NAP/ABZ	
	*23/07/2005	NAP/ABZ/LEV	NAP/ABZ/LEV	NAP/ABZ/LEV
Total treatments		28	27	15

2.3 Parasitology

Different transformations were used for the same variable in different experiments in order to correct the distribution problems of each individual data set and to better meet the assumptions of an analysis of variance (normal distribution and equal variance). This applies most directly to faecal worm egg counts, for which I have used two transformations; Log (Expt 1) and cubed-root (Expts 3 and 5), and the raw data (Expt 2). There are some valid arguments for the application of blanket transformation across experiments (Eady 1995), but I believe that each data set should be assessed on its unique distribution and the transformation applied that offers the best correction. The methods used to determine normality and equal variance of the data involved fitting a normal curve to the residuals and checking their pattern. In experiment 1, transformation of WEC did not solve the problem of the non-normal distribution, models performed on this data were very unreliable and the data was still in breach of the assumptions of the model. Thus, a two part analysis was employed. This involved analysis of a binomial representation of the data testing treatment effects on the presence or absence of WEC. In the second analysis all zero WEC were removed which resulted in more successful data transformation. This approach allowed a more robust analysis of WEC data from that experiment.

2.3.1 Faecal Worm Egg Counts

For faecal samples from all of the fixed challenge study (Experiment 2), the tracer study (Experiment 3), the capsule study (Experiment 4) and part of the longitudinal study (Experiment 1, from November 2003 until October 2004), the following technique was used for estimation of faecal worm egg count and were prepared and counted by myself. The remaining samples were submitted to a commercial laboratory for faecal worm egg count (WEC) determination (Paracount, Toowoomba, Qld).

1. A faecal sample was obtained from the rectum of each of the experimental sheep and placed in a plastic 20ml vial.
2. A label was applied with the following information: Animal tag number, class, experiment/ear tag colour and a predetermined random number for laboratory processing.
3. The faecal samples were stored in an esky containing ice for transport to the laboratory. Once at the laboratory the samples were placed on trays in order of their assigned random number and diluted immediately or placed in a 4^0C fridge overnight.

i. Each sample was diluted with 1 part faeces and 4 parts water

4. Once diluted, the samples were mixed using a bench drill fitted with a mixing rotor.
5. Universal slides were loaded with 600µl of saturated salt solution followed by a sieved sample of 150µl.
6. Examination of slides was at low power using a x4 objective thus magnifying the samples by 40 times.
7. One chamber was counted per sample and egg counts were then multiplied by 50 to obtain eggs per gram of faeces.

2.3.1.1 Pooled faecal worm egg counts

1. Mix rectally collected faeces thoroughly in jar.
2. Remove approximately 30g of the mixed faeces and place in a 250ml container
3. Weigh faeces and add distilled water at a rate of 1g faeces: 4g water
4. Mix thoroughly to break up faeces, if faecal matter is relatively dry place container, with a lid applied, on a mixer for 1-2 hours.
5. Follow steps 6 to 10 as for individual faecal worm egg counts
6. 5 slide chambers should be counted and averaged to give the pooled faecal egg count.

2.3.2 Pooled Larval Cultures

Pooled larval cultures were carried out regardless of WEC in all experiments. For the longitudinal study (Experiment 1) and the tracer study (Experiment 3) faecal samples were placed in jars according to management treatment and class. Faecal samples for the fixed challenge (Experiment 2) and the capsule study (Experiment 4) were bulked according to management treatment as only one class of stock was involved. The following method was then applied.

1. Add water to just cover the faeces and allow to soak if faeces are hard.
2. Mash to break up faecal pellets and mix into a slurry.
3. Add vermiculite to the same volume of the faecal slurry and mix thoroughly.
4. Lightly pack down the mixture and wash the sides of the jar with a water bottle being careful not to over wet the mixture.
5. Apply lid loosely to jar and place in a 27°C incubator
6. Remove from incubator after 7 days treat in either of two ways:
    a. If WEC was above 100 eggs/g of faeces tightly screw on lid of jar and leave at room temperature (or in incubator if room is cold) for ½ to 1 hour, then with the

jar on its side rinse the inside using a water bottle, being careful not to let the water touch the vermiculite/faecal mixture. Pour water into small container for further examination.

b. If egg count is zero or below 100 eggs per gram faeces fill jar with warm water to the brim, place a petri dish on top and invert the jar, fill the petri dish with water and leave at room temperature for at least 3 hours or overnight. Aspirate water from petri dish using a Pasteur pipette into a container for further examination.

7. Examine the washings from the jars under a dissecting microscope for presence of larvae. If there are many proceed to next step, if there are very few pour the water into a conical bottomed 50ml centrifuge tube and leave to sit for 15 minutes before removing sample from the bottom of the tube for examination.
8. Mix water by inversion, take a 0.5ml sample and place on a glass slide, add diluted Lugol's iodine to stain the larvae. Place a cover-slip over the droplet and examine under x10 objective for species/genera differentiation. A further magnification using a x20 objective was necessary for identification of *Teladorsagia circumcincta* and *Trichostrongylus* spp.

The species/genera differentiation was determined on the morphological characteristics of the third stage larvae based on established theory (Ministry of Agriculture Fisheries and Food 1986).

2.3.3 Faecal Egg Count Reduction Test on Farmlet C (IRG treatment)

A faecal egg count reduction test (FECRT) was carried out on Farmlet C lambs in May 2004 to determine the effectiveness of 4 anthelmintics against *Haemonchus contortus*. Due to the small number of lambs available only 4 treatment groups plus a control group were used. The control group of lambs were sampled at the beginning and the end of the experiment to determine the change of WEC over time. This method of FECRT was reported by Kemper and Walkden-Brown (2004) as the most accurate available. The experiment commenced when the WEC of the lambs reached a threshold of 400 eggs/g. The following anthelmintics were tested: Albendazole (Alben®, 3.8mg/kg), Levamisole (Levamisole Gold®, 8.0mg/kg), Ivermectin (Ivermec®, 0.2mg/kg), Closantel (Closicare®, 7.5mg/kg) at full dose rate.

On day 0:

1. 75 lambs were randomly allocated to 5 groups of 15

2. All lambs rectally sampled for faeces for individual WEC and bulked group larval cultures
3. Body weights were recorded and each lambs dosed, using a stomach tube, with anthelmintic calculated for their body weight or 30ml of water for the control group.

On day 13:

1. Individual WEC and bulked group cultures were taken at day 13 after treatment
2. Faecal egg count reduction calculated for *H.contortus*.

The equations for estimating the FECRT and 95% confidence intervals are as follows (Kemper and Walkden-Brown 2004):

$$\% \text{ Reduction} = 100 \left\{ 1 - \left[\frac{\text{Mean } \textit{treatment final} \text{ egg count (epg)}}{\text{Mean } \textit{treatment initial} \text{ egg count (epg)}} \right] \left[\frac{\text{Mean } \textit{control initial} \text{ egg count (epg)}}{\text{Mean } \textit{control final} \text{ egg count (epg)}} \right] \right\}$$

$$\pm 95\% \text{ CI} = 100 \left\{ \% \text{ Reduction} \cdot \exp(\pm 2.1 \sqrt{V}) \right\}$$

where,
$$V = \left[\frac{\text{variance } \textit{final treatment} \text{ WEC}}{n_t \cdot (\text{mean WEC } \textit{final treatment})^2} \right] + \left[\frac{\text{variance } \textit{initial control} \text{ WEC}}{n_c \cdot (\text{mean WEC } \textit{initial control})^2} \right]$$

where, n_t = number of animals in the treatment group

n_c = number of animals in the control group

2.3.3.1 Results of the Faecal Egg Count Reduction Test

The effectiveness of the drench is determined by both the percentage reduction in WEC and the lower confidence interval values. The presence of resistance to an anthelmintic was assumed if (i) the percentage reduction in WEC was less than 95% and (ii) the lower 95% confidence interval was less than 90%. Emerging resistance was suspected when only one of these criteria was met (Coles *et al.* 1992). Levamisole was the only anthelmintic treatment that returned a 100% susceptibility of *H. contortus* (Table 2-4), Closantel was suspected to have reduced efficacy on the Cicerone Project but returned a result of susceptible meeting both the selection criteria. However, Closantel is a persistent anthelmintic with a recommended protection period against *H. contortus* of 42 days, thus emergence of resistance is suspected as there were breakthrough egg counts only 13 days after treatment. *H. contortus* showed resistance to both Ivermectin and Albendazole. The resistance to Ivermectin may indicate the emerging resistance to its chemical relative moxidectin but this was not tested.

Table 2-4: Reduction of worm egg count with lower confidence interval for each anthelmintic treatment used in the faecal egg count reduction test on Farmlet C.

	Levamisole	Ivermectin	Closantel	Albendazole
% Reduction of WEC	100	86.1	96.8	61.1
Lower 95% C.I.	100	80	93	34

2.4 Haematology

Blood samples were obtained from sheep using an 18 gauge needle and holder into a 4.5ml purple topped vacutainer containing K_3EDTA to prevent clotting. The samples were kept on ice until transport to the CSIRO haematology laboratory. The samples were brought to room temperature, mixed by inversion on a mixer for 4 minutes before loading onto the racks. Blood cell parameters were measured using a Cell Dyn 3500 haematology unit (Abbott Diagnostics, USA) calibrated for sheep blood. In most instances samples were processed on the day of collection with a maximum time between sampling and processing of 24 hours. The Cell Dyn® measured white blood cell counts (neutrophils, lymphocytes, monocytes, eosinophils, basophils), red blood cell counts, mean corpuscular volume and haematocrit for each animal. The results were checked for low and high counts as well as missing data and re-run if irregularities were detected. The Cell Dyn® was serviced by an Abbott Diagnostics technician 6 monthly at which point quality controls are run through the machine to check that the various parameters are within acceptable ranges for their controls. Adjustments are made if required.

The blood samples were then transported to the UNE immunology laboratory and centrifuged at 4°C for 15 minutes at 1400g. The plasma was removed using glass Pasteur pipettes and stored in 2.5ml or 5ml screw top containers at -20°C.

The Cell Dyn® malfunctioned during experiment 3 in the summer challenge and could not be used on day 28, hence haematocrit was obtained using the microhaematocrit method described below. On day 35 the Cell Dyn® was working and samples were processed on it with a random selection of 10 animals also tested using the microhaematocrit method. The relationship between results from the Cell Dyn® and the microhaematocrit method was strong ($R^2 = 0.98$, Figure 2-4), thus the haematocrit measurements for day 28 were in included in subsequent analyses.

Microhaematocrit method.

1. Invert tube containing blood sample.
2. Place a microhaematocrit pipette into the blood and draw up using capillary action.

3. Place thumb over the top end protruding from the sample tube, wipe the exterior of the tube clean of blood and push the open end into plasticine plugging medium to seal.
4. Continue filling microhaematocrit pipettes until there are enough to fill the microfuge.
5. Place microhaematocrit pipettes into position making sure that the plugged end is facing outward. Always ensure that microfuge has an even number of microhaematocrits so it remains balanced.
6. Turn on the microfuge and spin blood for 5 minutes
7. Determine packed cell volume (haematocrit) by placing microhaematocrit in position on reader and reading the line which runs through the top of the red blood cell portion of the pipette.

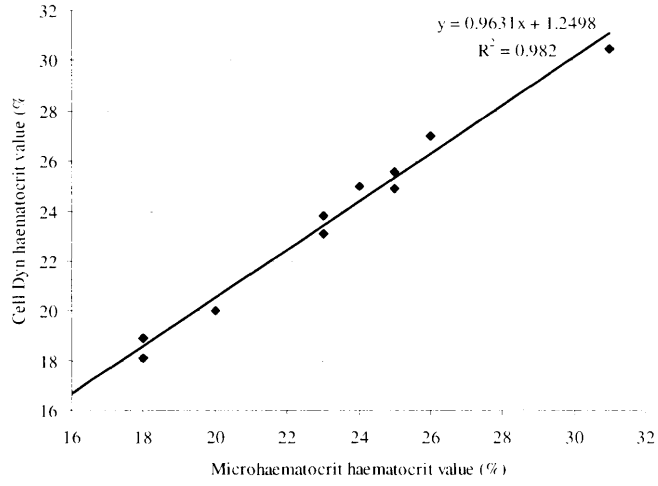


Figure 2-4: Linear regression of haematocrit determined by Cell Dyn® on haematocrit determined by the microhaematocrit method. Samples (n=10) are from day 35 of the summer fixed challenge in Experiment 2, (Chapter 4).

2.5 Measurement of anti-nematode IgG

Determination of anti-nematode IgG antibody levels in the retained plasma was performed only for the fixed challenge study (Experiment 2, Chapter 4) using an indirect ELISA based on antigen from L_3 of *Haemonchus contortus* developed at UNE (Gill, 1993). The assay has subsequently been shown to be non-specific for individual trichostonglyle genera with a high level of cross-reaction between *Haemonchus*, *Trichostrongylus* and *Teladorsagia* (Walkden-

Brown and Maslen 2002). Two thirds of the assays were performed by an assistant with the remaining portion completed by me.

The assay used antigen from L_3 of *Haemonchus contortus* to coat the wells. IgG in the plasma samples binding to this antigen was detected with monoclonal anti sheep/goat IgG raised in mouse. This second antibody was conjugated to horseradish peroxidase enzyme followed by the substrate (see section 2.5.1.1 for detail) providing a colorimetric indication of the amount of bound IgG from the sample. Quantification of bound IgG was by reading off a standard curve based on serial dilution of a high sample ascribed arbitrary units.

Three replicates of the standard curve (Table 2-5) were included in each plate, together with two replicates of high, medium and low quality control samples as positive controls and for determination of inter-assay variation. An example of the standard curves produced during the ELISA is illustrated in Figure 2-5. Two negative blanks were also included in each assay as negative controls. Samples were stratified so that all samples across time from each animal were assayed on a single plate.

Table 2-5: Standard curve dilutions for ELISA plates

Standard	Dilution
01	1:100
02	1:200
03	1:400
04	1:800
05	1:1600
06	1:3200
07	1:6400
08	1:12800

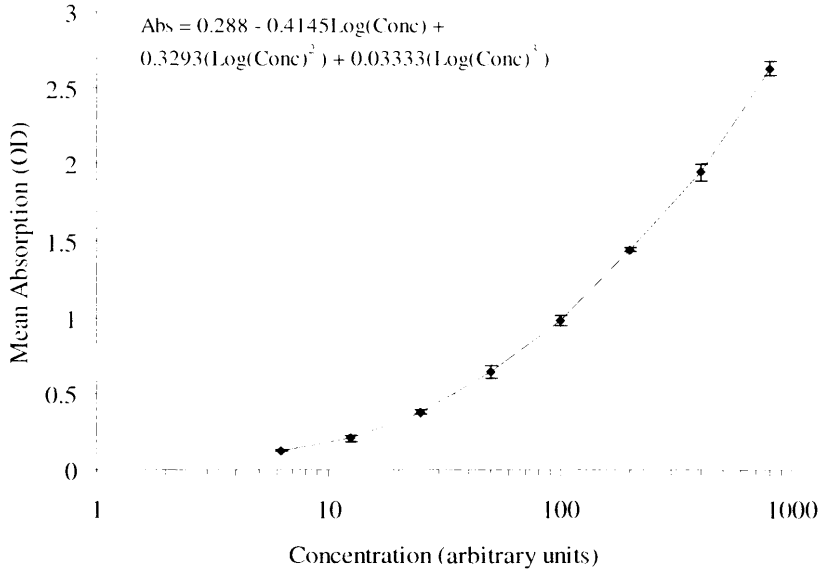


Figure 2-5: Example of a standard curve from ELISAs with a cubic log-linear curve fitted.

Samples were assayed in duplicate at an initial dilution 1:100, the same dilution as the highest standard. Samples were re-assayed at a different dilution if they fell outside the sensitive range of the assay at the initial dilution. When the effect of dilution on antibody concentration was tested it proved significant ($P<0.01$, Figure 2.6), but the significance was entirely due to higher values at the highest dilution rate of 1:5,000 well outside the range used for samples. All other dilution rates were not significantly different (Figure 2-6). The effect of dilution also accounted for only 1.3% of the total variance of the model whereas the between animal variation accounted for 63% indicating that dilution effects are unlikely to mask between animal variation.

The mean intra-assay coefficient of variation (CV) was 9.65% over all samples with the mean coefficient of variation for the quality control samples being 38% for the High QCs, 51% for the Medium QCs and 38% for the Low QCs. The CVs presented appear large because they are for backtransformed results on a linear scale (i.e.: Arbitrary units). Optical density is related to the log of the concentration and CV are much lower on the log scale.

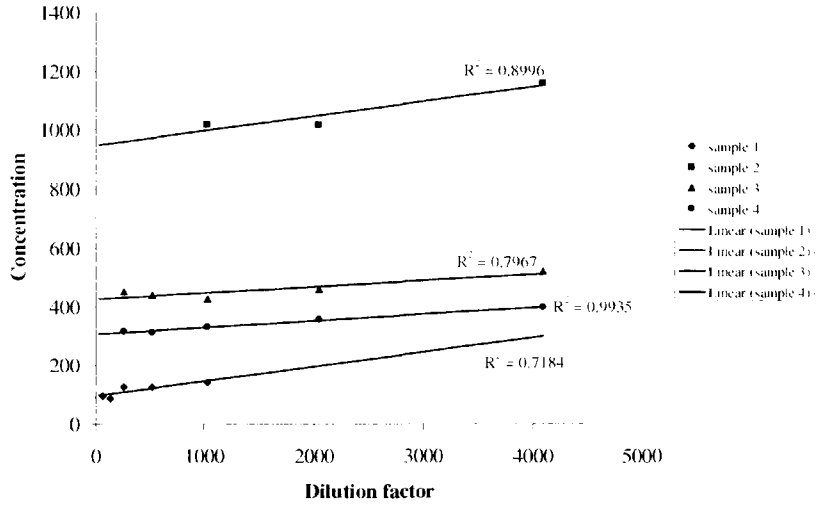


Figure 2-6: Serial dilution of four samples with high antibody titre demonstrating high repeatability of final concentration over a range of dilutions. Concentration values are adjusted for dilution.

2.5.1 ELISA method

1. Plasma samples were diluted using PBST to required concentration (1:100 dilution to start with, the dilution was adjusted only if the antibody levels were too high or too low for the standard curve).
2. ELISA plate was coated with *Haemonchus contortus* L_3 antigen with carbonate buffer (100µL/well).
3. Plates were covered and incubated overnight at 4°C.
4. Plates were washed three times with PBST (see below) in plate washer.
5. Samples, standards, quality controls and blanks were added to wells at 100µl/well and incubated for 1 hour at 37°C.
6. Plates were washed three times with PBST in plate washer.
7. Conjugate was added at 100µl/well to each plate: 1:3000 dilution in PBST (Horseradish peroxidase conjugated to monoclonal Anti-Goat/Sheep IgG, purified Mouse Immunoglobulin, Sigma).
8. Plates were incubated for 1 hour at 37°C.
9. Plates were washed three times with PBST in plate washer.
10. Substrate was added at 100µl/well, covered with foil and left at room temperature for 30 minutes. This provided the colour reaction to bound Horseradish peroxidase.
11. Reaction was stopped with 2N Sulphuric Acid at 50µl/well.
12. Wells were mixed for 3 seconds.

13. Wells were read at 490nm.

2.5.1.1 ELISA reagents

Carbonate Buffer: 0.05M pH 9.6

Anhydrous Carbonate (Na_2CO_3) 1.59g/L distilled water

Sodium Hydrogen Carbonate ($NaHCO_3$) 2.93g/L distilled water

PBST: Phosphate buffered saline pH 7.4 powder sachet (Sigma Diagnostics, St Louis, USA) made up to 1000ml with distilled water

0.5ml Tween20/L distilled water

Washing PBST: 0.15M pH7.2 to 7.4 in distilled water plus 0.5ml Tween20/L

NaCl 8g/L

KCl 0.2g/L

Na_2HPO_4 1.15g/L (di-Sodium Hydrogen Orthophosphate)

KH_2PO_4 0.2g/L (Potassium di-Hydrogen Orthophosphate)

Citrate Phosphate Buffer: pH 5.0

Citric Acid 7.3g/L

Na_2HPO_4 9.468g/L

Substrate:

Citrate Phosphate buffer 100ml

OPD (o-Phenylenediamine) 34mg

H_2O_2 (Hydrogen Peroxide 30%w/v) 50µL

Cover with Alfoil as is light sensitive, add Hydrogen peroxide just before use.

2N Sulphuric Acid(H_2SO_4):

98mls H_2SO_4 (98%) per litre distilled water.

2.6 Liveweights and Fat Scores

Liveweights were determined using a set of Ruddweigh electronic scales and holding crate. The liveweight of each animal was recorded manually onto sheets with tag numbers pre-recorded. An estimate of fat score was determined only for hoggets and ewes by myself by palpation of the

12th rib at the GR site (Figure 2-7) according to the guidelines in Table 2-6 (Holst and White 2001).

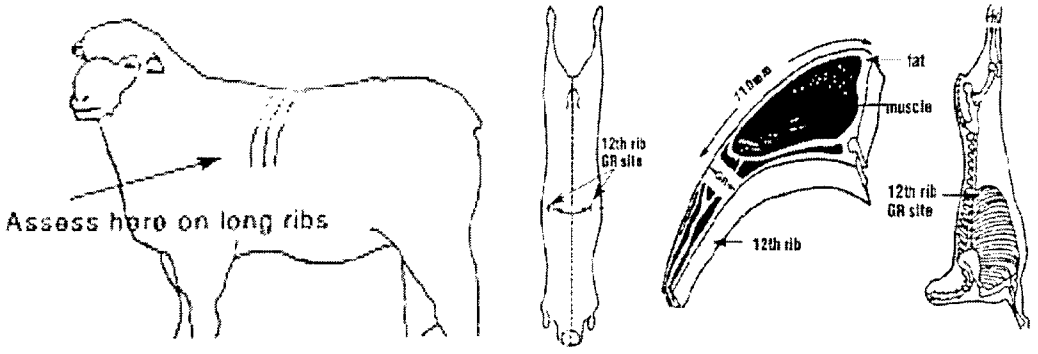


Figure 2-7: Best position for assessment of fat on sheep and lambs (Holst and White 2001).

Table 2-6: Fat scoring system based on 12th rib at the GR site (Holst and White 2001).

Fat Scores					
Score	1	2	3	4	5
GR tissue depth (mm)	0-5	6-10	11-15	16-20	21+
Assessment over the long ribs	Individual ribs felt very easily. Can not feel any tissue over the ribs.	Individual ribs easily felt however. some tissue is present.	Individual ribs can still be felt. Can feel more tissue over the ribs.	Can only just feel ribs. There is fluid movement of tissue.	Ribs can not be felt. Tissue movement is very fluid.
Dressing Percentage	41%	43%	45%	47%	49%
Dressing Percentages are for second cross lambs. Data is supplied by NSW Agriculture.					

2.7 Ultrasound scanning for pregnancy status

Pregnancy scanning was performed by Australian Livestock Technology in June 2004 and August 2005. Ewes were recorded as either empty, single bearing or twin bearing.

CHAPTER 3: Experiment 1. Longitudinal study of gastrointestinal nematode infection in sheep on the Cicerone Project Farmlets over two years

3.1 Introduction

The use of rotational grazing to control gastrointestinal nematode infection has been the subject of considerable experimental investigation (Morgan 1933; Morgan and Oldham 1934; Gordon 1948; Roe *et al.* 1959; Gibson and Everett 1968; Barger *et al.* 1994; Barger 1997; Barger 1999). Systems of rotational grazing have been proposed according to the understanding of the ecology of the free-living stages and tested against set-stocking for animal performance and nematode control. Rotational grazing strategies basically utilise one or both of two key principles. The first involves allowing sheep to graze for a short period of time with removal before the eggs laid down in the their faeces develop into infective larvae, thus preventing autoinfection from the current grazing. The other significant principle involves the paddock being spelled between graze periods for sufficient time to allow for the majority of the infective larvae to die off. However, in most earlier studies, particularly in cooler temperate climates, the length of the grazing and rest periods tested were not appropriate for worm control throughout the year. Thus graze periods of 1 week tend to be too long in warm weather and rest periods were usually too short (3-7 weeks).

In contrast to most studies, Robertson and Fraser (1933) did achieve significant differences in worm burdens between rotationally grazed sheep and set stocked sheep in the north of Scotland. Their grazing regime was 10 days graze, 100 days rest with the authors concluding that 10 days was a sufficient length of time to prevent autoinfection whilst 100 days was long enough to allow for adequate larval decay. Their main criticism of this rotational grazing system was the tendency of pasture to be over mature when sheep returned to graze, resulting in bodyweight losses not observed in the set stocked sheep.

Rotational grazing was implemented with much greater success in the tropics with a system termed rapid rotational grazing devised by Barger *et al* (1994) based on the ecological studies of Banks *et al* (1990). This system of rotations, initially applied to goats was based on a fixed time rotation with a grazing period of 3.5 days and a rest period of 31.5 days. The goats were housed at night to avoid predators and theft. This system worked well in a tropical environment where nearly constant heat and humidity combine to provide the perfect development conditions from

egg to L_3 and poor survival of infective larvae through rapid exhaustion of energy reserves. The authors predicted that rapid rotational grazing would not be economically viable in more temperate climates because seasonal variations in climate would render the system inappropriate in some seasons.

The introduction of more flexible intensive rotational grazing systems, based on pasture availability rather than fixed time rotations, has allowed graze and rest periods to alter naturally with the seasons (Savory and Parsons 1980; Earl and Jones 1996). This type of grazing management is used on approximately 6% of sheep producing farms, with a higher incidence of 10% in the Northern Tablelands of New South Wales (Reeve and Thompson 2005). As weather warms up the rotations become faster, and as weather cools the rotations slows. This is mainly based on pasture growth rates which slow and speed up according to temperature and rainfall. This type of intensive rotational grazing is employed on one of the 3 Cicerone Project farmlets as described in Chapter 2. As the conditions favouring pasture growth and larval development are broadly similar, such grazing systems may have the effect of regulating rotations appropriately for worm control as well. Indeed an analysis of historical WEC collected on the 3 Cicerone Project farmlet revealed a significant reduction of WEC on the intensive rotational grazing system (IRG) (Healey *et al.* 2004). The WEC were collected over a three year period of intermittent general farm monitoring of WEC on the 3 farmlets. The data were only suitable for analysis where all 3 farmlets had been monitored within the same month and same class of sheep (ie: ewe, lamb, wether, hogget) leaving the data set patchy and irregular.

In order to confirm these preliminary findings and investigate in more detail the effects of management system on gastro-intestinal nematodiasis, the present experiment was designed. Its aims were to a) determine whether management system influences the incidence and severity of GIN as determined by WEC and animal performance; b) determine whether the effects of management system differ for the different major gastrointestinal nematode species and c) to gain insight into possible underlying reasons for observed management system effects on WEC.

The hypotheses were a) that sheep on the intensive rotational grazing system will have a lower incidence and severity of gastrointestinal nematodiasis than the other two management systems, and that HI management system will have a greater magnitude of disease than TYP; b) That *Haemonchus contortus* will be more affected by the intensive rotational grazing system than *Trichostrongylus* spp. or *Teladorsagia* spp.; c) That any changes in gastrointestinal nematodiasis

on the intensive rotational grazing system will be due to mediation in the free-living stages of the parasite life cycle.

3.2 Materials and Methods

This experiment was a longitudinal study of GIN under 3 farm management systems over a 2 year period from November 2003 to October 2005. The key factors in the design were:

- Three management systems: High input (HI), Typical (TYP) and Intensive rotational grazing (IRG) (section 2.2).
- Three classes of sheep: Lambs, hoggets and ewes.
- Time. The study covered 2 years, Year 1 (November 2003 – October 2004) and Year 2 (November 2004 – October 2005).

3.2.1 Experimental sheep and their husbandry

The experimental sheep were superfine wool merinos. The classes of sheep monitored during the experiment were all females and defined as ewes (females over 2 years of age) hoggets (12 - 24 months of age) or lambs (<12 months of age). In November 2003, 20 ewes, 20 hoggets (Sept 2002 born) and 20 lambs (Sept 2003 born) from each management treatment were randomly selected and individually ear-tagged. These 180 experimental animals were sampled each month throughout the experiment, with lambs becoming hoggets and hoggets ewes in November 2004. In that month an additional 20 lambs (Sept 2004 born) from each management treatment were included thus bringing the total number sampled in year 2 up to 240. The size of the ewe group doubled in year 2 as the new hoggets were included and the original ewes retained. Mating occurred over a 5-week period in April/May each year with ewes and hoggets from all management treatments moved to a common periphery paddock for joining with common sires. Lambing started in mid-September and was finished by the end of October with lamb marking in mid-November and weaning in late January. Shearing of all sheep took place at the end of July each year in an off-site shearing shed.

Anthelmintic treatments given during the experimental period are provided in Chapter 2 (Table 2-3 section 2.2.3.1).

3.2.2 Measurements

The date of all stock movements into and out of paddocks was recorded. Each month all of the experimental sheep were weighed and faecally sampled per rectum for determination of WEC

and pooled faecal culture for larval differentiation as described in section 2.3. Every second month a blood sample was collected for haematological analysis as described in section 2.4. At shearing each year in July fleece weight was recorded and a mid-side fleece sample collected for fleece quality measurements. Greasy fleece weight of the un-skirted fleece with bellies and pieces was measured at shearing and fibre diameter measured on mid-side samples 1-2 weeks prior to shearing using an optical fibre diameter analyser (OFDA 2000, BSC Electronics Pty Ltd, Australia).

3.2.3 Statistical Analysis

Data for several variables was transformed prior to analysis: WEC, $Log_{10}(x+1)$; graze period, $Log_{10}x$; eosinophil data, cubed-root; haematocrit (%) and larval differentiation (%), ArcSine($\sqrt{}$proportion). In addition to analysing the percentage of larvae of each species in the pooled larval culture, WEC data for individual species or genera was derived by multiplying total WEC by the proportion of larvae of the relevant species from the pooled culture. WEC, bodyweight, wool parameters, fat scores, and blood parameters were analysed by fitting appropriate linear mixed models followed by analysis of variance using the statistical package JMP IN version 5.1 (SAS Institute Inc., NC, USA). The effects tested in the models were management treatment (HI, TYP, or IRG), Class (Ewe, Hogget or Lamb), Month, Season or Year and Tag Number (fitted as a random variable for repeated measures data). Seasons were spring/summer (September to February), autumn/winter (March to August). Significant two and three way interactions were retained in the model. Appropriate covariates were included in the models to either remove their effects or to explore their effect on the variable under analysis. Only significant covariates were retained in the final model except for anthelmintic interval. Interval between anthelmintics was analysed using Group (original Ewes, 2002-born, 2003-born and 2004-born sheep) in place of Class as the change over of animals between classes would confound the data otherwise. Tukey's post hoc tests and contrasts were used to separate means.

As no transformation made the entire WEC dataset amenable to analysis with a linear mixed model due to a high proportion of zero values, it was subjected to two analyses. Analysis 1 used a nominal logistic model to analyse the incidence of GIN (ie. WEC zero or >0). Analysis 2 analysed the magnitude of WEC in those animals where WEC > 0 using linear mixed models as described above.

A level of significance of $P<0.05$ is used throughout and data are generally presented as least square means ± standard error (LSM±SEM) or in the case of transformed data, back transformed LSM with 95% confidence intervals which are asymmetric due to back transformation.

3.3 Results

3.3.1 Implementation of treatments

3.3.1.1 Paddock rotations

There was a significant effect of management treatment on graze period with IRG having shorter graze periods (4.0 days, CI 3.9-4.3) than HI (41.3 days, CI 36.6-52.4) and TYP (53.8 days CI 46.9-70.2) (P<0.0001) which did not differ significantly. There was no overall effect of season (P~0.80) or year (P~0.54), however there was a significant interaction between management treatment and season (P<0.0001). This was due to shorter graze periods in spring/summer (2.9 days, CI 2.8-3.2) than autumn/winter (5.4 days, CI 5.1-6.1) on IRG but not the other management treatments (Figure 3-1).

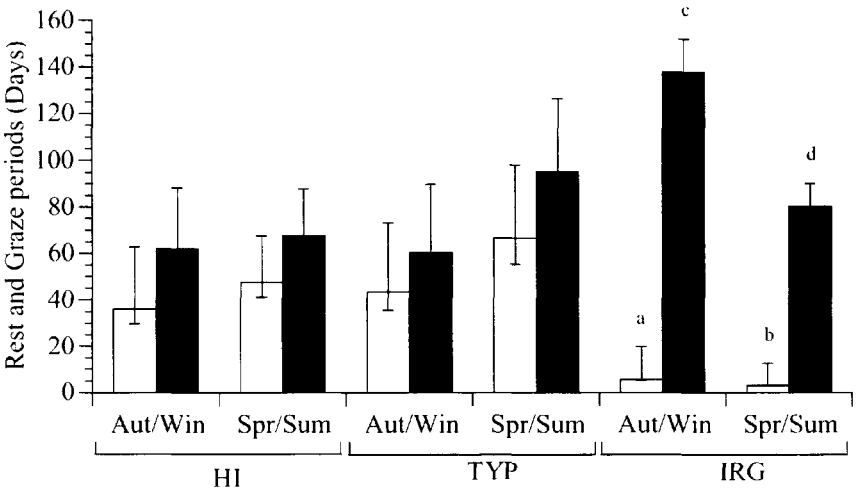


Figure 3-1: Mean rest period (black) and back-transformed mean graze period (white) with 95% CI by season and management treatment. Columns not sharing a common letter within management treatment differ significantly (P<0.05).

Management treatment also had a major effect on rest period between grazing events (P<0.0001) with IRG paddocks having a longer mean rest period (108.8 ± 4.1 days) than those under HI or TYP management (64.7 ± 7.9 and 77.7 ± 10.2 days, respectively). There was a significant effect of year (P~0.01) with shorter rest periods in Year 1 than Year 2 (72.1 ± 6.1 and 95.3 ± 6.7 days, respectively). There was no overall effect of season on rest period (P~0.56) but there was a significant interaction between management treatment and season (P<0.0001) due to significantly longer rest periods in autumn/winter (137.4 ± 6.8 days) than in spring/summer (80.2 ± 4.6 days) on the IRG paddocks, but not the other management treatments.

3.3.2 Parasitological variables

Figure 3-2 shows total WEC, *H. contortus* WEC and *Trichostrongylus* spp. WEC for each management treatment and class and indicates the time and type of anthelmintic treatment given.

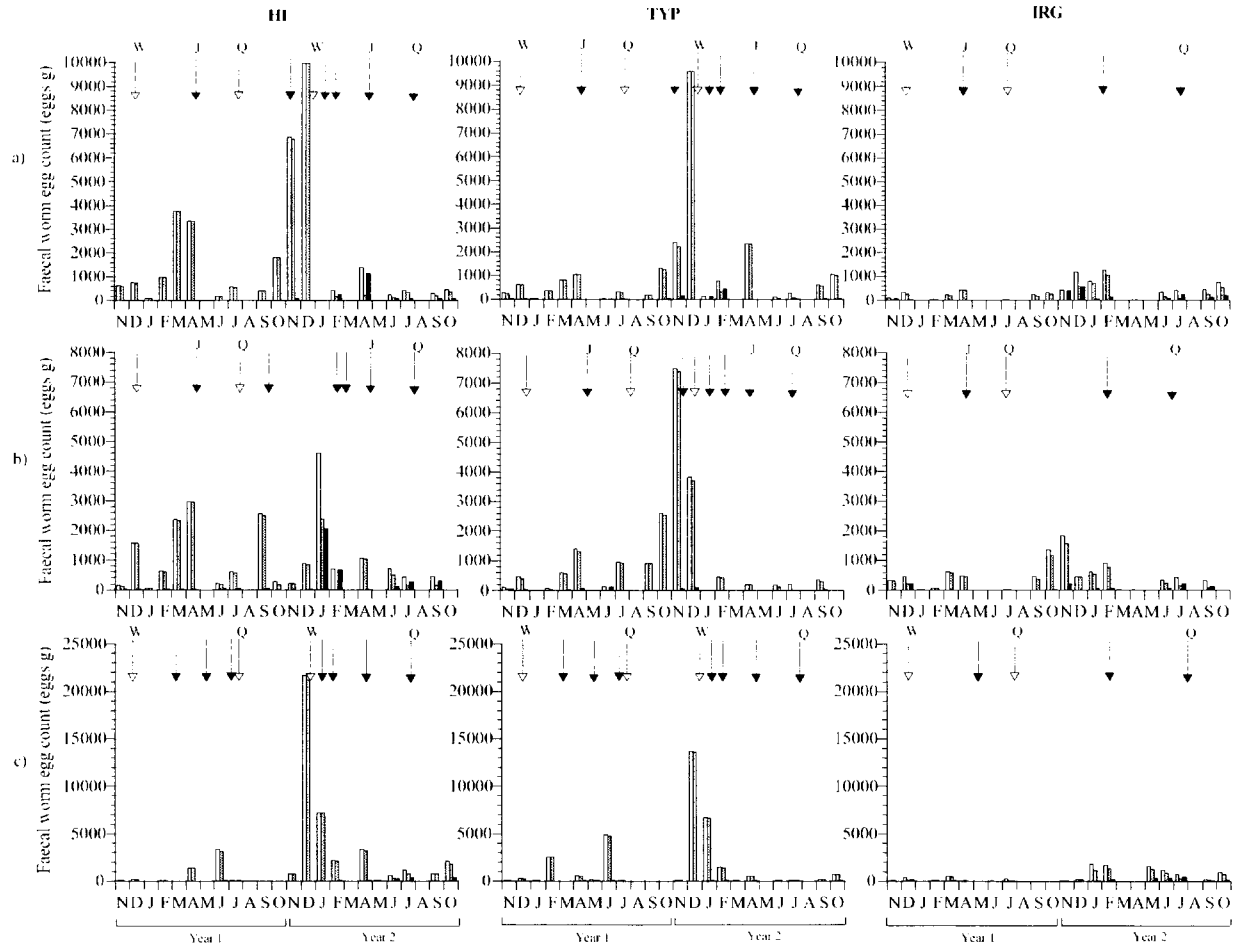


Figure 3-2: Mean raw a) Ewe b) Hogget c) Lamb total worm egg counts (white), *Haemonchus contortus* worm egg counts (grey) and *Trichostrongylus* spp. worm egg counts (black) over the experimental period with anthelmintic treatments indicated by arrows; moxidectin (white), short acting (black), weaning (W), mating (J) and quaratine (Q).

3.3.2.1 Larval differentiation

The overall proportions of nematode species and genera were *Haemonchus contortus* (70%), *Trichostrongylus* spp. (22%), *Teladorsagia circumcincta* (4%), *Oesophagostomum* spp. (3%) and *Cooperia* spp. (1%). The raw proportion of nematode species varied between management treatments as shown in Table 3-1.

Table 3-1: Overall raw proportions of parasitic nematode species found under each management treatment.

Nematode Species	High Input (HI) (%)	Conventional Management (TYP) (%)	Intensive Rotational Grazing (IRG) (%)
Haemonchus contortus	76.3	79.7	59.5
Trichostrongylus spp.	20.8	14.3	27.9
Teladorsagia circumcincta	2.2	2.1	8.6
Oesophagostomum spp.	0.5	3.9	3.9
Cooperia spp.	0.2	0.0	0.1

Analysis of the proportion of *H. contortus* in faecal cultures showed an effect of management treatment ($P<0.05$) month ($P<0.0001$) and year ($P<0.001$) with significant interactions between management treatment and class ($P<0.05$) and class and year ($P<0.01$). IRG sheep had a significantly lower proportion of *H. contortus* than those on HI or TYP management treatments which did not differ (59.7%, CI 49.4-69.8, 79.4% CI 70.6-87.4 and 80.9%, CI 72.4-88.7) respectively, Figure 3-3). There were lower proportions of *H. contortus* in year 2 than in year 1 (65.4%, CI 57.6-73.0 and 81.4%, CI 74.7-87.7 respectively). The management treatment by class interaction was because classes did not differ for management treatments HI and IRG, whereas TYP lambs had a significantly higher proportion of *H. contortus* than ewes and hoggets. The class by year interaction was because the proportion of *H. contortus* was similar for lambs over the both years (year 1: 71.4%, CI 52.4-89.2 and year 2: 78.4%, CI 60.1-94.9) whilst declining significantly in ewes from year 1 (87.7%, CI 72.2-100) to year 2 (61.8%, CI 41.1-81.9) and also in hoggets (year 1: 83.8%, 64.1-100 and year 2: 54.8%, CI 31.5-77.7).

Analysis of on the proportion of *Trichostrongylus* spp. again revealed significant effects of management treatment ($P<0.05$), month ($P<0.001$) and year ($P<0.01$) with an interaction only between class and year ($P<0.05$). There was a significantly higher proportion of *Trichostrongylus* spp. in IRG sheep (24.0%, CI 16.4-32.1) than in those on HI (14.5%, CI 8.3-21.2) or TYP (9.5%, CI 4.4-15.2) treatments (Figure 3-3). The interaction between class and year was due to ewes having a higher proportion of *Trichostrongylus* spp. in year 2 (32.5%, CI 21.2-44.3) than in year 1 (6.0, CI 0.7-12.8).

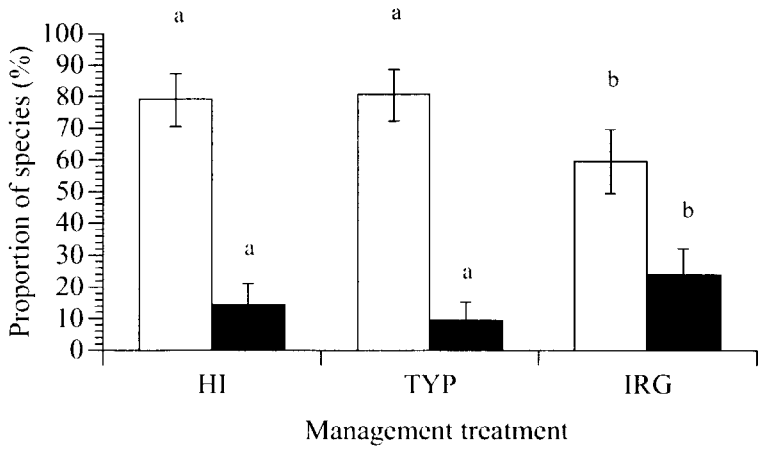


Figure 3-3: Backtransformed mean (with 95% CI) proportion of *Haemonchus contortus* (white) and *Trichostrongylus* spp. (black) by management treatment. Means not sharing a common letter within nematode species differ significantly (P<0.05).

3.3.2.2 Faecal worm egg count

3.3.2.2.1 Analysis 1 – incidence of positive WEC

Nominal logistic analysis revealed no overall effect of management treatment on the occurrence of positive total WEC, *H. contortus* WEC (*Hc*WEC) and *Trichostrongylus* spp WEC (*T*WEC) (P~0.99). However in each case there was a significant interaction between the effects of management treatment and class (P<0.0001) which is described below and illustrated in Figure 3-4.

For total WEC (i.e.: overall WEC which has not been divided into species) the occurrence of positive samples on management treatment HI differed significantly between each class of sheep with the highest incidence in hoggets. On the TYP treatment ewes and hoggets had a similar incidence, higher than that of lambs while on the IRG treatment ewes had a higher incidence than lambs with hoggets intermediate (Figure 3-4)

For *Hc*WEC the incidence of positive WEC was similar for all classes on management treatments CON and IRG. However, the HI treatment lambs had a lower incidence of positive WEC than ewes and hoggets.

For *T*WEC the incidence of positives in the HI treatment was higher in hoggets than ewes and lambs while for the TYP treatment ewes had a higher incidence than lambs with hoggets intermediate. There was no difference between classes on the IRG treatment (Figure 3-4).

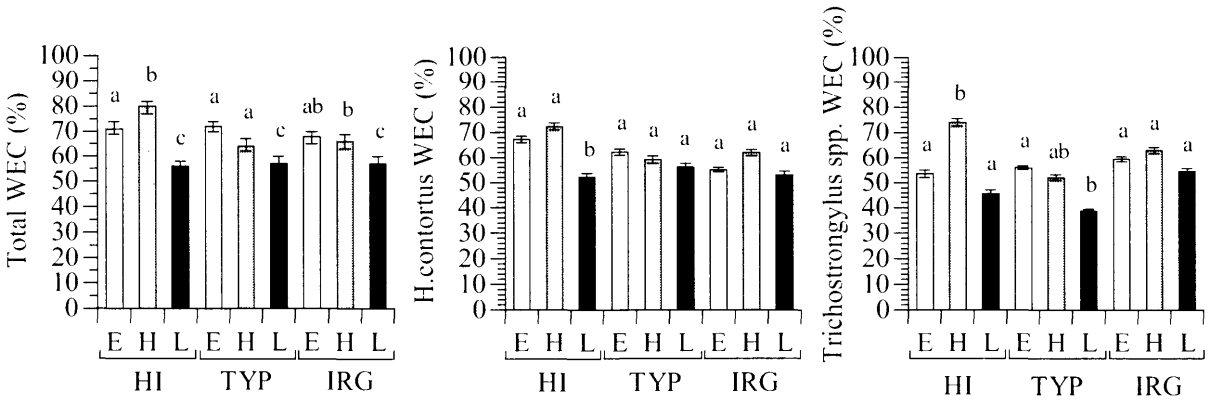


Figure 3-4: Mean (±SEM) proportion of positive faecal worm egg counts (WEC) showing the management treatment by class interaction for total WEC, *H. contortus* WEC and *Trichostrongylus* spp. Means within management treatments not sharing a common letter differ significantly (P<0.05).

3.3.2.2.2 Analysis 2 – Magnitude of WEC in WEC-positive sheep

Total WEC was significantly affected by management treatment (P<0.0001) with management treatments HI and TYP having higher WEC than IRG (546, CI 389-713; 582, CI 414-763 and 304 CI 200-414 eggs/g, respectively). All treatments were significantly different from each other for *Hc*WEC, HI sheep having the highest mean followed by TYP then IRG (555, CI 469-647; 438, CI 372-508 and 209 CI 173-249eggs/g respectively, P<0.0001). The situation was slightly different for *T*WEC with the HI management treatment having a significantly higher mean (51 eggs/g, CI 43-60) than IRG (39 eggs/g, CI 32-46) and TYP (37 eggs/g, CI 31-44, P<0.001). A significant interaction between management treatment, class and year was also revealed for total WEC (P<0.0001, Figure 3-5). Bodyweight, had a significant effect on *Hc*WEC (P<0.0001) with a weak, but significant negative association (R^2=0.03, P<0.0001). However, it did not significantly affect total WEC (P~0.11) or *Tc*WEC (P~0.61). Anthelmintic treatment interval (days since last treatment) also fitted as a regressor had a significant positive association with WEC (P<0.0001), as would be expected.

There was also a significant effect of month (P<0.0001) with April having the highest mean WEC (950 eggs/g, CI 779-1141) which was significantly higher than February, May, June, July and September (638, CI 521-768; 176, CI 49-130; 488, CI 405-578; 318, CI 260-382; and 524, CI 416-646 eggs/g, respectively). *T*WEC was highest in May, January, February, June and July while *Hc*WEC was highest in March, April, December, January and November (Figure 3-2).

The effect of Year was significant (P<0.0001) with higher mean WEC in year 2 (635 eggs/g, CI 541-737) than year 1 (445 eggs/g, CI 378-518).

There was no overall effect of class on WEC (Ewes: 447, CI 325-576; Hoggets: 424, CI 326-525 and Lambs: 508, CI 258-803 eggs/g, respectively P~0.56).

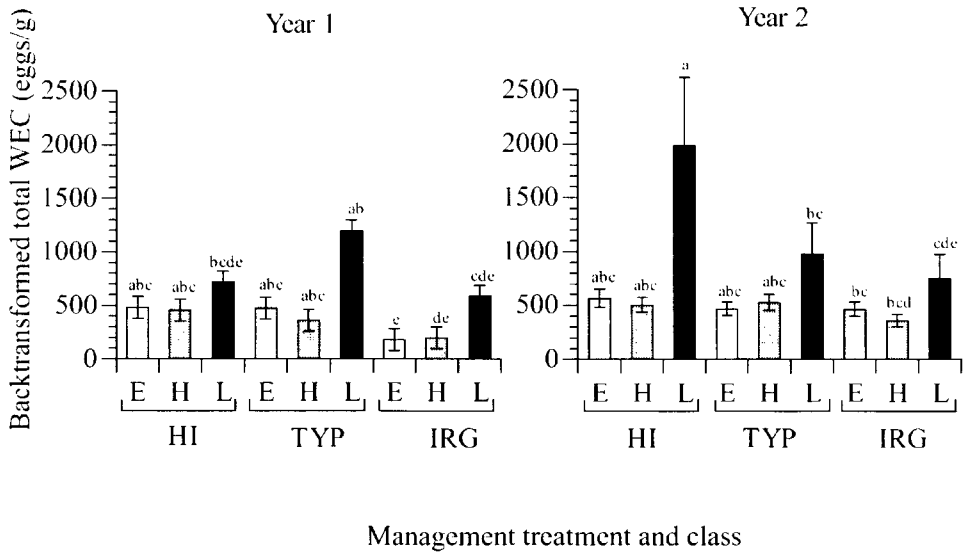


Figure 3-5: Backtransformed least square means with 95% confidence intervals for faecal worm egg count showing significant interaction between the effects of year, management treatment and class. Means within years not sharing a common letter differ significantly (P<0.05). E –ewe, L – Lamb, H – Hogget; HI – high management treatment, TYP – Typical management treatment, IRG – Intensive rotational grazing management treatment.

3.3.2.3 Interval between anthelmintic treatments

There was a significant overall effect of management system on anthelmintic treatment interval (P<0.0001) with IRG having the longest interval at 144 days, double that of management treatments HI and TYP (77 and 78 days). There was a significant interaction between management treatment and year with the interval between anthelmintics declining for HI and TYP from year 1 to year 2 (HI:96±11 and 58±7 days, TYP: 97±11 and 59±7 days) but increasing for IRG (114±14 and 173±12 days). There was no main effect of year (P~0.48), group (P~0.11), nor any interaction between management treatment and group (P~0.97).

3.3.3 Bodyweight

There was a significant overall effect of management treatment (P<0.05) with HI and TYP treatments having higher overall bodyweights than IRG (39.3±0.3, 39.1±0.3 and 38.1±0.3 kg, respectively). Initial bodyweight was a significant covariate (P<0.0001) and retained in the model. The overall effect of class was also significant (P<0.0001) with ewes heavier than

hoggets which were heavier than lambs (43.9±0.4, 41.3±0.3 and 31.4±0.5kg, respectively). The overall effects of month (P<0.0001) and year (P<0.0001) were also significant with higher bodyweights in year 1 (40.4±0.2kg) than year 2 (37.3±0.2kg). Fitting initial bodyweight as a covariate did nothing to change the lamb bodyweights, but it did correct some initial differences in the hoggets and in the ewes in year 2 (Figure 3-6).

These overall effects masked significant interactions between management treatment and year (P<0.0001), management treatment and month (P<0.0001) and management treatment and class (P<0.0001). The management treatment by year interaction was due to large declines in bodyweight between years 1 and 2 on HI and TYP treatments (HI year 1: 41.4±0.4, year 2: 37.2±0.3kg and TYP year 1: 41.1±0.3, year 2: 37.2±0.3kg) whilst bodyweights on the IRG treatment declined far less (year 1: 38.8±0.3, year 2: 37.4±0.3kg). The management treatment by class interaction is because ewe and hogget bodyweights on the IRG treatment were significantly lower than for HI or TYP, whilst the lamb bodyweights did not differ between management treatments (Figure 3-6).

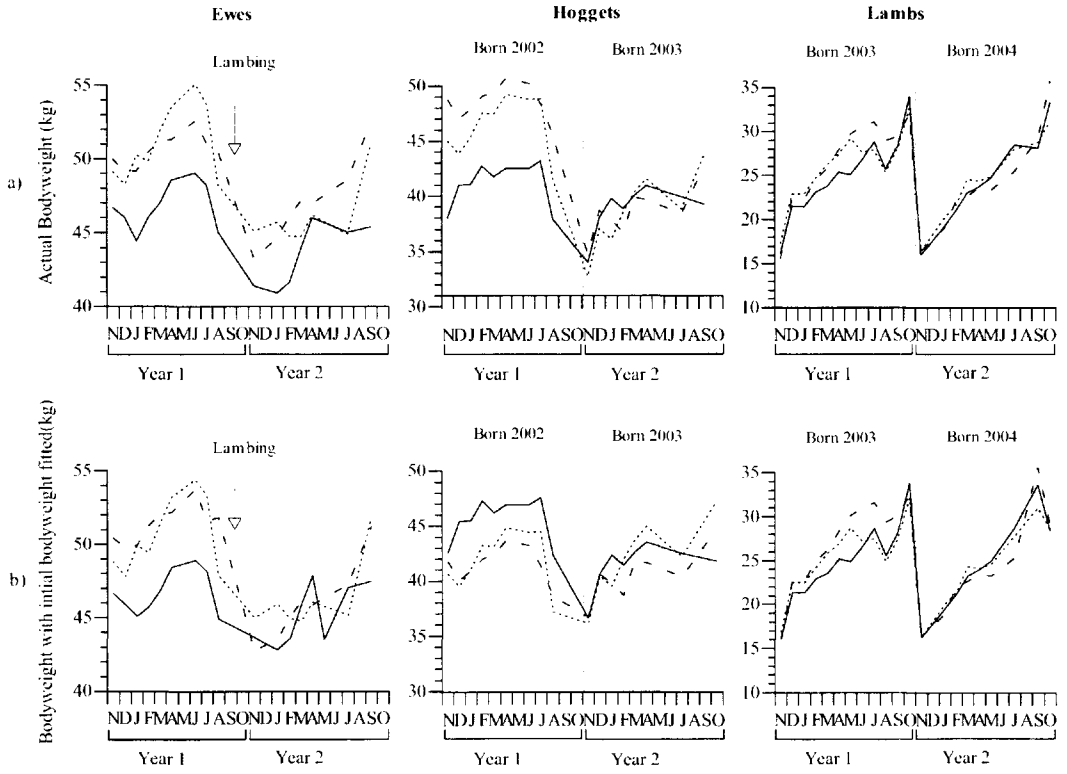


Figure 3-6: Monthly mean bodyweight per management treatment (HI – –, TYP ·····, IRG —) and class over the experimental period; a) Actual bodyweights; b) Bodyweights with initial bodyweight fitted as a covariate. Vertical dotted lines indicate the transition between classes.

Bodyweight gain was mostly positive for lambs on all management treatment in year 1 from November to April after which TYP lambs had considerable bodyweight losses in May and all lambs lost weight in July (Figure 3-7). In year 2 lambs on all farmlets remained in a positive or neutral bodyweight gain for the year. Bodyweight gain peaks in different months for each management treatment with the highest gains on TYP occurring in January and for HI and IRG in August.

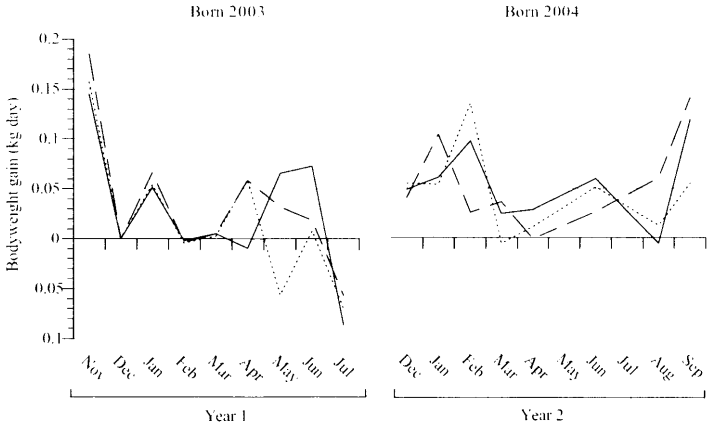


Figure 3-7: Daily bodyweight gain and losses for lambs over the experimental period for each management treatment (HI – –, TYP ·····, IRG —).

3.3.4 Fat score

Management system (P<0.05) had a significant main effect on fat score with HI sheep significantly fatter than TYP and IRG intermediate between the two (2.33±0.05, 2.15±0.05 and 2.21±0.05 score, respectively). There was also a main effect of class (P<0.0001) with hoggets having higher fat scores than ewes overall (2.44±0.04 and 2.01±0.03 score). Month had a significant effect (P<0.0001) with fat scores highest in April, November, December and March (2.72±0.04, 2.66±0.05, 2.62±0.05 and 2.620.04 score, respectively). The lowest mean fat scores were in the winter months with June the lowest (1.49±0.06 score) followed by August and July (1.81±0.06 and 1.70±0.04 score).

There was a significant interaction between management system, class, year (P<0.05) and management system, class, month (P<0.0001, Figure 3-8). The interaction between management system, class and year was due to HI ewes and hoggets having similar fat scores between years (Ewes year1: 2.25±0.07, year 2 2.08±0.07; Hoggets year1: 2.40±0.07 and year 2: 2.53±0.10 score). TYP ewes had higher fat scores in year 1 (2.06±0.07 score) than in year 2 (1.54±0.07 score), and TYP hoggets had similar fat scores in year 1 (2.28±0.07 score) and year 2 (2.6±0.10 score). Also IRG ewes did not differ between years (2.01±0.07 and 2.10±0.07 score), however

hoggets on IRG had lower fat scores in year 1 than year 2 (2.19±0.07 and 2.7±0.1 score). There was a strong positive between liveweight and fat score when liveweight was fitted as a covariate (P<0.0001).

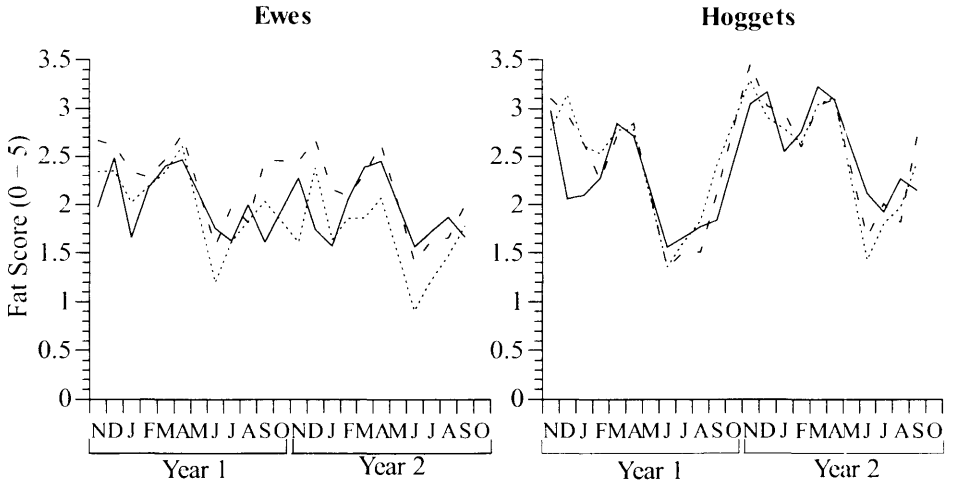


Figure 3-8: Mean fat score per farmlet by class over the experimental period (HI– –, TYP ·····,IRG —).

3.3.5 Wool measurements

Fleece weight was significantly affected by management treatment (P<0.0001), class (P<0.0001) and year (P<0.0001) with significant interactions between management treatment and year (P<0.01), management treatment and class (P<0.01), year and class (P<0.05) and management treatment, year and class (P<0.01). The HI and TYP management treatments had significantly heavier fleece weights than IRG (3.19±0.04, 3.08±0.04 and 2.61±0.04kg respectively, P<0.0001). Hoggets had the heaviest fleece weights followed by ewes then lambs (3.71±0.04, 3.34±0.04 and 1.84±0.04kg respectively, P<0.0001). Fleece weights were heavier in year 1 than year 2 (3.19±0.03 and 2.73±0.03kg, P<0.0001). The significant interaction between management system and class seems to be a difference in magnitude. In all classes HI and TYP had heavier fleece weights than IRG, but the difference was much greater for the ewes and hoggets than for the lambs (Figure 3-9).

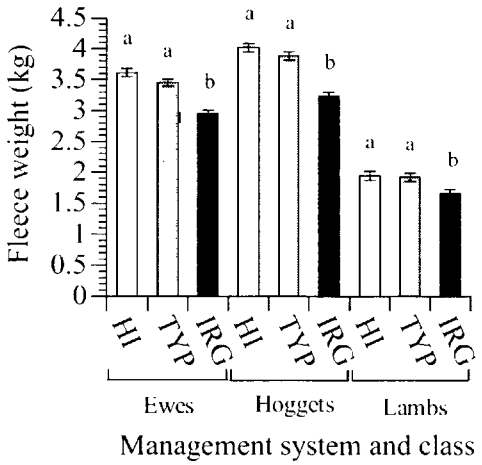


Figure 3-9: Least squared mean (±SEM) fleece weight by management treatment and class. Means within class not sharing letters are significantly different (P<0.05).

Mean fibre diameter was not affected by management treatment (P~0.12), but there was a significant effect of year (P<0.0001) with fibre diameter in year 2 lower than year 1 (16.88±0.08 and 17.60±0.09μm). There was also an effect of class (P<0.0001) with lambs being significantly finer than ewes which were finer again than hoggets (16.11±0.10, 17.61±0.10 and 18.00±0.10 μm, respectively). There was no interaction between management treatment and year (P~0.68) or management treatment and class (P~0.39), however there was a significant interaction between management treatment, class and year (P<0.0001). For all management treatments fibre diameter remained similar between year 1 and year 2 in the ewe and lamb classes (ewes: 17.5±0.31 and 17.61±0.25μm, lambs: 16.47±0.25 and 16.18±0.26μm). However for HI and TYP treatments hogget fibre diameter was broader in year 1 than year 2 (A: 19.38±0.26 and 17.30±0.25, B: 18.70±0.25 and 17.01±0.26μm), whereas it remained the same for IRG hoggets (17.88±0.25 and 17.78±0.25μm).

3.3.6 Haematology

3.3.6.1 Haematocrit

The effect of management treatment was significant (P<0.05) with sheep in the IRG treatment having higher HCT than those on the TYP treatment and those on the HI treatment being intermediate (34.5%, CI 33.5-35.5; 33.3%, CI 32.3-34.3; and 34.1%, 33.1-35.1, respectively). Overall, lambs had significantly higher HCT (36.9%, CI 35.5-38.4) than hoggets (33.3%, CI 32.5-64.1) which in turn had higher HCT than ewes (31.7%, CI 30.7-32.6) (P<0.0001). The effects of Month (P<0.0001) and year (P<0.0001) were also significant with significant interactions between management treatment and class (P<0.05), management treatment and

month (P<0.05), management treatment and year (P<0.05), and class and year (P>0.0001). Bodyweight had a significant positive relationship with HCT and was retained in the model (P<0.0001). The interaction between management treatment and class was due to HI ewes and hoggets having similar HCT values (32.4%, CI 31.8-32.9 and 33.2%, CI 32.7-33.7) which were lower than lambs on the same treatment (36.7%, CI 36.0-37.5), whereas TYP and IRG hoggets (32.6%, CI 32.1-33.2 and 34.1%, CI 33.6-34.6, respectively) had higher HCT than ewes (TYP: 30.4%, 29.9-30.9; IRG 32.3 (31.8-32.8). Both ewes and hoggets on TYP and IRG had values lower than lambs (TYP: 37.0%, CI 36.3-37.7; IRG: 37.1%, CI 36.4-37.8).

The management treatment by year interaction was due to HCT values decreasing from year 1 to year 2 for HI (Year 1:35.5%, CI 35.0-35.0; Year 2: 32.7%, CI 32.2-33.2) and TYP (Year 1:34.4%, CI 34.0-34.8; Year 2: 32.2%, CI 31.7-32.6) treatments but not the IRG treatment (Year 1:35.1%, CI 34.6-35.5; Year 2:33.9%, CI 33.1-34.4) (Figure 3-10).

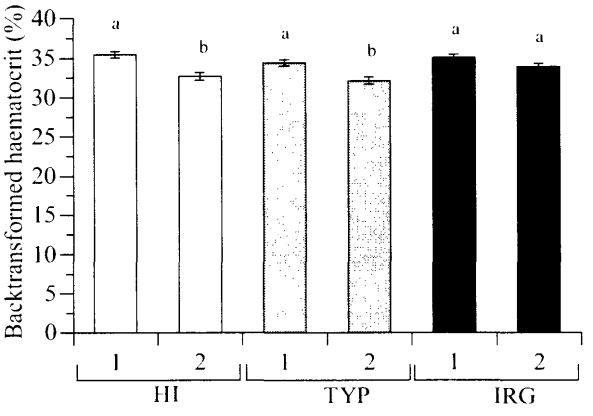


Figure 3-10: Backtransformed least squared mean haematocrit (with 95% CI) showing the interaction between management treatment and class. Means within management treatments not sharing a common letter differ significantly (P<0.05).

3.3.6.2 Eosinophil Count

There was no significant effect of management treatment on total eosinophil count (P~0.99) but there was a significant effect of class (P<0.0001) with higher eosinophil counts in hoggets (180 x 10^6/ml, CI 152-209) than ewes and lambs which did not differ (147 x 10^6/ml, CI 117-176 and 116 x 10^6/ml, CI 78-153, respectively). Eosinophil counts differed between years (P>0.0001) being higher in year 2 than year 1 (167 x 10^6/ml, CI 141-193 and 127 x 10^6/ml, CI 109-145 respectively). The effect of Month was also significant (P<0.0001), there was also a significant effect of bodyweight when fitted as a covariate in the model (P<0.0001) with a positive

relationship between it and eosinophil count. There were significant interactions between management treatment and class (P<0.001), management treatment and month (P<0.001), class and year (P<0.0001) and management treatment, class and year (P<0.05). Most of these interactions can be seen in Figure 3-11. Eosinophil counts for ewes and lambs did not differ between years on all management treatments while in each case hoggets had higher eosinophil counts in year 2 than year 1. The eosinophil counts were not particularly high and remained within the normal range of 0 to 1000 million cells/ml (Radostits *et al.* 2000).

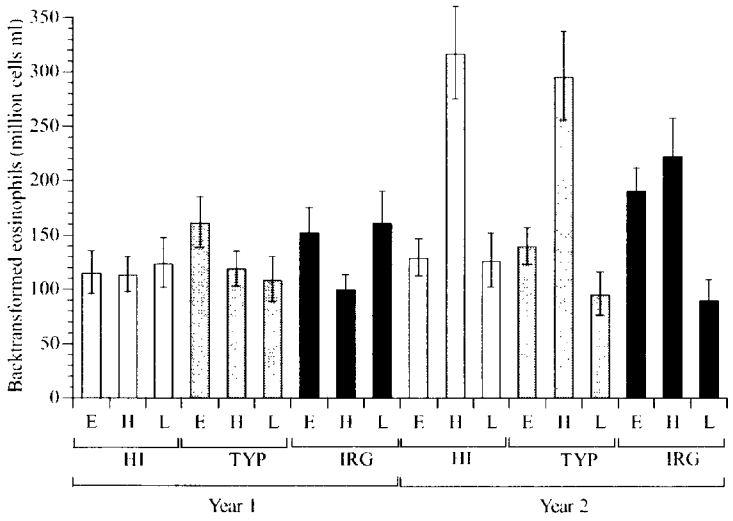


Figure 3-11: Backtransformed least square mean eosinophils with 95% CI showing interaction between management treatment (HI: white, TYP: grey, IRG: black), class and year.

3.4 Discussion

The main finding of this experiment was that sheep on the IRG treatment had significantly lower total WEC (46% lower) and *Hc*WEC (53% lower) than those under the HI and TYP management systems. This was despite receiving 15 anthelmintic treatments across the 3 classes of sheep over two years, compared with 28 treatments for both the HI and TYP treatments. Of these treatments 12 were mandatory treatments, given for reasons other than elevated WEC. *Trichostrongylus* WEC was not reduced by IRG, suggesting a specific action on *Haemonchus contortus*. Despite the difference in WEC, there was no advantage in production traits to sheep on the IRG system, and eosinophil counts did not differ, suggesting that the observed management system effects are less likely to be host-mediated than mediated by effects on the environmental phase of the nematode life-cycle. Support for this is found in the striking differences in grazing and rest periods between the IRG and the other treatments, differences which on the basis of existing epidemiological knowledge would predict large effects on infections with *Haemonchus*

contortus, but less so for *Trichostrongylus* species. These findings are discussed in more detail below in light of the original objectives.

The first objective was to determine whether management system influences the incidence and severity of GIN. The incidence of sheep with positive WEC did not differ with management system being approximately 65% overall. Similarly there were no differences in the occurrence of *Hc*WEC or *T*WEC. However, the number of anthelmintic treatments given on the management treatments needs to be considered. Management treatments HI and TYP had almost double the number of anthelmintic treatments given relative to IRG (Section 2.2.3.1, Table 2-3). This meant that these management treatments were sampled for WEC soon after an anthelmintic treatment on more occasions than sheep on the IRG management treatment which had much longer anthelmintic treatment intervals (Figure 3-2). Thus, although management treatments were statistically similar in the proportion of sheep with positive WEC, the IRG management treatment may actually have had a lower incidence of GIN had anthelmintic treatments been standardized across treatments. Interestingly lambs had a significantly lower incidence of positive WEC than either ewes or hoggets under each management system. Lambs are generally considered to be perhaps the most susceptible class of sheep to GIN and in this situation the lower incidence of positive WEC may be explained by good weaner management, with lambs allowed to preferentially graze pastures with low contamination. In the case of IRG sheep, weaners were allowed to graze pasture immediately ahead of the larger mob of ewes and wethers.

In contrast to the lack of effect on the incidence of worm infection, the magnitude of infection as determined by analysis of WEC values above zero was markedly affected by management system with significantly lower values for total WEC on the IRG treatment than the other two treatments which did not differ from each other. WEC was highest in the spring, summer and autumn months when temperature and rainfall are less limiting to development and survival of the pre-infective stages of *H. contortus* (Silverman and Campbell 1959; Rose 1963; Levine and Todd 1975; Besier and Dunsmore 1993b). However sheep in the IRG treatment completely avoided the very large peaks of WEC in late spring and summer observed in all classes of sheep in the other treatments. In November and December 2004 there were losses of lambs and ewes with acute haemonchosis in the HI and TYP treatments (10 to 15 ewes and lambs on each management system over 2 years) with lambs on the HI treatment reaching an average of 18,000 eggs/g and those on the TYP treatment 12,000 eggs/g. In the same months IRG lambs had an

average WEC of 300 eggs/g (November) and 1000 eggs/g (December) with no clinical signs of haemonchosis. Along with consistently low WEC, IRG lambs received only 2 anthelmintic treatments between the quarantine treatments in Year 1, compared to 4 on the other treatments, and only 1 anthelmintic treatment in year 2, compared with 5 on the other treatments. A similar pattern was observed with hoggets and ewes. So not only was WEC lower on the IRG treatment throughout the year with no deaths occurring, this was achieved with a much lower level of anthelmintic treatment. This is consistent with observations that graziers using IRG are able to maintain flocks with one or two anthelmintic interventions per year in this environment. The pattern of worm infestation over both years of the experiment followed established trends for this region (Gordon 1948; Roe *et al.* 1959; Barger *et al.* 1972; Southcott *et al.* 1976). High spring and early summer rainfall resulted in heavy infections of *H. contortus* in those seasons with relatively high *Trichostrongylus* spp. infections also. WEC was relatively low for all species in autumn and winter, due probably to low rainfall and anthelmintic treatments in autumn and low rainfall and cold inhibition in winter.

There was a higher mean WEC in year 2, than year 1 and it was associated with acute haemonchosis in HI and TYP sheep in the spring and summer. The reasons for this are not clear but may include the lack of a December 2005 moxidectin treatment in hoggets and lower feed availability in late summer and autumn particularly on the HI treatment, due to a very dry and hot late summer and autumn leading to a greater susceptibility to worm infection. Lamb WEC was lower overall than that of ewes and hoggets which was not expected. Lamb WEC did not appear in either year until December when they were 3 months old probably due to their low pasture intake in the early months of life. This plus weaning treatment in December with moxidectin, and the preferential treatment given to lambs for low infectivity pastures may explain their lower WEC overall.

The second major objective was to determine whether the effects of management system differed for the different major gastrointestinal nematode species. There was clear evidence for this with the reduction of WEC observed on the IRG treatment relative to the other treatments mostly attributable to the control of *H. contortus*. The significant shift in larval differentiation on the IRG treatment away from *Haemonchus* towards both *Trichostrongylus* and *Teladorsagia* relative to the other treatments indicates a preferential effect of the treatment on *Haemonchus contortus*. This was confirmed when larval differentiation was converted into genus-specific WEC, with the IRG treatment having significantly lower *Haemonchus* WEC than either of the other two

treatments overall. However the situation was less clear with *Trichostrongylus*. Despite a significantly higher proportion of *Trichostrongylus* in the larval differentiation on the IRG treatment, *T*WEC was equivalent to that the TYP treatment and significantly lower than on the HI treatment. This indicates that both the IRG and TYP treatments effectively reduced *T*WEC relative to HI. There are two lines of evidence suggesting an effect of IRG on *Trichostrongylus* spp., albeit a smaller effect than on *Haemonchus*. Firstly there is the significant reduction in *T*WEC relative to the HI treatment, and secondly there is the fact that *T*WEC on the IRG treatment was equivalent to that on the TYP treatment, despite the IRG treatment receiving 12 fewer anthelmintic treatments over the experimental period. This strongly suggests a suppressive effect of the IRG on *T*WEC had the anthelmintic treatments been the same across treatments.

The reasons for the higher *T*WEC on the HI treatment relative to the TYP treatment are not clear. They can possibly be attributed to the higher stocking rate, however, there is conflicting evidence of the effect of stocking rate on worm burdens with some studies reporting no effect whilst others report a significant effect related to worm species (Beveridge *et al.* 1985; Brown *et al.* 1985; Waller *et al.* 1987; Thamsborg *et al.* 1996). In Denmark, Thamsborg *et al.* (1996) found a significant positive effect of stocking rate on WEC especially for *Trichostrongylus* spp. even when pasture production was enhanced by fertiliser application, a similar situation to that of the HI management treatment in this study. The major *Trichostrongylus* species in the New England region is *T. colubriformis* (Donald *et al.* 1978b) although both *T. axei* (Cole 1986) and *T. vitrinus* (Wooster 1997) are also present. A limitation on our interpretation of the *Trichostrongylus* WEC is the association between WEC and worm counts in this species due to density-dependant effects on fecundity. Dobson *et al.* (1990c) showed a decline in the egg output of *Trichostrongylus colubriformis* adult females at higher infection rates (632 and 2000 L_3/day) as the population increased after 3 weeks of infection. At the lower infection rate (200 L_3/day) egg counts peaked at week 6 after which they declined while numbers of adult females remained stable. Thus we cannot determine with absolute certainty that the observed effects or lack of effects on *Trichostrongylus* in the present study are reflective effects on worm populations.

There were differences in the proportion of *Teladorsagia* spp. between management treatments with a higher occurrence on IRG (8.6%) than the other two treatments which were similar (~2.2%). These data were not analysed due to the small values rendering the statistical models unstable. It is not surprising that the IRG treatment had a higher incidence of *Teladorsagia* spp. with its ability to over winter, to develop at low temperatures (below 4°C) and to survive

desiccation in the pre-hatch stage (Kates 1950; Gibson and Everett 1972; Rossanigo and Gruner 1995).

The third aim of this experiment was to gain an insight into the possible underlying reasons for the observed differences between management effects on WEC. Our hypothesis was that the differences in WEC on the IRG management would be mediated primarily by interruption of the free-living stages of the parasitic lifecycle rather than differences in host immunity. It is not clear whether the highly effective tropical rapid rotational grazing system of Barger *et al* (1994) was effective against both *H. contortus* and *Trichostrongylus* spp. but the authors did note that all genera appeared to be remarkably similar in their ability to hatch and develop to L_3 and survive under Tongan conditions, Banks *et al* (1990) had similar results in Fiji. This suggests the hot, humid weather conditions may have killed *Trichostrongylus* spp. larvae as effectively as *H. contortus* larvae. The cool temperate climate of Armidale is more conducive to the survival of *Trichostrongylus* spp. eggs and larvae thus the use of IRG in this climate may not be as effective against that species (Anderson *et al.* 1966; Waller and Donald 1970; Levine and Anderson 1973; Levine *et al.* 1974). However, the susceptibility of *H. contortus* eggs to desiccation and low temperatures is likely to make them just as susceptible to rapid rotational grazing as they would be under tropical conditions but probably for different reasons. In the humid tropics a higher proportion of eggs deposited on pasture would hatch and develop into infective larvae than in this cool temperate climate, thus control of *H. contortus* would hinge more on rate of larval decay than it would in cooler climates. Because L_3 cannot feed they die off rapidly at high temperatures especially under humid conditions (Banks et al., 1990) and this would be a major mechanism operating in the tropics. On the other hand temperature and moisture are often limiting in the New England for development of *H. contortus* eggs to L_3 and failure of development is likely to be a major mechanism operating in this environment. The superior ability of *Trichostrongylus* species to survive in the embryonated egg stage during cool dry conditions relative to *Haemonchus* could account for the species difference in response to IRG observed in this experiment. It does not however, explain the higher *Trichostrongylus* WEC in sheep on the HI treatment compared to the TYP system. Of great importance in the IRG system both in the tropics and in cool temperate regions, is the short grazing period which precludes autoinfection from the current grazing. This ensures that the nematodes must run the gauntlet of high L_3 death rates in the tropics or intermittent development and/or high death rates in cool temperate regions, prior to being presented with host animals.

It is unlikely that the effects of IRG were mediated by improved host immunity. Nutrition is a major factor determining resistance and resilience against GIN (Abbott and Holmes 1990; Roberts and Adams 1990; Coop and Holmes 1996; Walkden-Brown and Kahn 2002). However the older sheep on the IRG treatment were 2 to 3 kg lighter than those on the other treatments and cut less wool, suggestive of an inferior nutritional status, yet these animals had consistently lower *Hc*WEC and higher HCT values. IRG sheep were also intermediate between HI and TYP for fat score, suggesting that the lower liveweights are the result of smaller framed sheep in IRG. The smaller frames could be due to lower birth weights in the earlier years of the Cicerone Project when the management of the IRG system was still in its learning stages. Lambs on the other hand did not differ in bodyweight between management treatments yet the effects of IRG were seen consistently across each class of sheep irrespective of differences in productivity. Mean circulating eosinophil count also did not vary between management treatments. The lower overall levels of sheep productivity seen on the IRG treatment appear to be due mainly to effects preceding the present experimental period. This is best illustrated in the bodyweights with major differences in ewe bodyweight present at the start of the experiment largely maintained thereafter. Similarly, bodyweight in hoggets differed widely prior to the start of the experiment with marked convergence between treatments by the end of the first year. In the second year there are no treatment differences between hoggets. For lambs, there are no major treatment effects in either year, suggesting that for animals born and reared during the experimental period, differences in productivity are minimal. Nevertheless there are important challenges with implementation of IRG treatments and it must be remembered that they are used primarily to improve animal production and the sustainability of grazing systems rather than to limit the effects of GIN. It is difficult to run breeding ewes in an IRG system with frequent moves during lambing detrimental to lamb survival. Thus during lambing in both years the IRG ewes were 'set stocked' for 6-8 weeks to reduce the risk of mismothering. Weaning poses another problem by increasing the number of mobs whilst reducing mob size. This impacts on pasture utilisation with insufficient grazing pressure and the greater possibility of feed over-maturing. Over-maturing of feed with consequent decline in quality is a constant threat in IRG systems, which is best managed by prevention, or alternatively the use of urea supplementation to increase animal utilisation of ingested pasture or inclusion of a higher proportion of wethers or cattle to also improve utilization. While IRG systems are implemented for animal and pasture management with any parasitological benefits are a bonus, they may become an important tool for integrated parasite management in the face of increased anthelmintic resistance and the demand for more sustainable animal production systems.

It is concluded that intensive rotational grazing systems such as that used in this experiment are able to markedly reduce WEC in all major classes of stock. This action appears to be preferential for *H. contortus* and we hypothesise that it is mediated by effects on the free-living stages of the lifecycle rather than on the host. The mechanisms behind the observed effects are currently under investigation. We believe that these findings demonstrate significant potential for the use of intensive rotational grazing for integrated parasite management for the control of *H. contortus* in temperate environments. Further studies on the impact of such systems in regions where *Trichostrongylus* spp. or *T. circumcincta* predominate is warranted.

CHAPTER 4: Experiment 2. Resistance and resilience to infection under a fixed larval challenge

4.1 Introduction

The significant differences in WEC between management treatments observed in experiment 1 (Chapter 3) could be due to either i) differences in host resistance to GIN due to treatment effects on the host phases of the lifecycle, or ii) differences in larval challenge due to treatment effects on the environmental phases of the parasite lifecycle. This experiment was designed to investigate the first of these possibilities by testing the general hypothesis that sheep on management treatments with the lowest WEC will exhibit the greatest resistance to infection.

Resistance to gastrointestinal nematode infection is the ability of the host to reduce the establishment, survival or reproductive rates of the pathogen (Walkden-Brown and Kahn 2002). Resistance to GIN can vary with age, sex, reproductive status, genotype, and the level and duration of larval challenge. Age has a marked effect on a host's ability to resist infection. Older sheep which have had prolonged or repeated exposure to the nematode pathogens, are better able to resist parasitic nematode infection than immunologically naïve lambs. The development of a mature immune response to nematode infection can take up to 1-2 years (Barger 1993). Female sheep are more resistant than entire males and possibly more resistant than emasculated males, however, there are no conclusive studies on the latter (Barger 1993). The acquired immunity of a ewe can be compromised by her reproductive status, periparturient relaxation of immunity is coupled with a rise in faecal worm egg count and worm burden (Connan 1967). This has a major impact on epidemiology with the periparturient relaxation of immunity in ewes being a significant contributor to lamb infection levels (Brunsdon 1970; Donald and Waller 1973).

There is heritable variation within sheep breeds in responsiveness to single infections of *H. contortus* and *T. colubriformis* as well as to mixed infections. The heritability of resistance appears to be moderate (h^2 = 0.23) based on one faecal egg count test, increasing when based on the mean of two faecal egg counts (h^2 = 0.35) (Morris *et al.* 1995). Estimates in Merinos of the heritability of resistance based on faecal worm egg counts range from 0.23 to 0.63 (Woolaston *et al.* 1991; Eady *et al.* 1994; Woolaston and Eady 1995; Woolaston and Piper 1996). Sheep bred

for resistance to parasites have fewer faecal egg worm counts and fewer worms, they have been shown to have significantly higher levels of IgA, IgE and IgG than non-resistant sheep (Gill *et al.* 1993; Bisset *et al.* 1996; Shaw *et al.* 1999). However, genetically resistant sheep do not necessarily have higher bodyweights or greater wool production and it is thought that mounting a greater immune response comes at a cost to production (Eady and Smith 2001).

Resistance is also influenced by exposure to nematode larvae and has been shown to be dose dependant for both *H. contortus* and *T. colubriformis* infections with higher dose rates inducing resistance where lower dose rates failed (Barger *et al.* 1985; Dobson *et al.* 1990a). For *T. colubriformis* the longer the period of exposure to infective larvae the greater the resistance to further infection (Gibson *et al.* 1970; Barnes and Dobson 1993). Acquired resistance to *H. contortus* appears to be more labile. In a study by Coyne and Smith (1992b) sheep exposed to infective larvae for 3 to 20 weeks maintained their immunity for up to 4 weeks after termination of the primary infection. After 7 weeks there was no apparent residual resistance to further infections.

Nutrition plays an important role in the development and manifestation of resistance to nematode infection and can be used to mediate the level of resistance. Dietary protein and energy are easily manipulated and there has been a focus on the benefits of protein supplements on the ability of sheep to resist infection. Numerous studies have shown that sheep on high protein diets have greater resistance to nematode infection, these have been reviewed extensively (Steel 1978; Topps 1983; Coop and Holmes 1996; Coop and Kyriazakis 1999; Walkden-Brown and Kahn 2002; Steel 2003). Protein supplementation is generally an expensive exercise and may not yield returns equal to the expenditure. Knox (2003) reviewed the use of non-protein nitrogen such as urea and urea-molasses blocks for enhancing resistance and resilience in sheep and concluded that they were successful and economically viable. The effect of energy supplementation has not been extensively studied, however, Kahn *et al.* (2000b) demonstrated a greater effect of energy supplementation than protein on resistance to infection with *T. colubriformis*. These studies indicate that sheep on a higher plane of nutrition have a greater ability to mount an immune response than those on lower quality diets.

It is not likely that there is a higher incidence of genotypically resistant sheep on the IRG treatment due to the use of common sires at mating and the common origin of the ewes (section

2.1.2). However, the lower WEC in IRG sheep observed in Experiment 1 (Chapter 3) may be due to greater resistance mediated through better nutrition. Intensive rotational grazing systems are established primarily to improve pasture utilisation, persistence and animal performance, so this is a reasonable proposition. However the liveweight data from experiment 1 (Chapter 3) is not suggestive of improved nutritional status of sheep on the IRG treatment.

The aim of this experiment was to determine whether there were differences between sheep on the different management treatments in resistance to challenge with bolus mixed infections of *H. contortus* and *T. colubriformis*. The general hypothesis was that sheep on management treatments exhibiting low WEC during natural challenge (Experiment 1) would exhibit superior resistance to artificial fixed infection, thus implicating host factors in mediating the effects of management system on WEC.

4.2 Materials and Methods

4.2.1 Experimental design and challenge protocol

Fixed larval challenges were conducted in spring (18th October to 22nd November 2004), summer (24th January to 28th February 2005), autumn (11th April to 16th May 2005) and winter (20th June to 25th July 2005) on 20 lambs or hoggets of each management treatment (TYP, HI, IRG) on the Cicerone project as previously described (Section 2.1).

The challenge protocol is summarized in Figure 4-1. A dose of *Haemonchus contortus (Hc)* and *Trichostrongylus colubriformis (Tc)* infective larvae (L_3) were seperately administered orally using a Roux revolver on day 0, following short-acting anthelmintic treatment 7 days earlier. Infected sheep remained in their usual mobs until day 21 when they were removed to a periphery paddock outside of the project until day 35 when infections were terminated. In Spring a dose of 8000 *Hc* and 12,000 *Tc* per sheep was used but following induction of severe infections the dose was reduced to 4000 *Hc* and 8000 *Tc* for all the subsequent challenges. The autumn infection was terminated at day 29 due to ill-thrift and low haematocrit levels on day 28. Bodyweights were recorded at days 0, 21, 28 and 35 for all seasons.

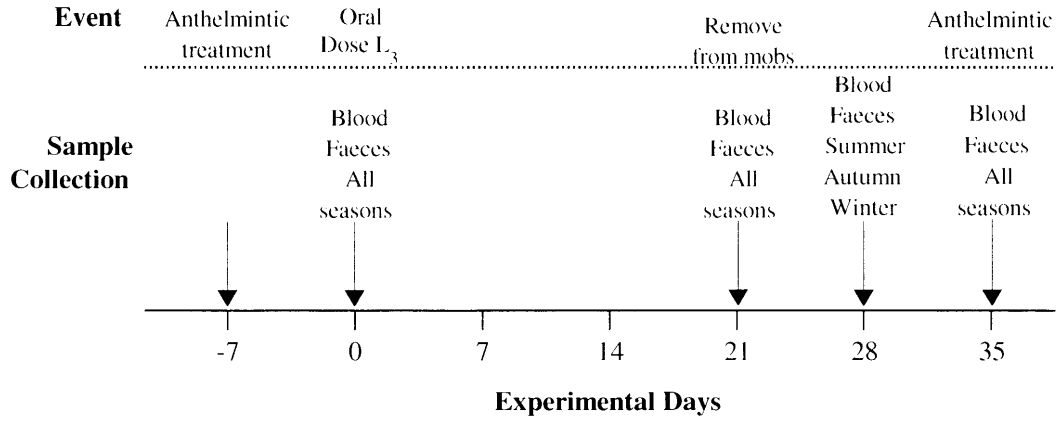


Figure 4-1: Timing of experimental events and sample collection for the fixed larval challenge.

For the spring and summer infections a combination of a double dose of levamisole and albendazole (1ml/10kg COMBI®, 34g/L albendazole oxide and 70g/L levamisole HCl, Novartis Animal Health Australasia Pty Ltd, Australia) was used to remove existing infections prior to challenge 7 days later. In summer, small numbers of *Trichostrongylus* spp. larvae were cultured from faeces on day 0 from the HI (2 L_3) and TYP (16 L_3) treatments. In response to this the autumn and winter fixed challenge sheep were given a double dose of ivermectin (1ml/4kg Ivomec, 0.8g/L, Merial Australia) along with the double dose levamisole and albendazole to remove existing infections. This had the desired effect with no more larvae cultured on day 0. The same anthelmintic treatment given on day -7 for each season was given again on day 35 to terminate the artificial infections.

4.2.2 Experimental animals

Sheep selected for the spring challenge (November 2004) were born in September 2003, sheep selected in summer, autumn and winter challenges in 2005 were born in September 2004. All were ewes except for those in the winter challenge which were wethers. There were insufficient ewes in the 2004 drop to conduct a third fixed challenge without some sheep undergoing a second infection so rather than bias the results in this way, wethers were used instead. The wethers were of the same age that had run together with the 2004 drop ewes. Young wethers should have a similar response to infection as ewes although this has not been proven (Barger 1993).

4.2.3 Sampling procedures and measurements

On each sampling day the challenged sheep were sampled rectally for faeces and individual faecal worm egg counts (WEC) and bulked faecal cultures performed in random order using the

methods outlined in section 2.3.1 and 2.3.2. Blood samples were collected as described in section 2.4 and all samples were processed in random order on the Cell Dyn® automated haematology analyser on the day of collection. In summer complete haematology data is only available for the day 0 sampling due to malfunctioning of the analyser for the subsequent samplings. Instead haematocrit was determined individually using the microhaematocrit method on days 28 and day 35. By day 35 the Cell-Dyn® was analysing red blood cell parameters again and there was a strong linear relationship between measurements made on the Cel Dyn® and manually using the microhaematocrit method (R^2=0.98, see section 2.4). Retained plasma for each sample was subjected to an ELISA for specific anti-trichostrongylid IgG antibody as described in section 2.5.

4.2.4 Statistical Analysis

A cubed-root transformation was used on eosinophil counts, a log transformation [Log_{10} (x+1] on white blood cells and antibody levels and ArcSine transformation ($\sqrt{}$proportion) was used for haematocrit. WEC was analysed as total WEC, *H. contortus* WEC (*Hc*WEC) and *T. colubriformis* WEC (*Tc*WEC). The total WEC was apportioned into species by applying the proportions obtained through faecal culture to the raw egg counts. Bodyweight gain was calculated as the gain or loss of bodyweight over the entire experimental period and expressed as grams of bodyweight per day. The proportion of anaemic sheep in each season was determined as those individuals with haematocrit levels below 25%, these were then analysed using a nominal logistic model and simple regressions. Each variable was analysed separately within seasons using linear mixed models using REML in JMP IN version 5.1 (SAS Institute Inc., NC, USA). The following terms were fitted in the model; management system (HI, TYP and IRG), day (0, 21, 28, 35), management system x day, animal tag number (Random effect), bodyweight and the day 0 value fitted as a covariate in the case of bodyweight, haematocrit, eosinophil counts and antibody levels. Tukey's post hoc tests and contrasts were used to determine the significance of differences between means. For transformed variables backtransformed least square means (LSM) are presented with 95% confidence intervals while all other data will be presented as LSM with standard errors. Associations between variables were determined by linear regression.

4.3 Results

In all seasons all Day 0 egg counts were zero, Day 0 faecal cultures did not yield larvae in spring, autumn and winter. However, in summer small numbers of *Trichostrongylus* spp. larvae were cultured from the HI (2 L_3) and TYP (16 L_3) but not the IRG treatment sheep.

4.3.1 Faecal worm egg count

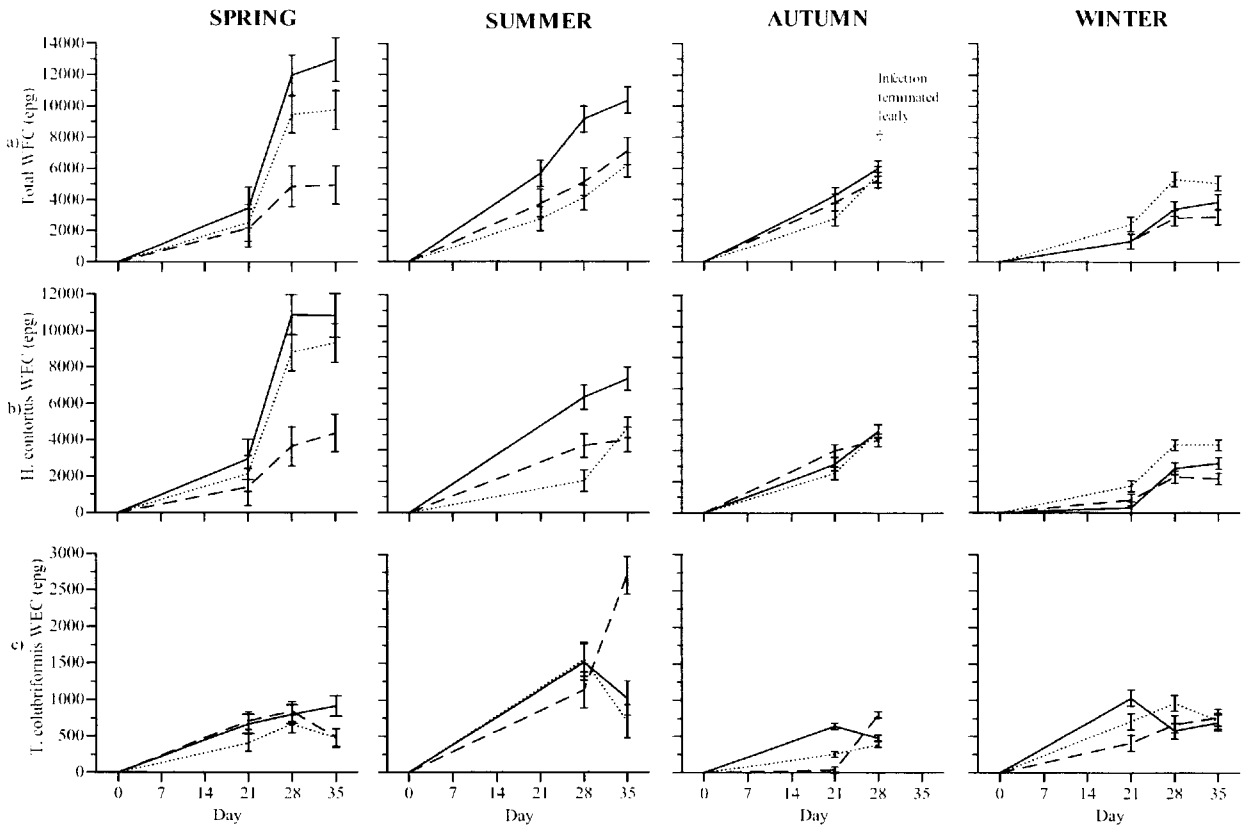


Figure 4-2: Fixed challenge data (LSM ± SEM) by management system (HI ----, TYP ····· , IRG ——) and season. a) Total faecal worm egg count (eggs/g faeces); b) *H. contortus* faecal worm egg count; c) *T. colubriformis* faecal worm egg count.

Spring

Analysis of variance showed that all terms fitted in the model were significant for total WEC and *Hc*WEC. HI had the lowest total WEC (4000±1000eggs/g) with TYP intermediate between HI and IRG (TYP: 7200±1000; and 9500±1000eggs/g, respectively, P<0.01). HI had lower *Hc*WEC than TYP and IRG (3100±850; 6800±800; 8200±900eggs/g, P<0.001). There was no effect of management system on *Tc*WEC (HI: 700±100; TYP: 500±100; IRG: 800±100eggs/g, P<0.21).

There was a management system and day interaction for all WEC analyses (P<0.001). The interaction for total WEC was due to TYP and IRG WEC increasing at a faster rate than HI. At Day 21 there was no difference between management treatments, but by day 28 IRG had the highest WEC, HI had the least with TYP intermediate between the two. By Day 35 HI was significantly lower than TYP and IRG which did not differ significantly (Figure 4-2a). A similar pattern occurred for *Hc*WEC (Figure 4-2b). There was no significant difference between management treatments on any day for *Tc*WEC, however IRG had slightly higher counts on day

35 (Figure 4-2c). There was a significant negative relationship between bodyweight and *Hc*WEC (P~0.01) and total WEC (Figure 4-3, P~0.01) but not *Tc*WEC (P~0.60), however, it did not remove the effect of management system. There was a significant negative relationship between total WEC and haematocrit (P<0.0001, Figure 4-3), but not with eosinophil count (P~0.66).

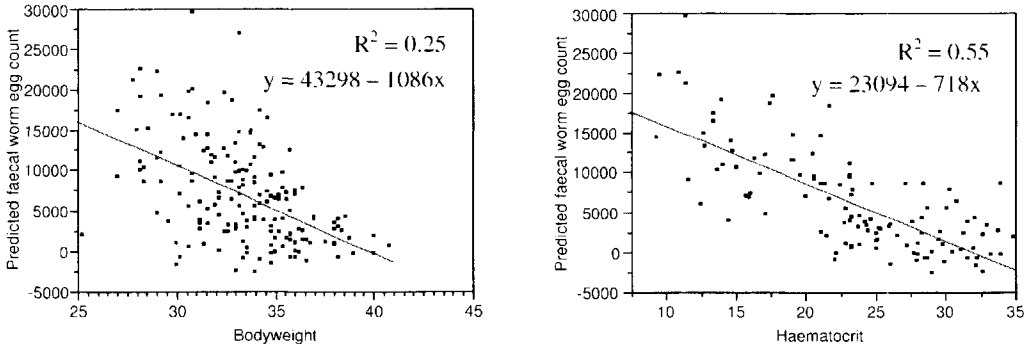


Figure 4-3: Linear regressions of predicted faecal worm egg count against bodyweight (left) and haematocrit (right) for the spring fixed challenge (dots are the raw data points).

Summer

During the summer challenge IRG sheep had a significantly higher total mean WEC (8400±750eggs/g, P<0.001) with HI and TYP not differing (5300±800, 4400±700eggs/g, respectively). The same occurred for *Hc*WEC (HI: 4500±700; TYP: 3800±600; IRG: 8000±700eggs/g, P<0.01). *Tc*WEC on HI was significantly higher than TYP with IRG intermediate (2000±200, 1100±200 and 1300±200eggs/g, respectively, P<0.05).

On each day of the challenge IRG sheep had significantly higher total WEC than HI and TYP (Figure 4-2) However, the rate of increase in total WEC on IRG was faster than on HI and TYP, thus causing a significant interaction between management and day. *Hc*WEC on IRG was greater than HI which was again higher than TYP on both days 28 and 35 (Figure 4-2b). *Tc*WEC had a different trend with *Tc*WEC on similar between management treatments on day 28. On day 35 HI *Tc*WEC was greater than TYP and IRG which did not differ (Figure 4-2c). There was also a significant effect of bodyweight on *Tc*WEC (P<0.05) with the relationship being slightly negative. There was no effect of bodyweight on total WEC (P<0.12) or *Hc*WEC (P<0.06) in summer, however there was a significant negative relationship between haematocrit and total WEC (P<0.0001, Figure 4-4).

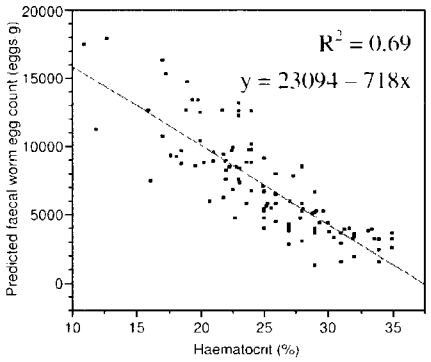


Figure 4-4: Linear regression of predicted faecal worm egg count against haematocrit for the summer fixed challenge.

Autumn

The analysis of autumn total WEC showed no significant effect of management (HI: 4500±450; TYP: 4200±450; IRG: 5100±450eggs/g P~0.36), however there was a significant effect of day with egg counts increasing from day 21 to day 28 (P<0.0001). There was no interaction between management system and day (P~0.08). The effect of management on *Hc*WEC was not significant (P~0.72). However, there was a higher significant effect of management treatment on *Tc*WEC with IRG having higher mean *Tc*WEC than HI and TYP (600±30, 400±35 and 300±30eggs/g, respectively).

There was a significant interaction between day and management treatment on *Hc*WEC (P<0.001), however there were no significant differences between treatments on any day. The interaction is most likely due to the fact that HI had a higher mean *Hc*WEC on day 21 than TYP. There was also a significant interaction between management system and day for *Tc*WEC with HI significantly lower than TYP which was lower than IRG (P<0.0001). On Day 35 HI had the highest mean *Tc*WEC whilst TYP and IRG did not differ. *Tc*WEC actually dropped on day 35 in IRG sheep (Figure 4-2c).

There was no effect of bodyweight on total WEC (P~0.41), *Hc*WEC (P~0.26) or *Tc*WEC (P~0.62) in autumn, nor any relationship with eosinophil count (P~0.20). However there was a negative relationship between haematocrit and total WEC (P<0.0001, Figure 4-5).

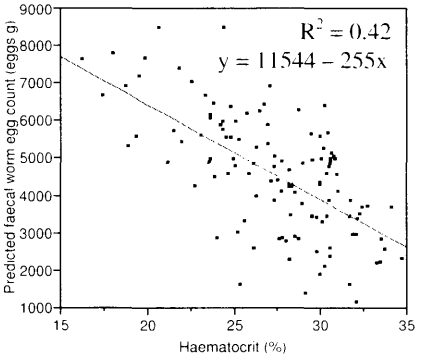


Figure 4-5: Linear regression of predicted faecal worm egg count against haematocrit for the autumn fixed challenge.

Winter

Analysis of winter total WEC again revealed a significant effect of management (P<0.01), day (P<0.0001) with no interaction (P~0.12). TYP had the highest mean total WEC (4300±400eggs/g) with IRG (2900±400eggs/g) intermediate between HI (2300±400eggs/g) and TYP. *Hc*WEC was highest in TYP sheep in winter, HI and IRG were similar (3500±300; 1800±300 and 2100±300eggs/g, respectively, P<0.0001). There was no significant difference between management systems for *Tc*WEC in winter (HI: 600±100; TYP: 800±100; IRG: 770±100, P~0.37). There was no effect of day on *Tc*WEC (P~0.94) and a significant effect on *Hc*WEC (P<0.0001) with means rising from day 21 to 28 for the latter.

On day 21 there was no difference between treatments for *Hc*WEC. On day 28 and 35 TYP had a greater mean *Hc*WEC than HI with IRG intermediate (Figure 4-2b). For *Tc*WEC, IRG had highest mean with TYP intermediate between IRG and HI. On days 28 and 35 there was no significant difference between management systems for *Tc*WEC (Figure 4-2c).

There was an effect of bodyweight on total WEC (P~0.05) and *Hc*WEC (P~0.03) with a weak negative relationship, there was no effect on *Tc*WEC (P~0.08) during the winter fixed challenge. There were also significant negative relationships between total WEC and haematocrit (P<0.0001) and eosinophil count (P~0.01, Figure 4-6).

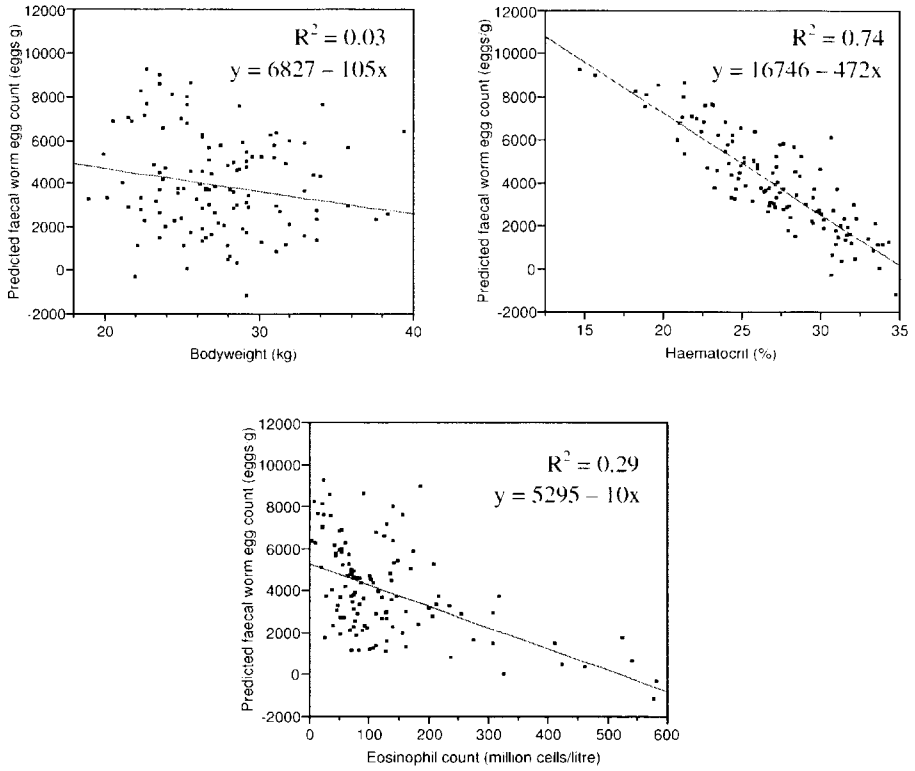


Figure 4-6: Linear regressions of predicted log of faecal worm egg count against raw bodyweight (top left), haematocrit (top right) and eosinophil count (bottom) for the winter fixed challenge.

4.3.2 Bodyweight

Spring

With Day 0 bodyweight fitted as a covariate, analysis of variance showed an effect of management system ($P<0.05$), day ($P<0.01$) and an interaction between the two ($P<0.0001$). Overall, HI sheep were heavier than IRG sheep with TYP intermediate (HI: 33.5±0.18; TYP: 33.0±0.17; IRG: 32.7±0.19kg). There was no significant difference between the management system on any day, however, the increase in bodyweight on IRG at Day 21 and Day 28 seemed low, whilst TYP increased at the same rate as HI until Day 28 when it declines along with IRG to Day 35 (Figure 4-7a).

Without Day 0 bodyweight fitted in the model there was a significant effect of management system with HI significantly heavier than TYP and IRG which did not differ (34.9±0.49, 32.6±0.52 and 31.5±0.53kg, respectively). On Day 0 HI sheep were heaviest with TYP intermediate ($P<0.0001$ HI: 33.3±0.5; TYP: 31.3±0.5; IRG: 30.4±0.6kg). HI remained

significantly heavier than IRG throughout the spring fixed challenge, whilst TYP was intermediate between HI and IRG on all days (Figure 4-7b).

The effect of management system on bodyweight gain over the entire experimental period in the spring challenge was borderline significant (P~0.07). Contrasts revealed that HI had significantly greater bodyweight gains overall than TYP and IRG (HI: 54.9±8.7; TYP: 30.2±9.1; IRG: 27.5±9.7g/day, P<0.05).

Summer

For the summer fixed challenge with Day 0 bodyweight fitted as a covariate, management system (P<0.0001) and day (P<0.0001) had a significant effect on bodyweight with a strong trend towards interaction between the two (P<0.06, Figure 4-7a). HI and TYP were significantly heavier than IRG (24.3±0.23, 23.6±0.21 and 22.8±0.21kg, respectively). Bodyweight increased steadily over the period of the experiment (day 0: 21.6±0.21, day 21: 24.0±0.21, day 28: 23.6±0.21 and day 35: 25.1±0.21kg). Day 0 bodyweight was a significant covariate (P<0.0001). When the model was run without Day 0 bodyweight fitted as a covariate, there was an effect of management system (P<0.0001) and day (P<0.0001), but no interaction between the two (P~0.06, Figure 4-7b). HI was significantly heavier than TYP which was again heavier than IRG (24.5±0.26, 23.5±0.24 and 22.7±0.24kg).

Bodyweight gain in summer was not affected by management system (P~0.29) with all systems showing similar gain, although IRG had slightly lower gain than HI and TYP (HI: 117.3±16.1; TYP: 100.9±14.9; IRG: 82.1±15.3g/day).

Autumn

When Day 0 bodyweight was fitted as a covariate for bodyweight in the autumn challenge, there was no effect of management system (P~0.3), no interaction between management system and day (P~0.1, Figure 4-7a) and a significant effect of day (P<0.0001). Bodyweights were the same for Day 0 and Day 21 (21.9±0.1), increasing on Day 28 to 23.3±0.1kg.

When Day 0 bodyweight was dropped from the model there was a significant effect of management system (P<0.05) and day (P<0.0001) but no interaction (P~0.13, Figure 4-7b). TYP sheep were heaviest overall with IRG sheep intermediate and HI sheep lightest (TYP: 23.4±0.7; IRG: 22.9±0.7; HI: 20.8±0.7 kg).

There was no difference between management systems for bodyweight gain in autumn (HI: 57.1±10.1; TYP: 38.3±10.3; IRG: 49.1±10.1g/day, P~0.43).

Winter

With Day 0 bodyweight fitted as a covariate, bodyweights in winter were not affected by management system (P~0.09), there was an effect of day (P<0.0001) with no interaction between management system and day (P~0.22), Figure 4-7a). Bodyweights increased from days 0 to 35, with each day being significantly different from the others (day 0: 25.4±0.2; day 21: 26.8±0.2; day 35: 27.7±0.2kg)

When Day 0 bodyweight was removed from the model, the effects of management system and day were significant (P<0.0001), but there was no interaction (P~0.23, Figure 4-7b). IRG sheep were heavier than HI but not TYP sheep (HI: 24.4±0.74, TYP: 26.6±0.73 and IRG: 28.9±0.74kg). Bodyweights increased from day 0 to 35 (Figure 4-3b). Again, there was no effect of management treatment on bodyweight gain in winter (HI: 48.7±10.3; TYP: 71.7±10.0; IRG: 69.5±10.6g/day, P~0.23).

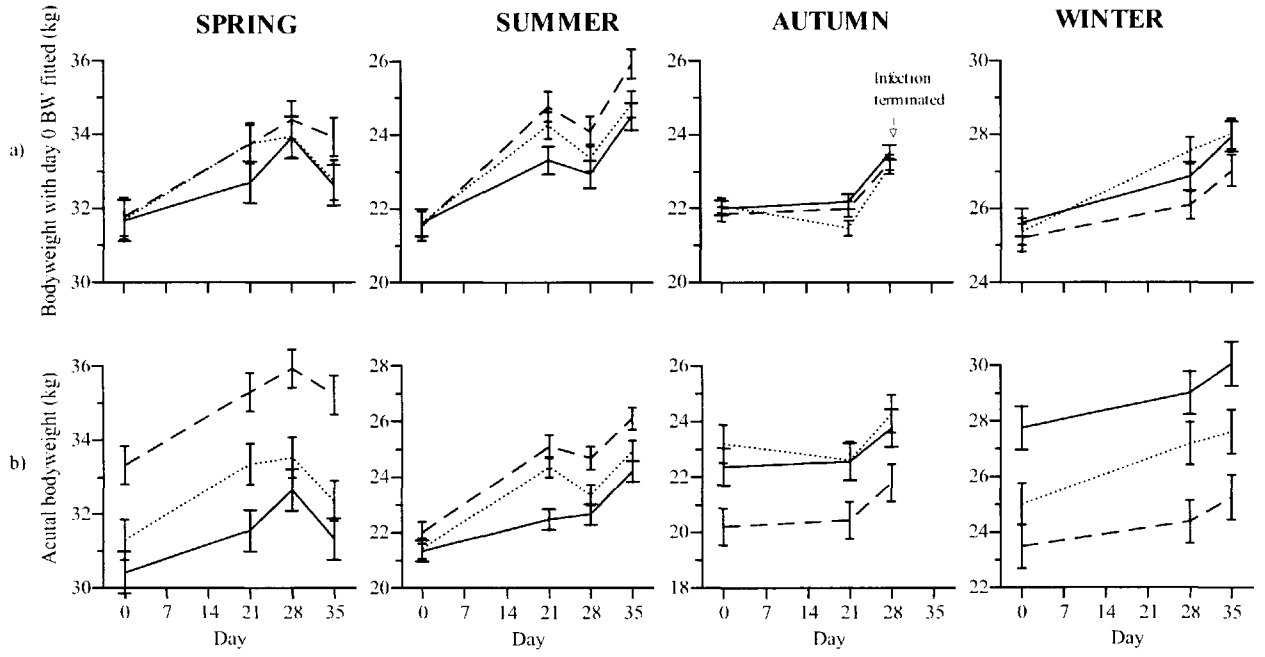


Figure 4-7: Bodyweight least squared means (± SEM) by management treatment (HI ---, TYP ·····, IRG ——)

a) with day 0 bodyweight fitted as a covariate; b) Actual recorded bodyweight.

4.3.3 Haematology

4.3.3.1 Haematocrit

Spring

Analysis of variance of haematocrit (HCT) showed no overall effect of management system (P~0.6), a significant effect of day (P<0.01) and a significant interaction between management system and day (P<0.01, Figure 4-8a). Management systems did not differ on day 21 (HI: 26.6±0.7; TYP: 27.6±0.5; IRG: 27.6±0.6%) with all treatments showing an increase relative to day 0. By day 35 mean HCT fell to 26.9±0.7% for the TYP and IRG treatments whilst for the HI treatment it increased to 29.6±0.6%. There was a borderline effect of bodyweight on HCT with a positive relationship (P~0.06, Figure 4-9, WEC also had an effect on HCT (P<0.0001) with a strong negative relationship.

An analysis of the proportion of sheep deemed anaemic (HCT<25.0%) during the challenge showed an effect of management system (P<0.01) with IRG sheep having a higher proportion of anaemic sheep than HI but not significantly higher than TYP (HI: 27.1; TYP: 46.3 and IRG: 58.8%). TYP sheep also had significantly higher proportions of anaemic sheep than HI. There was no difference between management systems for proportion of anaemic sheep on day 0 (HI: 0, TYP: 0 and IRG: 5.88%), but by day 21 both TYP and IRG had a higher proportion of anaemic animals than HI with IRG also higher than TYP (HI: 40.0; TYP: 61.1 and IRG: 82.4%). On day 35 TYP and IRG were not statistically different but had significantly more anaemic sheep than HI (HI: 42.1; TYP: 77.8 and IRG: 88.2%).

Summer

There was no effect of management system on HCT (P~0.55) in summer, a significant effect of day (P<0.0001) and no significant interaction between the two (P~0.09, Figure 4-8a However, a contrast between HI and IRG at day 35 and was significant (HI: 32.8, CI 30.1-35.6; IRG: 30.4, CI 27.6-33.2%, P~0.02). Mean HCT increased from day 0 to 28 (23.3, CI 20.7-25.9% and 34.0, CI 32.3-35.7%, respectively) and dropped slightly on day 35 (31.5, CI 29.5-33.5%). There was no effect of bodyweight on HCT (P~0.11) and an effect of WEC again with a strong negative relationship (P<0.0001).

The proportion of anaemic sheep was significantly higher on IRG than HI and TYP in the summer fixed challenge (HI: 37.2; TYP: 39.0 and IRG: 54.6%, P<0.05). There was no difference at day 0 but IRG had more anaemic sheep than HI and TYP by day 28 while HI and TYP were similar (HI: 42.9; TYP: 45.0; IRG: 76.5%). By day 35 IRG had a higher proportion of anaemic sheep than TYP which had more than HI (HI: 50.0; TYP: 65.0; IRG: 89.5%).

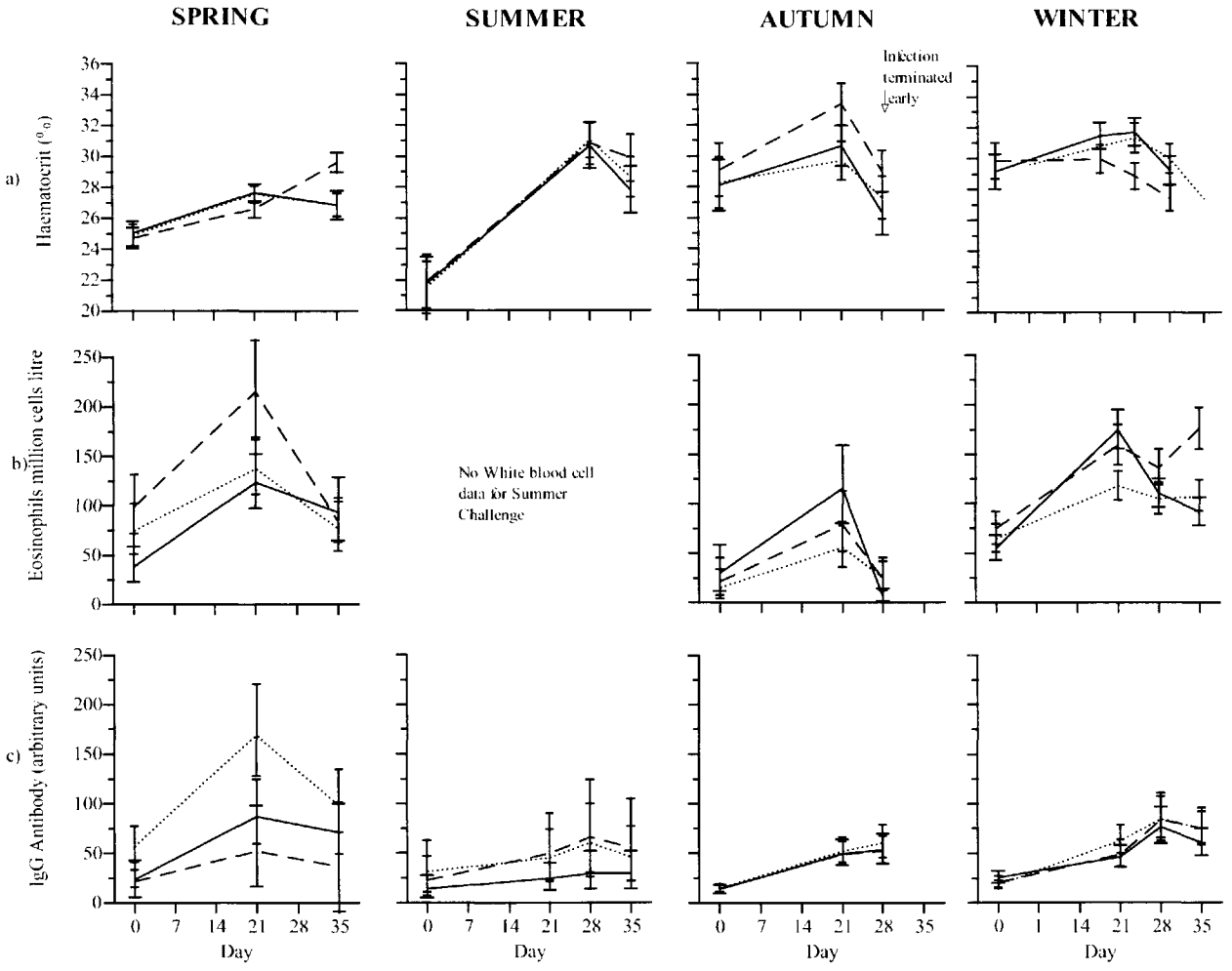


Figure 4-8: Haematology least squared means by management system (HI---, TYP..... , IRG ——) for a) Haematocrit (%); b) Circulating eosinophils (cells x 10^6/ml); Peripheral IgG antibody level (Arbitrary units).

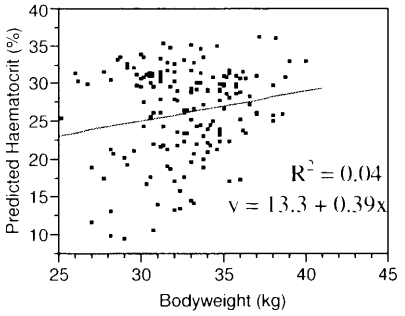


Figure 4-9: Linear regression of predicted haematocrit against bodyweight for the spring fixed challenge.

Autumn

In autumn, there was a significant effect of management system (P<0.01), day (P<0.0001) and an interaction between them (P<0.001, Figure 4-8a). All management systems had similar day 0 HCT (HI: 31.9, CI 28.8-35.0; TYP: 30.9, CI 27.9-34.0; IRG: 30.7, CI 27.8-33.8%) which increased by day 21. HI had a higher day 21 HCT than TYP with IRG intermediate (HI: 37.0, CI 34.5-39.6; TYP: 32.6, CI 30.3-34.9; IRG: 33.7, CI 31.3-36.2%). IRG had the lowest HCT at day 28 (28.6, CI 26.1-31.1%) and was significantly lower than HI (31.8, CI 29.3-34.2%) but not lower than TYP (29.8, CI 27.2-32.3%). There was an effect of bodyweight on HCT (P~0.004) which was positive, and an effect of WEC which was negative (P<0.0001).

The TYP and IRG treatments had a similar significantly higher proportion of anaemic sheep which was higher than that on the HI treatment (HI: 19.0; TYP: 31.6; IRG: 36.7%, P<0.05). There was no significant difference between management at day 0 (HI: 0, TYP: 0, IRG: 5%) but by day 21 TYP and IRG had significantly higher proportions of anaemic sheep than HI (HI: 5.6; TYP: 31.6; IRG: 25.0%). On day 28 IRG had the highest proportion of anaemic sheep which was greater than both HI and TYP which did not differ (HI: 50.0; TYP: 63.2; IRG: 80.0%).

Winter

In winter there was a main effect of management system (P<0.05), day (P<0.0001) and an interaction between management system and day (P<0.0001, Figure 4-8a). The interaction was due to all farmlets starting equally (HI: 32.8, CI 30.7-34.9; TYP: 32.5, CI 30.5-34.5; IRG: 32.0, CI 29.9-34.0%) while IRG had higher HCT at days 28 (HI: 31.6, CI 30.0-33.2; TYP: 33.0, CI 31.2-34.7; and IRG: 35.0, CI 33.3-36.7%) and day 35 than HI and TYP (HI: 29.9, CI 28.4-31.4; TYP: 29.9, CI 28.2-31.5; and IRG: 32.0. CI 30.4-33.7%). Again there was an effect of

bodyweight on HCT in winter with a positive relationship (P<0.05) and an effect of WEC which had a negative relationship (P<0.0001).

There was no overall difference between management systems for the proportion of anaemic sheep (HI: 24.0; TYP: 29.7; IRG: 26.2%), however, there was a management system and day interaction with HI and TYP having higher proportions on day 21 than IRG (18.8, 18.8 and 5.9%), on day 28 all management systems were similar again (HI: 31.6; TYP: 27.8; IRG: 42.1%), then on day 35 TYP sheep had a significantly higher proportion of anaemic sheep than the other 2 management systems (HI: 45.0; TYP: 70.0; IRG: 50.0%).

4.3.3.2 Circulating eosinophil count

Spring

There was no effect of management system on blood eosinophil counts (P~0.12) however, there was an effect of day (P<0.0001) and an interaction between day and management system (P<0.0001, Figure 4-8b). All management systems had similar eosinophil counts on day 0 (HI: 99, CI 64-141; TYP: 74, CI 45-110; IRG: 39, CI 19-64cells $x10^6$/L) and all rose on day 21. However HI sheep had significantly higher eosinophil counts than IRG at day 21 with TYP intermediate between the two (HI: 215, CI 158-281; TYP: 138, CI 105-175; IRG: 123, CI 91-161cells $x10^6$/L). By day 35 eosinophil levels dropped on all management systems with no significant difference between them (HI: 84, CI 58-114; TYP: 77, CI 48-112; IRG: 94, CI 57-138 cells $x10^6$/L). There was no effect of bodyweight on eosinophil count (P~0.62), however there was an effect of WEC with a strong negative relationship (P<0.001). There was also a positive linear relationship between eosinophil counts and haematocrit for all management treatments, except IRG (HI: c= 0.34, P~0.03; TYP: c=0.57, P~0.00; IRG: c=0.29, P~0.09).

Summer

There were no values for white blood cells in the summer challenge due to malfunction of the Cell-Dyn® automated haematology analyser.

Autumn

Eosinophil counts in autumn were generally low with no effect of management system (P~0.8), an effect of day (P<0.0001) and an interaction between management system and day (P<0.05, Figure 4-8b). There was no significant difference in mean eosinophil counts between

management systems on days 0 (HI: 22, CI 4-52; TYP: 15, CI 2-40; IRG: 30, CI 7-67cells x 10^6/L) and 21 (HI: 79, CI 45-122; TYP: 56, CI 31-89; IRG: 116, CI 70-171cells x10^6/L), however, TYP had significantly higher counts on day 28 than IRG with HI intermediate (HI: 24, CI 9-46; TYP: 26, CI 8-51; IRG: 6, CI 0-16cells x10^6/L). There was no relationship between bodyweight (P~0.99) or WEC (P~0.12) and eosinophil counts in autumn.

Winter

In winter there was as significant effect of management system (P<0.05), day (P<0.0001) and an interaction between the two (P<0.05, Figure 4-8b). Mean eosinophil counts were similar between management systems for days 0 (HI: 75, CI 55-97; TYP: 64, CI 48-84; IRG: 55, CI 40-72 cells x 10^6/L) and 28 (HI: 137, CI 115-160; TYP: 105, CI 85-126; IRG: 110, CI 92-130cells x 10^6/L), however, TYP had significantly lower counts on day 21 than HI and IRG (HI: 159, CI 134-186; TYP: 118, CI 100-137; IRG: 175, CI 151-200cells x10^6/L). By Day 35 IRG eosinophil levels had dropped to half that of HI and were similar to those of TYP sheep which also had significantly lower values than HI sheep (HI: 176, CI 150-204; TYP: 108, CI 88-129; IRG: 92, CI 75-110cells x10^6/L). There was a negative relationship between WEC and eosinophil counts (P<0.05) and no relationship between bodyweight and eosinophil counts in winter (P~0.40).

4.3.4 Circulating anti-trichostrongylid IgG

Spring

Backtransformed IgG levels are expressed in arbitrary units. There was no effect of management system on IgG antibody levels in spring (P~0.10), a main effect of day (P<0.0001) and no significant interaction between management and day (P<0.24, Figure 4-8c) although contrasts showed some significant differences between management on certain days. Levels in HI sheep were lower than those of TYP and IRG sheep on day 0 (HI: 23, CI 14-36; TYP: 57, CI 37-83; IRG: 55, CI 35-82 units of IgG), even though day 0 was fitted as a covariate in the model. HI tended to remain lower than the other two management systems over the course of the experiment and was significantly lower than TYP on day 21 (HI: 87, CI 52-135; TYP: 169, CI 117-235; IRG: 140, CI 95-199 units of IgG), There was no significant difference between management systems on day 35 although IRG had double the level of antibody than HI (HI: 71, CI 44-110; TYP: 98, CI 64-145; IRG: 144, CI 86-225 units of IgG). There was no effect of bodyweight on IgG in spring (P~0.89) or any relationship with WEC (P~0.13) or HCT (P~0.08).

Summer

There was no effect of management system (P~0.1) or day (P~0.2) on IgG antibody levels in summer and no interaction (P~0.8, Figure 4-8c) although IRG sheep showed a trend towards lower antibody levels overall (HI: 45, CI 28-68; TYP: 44, CI 30-62; IRG: 23, CI 17-32 units of IgG). There was no effect of bodyweight (P~0.78) or any relationship with WEC (P~0.67). There was a negative relationship between IgG and haematocrit for IRG sheep in summer but no relationship between the two for HI and TYP (HI: c=0.11, P~0.48; TYP: c=0.11, P~0.40; IRG: -0.29, P~0.03).

Autumn

In autumn there was no effect of management system (P~0.9) on IgG antibody levels nor any significant interaction between management system and day(P~0.9, Figure 4-8c). There was a significant effect of day (P<0.0001) with IgG levels significantly lower on day 0 than days 21 and 28 (21(2-74), 66(6-232) and 71(7-254) units of IgG, respectively). Again there was no effect of bodyweight on IgG (P~0.73) or any relationship with WEC (P~0.37). There was a positive relation ship between IgG and HCT (P~0.02)

Winter

There was no effect of management system in winter on IgG antibody level (P~0.9), a significant effect of day (P<0.0001) and a significant interaction between management system and day (P<0.05, Figure 4-8c). There is no significant difference between management systems on any of the experimental days, so the interaction can be explained by subtle changes in rank of the management system over the course of the experiment. On day 0 IRG sheep had a higher mean IgG level than HI and TYP, on day 21 TYP had slightly higher levels, on day 28 IgG levels were more even and on day 35 IRG tended to have lower IgG levels. There was no effect of bodyweight (P~0.12) or HCT (P~0.37) but there was a significant positive relationship with WEC (P<0.01).

4.4 Discussion

Young sheep on the IRG system showed resistance to GIN that was no better, and in two seasons was worse, than the HI and TYP systems. In both the spring and summer studies IRG sheep had higher total WEC, lower bodyweights but similar bodyweight gains, higher proportions of anaemic sheep and lower day 21 eosinophil counts (spring). There were fewer differences between management systems in autumn and winter. Circulating anti-trichostrongylid IgG did nor differ significantly between management treatments in any season. However, there was a

trend in the summer challenge for higher IgG in HI and TYP sheep with IRG having the lowest mean IgG on all days. These were reflected in the differences in WECs for summer such that lower WEC was accompanied by higher IgG.

The overall hypothesis that management effects on WEC observed in Experiment 1 (which ran contemporaneously with the current experiment) are due to improved host resistance is unequivocally rejected as the IRG treatment which had consistently lower WEC in Experiment 1 exhibited markedly greater susceptibility to fixed challenge in spring and summer. Following fixed challenge in spring IRG sheep had significantly higher total WEC than those on the HI or TYP treatments, a higher proportion were anaemic, they had lower numbers of circulating eosinophils and lower bodyweights but similar bodyweight gain. WEC was dominated by *H. contortus*. These findings all support the conclusion that during the spring fixed challenge, sheep on IRG mounted a less effective immune response to infection than those on the other management systems. Circulating anti-trichostrongylid IgG did not differ significantly between management systems in both spring and summer, however, the large standard errors mask a trend for higher levels in the TYP and HI treatments, with IRG have the lowest means over all days in summer (Figure 4-8c). Following the summer fixed challenge the IRG treatment again had higher WEC, lower HCT at day 35, a higher proportion of anaemic sheep, lower bodyweights and lower bodyweight gain than HI. The sheep involved in the summer challenge had been weaned only 2 weeks prior to the start of the fixed challenge, the stress of which has been shown to contribute to delayed development of an effective immune response against both *H. contortus* and *T. colubriformis* (Watson and Gill 1991). However, this did not seem to translate to this study with clear differences in WEC observed between management systems. WEC from IRG sheep was again dominated by *H. contortus*. Both the spring and summer fixed larval challenges give strong evidence that sheep on IRG had a lower immune response to *H. contortus* infections. The immune response of IRG sheep against *T. colubriformis* did not differ greatly from the other two management systems in spring and was greater than HI in the summer challenge, suggesting that prior exposure to that worm species was high enough to generate a greater level of resistance in IRG sheep than to *H. contortus*.

There was a negative relationship between bodyweight and total WEC and *Hc*WEC in spring with WEC declining with increasing bodyweights. There was also an effect of bodyweight on HCT with HCT increasing with increasing bodyweight which is consistent with the relationship

between *Hc*WEC and bodyweight. These data suggest that heavier sheep had lower *Hc*WEC and total WEC and thus reduced blood loss due to *H. contortus* as evidenced by their higher HCTs. This effect of bodyweight on HCT and total WEC was not seen in summer. There was no effect of bodyweight on circulating eosinophil count or anti-trichstrongylid IgG concentrations in any season.

The other 2 fixed challenge experiments did not follow the same trend as the spring and summer challenges. Paddock rotations on the IRG treatment were altered in autumn 2005 to provide longer graze periods and shorter rest periods in an attempt to increase bodyweights. This may have increased the level of exposure to infective larvae on the IRG treatment, possibly boosting immunity on that management system. All IRG lambs had substantial natural infections in late January and February 2005 of approximately 1000 eggs/g (Expt 1, Chapter 3) possibly triggering the development of an immune response which would have been apparent in the autumn challenge experiment. Following the autumn fixed challenge IRG sheep did have significantly higher WEC than TYP sheep but they had similar HCT and proportions of anaemic sheep. HI sheep had similar WEC to IRG but had significantly lower levels of anaemia despite the fact that *H. contortus* was the dominant infection on all treatments. This suggests that although HI sheep were more resistant in the spring and summer challenges they were less resistant in autumn whilst maintaining their resilience to GIN even though they had lighter bodyweights than the other 2 management systems at this time. There was no relationship between bodyweight and WEC in autumn possibly due to the smaller differences in these two traits. However, there was a positive relationship between HCT and bodyweight as seen in spring.

It is possible that the anthelmintic treatment history of the management systems also promoted similar resistance levels in autumn. The HI and TYP management sheep used in the autumn challenge had all received 3 anthelmintic treatments (moxidectin on 12th December 2004; levamisole on 24th January 2005; and albendazole with napthalophos 24th February 2005). The IRG sheep only received the latter treatment in February 2005. These drenches may have reduced the ability of the HI and TYP sheep to maintain their resistance that had developed to *H. contortus* in early summer by restriction of exposure to larval antigens. Along with increased nutritional stress on the HI treatment and greater exposure to infective larvae on the IRG treatment in January 2005, there was very little rainfall and very warm temperatures from February to June 2005. These weather conditions are not conducive to the development of eggs

or for the survival of infective larvae of any of the major nematode species, thus lowering pasture infection levels (Waller and Donald 1970; Southcott *et al.* 1976; Hsu and Levine 1977). The fact that all management systems received anthelmintic treatment together in late February, followed by dry, hot weather, may have put them all on a level playing field in terms of larval uptake and possibly of resistance to *H. contortus* infections (due to their labile nature).

There were also smaller differences in antibody levels and eosinophil counts between management treatments in autumn compared to spring. Reduced infections (indicated by lower WEC) were observed in both the autumn and winter fixed challenges compared to the spring and summer infections. In the case of the spring challenge the considerably higher challenge dose of larvae used is probably the explanation for the high WEC observed. However the decline in WEC in following autumn and winter challenges relative to the summer challenge is probably due to the increasing age of the challenged sheep (which were all from the same lamb drop) and thus greater development of immunity to infection. However, age did not benefit IRG sheep in the spring challenge which were 12 months old (2003 drop). The most likely explanations for this are reduced exposure to larval challenge over the course of their life (Healey, 2004) resulting in lesser developed resistance and possibly lower nutritional status (Gibson *et al.* 1970; Barger *et al.* 1985; Dobson *et al.* 1990a; Barnes and Dobson 1993; Walkden-Brown and Kahn 2002).

During the winter challenge there was a significant positive relationship between circulating anti-trichostrongylid IgG and WEC, although there was no significant difference between management systems for IgG or WEC. The direction of this relationship is opposite to that observed in the spring and summer challenges, although in the latter 2 seasons the relationships were not statistically significant, most probably due to high standard errors for IgG in those seasons. As the magnitude of IgG levels was so much lower in winter than in spring, the positive relationship with WEC is likely due to IgG increasing in response to artificial challenge after a period of low natural challenge, whereas the negative relationship in spring and summer more probably reflects the fact that sheep with pre-existing high levels immunity reflected in high IgG levels, resisted infection better. In other words, the winter infection (as determined by WEC) caused changes in IgG whereas summer, differences in IgG (indicating differences in immune status) caused changes in WEC.

During the winter fixed challenge trial TYP and IRG received a supplementary ration with a high protein content, whilst HI sheep received high energy and high protein supplement. HI sheep had significantly lower WEC than TYP, with IRG intermediate between the two. However, actual bodyweights on the HI treatment were lower than TYP and IRG. This is most likely due to the dry conditions in the preceding autumn and greater pressure on limited feed on offer due to higher stocking rates and inaccessible paddocks due to pasture improvement. However, the lower WEC coupled with lower bodyweights suggest that resilience to parasitic infection on HI could have been greater through nutritional modulation, as their diet was supplemented with both energy and protein whereas TYP and IRG diets were supplemented with protein only. Much work has been done to show the beneficial effects of high protein feeds on GIN but energy too is important as shown by Kahn *et al.* (2000b). These authors demonstrated a greater effect of energy in mediating GIN than protein. There was greater pasture dry matter on TYP and IRG, but being winter pastures a small proportion of that was green and digestibility would have been low. In winter, there was a negative relationship between bodyweight and WEC and *Hc*WEC, and a positive relationship between bodyweight and HCT as observed in spring.

The measurement of circulating eosinophil counts appeared to be a useful indicator of resistance in the spring challenge, with the HI sheep having the highest eosinophil counts on day 21 and the lowest overall WEC. IRG sheep had the lowest eosinophil counts on day 21 but the highest overall WEC, TYP was intermediate for both measurements. Unsurprisingly, there was a significant negative relationship between circulating eosinophil counts and WEC, higher eosinophil counts were accompanied by lower WEC. There was also a significant positive correlation between HCT and circulating eosinophils in spring, with eosinophil count increasing with increasing HCT, reinforcing the argument that higher eosinophil counts were indicators of greater resistance to nematode infection (Buddle *et al.* 1992; Woolaston *et al.* 1996). However, eosinophil counts may have fallen along with the HCT as a result of blood loss and the increased eosinophil levels in lower WEC sheep may simply be correlated with lower blood loss resulting from lower infection levels. Eosinophil counts did not show any correlation with WEC in autumn or winter and, unfortunately they could not be measured during the summer challenge in which there were large differences in WEC between management systems.

The lower resistance to infection in IRG sheep in the spring and summer fixed challenges could be due either or both of these things; i) lower host nutritional status or ii) lower exposure to

larval pathogens. Sheep on HI have access to pastures that have a higher proportion of green digestible dry matter and a higher proportion of sown species which are more digestible and more palatable then the pasture on IRG and TYP management systems (Chapter 2, section 2.2). There was also a higher proportion of native grasses on IRG than on HI, which are not as high in digestible dry matter as the pasture species on HI (Mpiti-Shakhane 2006). Thus the sheep on HI could have access to a higher plane of nutrition than IRG sheep although they are maintained at a significantly higher stocking rate. The IRG sheep had lower bodyweights in spring and summer but there was not the same difference in bodyweight gains. So whilst lower bodyweights and a higher proportion of native grasses might suggest a lower plane of nutrition on the IRG treatment, the lack of difference in bodyweight gain does not.

The second proposition, that lower resistance on IRG is due to lower exposure to larval pathogens, is based on the relationship between level and continuity of larval challenge and the development and maintenance of immunity to GIN. Resistance to nematode infection is dose dependant for both *H. contortus* and *T. colubriformis* with greater resistance resulting from higher exposure to the infective larvae (Barger *et al.* 1985; Dobson *et al.* 1990a). Continuous larval challenge with *T. colubriformis* translates into greater resistance to further infection (Gibson *et al.* 1970; Barnes and Dobson 1993), unfortunately this does not seem to hold true for *H. contortus*. Acquired resistance to *H. contortus* does not last much longer than 4 weeks after termination of the infection, so if larval challenge is not continuous then resistance will wane (Coyne and Smith 1992b). This could go some way to explaining the large differences in *H.contortus* WEC and very little difference in *T. colubriformis* WEC between IRG and the other two management systems in spring and summer. Low and inconsistent exposure to *H. contortus* on IRG, reflected in the low levels of that worm in IRG sheep in Experiment 1 (Chapter 3), could result in weaker resistance to that nematode and consistent exposure to *T. colubriformis* resulting in better resistance to the latter.

Of course the lower resistance on IRG could be due to a combination of both lower exposure to larvae and lower nutrition. If the sheep had not been exposed to threshold levels of infective larvae, also exposed intermittently rather than constantly to infective larvae, and had a lower plane of nutrition, those sheep would not only have a reduced stimulus for mounting an immune response but would also have a deficiency in the energy and protein requirements for mounting a full blown immune response. Our understanding of the ecology of the free-living stages of the

nematode parasites suggests that the IRG treatment may indeed reduce levels of larval challenge and this is explored in detail in Experiment 3, Chapter 5.

In conclusion, sheep on the IRG system showed reduced resistance to GIN in spring and summer relative to the HI and TYP systems, and similar levels of resistance in autumn and winter. Thus it is highly unlikely that the reduced WEC observed on IRG is due to an enhanced host immune response. The summer fixed challenge is of particular importance when gauging the differences in resistance between the management systems as this was a period in which there were very large differences in WEC between IRG and the other two management systems in experiment 1 (Chapter 3). It was during this summer fixed challenge that the greatest differences in resistance to infection were observed. There was also a differential effect of species with IRG sheep displaying lower resistance to *Haemonchus contortus* than *Trichostrongylus colubriformis* in the spring and summer challenges. When IRG rotations were maintained, sheep on that system had a lower resistance to *Haemonchus contortus*, as graze periods lengthened and rest periods shortened (in autumn), resistance to infection became similar to the other two management systems. This further supports the hypothesis that lower WEC observed during Experiment 1 on IRG was not due to better host resistance.

CHAPTER 5: Experiment 3. Effect of management system on pasture infectivity with infective larvae – Tracer sheep study

5.1 Introduction

The significant differences in WEC between management treatments observed in Experiment 1 (Chapter 3) could be mediated by either/or i) differences in host resistance to GIN due to treatment effects on the host phases of the lifecycle, or ii) differences in larval challenge due to treatment effects on the environmental phases of the parasite lifecycle. The first of these possibilities was tested in Experiment 2 and reported in Chapter 4. To investigate the second possibility the present experiment was designed to test the general hypothesis that management treatments with the lowest WEC in Experiment 1 will exhibit reduced levels of pasture infectivity. This is particularly relevant for the IRG treatment which exhibited markedly lower WEC than the HI or TYP treatments overall.

Over the last century, rotational grazing and set stocking have been discussed in detail in relation to control of gastrointestinal nematode infection (Morgan 1933; Morgan and Oldham 1934; Gordon 1948; Gibson and Everett 1968; Barger *et al.* 1994; Barger 1997; Barger 1999). Systems of rotational grazing were suggested according to the understanding of the free-living stages and tested against set-stocking for animal performance and for nematode control. Rotational grazing strategies basically utilise one or both of two key principles. The first involves allowing sheep to graze for a short period of time with removal before the eggs laid down in the their faeces develop into infective larvae, thus preventing autoinfection from the current grazing. The other significant principle involves the paddock being spelled between graze periods for sufficient time to allow for the majority of the infective larvae to die off. However to date only one rotational grazing strategy developed for a cool temperate climate has been successful in reducing worm burdens. That strategy (termed "progressional grazing") was devised by Robertson and Fraser (1933) in the North of Scotland and involved grazing periods of 10 days with 100 days rest. The main downfall of this experiment was the quality of feed on the rotational system with rest periods meaning that grass became over-matured and of lower quality. Other rotations tested by Morgan (1933), Morgan and Oldham (1934), Roe *et al* (1959)

and Gibson and Everett (1968) all found no advantage of rotational grazing for worm control. Their rotations were generally 1 week of grazing followed by rest periods ranging from 3 to 7 weeks. The common failings of these experiments seem to be in the length of the rotation and inflexibility of rotations with the changes in season. One week of grazing may be too long in summer as L_3 may develop within that time, but fine in winter. The rest periods are too short given evidence from Donald (1967) that infective *H. contortus* and *Trichostrongylus* spp. larvae can persist on pasture in significant numbers at until at least 9 weeks after egg deposition which was the duration of that experiment. This work was carried out from summer to early autumn, thus the results suggest that pasture should be rested for at least 8 weeks in summer. These results also showed a peak of L_3 availability 5 weeks after contamination, with substantial numbers of L_3 at two and three weeks. In fact Donald (1967) states that there is no sound evidence that pastures should be spelled for periods shorter than 2 months.

The grazing periods used in the studies mentioned above seem reasonable in some cases as a study in the British Isles concluded that *H. contortus* eggs require upwards of 2 weeks to develop in summer and considerably longer at other times of the year (Silverman and Campbell 1959). However the study by Roe *et al.* (1959) was carried out in Northern NSW, Australia, where larval development can occur within 5-7 days in summer (Monnig 1930; Rose 1964), thus the system of rotation used by these authors (1 week graze, 3 weeks rest) provided perfect conditions for the perpetuation of the lifecycle of parasitic nematodes.

The dynamics of the free-living stages of gastrointestinal nematodes is slightly different in humid tropical regions as the factors most limiting to egg development in temperate climates (i.e.: cold temperatures and lack of moisture) generally do not apply. A series of experiments by Banks *et al.* (1990) and Barger *et al.* (1994) led to the development and adoption of a rapid rotational system for grazing small ruminants in tropical climates. The rotation interrupted the parasite lifecycle in its free-living stages and was based on prevention of autoinfection by rotating sheep prior to development of L_3 from the current faecal contamination (3.5 days), and by exploiting the high death rate of larvae under tropical conditions by having a moderately long rest period between grazing episodes (31.5 days). This rapid rotational grazing has been successfully used in other tropical regions such as Malaysia and the Philippines (Chandrawathani *et al.* 1995; Sani *et al.* 1996; Chandrawathani 1997; Gray *et al.* 2000). These studies along with Robertson and Fraser (1933) demonstrate the that rotational grazing can be used for worm

control, although appropriate methods in temperate climates have generally eluded other researchers. It is possible that reduced WEC on the IRG treatment is mediated through the same mechanism as that demonstrated by Barger *et al.* (1994) in the tropics and by Robertson and Fraser (1933) in Scotland.

Estimates of pasture infectivity in the past have relied on two methods, direct pasture sampling (Taylor 1939; Donald 1967; Donald and Waller 1973) or use of worm-free lambs or "tracers" grazing for short intervals prior to slaughter for total worm counts (Kates 1950; Anderson 1972; Donald *et al.* 1978a). Pasture sampling provides an estimate of the concentration of infective larvae on the herbage in a given area at a certain time point. The tracer animals provide worm counts which are the result of larval intake by that animal and worm-expulsion or losses. They can also give information on hypobiosis and arrested development of ingested larvae (Waller *et al.* 1981; Amarante and Barbosa 1998). Tracer sheep are generally allowed to graze on a given pasture for 2-4 weeks, after which they are housed for a further 2-4 weeks and slaughtered for total worm counts. The tracers used in this study were used to obtain faecal worm egg counts and could not be slaughtered to provide total worm counts as the experiment took place in a commercial environment. This approach has been used effectively in the past (eg. Kahn *et al.* (2003a; 2003b). However, these studies were investigating host responses to infection and not parasite epidemiology.

This experiment was conducted to determine whether there were differences in pasture infectivity and larval uptake by sheep on the 3 management systems. The main hypothesis was that tracers on the IRG system would have a lower larval uptake from pasture (as determined by WEC after two weeks exposure to pastures) than those on HI and TYP management systems.

5.2 Materials and Methods

5.2.1 Experimental design

The experiment was a seasonal study of the level of larval uptake of tracer sheep (as determined by WEC) on three different management systems. The main factors in the design were:

- Three management systems: High input (HI), Typical (TYP) and Intensive rotational grazing (IRG) (section 2.2).
- Three classes of sheep: Lambs, hoggets and ewes.

- Season: winter (24th June to 30th July 2004), spring (18th October to 22nd November 2004), summer (24th January to 28th February 2005), autumn (11th April to 16th May 2005)

Seven days after short acting anthelmintic treatment, 3 to 5 tracer animals were allocated to the ewe, hogget and lamb mobs on each management system randomly with the stipulation that each mob receive at least one tracer originating from each of the 3 management systems. The tracers were run with these mobs for 2 weeks then removed to a clean periphery paddock reserved for this purpose. Only cattle had grazed the periphery paddock for a period of 1 year prior to the initial winter experiment so it should have been effectively free of ovine infective L_3. Faecal samples were taken at 2 and 4 weeks after tracers were removed from mobs (ie. 4 and 6 weeks after being placed on the treatment pastures). The tracers were then dosed with anthelmintics and returned to another periphery paddock where they were maintained until use in the next tracer experiment. A schematic representation of experimental events for each seasonal tracer study is shown in Figure 5-1.

The same anthelmintic treatments were used as for the fixed challenge studies (Experiment 2, Chapter 4, section 4.2) and the spring, summer and autumn tracer studies were run in conjunction with the fixed challenge studies of those seasons. Because tracer sheep were removed at day 14 of the concurrent fixed challenge study, pasture larval contamination from the fixed challenge study could not influence the tracer study. During the winter challenge tracer sheep received the same anthelmintic treatment as the spring and summer tracers.

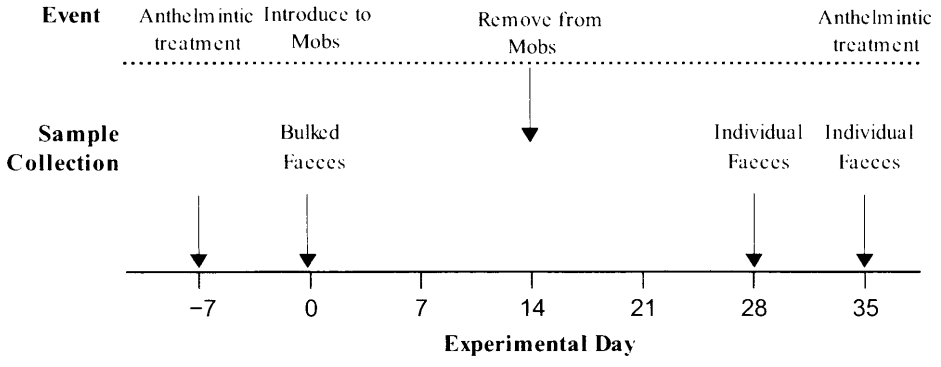


Figure 5-1: Timing of experimental events and sample collection for each tracer study.

5.2.2 The tracer animals

The tracer animals for the winter and spring tracer events were wethers born in September 2003 that had been run with their mobs in each management system prior to the winter 2004 experiment. The tracers for the summer and autumn experiments were wethers born in September 2004 that had been removed from the lamb mobs by the Cicerone Project manager due to low liveweights at weaning in January 2005. Although it is not ideal to use tracers that have had prior exposure to parasitic nematode infection, it was unavoidable in this instance as the Cicerone Board would not approve the introduction of sheep from an outside source.

Tracer faecal worm egg counts only were determined as there were insufficient animals to allow euthanasia and total worm counts. Tracers were also not housed after their 2 week exposure period due to a lack of facilities on site. Removal from the Cicerone Project site would have involved anthelmintic treatment which was counter to the requirements of this experiment. Therefore, there would have been no detection of larvae in arrested development under these experimental conditions.

5.2.3 Sampling procedures and measurements

On each sampling day the tracer sheep were sampled rectally for faeces and individual faecal worm egg counts (WEC) and bulked faecal cultures performed in random order using the methods outlined in 2.3. Bulked WEC (section 2.3.1.1) and faecal cultures were performed on day 0 to confirm the worm-free status of the tracers.

5.2.4 Statistical Analysis

Two main analyses were considered: i) across season comparisons and ii) within season comparisons. A cubed-root transformation was used for all faecal worm egg count data and backtransformed means are presented with 95% confidence intervals. For analysis i) The data structure did not allow the effect of season to be included in the full model so to determine the significance of seasonal effects a separate model was fitted averaged over all classes and days for total faecal worm egg count (WEC), larval differentiation, *Haemonchus contortus* WEC (*Hc*WEC), *Trichostrongylus* spp. WEC (*T*WEC) and *Teladorsagia circumcincta* WEC (*Ost*WEC). A linear mixed model was performed in JMP IN version 5.1 (SAS Institute Inc., NC, USA) using REML fitting season (winter, spring, summer, autumn), management system (HI, TYP, IRG), significant interactions between these effects and tag number as a random variable.

For analysis ii) data were analysed within seasons with following terms fitted; management system (HI, TYP, IRG), day (21, 28, 35), class (ewe, lamb, hogget), significant interactions between these effects and tag number as a random variable. Class was not fitted in interaction terms for the autumn tracer study as the hogget and ewe classes were run in the same mob on IG but were run separately on HI and TYP management systems. Tukey's HSD post hoc tests and contrasts within the model were used to determine significant differences between means. As the larval differentiation data from the faecal cultures is not replicated within season it was not analysed and arithmetic means are presented.

5.3 Results

Tracers had zero faecal worm egg counts and no larvae cultured at day 0 for all of the seasonal experiments. They were therefore assumed to be free of gastrointestinal nematode infection at the start of each experimental period.

5.3.1 Seasonal comparisons

5.3.1.1 Total faecal worm egg counts

Tracers during summer had significantly higher WEC than in winter, spring and autumn which did not differ (780, CI 600-970; 230, CI 150-310; 130, CI 80-190; and 120, CI 70-180eggs/g, respectively, $P<0.00001$). Overall tracers on IRG had lower WEC than HI and TYP tracers (90, CI 55-135; 330, CI 240-430; and 420, CI 320-530eggs/g, respectively, $P<0.0001$). There was a significant interaction between management system and season ($P<0.0001$) with tracers on IRG lower than HI and TYP in winter, spring and summer, with no significant difference between tracer WEC on management systems in autumn (Table 5-1).

Table 5-1: Backtransformed least square mean tracer faecal worm egg counts by management treatment and season with 95% confidence intervals. Different letters within season denote a significant difference (P<0.05).

Season	Management Treatment	Mean faecal worm egg count	95% Confidence Interval
Winter	HI	290[a]	150-470
	TYP	400[a]	230-620
	IRG	80[b]	20-150
Spring	HI	420[a]	240-640
	TYP	170[a]	70-280
	IRG	10[b]	0-40
Summer	HI	560[a]	330-820
	TYP	2100[a]	1500-2740
	IRG	280[b]	140-450
Autumn	HI	150[a]	60-270
	TYP	80[a]	20-160
	IRG	130[a]	40-230

5.3.1.2 Larval culture results

The proportion of nematode species found in tracer faecal cultures differed significantly between season with higher proportions of *H. contortus* in spring and summer than autumn and winter (P<0.0001). For *H. contortus* there was an interaction between management treatment and season (P<0.0001) with all management treatments not differing in summer and autumn and IRG tracers having lower proportions of *H. contortus* than HI and TYP in winter and spring (Table 5-2).

There were higher proportions of *Trichostrongylus* spp. in winter and summer than in autumn and spring (P<0.001). There was no significant interaction between management treatment and season for *Trichostrongylus* spp. proportions with IRG tracers having significantly higher proportions than HI and TYP in autumn, summer and winter (P~0.12). However, contrasts revealed that IRG and TYP did not have different proportions of *Trichostrongylus* spp. in spring (Table 5-2, P~0.88).

In spring there were significantly higher proportions of *T. circumcincta* than in autumn, but there were no differences between the seasons of spring, summer and winter, or between winter, summer and autumn. Again there was a significant interaction between management treatment and season (P<0.05), however, IRG sheep had significantly higher proportions of *T. circumcincta* than HI and TYP in all seasons (Table 5-2). The interaction is most probably due to the magnitude of the differences between management treatments between seasons.

Table 5-2: Backtransformed mean proportions of the 3 major parasitic nematode species recovered from tracer faecal cultures. Different letters within season indicate a significant difference (P<0.05).

		Haemonchus contortus		*Trichostrongylus* spp.		*Teladorsagia circumcincta*	
Season	Management treatment	%	95% CI	%	95% CI	%	95% CI
Winter	HI	95[a]	86-100	4[a]	0-10	0[a]	0-3
	TYP	92[a]	80-100	5[a]	0-12	0[a]	0-3
	IRG	19[b]	12905	27[b]	10-46	14[b]	3-27
Spring	HI	100[a]	96-100	0[a]	0-1	0[a]	0-1
	TYP	96[a]	87-100	2[b]	0-7	1[a]	0-5
	IRG	0[b]	0-5	2[b]	0-6	58[b]	34-82
Summer	HI	90[a]	80-99	7[a]	2-14	2[a]	0-7
	TYP	98[a]	93-100	2[a]	0-5	0[a]	0-3
	IRG	72[a]	58-86	14[b]	6-22	9[b]	1-17
Autumn	HI	99[a]	94-100	1[a]	0-3	0[a]	0-2
	TYP	100[a]	99-100	0[a]	0-1	0[a]	0-1
	IRG	79	62-94	8[b]	1-17	12[b]	2-24

5.3.1.3 Faecal worm egg count by species

There was significantly higher *H. contortus* WEC (*Hc*WEC) in summer (660, CI 520-820eggs/g, P<0.0001) than in any other season. *Hc*WEC was higher in winter than in spring and autumn which did not differ (winter: 160, CI 110-220; spring: 85, CI 50-125; autumn: 35, 15-60 *H. contortus* eggs/g). Tracers on IRG had the lowest *Hc*WEC overall with HI and TYP not differing (IRG: 40, CI 20-60; 240, 180-310; and 310, CI 240-390eggs/g, respectively, P<0.0001). There was also an interaction between management system and season with tracers on IRG having lower *Hc*WEC in winter, spring and summer than HI and TYP, tracers on TYP had higher *Hc*WEC than HI in summer and no significant differences in autumn (Table 5-3).

There was a significant effect of season (P<0.0001) on *Trichostrongylus* spp WEC (*T*WEC) in tracers with the highest counts in summer (25, CI 15-40eggs/g) and winter (15, CI 7-25eggs/g). Spring and autumn tracer *T*WEC were lower than summer and winter (0, CI 0-1 and 1, CI 0-2eggs/g). There was no significant effect of management treatment (P~0.17) and a significant interaction (P<0.05). All management systems had similar *T*WEC in winter and summer, but TYP had higher *T*WEC in spring than HI and IRG, and IRG had higher *T*WEC in autumn than HI and TYP (Table 5-3).

There was a higher incidence of *T. circumcincta* WEC (*Ost*WEC) in the summer tracers than in any other season (9, CI 5-15, P<0.0001). Winter had higher levels of *Ost*WEC than spring with

autumn intermediate between the two (winter: 2, CI 1-4; spring: 0, CI 0-1; autumn: 1, CI 0-2eggs/g). There was no interaction between season and management treatment with IRG tracers having greater *Ost*WEC over all seasons than HI and TYP (Table 5-3). Given the low numbers of *T. circumcincta* recovered it is reasonable to conclude that there was no biological difference between seasons even though there is a statistical difference.

Table 5-3: Backtransformed faecal worm egg counts with 95% confidence intervals (95% CI) for the three major nematode species by season. Different letters within season indicate a significant difference (P<0.05).

		Haemonchus contortus		*Trichostrongylus* spp.		*Teladorsagia circumcincta*	
Season	Management system	WEC	95% CI	WEC	95% CI	WEC	95% CI
Winter	HI	280[a]	150-420	12[a]	2-25	0[a]	0-2
	TYP	380[a]	220-560	18[a]	5-35	0[a]	0-2
	IRG	15[b]	0-40	15[a]	4-30	15[b]	5-30
Spring	HI	420[a]	250-610	0[a]	0-0	0[a]	0-0
	TYP	150[a]	70-250	3[b]	0-9	0[a]	0-0
	IRG	0[b]	0-1	0[a]	0-1	5[b]	0-10
Summer	HI	460[a]	280-660	26[a]	Oct-50	6[a]	1-14
	TYP	1970[b]	1470-2520	16[a]	3-30	3[a]	0-5
	IRG	210[c]	100-330	35[a]	15-60	24[b]	Oct-40
Autumn	HI	30[a]	4-70	0[a]	0-2	0[a]	0-1
	TYP	8[a]	0-25	0[a]	0-0	0[a]	0-0
	IRG	100[a]	40-180	5[b]	0-13	17[b]	5-30

5.3.2 Within season comparisons

Figure 5-2 shows the tracer mean WEC for each class by management system and season.

5.3.2.1 Winter

During the winter tracer study (June/July 2004) there was a significant effect of management system (P<0.01), day (P<0.0001) and class (P<0.01) on tracer WEC, with a significant interaction between management system and class (P<0.05). The interaction between management system and class was due to higher WEC in tracers from the lamb mob than the ewe mob on both TYP and IRG treatments but not the HI treatment (Figure 5-2). Overall, tracers on IRG had significantly lower WEC (94, CI 29-177eggs/g) than those on HI and TYP treatments which did not differ (328, CI 173-511 and 471, CI 271-701eggs/g respectively, P<0.0001). Overall, tracers with lambs had higher total WEC than ewes (420, CI 250-620; 150, CI 85-225 eggs/g, respectively, P<0.01).

Faecal culture revealed a higher proportion of *Trichostrongylus* spp. and *Teladorsagia* spp. and lower proportions of *H.contortus* on the IRG treatment than the HI and TYP treatments which were dominated by *H. contortus* infections (Table 5-4).

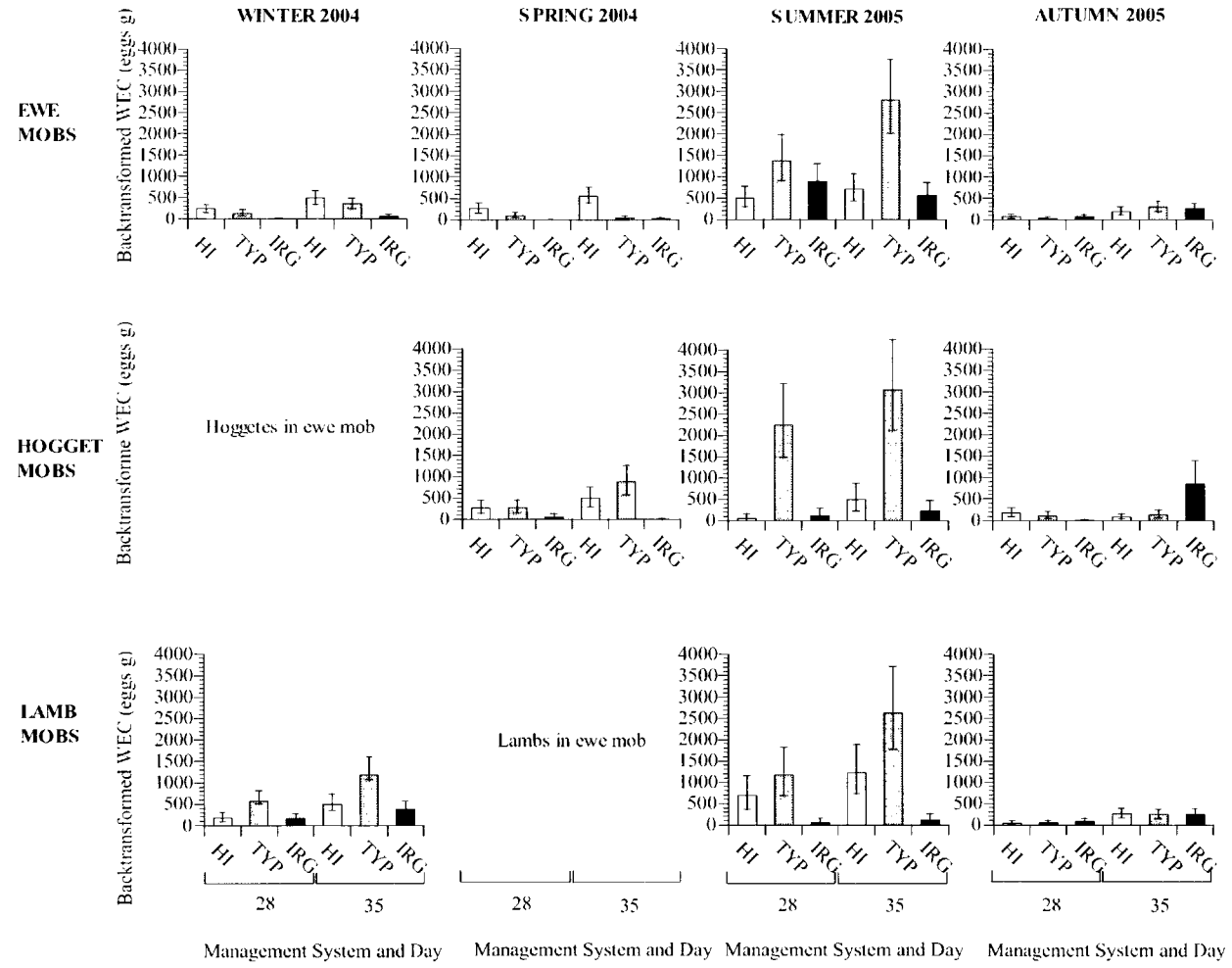


Figure 5-2: Backtransformed mean faecal worm egg count with 95% confidence intervals for tracers on days 28 and 35 by class, management treatment and season.

There was lower *Hc*WEC on IRG in winter than HI and TYP (20, CI 2-50; 310, CI 180-460; and 440, CI 270-640eggs/g, P<0.0001) and lower *Hc*WEC in tracers in with ewes than in lamb mobs (95, CI 50-140; 330, CI 200-470eggs/g, P<0.001). There was a management system, class interaction with tracers in with lambs on TYP and IRG both having higher *Hc*WEC than those with ewes, whereas there was no difference between the classes on HI (Table 5-5).

There was no main effect of management system (P~0.23) or class (P~0.08) on tracer *T*WEC during winter. There was a significant interaction between management system and class

(P<0.0001) with tracers in the ewe mob having lower *T*WEC than lambs on IRG, ewe and lamb mobs similar on TYP and lambs having lower *T*WEC than ewes on HI (Table 5-5).

There were higher *Ost*WEC on IRG during winter than on HI and TYP (22, CI 14-30; HI and TYP both 0, CI 0-0 eggs/g, P<0.0001). There was no main effect of class (P~0.32), however there was an interaction between management system and class. Tracers in ewe mobs on HI and TYP had higher *Ost*WEC than lambs, whilst tracers in with lambs on IRG had greater *Ost*WEC than ewe tracers (Table 5-5).

Table 5-4: Mean proportions of the three major parasitic nematodes collected from bulked tracer faecal cultures by season and management treatment. Numbers in brackets represent actual numbers counted when less than 100 larvae where counted.

		Day 28			Day 35		
Season	Mgt System	*H. contortus*	*Trichostrongylus* spp.	*Teladorsagia circumcincta*	*H. contortus*	*Trichostrongylus* spp.	*Teladorsagia circumcincta*
Spring	HI	100	0	0	98	0	0
	TYP	91	2	7	98	1	1
	IRG	1	3	96	30	1	12
Summer	HI	85	11	4	90	7	1
	TYP	93	6	1	96	4	1
	IRG	59	17	24	83	12	2
Autumn	HI	100	0	0	(19)	(7)	(1)
	TYP	100	0	0	100	0	0
	IRG	81	11	8	84	7	9
Winter	HI	84	0	0	95	5	0
	TYP	92	9	0	96	3	2
	IRG	(2)	(1)	(1)	20	54	27

5.3.2.2 Spring

There was a significant effect of management system (P<0.0001) and a strong trend towards interaction between management system and class (P<0.06) on tracer WEC in spring (Oct/Nov 2004). There was also significant three way interaction between management system, class and day (P<0.01) with all other effects being non-significant. The interaction is due to the WEC of tracers in the TYP ewe and IRG hogget mobs not increasing on Day 35 unlike the other mobs (Figure 5-2). Overall tracers on IRG had significantly lower WEC (15, CI 0-43eggs/g) than tracers on HI and TYP (380, CI 203-588 and 227, CI 103-377eggs/g, respectively).

Spring faecal culture results again showed a dominance of *H. contortus* infection in HI and TYP tracers with IRG tracers having high ratios of *T. circumcincta* on Day 28 and *H. contortus*

showing through on Day 35, but at lower levels than the other two management treatments (Table 5-4).

Tracers on IRG had significantly lower *Hc*WEC than those on HI and TYP which did not differ (0, CI 0-1; 380, CI 230-330; and 210, CI 110-330 eggs/g, P<0.0001). Tracers in with hogget mobs had greater *Hc*WEC than ewes (140, CI 70-230l and 60, CI 30-90, P<0.05). There was a significant interaction between class and management treatment with no significant difference between ewes and hogget mob tracers on both HI and IRG, and tracers with hoggets on TYP having greater *Hc*WEC than ewes (P<0.05, Table 5-5).

Tracer *T*WEC was very low during the spring study with tracers on TYP had the highest *T*WEC in spring, followed by IRG and HI tracers (5, CI 3-7; HI and TYP both 0, CI 0-0eggs/g, P<0.0001). Tracers with hogget mobs were higher for *T*WEC than ewe mob tracers (1, CI 0-2 and 0, 0-0eggs/g, P<0.05). The main effects of class seem are entirely contributable to the TYP management system with no difference between ewes and hogget mob tracers on HI and IRG and hoggets higher than ewe mob tracers on TYP (P<0.01, Table 5-5).

There was significantly more *Ost*WEC on IRG than on HI and TYP in spring (6, CI 1-12; HI and TYP both 0, CI 0-0eggs/g, P<0.0001). There was no effect of class (P~0.13) and no interaction between management system and class (P~0.11).

5.3.2.3 Summer

During the summer period (Jan/Feb 2005) there was a significant effect of management system (P<0.0001) and day (P<0.001) but not class (P=0.2), on tracer WEC with a significant interaction between management system and day (P<0.05). There was a trend towards interaction between the effects of management system and class (P~0.08), with other interactions being non-significant. The interaction between management system and day was due to tracer WEC on the IRG treatment not increasing significantly from day 28 to 35 unlike HI and TYP (Figure 5-2). Overall tracer WEC on the HI and IRG treatments were not significantly different (515, CI 238-849 and 243, CI 80-457eggs/g, respectively) with both significantly lower than TYP (2130, CI 1370-2987eggs/g).

Generally, HI and TYP tracer faecal cultures were dominated by *H. contortus* with lower proportions of that species on the IRG treatment. There were higher proportions of *Trichostrongylus* spp and *T. circumcincta.* on IRG (Table 5-4).

Tracers on TYP had significantly higher *Hc*WEC than those on HI and IRG which did not differ (2010, CI 1340-2770; 450, CI 220-730; 170, CI 50-320eggs/g, P<0.0001). There was no main effect of class (P~0.56) but there was a significant interaction between management system and class (P<0.05). Tracers on TYP had the highest *Hc*WEC for ewes and hoggets, but were similar to HI in the lamb mobs. HI and IRG had similar *Hc*WEC in ewes and hogget mobs but HI had greater *Hc*WEC in the lamb tracers (Table 5-5).

There was no significant effect of management system on *T*WEC in summer (P~0.09), but there was a significant effect of class with ewes having much higher *T*WEC than hoggets and lambs (130, CI 90-170; 5, CI 0-13; 0, CI 0-2eggs/g, P<0.0001). There was a significant interaction between management system and class (P<0.0001) with tracers in TYP ewes showing higher *T*WEC than HI and IRG which were similar; tracers in TYP hogget mobs had lower *T*WEC than HI and IRG; and tracers in IRG lamb mobs had higher *T*WEC than HI and TYP (Table 5-5).

There was significantly higher *Ost*WEC in IRG tracers in summer whilst HI was higher than TYP (14, CI 9-20; 6, CI 3-9; and 2, CI 1-3eggs/g, P<0.001). There was also an effect of class with tracers in ewe mobs showing higher *Ost*WEC than lamb and hogget mob tracers (33, CI 25-40; 1, CI 0-2; 2, CI 1-4eggs/g, P<0.0001). There was also a significant interaction between management treatment and class for *Ost*WEC (P<0.0001) with tracers in HI ewe mobs displaying lower *Ost*WEC than TYP and IRG, whilst TYP tracers showed lower *Ost*WEC in both hoggets and lambs than IRG and HI (Table 5-5).

5.3.2.4 Autumn

During the autumn period (April/May 2005) tracer WEC were extremely low due to an extended dry period. There was a significant effect of day (P<0.01) with counts rising from day 28 to day 35 (58, CI 26-97 and 251, CI 163-351eggs/g, respectively, Figure 5-2) but no effect of management treatment (P~0.9), class (P~0.15) or significant interaction between the main effects.

H. contortus was dominant in the autumn cultures with low levels of *Trichostrongylus* spp. and *T. circumcincta.* on IRG and HI at day 35 (Table 5-4).

There were significantly higher *Hc*WEC counts on IRG in autumn than in HI and TYP tracers (114, CI 60-180; 30, CI 10-50; 9, CI 1-20eggs/g, P<0.0001). Tracers in lamb mobs had the highest *Hc*WEC with Hoggets intermediate between lambs and ewes (ewes: 20, CI 7-35; hoggets: 32, CI 6-70; lambs: 74, CI 35-120eggs/g, P<0.05).

There were higher *T*WEC counts from IRG tracers than HI and TYP in autumn (9, CI 4-15; HI and TYP both 0, CI 0-10eggs/g, P<0.0001) and higher *T*WEC in lambs than in the other two classes (lambs: 4, CI 1-7; ewes and hoggets both 0, CI 0-0eggs/g, P<0.001).

There was also a higher level of *Ost*WEC on IRG than on HI and TYP (16, CI 9-24; HI and TYP both 0, CI 0-0eggs/g, P<0.0001). There was no effect of class on *Ost*WEC in autumn (P~0.07).

5.4 Discussion

The hypothesis that tracers on the IRG system will have lower WEC than those on the HI and TYP management systems was strongly supported with significantly lower tracer WEC on the IRG treatment. During autumn HI and TYP systems fell to IRG levels due to dry, hot weather and a higher number of anthelmintic treatments on the latter management systems restricting larval development. During the winter of 2004, spring of 2004 and summer of 2005 the IRG treatment maintained short graze and long rest periods and it was during these seasons that the IRG tracers had consistently lower WEC than those grazed on the HI and TYP treatments. The management treatment effects during these periods appeared to hold true for all classes of sheep.

The domination of *H. contortus* in faecal cultures from tracers on the HI and TYP treatments is generally consistent with that seen in the sheep in Experiment 1 (Figure 3-2, Chapter 3). The WEC from Experiment 1 were influenced by the type and timing of the last anthelmintic treatment, thus there are times when one must look at WEC in the months prior to and post tracer experimental periods to gain a comparison with the tracer egg counts. One major exception to conformity with Experiment 1 was for the HI management treatment hoggets in January 2005. Their WEC at this time was around 5000 eggs/g which was ~50% *Trichostrongylus* spp., this was not reflected in the tracer WEC for this mob during that month which also showed high

WEC but 100% *H. contortus* in culture. The autumn 2005 tracer WEC was also dominated by *H. contortus* which would have been deposited during the late summer months.

Table 5-5: Backtransformed mean faecal worm egg counts of tracers with 95% confidence intervals (95% CI) apportioned into the three major nematode parasites using the proportions of each parasite counted in faecal cultures by management treatment and class (E- ewes, H- hoggets, L- Lambs) within season.

			Haemonchus contortus		*Trichostrongylus* spp.		*Teladorsagia circumcincta*	
Season	Management system	Class	WEC	95% CI	WEC	95% CI	WEC	95% CI
Winter	HI	E	310	160-500	24	10-40	1	0-3
		L	300	100-550	4	0-10	0	0-0
	TYP	E	200	90-350	17	7-30	1	0-3
		L	820	420-1290	19	5-40	0	0-0
	IRG	E	1	0-7	3	0-7	3	1-6
		L	100	10-220	70	30-110	74	45-100
Spring	HI	E	387	200-600	0	0-0	0	0-0
		H	365	140-650	0	0-0	0	0-0
	TYP	E	71	20-140	1	0-2	0	0-0
		H	477	200-800	12	6-20	0	0-0
	IRG	E	0	0-1	0	0-0	1	0-4
		H	0	0-7	0	0-1	15	12-33
Summer	HI	E	423	115-830	111	60-180	3	0-6
		H	187	0-520	9	0-30	5	0-10
		L	920	250-1800	0	0-0	13	4-25
	TYP	E	1686	780-2780	270	160-400	48	30-70
		H	2635	1230-4330	0	0-0	0	0-0
		L	1801	720-3140	0	0-0	0	0-0
	IRG	E	518	160-980	59	30-100	97	70-130
		H	111	0-350	29	0-80	2	0-6
		L	56	0-220	12	0-30	3	0-8
Autumn	HI	E	13	1-30	13	1-30	0	0-0
		H	21	1-50	21	1-50	0	0-0
		L	78	30-140	78	30-140	2	1-3
	TYP	E	3	0-10	3	0-10	0	0-0
		H	9	0-25	9	0-25	0	0-0
		L	21	1-50	21	1-50	0	0-0
	IRG	E+H	77	25-140	4	1-8	77	25-140
		L	170	40-340	29	10-50	170	40-340

In winter, spring and summer tracers from the IRG treatment had a lower proportion of *H. contortus* and higher proportions of *Trichostrongylus* spp. and *T. circumcincta* in their faecal cultures (Tables 5-2 and 5-3). This suggests that the IRG treatment had a differential effect with

the major impact being on *H. contortus*. The eggs of *H. contortus* hatch between 3-5 days after deposition at 25-26°C and between 15 and 30 days after deposition at 10-11°C (Rose 1963) and require additional moisture within a short time of deposition (~5 days) to survive and develop (Donald 1973; Rossanigo and Gruner 1995). This supports the theory that IRG sheep have lower levels of *H. contortus* larvae on pasture because they are removed from each paddock prior to autoinfection from faeces deposited during the current grazing period, and because the long period between grazing allows significant larval die off. On the other hand the eggs of *Trichostrongylus* spp. and *T. circumcincta* are significantly more resistant to the effects of desiccation and cold than those of *H. contortus* and thus survive for a much longer period (Waller and Donald 1970; Rossanigo and Gruner 1995). The higher proportions of *Trichostrongylus* spp. and *T. circumcincta* on IRG could therefore be due to their superior ability to survive as eggs for longer and develop into infective larvae when conditions are favourable. Nevertheless one might expect that there would be some impact of prevention of autoinfection and long rest periods on *Trichostrongylus* spp. and *T. circumcincta*. However, based on the *T*WEC and *Ost*WEC analyses, there did not seem to be a major impact of the short grazing and long rests on these nematode species. There was also a much lower anthelmintic treatment frequency on IRG which means that if these species survive the IRG grazing treatment that they would be less frequently affected by anthelmintics compared to the same nematode species on HI and TYP.

There was a higher proportion of *H. contortus* in faecal cultures in spring and summer than in autumn and winter. This is as would be expected given the temperature and moisture requirements for *H. contortus* eggs to develop into infective larvae and complies with the established epidemiology of GIN in this region (Gordon 1948; Donald *et al.* 1978b). Although *T. circumcincta* and *Trichostrongylus* spp. also generally show peaks in spring. The winter and autumn tracer periods were both periods of low rainfall with very low overnight temperatures in winter and moderate temperatures interspersed with frosts in autumn. The summer and spring months were warmer and both included significant rainfall events. The highest levels of *Trichostrongylus* spp. were seen in winter and summer with the summer increase mostly due to higher levels of this worm in the tracers from the IRG mobs. *Teladorsagia* spp. proportions were greatest in autumn when temperatures are cooler with multiple frosts and low rainfall. This worm has been shown to best resist freezing and low moisture conditions due to the higher lipid

content of its eggs providing a cryoprotectant effect and greater energy stores greatly enhancing the survival of the eggs of this species (Jasmer *et al.* 1986; Jasmer *et al.* 1987).

Generally there were very few differences between the HI and TYP management treatments, both displaying similar WECs in winter, spring and autumn and similar proportions of *H. contortus*, *Teladorsagia* spp. and *Trichostrongylus* spp. in faecal culture. Summer was the only season in which these two treatments differed with TYP having significantly higher WEC, but even then both treatments had similar proportions of the major worm species. This similarity of HI and TYP is also reflected in experiment 1 where, in general, the timing and magnitude of peaks in WEC were similar on the 2 management systems (Figure 3-2, Chapter 3). This further singles out the IRG management system as having something that is notably different from the other two. Given that the major difference between IRG and the other two management systems is in grazing management, and given what is known about parasitic nematode ecology, it is almost certain that intensive rotational grazing is driving the differences in gastrointestinal nematodiasis seen between the IRG and other management treatments.

The grazing management on IRG, consisting of short graze periods (~3 days) and long rest periods (>90 days), possibly breaks the nematode lifecycle in two ways. Firstly by preventing autoinfection and secondly by allowing a rest period that is sufficient to enable significant larval die off before sheep return to graze (Donald 1973). It is these two criteria that are the driving force behind the success of rapid rotational grazing in the tropics (Barger *et al.* 1994; Chandrawathani *et al.* 1995; Sani *et al.* 1996; Chandrawathani 1997; Gray *et al.* 2000) and "progressional" grazing in northern Scotland (Robertson and Fraser 1933). Both Banks *et al.* (1990) and Barger *et al.* (1994) state that the rapid rotational grazing they employed in the tropics would not work in more temperate climates and they were most probably right. The rigid time periods imposed on the rapid rotations work well in the tropics where temperatures are always warm and rainfall relatively constant resulting in constant development of eggs into infective larvae. They would be unsuitable in temperate climates due to the large variability in rainfall and temperature throughout the year, thus large fluctuations in development time and survival of the free-living stages.

The grazing management used on IRG is more flexible with animal movements from paddock to paddock based on feed on offer, thus rotations speed up or slow down according to pasture

growth and recovery rates. So when development of egg to L_3 and larval decay is rapid (i.e.: during summer and late spring) paddock rotations are also rapid. Conversely, when egg development and larval decay is slow (i.e.: autumn and winter) paddock rotations slow down.

In, conclusion, this experiment has shown that levels of pasture contamination with infective L_3, as determined by tracer WEC, are markedly lower on the IRG treatment than the other management treatments in winter, spring and summer. It has also shown that the IRG treatment has a strong differential effect on *H. contortus* relative to *Trichostrongylus spp.* and *Teladorsagia circumcincta*. Taking into account the finding of Experiment 2 that sheep on the IRG treatment are, if anything, more susceptible to GIN than those on the other treatments, these two experiments provide strong evidence that the reduction in WEC on IRG observed in the longitudinal study (Chapter 3) is due to effects on environmental phases of the nematode lifecycle. These observations are consistent with our understanding of the free-living ecology of the different GIN species and also the work of Banks *et al.* (1990) and Barger *et al.* (1994), leading to the conclusion that intensive rotational grazing is the causal factor in the reduction of WEC observed on the IRG treatment.

CHAPTER 6: Experiment 4. Determinants of larval development and survival of *Haemonchus contortus* on the Cicerone project

6.1 Introduction

Availability of infective larvae on pasture is a function of the rate of development of the egg to infective larvae, mortality of the free-living stages, and the survival of infective larvae. The tracer study in Chapter 5 demonstrated that pasture infectivity on the IRG treatment was lower than that of on the other two management treatments in 3 out of 4 seasons. This finding, together with that of no better resistance to infection on this treatment (Chapter 4) indicates that the main effect of IRG on GIN is on the free-living phase of the nematode lifecycle. This prompted this study into the dynamics of larval development and survival in the field in spring, summer and autumn.

The focus of this study was on the free-living stages of *Haemonchus contortus* as it is the nematode species that was most affected by the intensive rotational grazing management (Healey et al., 2004; Experiment 1, Chapter 3). The eggs of *H. contortus* have a high requirement for moisture to develop, hatch and survive. Donald and Waller (In Donald 1973) observed that if rainfall or heavy dew does not occur within 4 days of deposition, the eggs of *H. contortus* will perish. All free-living stages of *H. contortus* are also susceptible to cold temperatures meaning they have a limited ability to over winter as either embryonated eggs or infective larvae (Jasmer *et al.* 1986). These susceptibilities of this species leave it vulnerable to control through IRG. Short graze periods (3-7 days) cause sheep to be removed before eggs in freshly deposited faeces can hatch and develop into infective larvae. Also long rest periods (75-130 days, depending on season) allow a high proportion of the eggs that made it to the infective larval stage to die off before sheep return to graze. The stringent development requirements of *H. contortus* suggest that for many paddocks in an IRG system, there will be no effective contamination following grazing due to failure of development due to cold or desiccation. This is less likely to be so for *Trichostrongylus* spp. and *T. circumcincta* given their ability to survive for longer as eggs, and their greater tolerance of desiccation and low temperatures (Dinaburg 1945; Kates 1950; Crofton and Whitlock 1965b; Jasmer *et al.* 1986).

To confirm these general principles and gain insight into the free-living ecology of a local isolate of *H. contortus* in this environment, an experiment was designed to determine the rates of development and survival of *H. contortus* on the Cicerone project in spring summer and autumn. Winter was not included as the literature (Rose 1963; Todd *et al.* 1976; Jasmer *et al.* 1986) and previous studies in our group (Sakwa PD, unpublished, O'Connor LJ, unpublished), indicated that cold inhibition prevents development of *H. contortus* in these months. The experiment used watered and unwatered plots. Unwatered plots were to enable determination of development under normal field conditions while the watered plots were to enable determination of development when moisture was not limiting (ie effects of temperature alone) and to provide sufficient larvae to allow measurement of larval death rates over time.

The overall hypothesis was that the local strain of *H. contortus* would conform to previously published requirements for development resulting in development constrained mainly by cold in spring and autumn, and lack of moisture in summer with high rates of development in summer when moisture is not limiting. It was also hypothesised that death rates of infective larvae would be slower in the cooler autumn and spring months than in summer.

6.2 Materials and Methods

The experiment was carried out in 3 seasons (spring, summer, autumn) each in a different paddock of the IRG treatment of the Cicerone project. The paddock used was that which was most recently grazed by sheep with the grazing period in each instance being 3 to 6 days in duration. The sheep, in each instance, were removed on the day before artificial contamination so that the plots could be set up. The experiment was set up on the IRG treatment because the long period between grazing would allow sequential measurements of larval development and survival without interference by sheep. It also allowed comparison of larval availability on the natural infection plots with the infections of tracer sheep run on the IRG system during Experiment 3 (Chapter 5).

6.2.1 Experimental design

The design of each seasonal experiment was a 2x2 factorial with an added factor of time, with 4 replicates of each combination blocked on geographical location. The first factor was level of contamination; natural (resulting from previous grazing) or artificial (Kirby strain of *H. contortus*). The second factor was irrigation; unwatered or watered (25mm on days 0 and 2 post contamination). The 5 time points for assessment of pasture contamination were days 7, 14, 28, 2

months and 3 months post-contamination. Because sample collection was destructive, separate plots were required for each sampling. Thus with the 4 treatment combinations, 5 time points and 4 blocked replicates there were 80 individual plots of 80cm x 80cm in each season. Within each block there were 4 main plots in with 5 sub-plots, one for each sampling of time period (Figure 6-1).

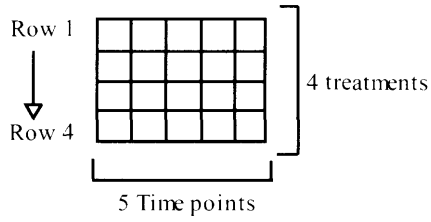


Figure 6-1: Graphical representation of a block containing 5 replicates of each treatment for destructive sampling (20 plots). Plots were 80cm x 80cm and separated from each other by metal inserts.

The treatments were randomly allocated to a row within the block. The time periods were then randomly allocated within the rows for each treatment.

6.2.2 Arrangement of plots

Four blocks each consisting of 20 plots were set up within the one paddock on the day sheep were moved off the pasture. They were located around the paddock on a stratified basis to account for differing geography, vegetation type or height. The individual plots were delineated by galvanized metal squares (80 x 80 x 10cm height) which were hammered lightly into the soil with metal tent pegs holding the plots in place. After contamination and watering treatments were applied on day 0, 50% shade cloth was applied to simulate cloudy conditions for 3 days.

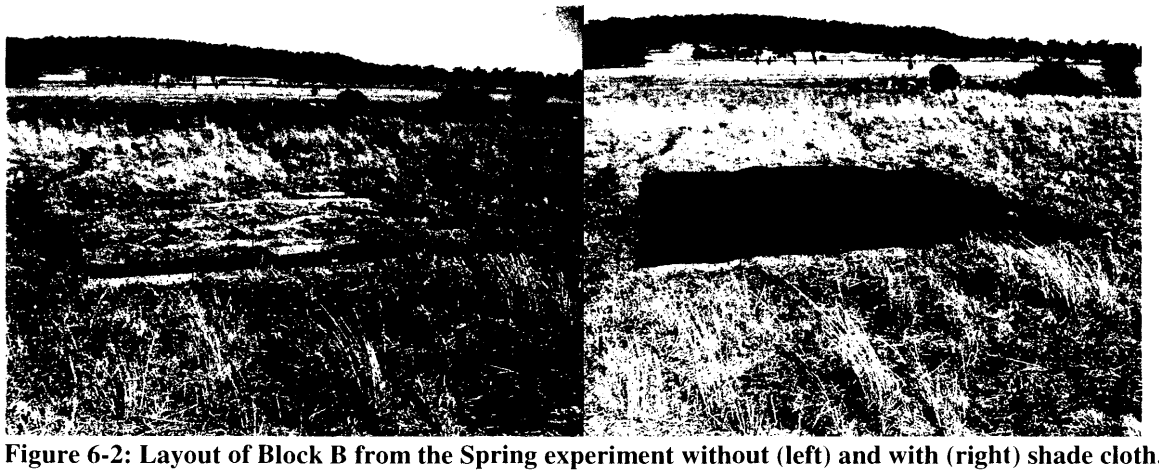


Figure 6-2: Layout of Block B from the Spring experiment without (left) and with (right) shade cloth.

6.2.3 Contamination of plots

6.2.3.1 Natural contamination

Paddocks had been grazed immediately prior to day 0 (day of artificial contamination) by sheep on the IRG management treatment. In spring, 12 month old sheep had grazed on the paddock for 3 days and were removed on day -1. In the summer experiment, sheep were mixed age ewes and wethers (>2 years old), they had grazed the paddock for 4 days and were removed on day -1. The autumn experiment had mixed age ewes (<2 years old) grazing for 6 days prior to removal on day -1. Mean faecal worm egg counts and larval culture results for these mobs taken from Experiment 1 (Chapter 3) are given in Table 6-1. Faeces deposited by IRG sheep were left *in situ*, thus the contamination of any one plot by sheep was random. Originally the faeces deposited within the sample areas by the grazing sheep were to be quantified, mixed, worm egg count estimated and redistributed evenly across the 'natural infection' plots to reduce error. This was not possible as faecal pellet recovery could not be achieved unless the pasture was removed. An estimate of the extent of natural faecal contamination was obtained by weighing recovered faeces for each plot at the time of sampling.

Table 6-1: Faecal worm egg counts and larval differentiation results by season for the sheep grazing paddocks immediately prior to day 0.

Season	Date of WEC	Class of sheep	Days grazed	Mean Raw WEC	Range	*Haemonchus contortus* (%)	*Trichostrongylus* spp. (%)	*Teladorsagia* spp. (%)
Spring	28/09/2004	Hoggets	3	208	0-1150	82	2	16
Summer	24/01/2005	Ewes	4	782	0-3900	91	7	1
Autumn	11/04/2005	Ewes	6	39	0-400	100	0	0

6.2.3.2 Artificial contamination

Fourteen passage sheep (wethers) were used to obtain faeces contaminated with *H. contortus* eggs. A time line for preparation of passage sheep is given in Figure 6-3. Passage sheep were given a double dose of levamisole, benzimidazole and ivermectin at day -35 (2ml/10kg COMBI®, 34g/L albendazole oxide and 70g/L levamisole HCl, Novartis Animal Health Australasia Pty Ltd, Australia; IVOMEC®, 2ml/4kg, 0.8g/L ivermectin, Merial Australia, Australia). They were infected with *H. contortus* Kirby strain infective larvae (Spring: 15 600; Summer: 15 000; Autumn: 10 000L_3/sheep) on day -28 and housed indoors on a pelleted ration with Lucerne chaff. The Kirby strain of *H. contortus* was isolated from the University of New England Kirby Research Station near Armidale, NSW in 1982 and is susceptible to all major

broad-spectrum anthelmintics. On day -11 the passage sheep were removed to a grassed paddock until day -4 so that the pellets would have a similar consistency to those in the natural infection. Bulked WEC was determined on days -7 (21 days after infection) to monitor the success of the infection. On day -1 faecal collection bags were applied to the sheep and left on overnight. Faecal collection bags were emptied and removed on day 0, faecal worm egg counts (WEC) were performed and faeces from sheep with low WEC were discarded. The remaining faeces were then thoroughly mixed in a large tub and divided evenly by weight into 40 portions. A sub-sample was cultured to confirm the viability of the eggs.

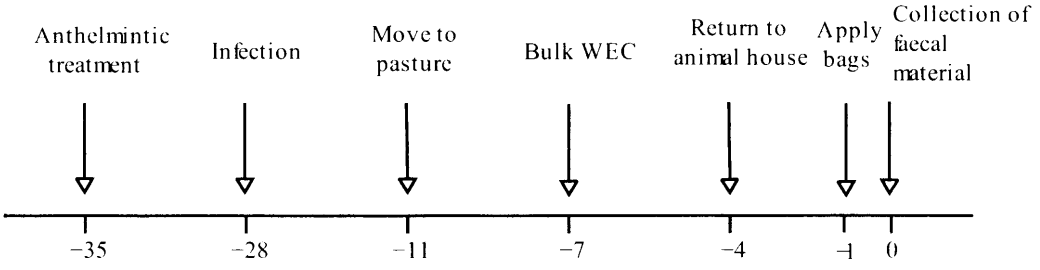


Figure 6-3: Timing of events for preparation of passage sheep.

Between 84 and 745 x 10^3 eggs were deposited per plot (Table 6-2). Faeces were placed in the centre of the plot to minimise larval migration out of plots. The amount of faeces placed on plots in each season was dependant upon the amount of suitable faeces collected from the passage sheep i.e.: not squashed, well formed pellets that had not been contaminated with urine.

Table 6-2: Average weight of faeces, average number of eggs per gram of faeces and average number of eggs deposited on plots.

Season	Start and end dates of experiment	Weight of faecal deposition (g)	Average number of eggs/g	Average number of eggs/plot
Spring	5th October 2004 to 30th November 2004	135	3947	532 800
Summer	18th January 2005 to 12th April 2005	100	7450	745 000
Autumn	19th April 2005 to 12th July 2005	30	2800	84 000

6.2.4 Irrigation

The irrigated plots were watered with 25mm of rainfall (15 litres/plot) on days 0 and 2 using a watering can with a fine spray nozzle.

6.2.5 Microclimate and climactic data

Tiny Tag® data loggers (Gemini Data Loggers, Chichester, UK) were used to record temperature at ground level in the different treatments. Tiny tags® were placed on 4 watered and 4 unwatered plots.

The nearest weather station for collection of climatic data was at CSIRO, Chiswick which used an automated system. However the rain gauge was not functional and no rainfall data was recorded for the duration of this experiment. Rainfall and temperature data from the University of New England weather station was used instead (20km north of the plots).

6.2.6 Sampling and laboratory procedures

In spring, when the bulk of pasture on the plots was very large, a sub-sample of ~50% of the total fresh weight of herbage was taken at all time periods for all plots after thoroughly mixing the pasture. The entire sample was used for washing in both summer and autumn.

6.2.6.1 Sample collection from plots

Collection of samples started at 8am and generally took until 11am-12pm to complete. Pasture height was recorded in the centre and at the 4 corners of each plot using a metric ruler before faecal pellets were collected into plastic jars with the lid loosely applied. The plots were then destructively sampled using Makita electric grass shears removing all vegetation and including litter. Invariably some soil was also collected. The pasture samples were placed in a plastic bag each marked with the plot number, some plots required more than one bag. The samples were then placed in a large cardboard box in the vehicle out of direct sunlight with the tops of the bags unsealed. On each sampling day a sample of grass was collected from a tree guard area where animals have not grazed for use as a recovery control during the washing process.

6.2.6.2 Larval recovery from faeces

The weight of faeces recovered from each plot was recorded, the faeces placed in a culture jar and submerged in water overnight. The next morning the pellets were broken up using a metal spatula and mixed with vermiculite. The faecal cultures were then placed in a 27°C oven for 7 days. After 7 days the jars were filled with warm water and inverted onto a Petrie dish and allowed to stand for at least 3 hours or overnight. The water in the petri dishes was pipetted off and weighed, 2 x 500µL placed in a Universal slide chamber, Lugol's iodide was added and the

sample examined for infective larvae. The following formula was used to calculate the number of larvae per plot recovered from faecal depositions:

Larval count per plot:

L_3/plot = L * (A/B)

Where, L = number of infective larvae counted

A = weight of solution pipetted from petri dish (g)

B = volume of sediment examined (total vol of aliquots in Universal slide chambers, µl)

Number of larvae per 10 000 eggs deposited:

Recovery rate = L_3/plot x (10 000/average number of eggs deposited)

6.2.6.3 Larval recovery from pasture samples

The following technique, adapted from Martin *et al.* (1990) was used for larval recovery from pasture samples:

1. Control samples were spiked with 3000 infective *Haemonchus contortus* larvae and left aside until the weighing of other samples was completed (generally 1 hour). The 3ml of containing the larvae was squirted as evenly as possible into the grass in its plastic bag. The bag was then shaken to mix the infected larvae through the sample.
2. Pasture samples were weighed
3. In the spring experiment it was necessary to take a sub-sample of the pasture as there was too great a volume of grass for the buckets. The sub-sample was taken by thoroughly mixing the pasture in a large tub dividing in two, one part was discarded and the process repeated until a small enough sample was obtained for washing. The sub-sample and total sample were weighed and the final concentration of larvae recovered was adjusted to reflect the total sample size.
4. 10L of water was added to 20L buckets with non-ionic detergent (Pyroneg, SUMO, JohnsonDiversey Pty Ltd, Smithfield, Australia) added at a rate of 1g/2L.
5. The herbage was placed in mesh bags suspended over the buckets.
6. Herbage was then agitated by hand, and mesh lifted clear of the water several times.
7. Herbage was left to soak overnight or for 4-6 hours depending on the time of initiation of soaking. Bags were be mixed at least once during this time.

8. Following soaking, the mesh bag was lifted from the bucket and drained. Herbage in the bag was then rinsed using a hose, periodically squeezing the herbage whilst washing until the bucket was full or washings appeared clear. As much water as possible was finally squeezed from the bag and the herbage placed in a labelled aluminium tray for herbage estimates.
9. Sediments and washings were left to settle either overnight or for at least 6-7 hours.
10. Supernatant was pumped off through a filter so as not to disturb the sediment, ~4L of water and sediment was left in the bucket.
11. The mixed water and sediment were passed through a coarse sieve (1mm x 1mm) into a 10L bucket, hosing the sieve thoroughly to wash sediment through.
12. A length of polytubing (~1.2m), which had been heat sealed to form a bag with a cone at the base, was placed into a holding apparatus (large rigid PVC pipe (~15-20cm wide) attached to a retort stand). The water and sediment were poured into the bag and the bucket rinsed to ensure all sediment was washed into the bag.
13. The polytube bag was hung on a hook overnight to allow sediment to settle.
14. The following morning I ran my fingers down the sides of the bags to release any sediment caught in crevices, split the upper portion of the bag above the 'v' and released the bulk of water.
15. The cone section on the bottom was cut from rest of bag retaining at least 2.5cm of water above the sediment. Sediment and water was poured into a 250-500ml container washing with 70% alcohol to preserve any larvae collected. Samples were then left at room temperature until processing.

6.2.6.4 Laboratory Procedure after Larval Extraction from Pasture

1. Jars were left to sit overnight to allow the sediment to settle.
2. Supernatant was removed using a Pasteur pipette attached to a vacuum pump, leaving ~5mm above the sediment.
3. The volume of sediment was recorded including the layer of alcohol, the sample was mixed well by inversion and 3ml of sediment transferred into a 15ml centrifuge tube.
4. The volume in the tube was made up to 7ml with a solution of potassium iodide (KI) (rd 1.4). Mix well by inversion.
5. Two additional control samples (post-washing recovery controls) were prepared to test recovery rates for the post-washing steps by mixing 2ml of clean sediment slurry with 1ml containing 1000 *H.contortus* L_3. Step 4 was then completed for each control.

6. Samples were centrifuged at 1400g for 6 minutes.
7. The supernatant (containing the larvae) was poured off into a 50ml centrifuge tube, the sides of the 15ml tube were washed into the larger tube using de-ionised water.
8. The volume was made up to 50ml using de-ionised water and mixed by inversion.
9. Samples were centrifuged at 1400g for 6 minutes.
10. Supernatant was drawn off with a Pasteur pipette which had been shaped into a hook at the end, leaving ~1.5ml sediment and remaining sample was weighed.
11. 4 x 200µl aliquots of sediment were pipetted into Universal slide chambers, 400µl of KI (rd 1.6) and 1 drop of Lugol's iodide was added to each aliquot.
12. Examine at 100x to count L_3. Infective larvae were identified, using the head and tail shape and length of the nematode into, species for *Haemonchus* and to genera for *Trichostrongylus*, *Teladorsagia* and *Oesophagostomum*.

6.2.6.5 Enumeration of infective larvae recovered from pasture samples

The following equations were used to determine number of larvae per plot and larvae per kg DM.

Number of larvae per plot:

$$\mathbf{N_1 = (C \times A/B) \times (S/3ml) \times (D/E)}$$

Where, N_1 = number of larvae per plot
A = Final weight of sediment (g. step 10)
C = number of larvae counted
B = volume of sediment examined (total vol of aliquots in Universal slide chambers, µl) Step 11)
S = total volume of sediment (Step 3, divide by 3 for 3 ml aliquot)
D = Fresh weight
E = Sub-sample weight

Number of larvae adjusted for the average recovery from pasture control:

Percentage recovery of spiked controls was averaged for each season.

$$\mathbf{N_2 = (100/F) \times N_1}$$

N_1 = unadjusted number of larvae per plot calculated as above
N_2 = number of larvae adjusted for control recovery/ plot
F = percentage of control larval recovery averaged per season

Number of larvae per 10 000 eggs deposited

$N_3 = N_2$ x (10 000/average number of eggs deposited)

N_3 = number of larvae per 10 000 eggs deposited

N_2 = number of larvae adjusted for control recovery/ plot

6.2.6.6 Calculation of larval recovery rate of spiked pasture control samples

The larval recovery rate of the entire larval recovery procedure was estimated using the control pasture samples described in step 1 of section 6.2.7.3. The recovery rate was calculated using the equations below:

Number of infective larvae required for 100% recovery:

G = (H x (3ml/S)) x B/A)

Where, G = Number of L_3 required to be counted if recovery rate is 100%

H = Number of L_3 in spike

S = total volume of sediment (Step 3, divide by 3 for 3 ml aliquot)

B = volume of sediment examined (total vol of aliquots in Universal slide chambers, µl) Step 11, section 6.2.6.4)

A = Final weight of sediment (g. step 10, section 6.2.6.4)

Recovery rate:

Recovery rate = C/G x 100

Where, G = Number of L_3 for 100% recovery rate

C = Number of L_3 counted in slides

The average recovery of spiked controls from the whole pasture washing procedure and from the laboratory stage are given in Table 6-3. The recovery rates from the sediment or post-washing step controls described in step 5, section 6.2.6.4 are also presented in Table 6-3.

Table 6-3: Mean recovery rates and coefficient of variation (%CV) for the pasture controls (entire recovery procedure) and the sediment controls (post-washing steps only) by season.

		Mean recovery rate (%)	%CV
Spring	Pasture Control	18	79
	Sediment control	59	35
Summer	Pasture Control	27	52
	Sediment control	92	10
Autumn	Pasture Control	54	60
	Sediment control	89	15

6.2.6.7 Estimation of Percentage Green in pasture sample

After step 8 of the pasture larval washing (6.2.6.3) the percentage of green matter in the pasture was estimated using the following method:

1. An aluminium tray was tared on scales and washed pasture placed into the tray out of the mesh bag and the weight recorded.
2. Pasture was tipped onto a large bench surface and mixed thoroughly
3. Pasture sample was divided into half, then in half again so the grass was in quarters, two diagonal quarters were removed and the remaining two mixed.
4. Step 3 was repeated until a 30g sample of pasture was obtained
5. The green matter was separated from the brown using tweezers.
6. Aluminium tray was tared the green matter weighed, the brown matter was added and the total weight recorded.
7. For percentage green matter the following equation was used:

$$GM\% = (\text{green matter/total pasture weight}) \times 100$$

6.2.6.8 Estimation of Dry Matter

The sample used to determine the percentage green was reserved for dry matter estimation and place in an 80°C oven for at least 5 days. The weight of the sample was recorded after drying and the dry matter percentage determined using the following equation:

$$DM\% = (\text{dry weight /fresh weight}) \times 100$$

6.2.7 Statistical analysis

The statistical package JMP IN version 5.1 (SAS Institute Inc., NC, USA) was used for all the following analyses.

6.2.7.1 Climatic variables

Mean daily maximum and minimum air temperatures, ground temperatures, rainfall and evaporation were analysed across seasons using a linear mixed effects model with season the only effect fitted. The effect of irrigation treatment on ground temperatures was tested within season, daily minimum and maximum ground temperatures were determined for individual tiny tags for each day of the experiment and analysed using a simple linear mixed model fitting irrigation (watered, not-watered) and date as main effects and block as a random effect. Maximum and minimum air temperatures were regressed against ground temperatures to determine the overall relationship between them. The daily average maximum and minimum ground and air temperatures from days 0 to 7 were also analysed using a linear mixed effects model with season and day fitted.

6.2.7.2 Larval recovery from artificially contaminated plots

Due to the inability to collect all the naturally deposited faeces from the plots, there was no measurement of initial infection on those plots that were not artificially contaminated. Thus, although the experiment was conceived as a factorial experiment and run as such, the natural and artificial contaminations will be analysed separately as recovery rates can only be calculated for the artificially contaminated plots. Thus recovery rate is the key variable on the artificially contaminated plots while larval counts are used for the naturally contaminated plots. The true recovery rates on artificially contaminated plots would have been slightly lower than the calculated recovery rates as larvae recovered would have included a small proportion arising from the background natural deposition on these plots.

6.2.7.2.1 Larval recovery from pasture samples

H. contortus pasture larval counts were calculated per plot and corrected for the recovery rate of larvae during sample processing of that batch as described in section 6.2.6.3. The effects of irrigation, day, their interaction on the presence or absence of infective larvae on a plot was tested using a nominal logistic model, block was fitted as a random factor. Season was not fitted as infective larvae were only found on pasture during the summer experiment.

The recovery rates of L_3 expressed per 10 000 eggs deposited were analysed using a linear mixed model. Irrigation and day were fitted along with their interaction and block was fitted as a random term. Average pasture height, percentage green matter, percentage dry matter and fresh weight of cut pasture were added to the model as covariates and dropped where not significant.
A decay curve for L_3 survival was also determined by fitting an exponential curve to the mean L_3 recovered per sample day. Day 7 was the reference point with all other sample days expressed as a proportion of L_3 recovered on day 7.

6.2.7.2.2 Larval recovery from faeces

Faecal L_3 per plot was estimated using equation in section 6.2.6.2. The presence or absence of L_3 on individual plots was analysed using a nominal logistic model with the effects of irrigation, contamination, day, season and block (random) all fitted in the model.

The recovery rate of infective larvae from faecal culture was analysed using a linear mixed model fitting irrigation, day, season, irrigation by day, irrigation by season and block as a random factor.

6.2.7.3 Larval counts from naturally contaminated plots

6.2.7.3.1 Pasture larval counts

A linear mixed model was used to determine the effects of irrigation, day, and block (random) on the log transformed infective larvae per plot for *H. contortus*, *Trichostrongylus spp.* and *Teladorsagia* spp separately. The infective larvae of *H. contortus* were only analysed for naturally infected plots, but the other nematodes were analysed across infection plots as their occurrence on all plots was entirely derived from the faeces put down by grazing sheep.

6.2.7.3.2 Faecal larval counts

Faecal L_3 recovery was also analysed as number of larvae per plot using a series of linear mixed models for the 3 major nematode species observed (*Haemonchus contortus*, *Trichostrongylus* spp., *Teladorsagia* spp.). The faecal L_3 area data were transformed using $Log_{10}(L_3 + 1)$. Factors fitted were irrigation, day, irrigation by day and block (random effect).

The treatment combinations will be referred to as watered/artificial contamination (WA), unwatered/artificial (UA), watered/natural contamination (WN) and unwatered/natural (UN).

Data is generally presented as least square means with standard errors with backtransformed means presented as least square means with 95% confidence intervals. A significance level of $P<0.05$ is used throughout.

6.3 Results

6.3.1 Rainfall and air temperature

Raw climatic data during the experimental period is presented in Figure 6-4. Mean maximum air temperature was highest in summer (25.2±0.4°C) then spring (22.3±0.5°C) and autumn (16.3±0.4°C) with all seasons being significantly different from each other ($P<0.0001$). Summer also had the highest mean minimum air temperature (10.4±0.5°C) followed by spring (7.6±0.6°C) and autumn (2.3±0.5°C), again all seasons were significantly different ($P<0.0001$).

For days 0 to 7, maximum air temperature was highest in summer (26.2±1.1°C) while spring and autumn did not differ (21.2±1.1°C and 21.7±1.1°C, $P<0.0001$). Summer had the highest mean minimum temperature during days 0 to 7 (13.6±1.0°C), again spring and autumn did not differ (3.5±1.0°C and 6.4±1.0°C, $P<0.0001$, Figure 6-5).

There was no significant difference in rainfall between seasons when averaged over all days ($P\sim0.30$) although the summer experimental period had the highest total rainfall (199.4mm) followed by the spring (173mm) and autumn (111mm) experimental periods. The total rainfall for days 0 to 7 for each season also differed with 64mm in summer, 1mm in autumn and 0mm in spring (Figure 6-6). All seasons had significantly different daily evaporation rates with spring being higher than summer which was higher than autumn (4.5, 4.1 and 1.6mm/day, respectively, $P<0.0001$). Summer had the highest total evaporation rate (345mm) followed by spring (263.8mm) and autumn (139.6mm). Total evaporation from days 0 to 7 was the same in summer and spring (39.4mm) and lower in autumn (21.6mm, Figure 6-5). Thus the difference between precipitation and evaporation (precipitation minus evaporation) for days 0 to 7 was positive in summer (24.6mm) and negative in spring (-39.4mm) and autumn (-20.6mm).

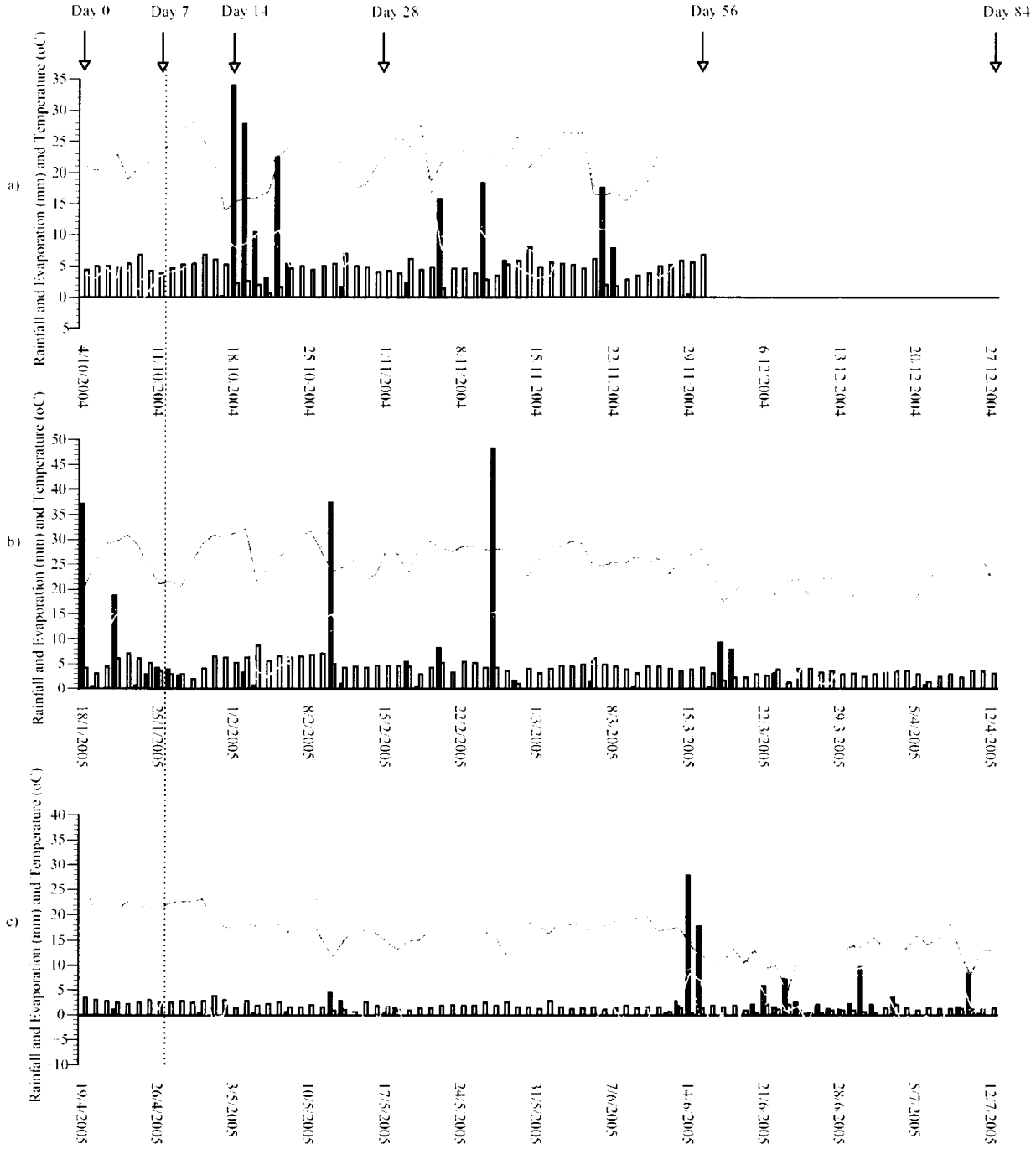


Figure 6-4: Rainfall (black column), evaporation (white column), maximum air (——), maximum ground ( ), minimum air (——) and minimum ground ( ) temperatures for each experimental period a) spring, b) summer and c) autumn. Ground temperature data are from the experimental site itself while other climatic data is from Armidale, 20km away (Burr 2006).

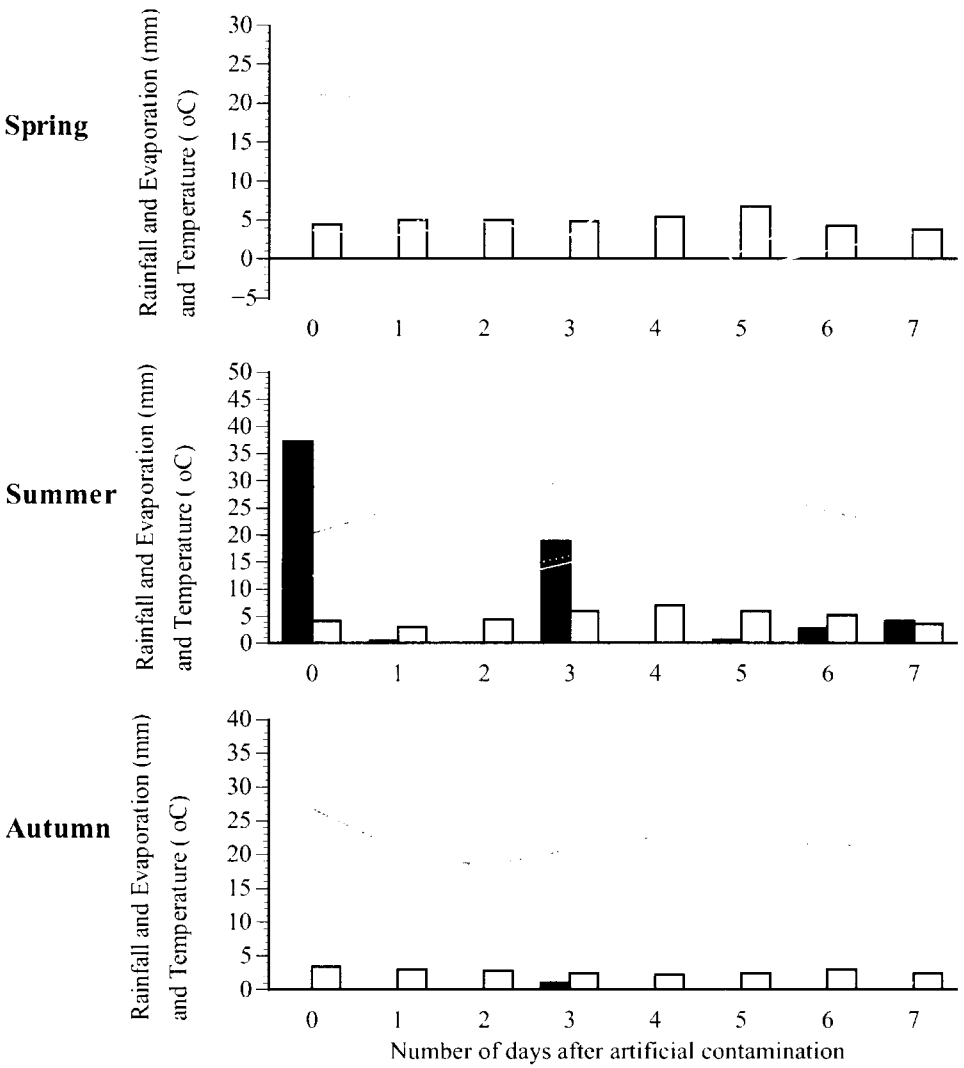


Figure 6-5: Rainfall (black column), evaporation (white column), maximum air (—), maximum ground ( ), minimum air (—) and minimum ground ( ) temperatures from day 0 to day 7 for each experimental period a) spring, b) summer and c) autumn. Ground temperature data are from the experimental site itself while other climatic data is from Armidale, 20km away (Burr 2006).

6.3.2 Ground Temperature

Average daily maximum and minimum ground temperatures were positively associated with the average daily air temperatures (maximum temperatures R^2=0.70 and minimum temperatures R^2=0.84, P<0.0001, Figure 6-6). The relationship between ground and air temperatures was not as strong in spring as in autumn, and the association between ground and air temperatures was stronger for maximum than minimum temperatures in summer (Figure 6-6). In all seasons, both maximum and minimum ground temperatures were warmer than their respective air temperatures.

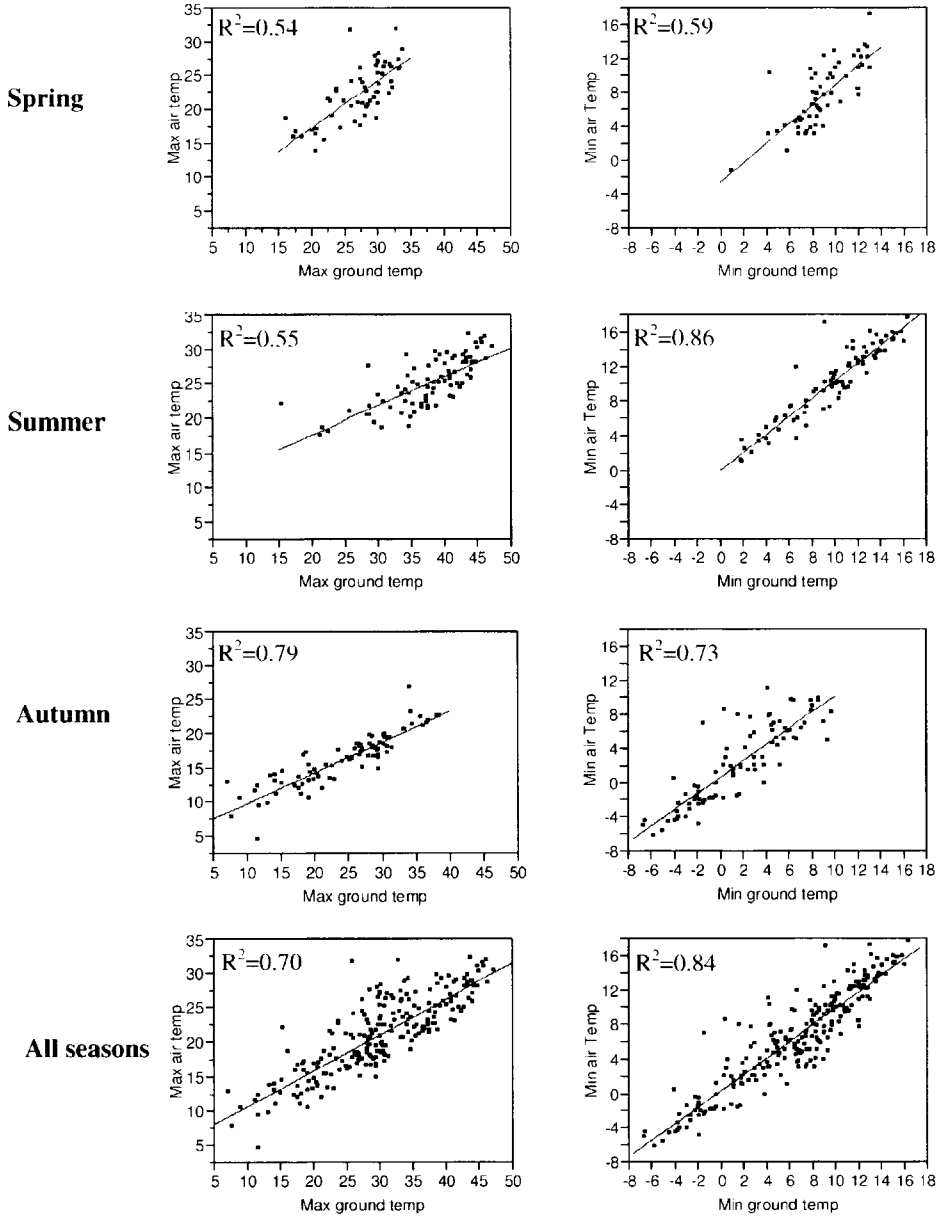


Figure 6-6: Regressions for maximum and minimum ground and air temperatures by season and for seasonal data combined.

There was a significant effect of season on mean minimum ground temperature with summer and spring being similar (10.1±0.4 and 8.9±0.5°C) with both seasons higher than autumn (1.8±0.4°C, P<0.0001). Mean maximum ground temperatures were higher in summer (37.8±0.7°C) than in spring (27.2±0.9°C) which were higher again than autumn (24.5±0.7°C, P<0.0001).

Ground temperatures from day 0 to 7 are shown in Figure 6-6. For the period from day 0 to 7, maximum ground temperatures were similar for summer and autumn (35.4±1.6 and 34.1±1.6°C),

but were significantly hotter than those recorded in spring (26.4±1.6°C, P<0.01). The minimum ground temperatures for days 0-7 were significantly warmer in summer (13.7±0.7°C) than in autumn and spring (6.9±0.7 and 5.2±0.7°C, P<0.0001).

6.3.2.1 Effect of watering treatment on ground temperatures

In spring there was a significant effect of day on minimum temperatures (P<0.0001), but no effect of irrigation (watered 9.0±0.1°C and un-watered 8.9±0.1°C, P~0.82). Minimum temperatures fluctuated from 1.7 to 13.1°C throughout the experimental period. For maximum temperatures there was a significant effect of date (P<0.0001) and irrigation (P<0.001) with watered plots being cooler than non-watered plots (26.4±3.4 and 28.1±3.4°C). Maximum temperatures fluctuated throughout the experiment with no real trend from 16.1 to 33.7°C. Analysis of ground temperatures from day 0 to 7 revealed no effect of irrigation on either maximum (P~0.16) or minimum temperatures (P~0.33).

In summer there was a significant effect of date (P<0.0001) and irrigation (P<0.01) on minimum and maximum temperatures. Both minimum and maximum temperatures were higher in watered plots (10.2±0.1 and 38.2±0.1°C) than non-watered plots (9.9±1.0 and 37.4±1.0°C). Maximum temperatures fluctuated from 15.4 to 47.3°C and minimum temperatures fluctuated from 1.8 to 16.4°C. Analysis of ground temperatures from day 0 to 7 revealed no effect of irrigation on either maximum (P~0.30) or minimum temperatures (P~0.20).

In autumn there was a significant effect of irrigation (P<0.0001) and date (P<0.0001) on both minimum and maximum ground temperatures. Watered plots had a significantly lower mean minimum temperature (1.5±0.2°C) and a significantly higher mean maximum temperature (25.5±0.8°C) than un-watered plots (2.1±0.2 and 23.6±0.8°C). Maximum temperature at ground level declined steadily over the course of the experiment from the high thirties to below 10°C.

In autumn irrigation had a significant effect on ground temperatures from day 0 to 7 with watered plots having lower minimum ground temperatures than un-watered plots (6.5±0.4 and 7.3±0.4°C P<0.0001). The mean maximum ground temperature on watered plots was higher than on un-watered plots (35.0±0.8 and 33.2±0.8°C, P<0.01).

6.3.3 Parasitology – Third stage (infective) larvae

In each season the viability of the eggs deposited was confirmed by faecal culture in the laboratory. Large numbers of larvae were recovered on each occasion and these were used to spike the recovery controls but unfortunately they were not quantified, so absolute measures of egg viability cannot be reported.

6.3.3.1 Infective larvae recovered from pasture washings - Summer

No third stage infective larvae (L_3) were recovered from plots in spring and L_3 were recovered on only one occasion on only one plot in autumn. Thus only summer pasture L_3 data were analysed. In summer the average recovery of infective larvae from eggs deposited on artificially contaminated plots was 0.06%.

6.3.3.1.1 Haemonchus contortus recovery from artificially contaminated plots

In summer, L_3 were recovered on day 7 from three of four WA plots and from one DA plot (Figure 6-7). The peak of L_3 recovery differed between treatment combinations with the peak for WA plots being at day 7 with a smaller peak at day 56. For the UA plots the peak of L_3 recovery occurred on day 56. Infective larvae were recovered on both treatments on day 84.

The nominal logistic analysis revealed a no significant effect of irrigation on the presence or absence of L_3 on plots (P~0.29). Although there were slightly more positive plots after watering than on the unwatered plots (60 and 45%, respectively). There was no effect of day (P~0.60) and no significant interaction between the main effects (P~0.40).

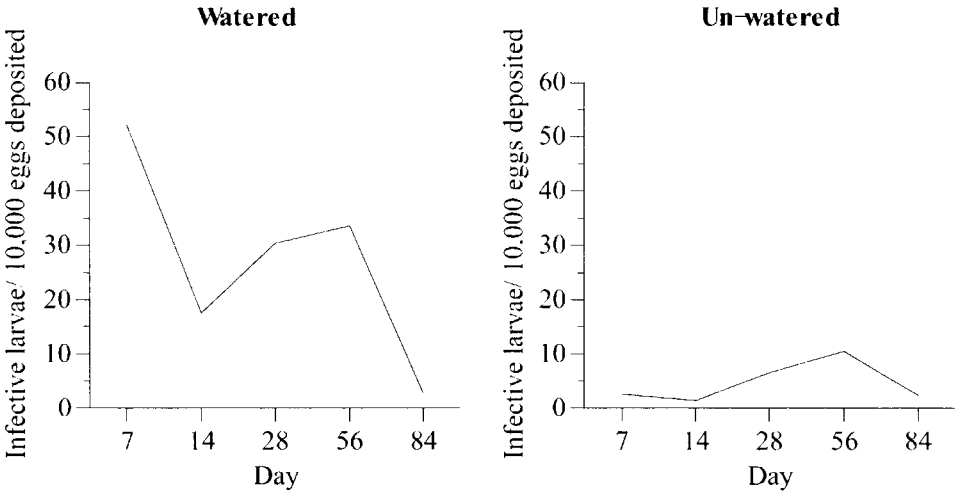


Figure 6-7: Summer experiment – Artificial contamination. Average *Haemonchus contortus* infective larvae recovered from herbage (adjusted for spiked control recovery) per 10 000 eggs deposited on watered and un-watered plots.

Analysis of *H. contortus* recovery rates revealed an almost significant effect of irrigation (P~0.06), no effect of day (P~0.52) and no interaction between irrigation and day (P~0.54). There was a strong trend for greater numbers of larvae to be collected from the watered plots than from unwatered plots (66, CI 1-295 and 8, CI 0-41 larvae/10 000 eggs deposited). There was no effect of any pasture parameters fitted in the model: pasture height (P~0.23), fresh weight (P~0.28), percentage green (P~0.61), percentage dry matter (P~0.69).

A fitted exponential larval survival curve is illustrated in Figure 6-8, day 7 was set as 100% of larvae present. The low recovery on day 14 and high recovery on day 56 reduce the accuracy of the fit (R^2=0.61). Based on this fit the half-life of *H. contortus* infective larvae on pasture during the summer experiment was 19 days.

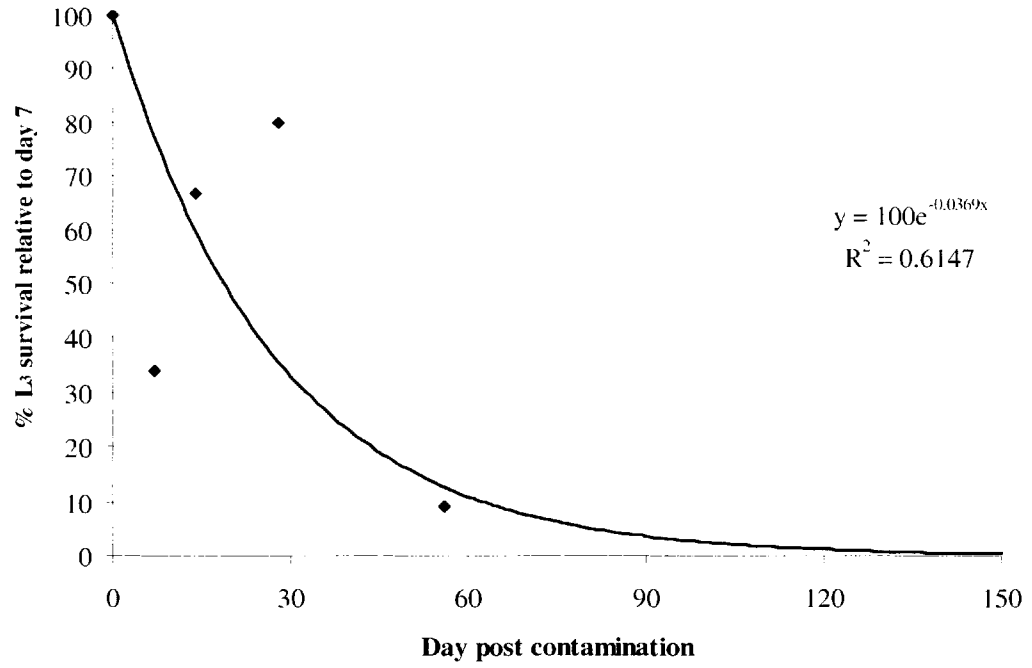


Figure 6-8: Survival of *Haemonchus contortus* infective larvae on pasture over the experimental period represented as a proportion of day 7 larval recovery with a fitted exponential curve.

6.3.3.1.2 Haemonchus contortus recovery from naturally contaminated plots

In summer infective larvae were recovered on the watered plots on all days with the highest recovery on day 84 (Figure 6-9). Infective larvae were only recovered on day 28 from the un-watered plots.

The nominal logistic analysis revealed no effect of irrigation (P~0.95) or day (P~1.0) on the presence of *H. contortus* L_3 on pasture and no interaction between the two (P~1.0). The linear mixed effects model also revealed no effect of irrigation (watered: 0.5, CI 0-2.3 and unwatered 0.3, 0-2.1 L_3/10 000 eggs deposited, P~0.62) or day (P~0.35) on the recovery rate of *H. contortus* in naturally contaminated plots. There was no interaction between the two (P~0.74).

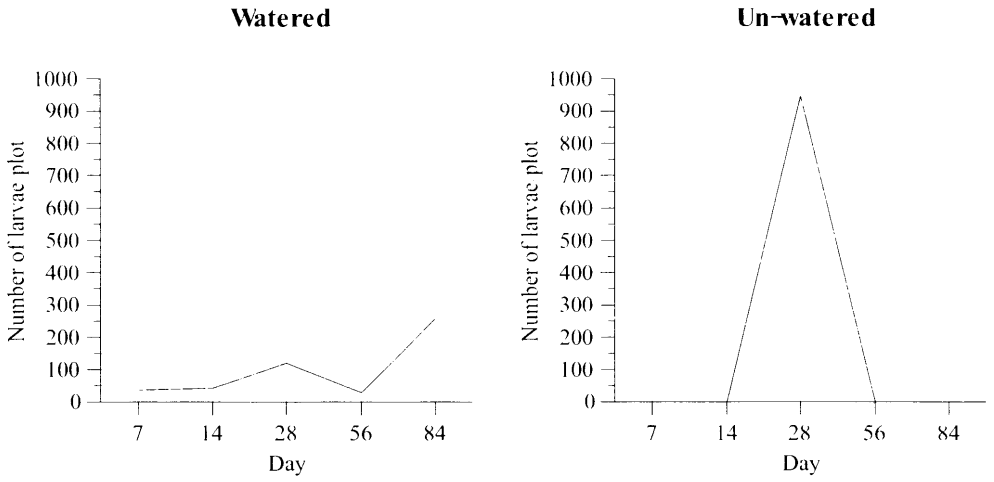


Figure 6-9: Summer experiment – naturally contaminated plots. Average *Haemonchus contortus* infective larvae recovered (adjusted for spiked control recovery) per plot (80 x 80cm) from the natural infection for watered and un-watered treatments.

6.3.3.2 Infective larvae recovered from faecal culture

Infective larvae were recovered by culture of naturally and artificially contaminated faeces in all seasons. The average weight of faecal material collected from all treatments is presented in Table 6-4 Four main nematodes were recovered; *Haemonchus contortus*, *Trichostrongylus* spp., *Teladorsagia* spp. and *Oesophagostomum* spp. The latter 3 genera were pooled for the artificial and natural contamination plots as they all occurred naturally from faecal deposition by grazing sheep.

Table 6-4: Average weight of faeces collected from plots by treatment for each season. UA – unwatered, artificial contamination; WA – Watered, artificial contamination; UN – Unwatered, natural contamination; WN – watered , natural infection.

	Average weight of faeces (g)		
Treatment	Autumn	Spring	Summer
UA	55	55	80
WA	50	61	88
UN	18	8	43
WN	27	9	36

6.3.3.2.1 Haemonchus contortus recovery from artificially contaminated plots

The binomial analysis of the presence or absence of *H. contortus* L_3 cultured from faecal samples showed no significant effect of irrigation (P~0.93), but a significant effect of season

(P<0.05) with L_3 recovered from more plots in summer (32.5% positive) than in autumn (12.5%) with spring intermediate between the two (21.9%). There were more positive plots on day 7 (62.5%) than any other day (day 14: 12.5; day 28: 8.3; day 56: 16.7; day 84: 6.3%). There was no interaction between irrigation and season (P~0.60) or irrigation and day (P~0.35).

The linear mixed effects analysis of recovery rate of faecal *H. contortus* L_3 showed a significant effect of irrigation (P<0.001), season (P<0.001) and day (P<0.0001). There was greater recovery of *H. contortus* L_3 from watered plots than unwatered plots (0.57, CI 0-2.14 and 0.11, CI 0-1.64 L_3/ 10 000 eggs deposited respectively). There were greater numbers of *H. contortus* L_3 recovered from faecal culture in autumn and summer than in spring (autumn: 0.5, CI 0-2.1; summer: 0.6, CI 0-2.2; spring: 0, CI 0-1.5 L_3/10 000 eggs deposited). Day 7 had the highest recovery of L_3 from faecal culture (2.6, CI 1.8-4.7 L_3/10 000 eggs deposited), with close to zero recovery on the other sample days. There was no interaction between season and irrigation (P~0.09) but there was a significant interaction between irrigation and day (P<0.0001). Watered plots had much greater recovery on day 7 than unwatered plots, whereas there was no effect of irrigation on recovery rates on any other sample day (Table 6-5).

Table 6-5: Average number of *Haemonchus contortus* infective larvae per 10 000 eggs deposited recovered from faecal culture on artificially contaminated plots, by season and irrigation treatment, with the number of plots positive for infective larvae.

		Spring		Summer		Autumn	
Irrigation treatment	Day	+ve plots[a]	*H. contortus*[b]	+ve plots[a]	*H. contortus*[b]	+ve plots[a]	*H. contortus*[b]
Un-watered	7	1	0.02	2	35.8	2	16.01
	14	2	0.03	0	0.00	0	0.00
	28	0	0.00	1	0.38	0	0.00
	56	0	0.00	2	0.60	0	0.00
	84	-	-	0	0.00	0	0.00
Watered	7	4	1.34	3	15.9	3	41.7
	14	0	0.00	1	0.17	0	0.00
	28	0	0.00	1	0.09	0	0.00
	56	0	0.00	2	2.56	0	0.00
	84	-	-	0	0.00	0	0.00

[a] Four plots per treatment combination

[b] Mean number of larvae/10,000 eggs deposited

6.3.3.2.2 Haemonchus contortus recovery from naturally contaminated plots

There was no effect of irrigation (P~0.99) or day (P~0.99) on the number of positive plots for the natural infection. However there were more positive plots on days 7 (4.4%) and 14 (12.5%) than the remaining days all of which were negative.

The LME analysis revealed that irrigation and day had no effect on the number of faecal *H. contortus* L_3/plot (P~0.77 and P~0.12), nor was there any interaction (Table 6-6).

Table 6-6: Average number of *Haemonchus contortus* larvae per plot from the natural infection recovered from cultured faeces by season and irrigation treatment with number of plots that were positive for infective larvae, with number of plots positive for infective larvae.

		Spring		Summer		Autumn	
Irrigation treatment	Day	+ve plots	*H. contortus*	+ve plots	*H. contortus*	+ve plots	*H. contortus*
Un-watered	7	0	0.0	1	5.0	0	0.0
	14	1	1.1	0	0.0	0	0.0
	28	0	0.0	0	0.0	0	0.0
	56	0	0.0	0	0.0	0	0.0
	84	0	-	0	0.0	0	0.0
Watered	7	0	0.0	0	0.0	0	0.0
	14	1	0.6	1	06.4	0	0.0
	28	0	0.0	0	0.0	0	0.0
	56	0	0.0	0	0.0	0	0.0
	84	0	-	0	0.0	0	0.0

6.3.3.2.3 Other nematode species recovered from faecal culture

The average numbers of infective nematodes of *Trichostrongylus* spp, *Teladorsagia* spp and *Oesophagostomum* spp. recovered during each experiment are given in Table 6-7.

Table 6-7: Average number of larvae per plot recovered from cultured faeces from naturally contaminated plots for nematodes other than *Haemonchus contortus* (Tr- *Trichostrongylus* spp., Te- *Teladorsagia circumcincta*, Oes- *Oesophagostomum* spp.) by season and irrigation treatment, with number of plots that were positive for infective larvae of these genera.

		Spring				Summer				Autumn			
Irrigation treatment	Day	+ve plots	Tr	Te	Oes	+ve plots	Tr	Te	Oes	+ve plots	Tr	Te	Oes
Unwatered	7	0	0.0	0.0	0.0	7	12.2	12.8	14.0	3	0.0	8.9	8.9
	14	4	0.0	1.3	0.0	1	0.0	17.1	0.0	0.0	0.0	0.0	0.0
	28	2	3.7	8.7	1.05	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	56	0	0.0	0.0	0.0	4	6.3	3.1	0.0	0.0	0.0	0.0	0.0
	84	-				0	0	0.0	0.0	0.0	0.0	0.0	0.0
Watered	7	4	3.7	3.0	0.0	4	9.5	5.9	17.9	3	0.0	0.0	8.9
	14	2	1.1	2.2	1.1	2	0.0	0.0	3.8	2	0.0	0.0	3.8
	28	2	0.0	0.0	5.4	1	0.0	0.0	0.0	1	8.0	0.0	0.0
	56	0	0.0	0.0	0.0	5	72.5	16.1	0.0	0.0	0.0	0.0	0.0
	84	-	-	-	-	2	0.0	0.0	0.0	0.0	0.0	0.0	0.0

There was no effect of irrigation (P~0.61), nor day (P~0.09) for faecal *Trichostrongylus* spp. L_3 per plot and there was no interaction. From Table 6-7, there were more *Trichostrongylus* spp. in faecal material in summer than in spring and autumn.

There was no effect of irrigation (P~0.90), or day (P~0.84) on faecal *Teladorsagia circumcincta* infective larvae per plot and there was no significant interaction. From Table 6-6, there were more *T. circumcincta* L_3 in summer than spring with very little recovered in autumn.

There was also no effect of irrigation on *Oesophagostomum* spp. (P~0.55), however there was an effect of day (P<0.05) but no interaction (P~0.99). Day 7 had the highest mean *Oesophagostomum* spp. L_3 with very little recovered from the other sample days. From Table 6-7, there were more *Oesophagostomum* spp. L_3 recovered in summer followed by spring and then autumn.

6.4 Discussion

As expected, summer was the most favourable season for the development of eggs to L_3 and survival of eggs and pre-infective stages. Although the spring and autumn temperatures appeared favourable for development on most days for the first 14 days of the experiment no L_3 were recovered from pasture. This could be due to frost events in these seasons or cold temperatures limiting development whilst moisture was available leading to desiccation later on, but it could

also be due to the sensitivity of the pasture washing technique, as infective larvae were cultured from faeces in all seasons. There was also no significant rainfall in either autumn or spring up to day 7 with summer being the only season where precipitation outweighed evaporation in that early developmental period. The watering treatment was applied to account for a possible shortfall in rainfall in the early developmental period (day 0 to 7). But the extended lack of rainfall in spring and autumn coupled with failure to recover L_3 even from watered plots suggests that temperature was the most limiting factor. Whilst moisture was not limiting initially, cold temperatures could have slowed development such that desiccation became a limiting factor when temperatures warmed up enough for further development. Watering did not significantly increase the proportion of plots with L_3 recovered from them but there was a strong trend for higher recovery rates from both pasture and faecal culture on watered plots than un-watered plots. Thus moisture was sufficient for some development to L_3 on un-watered plots but the number of larvae developing on watered plots was greater. Watering also affected the peak of *H. contortus* L_3 recovery in summer on artificially contaminated plots with peak larval recoveries on days 7 for watered and day 56 for un-watered plots. This indicates that the incident rainfall supplied enough moisture for development, but that development of the majority of eggs and larvae was slower than those on plots that received the extra 50mm. The effect of moisture on development and survival of *H. contortus* eggs is well documented (Waller and Donald 1970; Rossanigo and Gruner 1995), however, the only published data to date that describes the effects of timing and volume of rainfall events is from O'Connor *et al* (2005). Those authors found recovery of first and second stage larvae, in summer, was greater when watered on days 1 and 4 after contamination. However, there was no recovery of infective larvae and mention of the effect of the different volumes of rainfall on larval recovery. Infective larvae were also recovered from faecal cultures of artificially contaminated plots up to day 56 on un-watered plots and day 84 on watered plots. The reservoir in the faecal pellet would most likely be pre-infective or infective larvae as most *H. contortus* eggs either develop before day 5 or die (Donald 1973). It is most likely that the reservoir was pre-infective larvae with infective larvae more likely to have migrated from the pellet by this stage, however, migration of L_3 may be hindered if the pellet had hardened. Thus a portion of the reservoir could also have been infective larvae.

The survival of L_3 on pasture could only be determined for the summer experiment as there were no recoveries from pasture in the other seasons. The relatively high recovery of infective larvae at day 56 after contamination reduced the fit of the survival curve and may be a chance

phenomenon. However, Donald (1967) observed a secondary peak in L_3 in summer (near Sydney, NSW) at 49 days post contamination. Thus an event of a peak 56 days after faecal deposition is not unlikely. This peak in *H. contortus* infective larvae 56 days after deposition could have been the result of further larval development within the pellet resulting in migration of L_3 after a significant rainfall event on day 38 (48mm). This highlights the confounding of development and survival in this study and limits the true estimation of larval survival. The predicted half-life of infective larvae during summer was 19 days. This is slightly longer than the half-life obtained from the model described by Barger *et al.* (1972) of 13 days when solved for the mean maximum temperature of 25°C observed in this study and the long term average humidity in Armidale for January of 45% (Australian Bureau of Meteorology 2006 site accessed August 2006). Thus there is a small discrepancy between the data obtained in this experiment and the model proposed by Barger *et al.* (1972). However, confounding of development with survival in this experiment could account for the longer perceived survival rate. The half-life obtained in this study was lower than that obtained by the model outlined by Leathwick *et al.* (1992) who applied a daily survival rate of 0.977 resulting in a half-life of around 31 days. Their model was not based on a single species as that of Barger *et al.* (1972), rather, it was based on general nematodosis patterns in New Zealand involving mixed genera infections which included *H. contortus*, *Trichostrongylus colubriformis* and *Teladorsagia circumcincta*. Thus, their survival rate is not entirely applicable to the data from this experiment.

Summer temperatures fell favourably within the range of optimum and minimum temperatures for egg hatching and subsequent development. The mean maximum temperature was within the optimal range for development of all major worm species which is between 20 and 35°C for *H. contortus* (Veglia 1915; Kauzal 1941; Silverman and Campbell 1959; Gibson and Everett 1976; Coyne and Smith 1992a), between 26 and 35°C for *Trichostrongylus colubriformis* (Monnig 1930; Ciordia and Brizzell 1963; Levine and Anderson 1973) and ~27°C for *Teladorsagia circumcincta* (Furman 1944). The mean minimum temperature was also warm enough in summer to allow some development of *H. contortus* overnight. The minimum temperature for the development of *H. contortus* eggs is generally agreed to be 10-11°C (Gibson and Everett 1976). Therefore, daily minimum and maximum temperatures would not have been limiting for the development of *H. contortus* eggs and pre-infective larvae although the rate of development during the cool evenings would have been slow. The Kirby strain of *H. contortus* used in the artificial contamination and the local strain present on the Cicerone project seemed to conform to

the general understanding of the ecology of this nematode species with restricted development in spring and autumn at lower temperatures and rapid development in the warm summer months when moisture was not limiting. The numbers of larvae recovered from the naturally infected plots were much lower than those recovered from a February contamination by Donald (1967) near Sydney. Their starting contamination would have been much higher than this study as they used "heavily" contaminated lambs whereas the sheep contaminating pastures in this study had relatively low nematode infections. Recovery rates from un-watered artificially contaminated plots were similar to those reported by Levine *et al* (1974) on outdoor plots near Urbana and slightly better than those reported by Dinaburg (1944a) near Washington D.C., USA. Although Dinaburg ground the faecal pellets before subjecting them to outdoor conditions. The watered plots in this experiment yielded higher recoveries than Levine *et al.* (1974).

Spring and autumn maximum temperatures may, at a glance, seem warm enough for development of eggs to L_3 during the day, but both seasons had frost events during the early post-contamination period. In spring there was only one mild frost event during the experimental period but it was during a crucial period of development (on day 5) during which only eggs and pre-infective larvae would have been present. These stages are more susceptible to cold temperatures than infective larvae (Ransom 1906; Silverman and Campbell 1959). Whilst no infective larvae were found from pasture washing, *H. contortus* L_3 were recovered in small numbers from faecal cultures during spring and autumn. Faecal L_3 were only recovered in spring on day 7 in watered plots and, in lower numbers on days 7 and 14 in un-watered plots. With the frost event occurring on day 5, it seems that the some *H. contortus* survived as eggs, pre-infective larvae or infective larvae within the pellet. It is possible that the pellet provided insulation from the cold with other studies reporting over-wintering of *H. contortus* near Armidale (Southcott *et al.* 1976). Cool temperatures would have restricted development in the earlier part of the study whilst moisture was not limiting, after temperatures warmed up the lack of rainfall then would have left first and second stage larvae susceptible to desiccation. Thus, only very small numbers of infective larvae would have developed and migrated onto pasture from this contamination point.

The frost events would have played a greater role in the autumn experiment where a number of viable *H. contortus* L_3 were recovered from faecal cultures at day 7, with none thereafter. On day 7 there was a light frost with ground temperatures of around 1°C, this was followed by heavier

frosts on days 12 and 13 of -2°C. Further frosts followed on days 20 and 24 to 52 and then regularly thereafter until day 84. There was also a negative difference between precipitation and evaporation for the first 7 days (-20.6mm), with most of the precipitation fell towards the end of the autumn experiment in June 2005. As such, watered plots were the only plots receiving sufficient moisture for development of eggs and pre-infective larvae. The data from both spring and autumn experiments suggest that, whilst there was enough moisture to begin development of eggs, under cool conditions follow up moisture is required to maintain development to the infective larval stage.

There were fewer *H. contortus* larvae recovered from the naturally contaminated plots and the peaks differed quite markedly from those observed in the artificially contaminated plots. There were L_3 recovered on day 7 and 14 from watered plots, but the peaks were observed at days 28 and 84. Infective larvae were only recovered on day 28 from the unwatered, naturally contaminated plots. Donald (1967) found moderate levels of infective larvae at day 28, but his observed peaks from the natural infection were at days 21 and 49 after sheep were removed, a lot earlier than the 84 day maximum in this experiment. The maximum at day 84 could be due to a larger number of larvae having migrated onto pasture from the soil due to favourable climatic conditions on that particular day than on other sample days. Indeed, maximum ground temperature on that day was cooler than the previous few days (15°C versus 40°C). It seems a very long time after contamination for such a large number of larvae to be recovered especially when our estimated half-life from the artificial contamination was 32 days. These data could also be a result of the insensitivity and unreliability of the pasture washing technique for quantification of infective larvae on pasture. There were only small numbers of *H. contortus* larvae cultured from faeces from the naturally infected plots on day 7 on both watering treatments with a larger number recovered on day 56. So some faecal deposits could be acting as reservoirs for infective larvae, although there was substantial rainfall prior to this sampling event. Another, possible explanation for this anomaly in the data is the lack of equal initial contamination for the naturally infected plots which were contaminated randomly by sheep. Only one plot yielded *H. contortus* infective larvae on day 84, thus that plot may have had a higher concentration of sheep faeces than plots sampled on the earlier sample days. The fact that infective larvae were recovered from both watered and un-watered plots on day 28 would suggest that this may be the real peak in infective larvae on the naturally contaminated plots.

However, as there is no point of reference it is difficult to attribute any defined outcomes for development and survival on the naturally infected plots.

H. contortus eggs and larvae are the most susceptible of all the 3 major parasitic nematodes to cold temperatures with *T. circumcincta* pre-infective stages being much more resilient (Jasmer *et al.* 1986). Dinaburg (1945) found overnight temperature of -1°C detrimental to *T. colubriformis*, so this species may also have been affected by a frost event. Given the ability of *T. circumcincta* to develop and survive at low temperatures is surprising that infective larvae of this species were not recovered in spring or autumn from pasture. The spring tracer sheep in IRG from Experiment 3 (Chapter 5), which commenced 14 days after the start of this experiment revealed very low WECs that were dominated by *Teladorsagia* spp. with low levels of *H. contortus* and *Trichostrongylus* spp. The WECs on the IRG treatment in Experiment 1 (Figure 3-2, Chapter 3) also reflect the low infectivity of the pasture during September and October 2004 and the presence of *Teladorsagia* spp. The presence of *Trichostrongylus* spp. and *Teladorsagia* spp. infective larvae on pasture in summer is also reflected in the tracer study results for this season (Expt 3, Chapter 5). The highest counts for IRG sheep in the tracer studies was in summer with a mean count of 243eggs/g, 73% *H. contortus*, 14% *Trichostrongylus* spp and 9% *Teladorsagia* spp. The higher pasture infectivity during January/February 2005 was also reflected in the WEC of sheep from experiment 1 (Figure 3-2, Chapter 3). The presence of low numbers of *Trichostrongylus* spp. and *Teladorsagia* spp. eggs in sheep faeces in summer were also observed by Roe *et al.* (1959) and Gordon (1948). The presence of *Teladorsagia* spp. in tracers and longitudinal study sheep highlights the inadequacies of the pasture sampling method for detecting low numbers of infective larvae on pasture. In the present studies, the tracer sheep were more sensitive indicators of pasture infectivity picking up infective larvae that were not detected with pasture sampling. The recovery rates determined by spiked pasture controls were extremely variable with very large coefficients of variation in all seasons (Table 6-3). Faecal culture revealed the potential larval infection present in the faecal pellet and showed greater sensitivity in detection of infective larvae than the pasture washing technique by recovering infective larvae in autumn and spring when the pasture washing method did not.

The low natural infections observed in this experiment and experiment 3 (Chapter 5) lend further support to the hypothesis that the reduced resistance to nematode infection observed in IRG sheep during experiment 2 (Chapter 4) was due to low exposure to infective larvae. This is

consistent with the failure of sheep on the IRG system to develop strong resistance to infection relative to sheep on the other systems where WEC was consistently higher. From these three experiments (Expts 2-4, Chapters 4-6) it is clear that any reduction in WEC on and IRG system is most likely due to reduced pasture infectivity and not mediated by better host resistance.

The very capriciousness of what happens to egg contamination on any given day is a key aspect of intensive rotational grazing. The strict development criteria required for *H. contortus* mean that many paddocks in an intensive rotational grazing system will have had no egg development arising from the most recent faecal deposition. This situation is far less likely to occur with *Trichostrongylus* spp. or *Teladorsagia* spp. which are much more likely to survive until favourable conditions for egg development prevail. It would also be less likely to occur in other grazing systems which employ longer rotations or set stocking. In those systems *H. contortus* can take advantage of any favourable conditions at any time and build on them exponentially via the sheep, resulting in permanently contaminated pastures. However, in an intensive rotationally grazed system, any benefits of favourable conditions are solely on development of faeces in that window of time on that paddock. There is no opportunity for build up of *H. contortus* contamination as the sheep move on before the emergence of infective larvae. Thus on an intensive rotational grazing system it is likely that a large proportion of paddocks had very low infection levels.

In conclusion, the local strain of *Haemonchus contortus* (natural infection) and the Kirby strain (used for artificial infection) seemed to conform to previously published requirements for development resulting in development constrained mainly by cold in spring and autumn, and moisture in summer with high rates of development in summer when moisture was not limiting. Cold temperatures in spring and autumn most likely slowed development of pre-infective stages which were then inhibited by a lack of subsequent rainfall to prevent desiccation when temperatures were warm enough for further development to the infective larval stage Frost events autumn could also have been responsible for no larvae being recovered from pasture in that seasons, with some nematodes surviving in the faecal pellet. The death rate of *Haemonchus contortus* was estimated for the summer contamination and proved to be lower than that proposed by some others.

CHAPTER 7: Experiment 5. Cost of gastrointestinal nematode infection on sheep production performance on the Cicerone Project Farmlets

7.1 Introduction

Gastrointestinal nematode infection is generally associated with a loss of animal production through bodyweight losses, poor growth rate, lost wool production, reduced reproduction rate and animal deaths (Steel 1974). The cost of these production losses to the Australian sheep industry was estimated by McLeod (1995) to be in the order of $222 million although the current cost could be higher due to the increased prevalence of anthelmintic resistance (Love and Coles 2002; Besier and Love 2003). Three main mechanisms are thought to underlie production losses during parasitic nematode infection. These are appetite depression, changes in gastrointestinal function and alterations in protein metabolism (Fox 1997). The manifestation and degree of the disease is dependent upon the species involved, and in the case of *Haemonchus contortus*, anaemia induced by blood loss in an important additional mechanism. A comprehensive review of laboratory studies by Donald (1979) provides strong evidence that reductions in wool production are mainly associated with *Trichostrongylus* spp. and *Teladorsagia circumcincta* infections. A number of experiments involving artificial and natural infections in sheep have demonstrated a depression in wool production (from 18% to 66%) and bodyweight (Carter, 1946 ; Barger, 1975; Thompson, 1978; Leyva, 1982; Steel, 1982). *Haemonchus contortus* has also been shown, in a field trial with an artificial infection, to cause a significant reduction in wool growth (6 to 8%), fibre diameter (0.39 to 0.79μm) and bodyweight gain (12-64%) (Albers *et al.* 1988).

Conventional worm control measures may not eliminate these production losses. A number of studies, conducted in the southern states of Australia, compared production of sheep on differing anthelmintic control programmes; Thompson *et al.* (1978) reported a 21-23% reduction in bodyweight of lambs with a natural infection (predominantly *T. colubriformis* and *T. circumcincta* in Victoria) drenched as recommended relative to those drenched weekly. The details of the recommended drenches were not provided. In a study replicated in Victoria and the Northern Tablelands of NSW, Johnstone *et al.* (1979) compared the performance of weaners given four levels of anthelmintic treatment; 1. Salvage (given only to prevent death), 2. Curative

(4 thibendazole, 1 rafoxanide), 3. Preventative (5 thibendazole, 5 rafoxanide). 4. Suppressive (11 thibendazole, 5 rafoxanide). The highest level of production gain in both wool and bodyweight gain was with the Suppressive programme followed by the Preventative then Curative programme, thus production gains increased with increasing level of anthelmintic treatment and worm control. Barton and Brimblecombe (1983) had similar results with wool growth being lowest in the group given only one drench and increasing with increasing drench frequency. Larsen *et al.*(1995) compared breeding and non-breeding ewes with 2 levels of treatment (no anthelmintic or a controlled release benzimidazole capsule with an ivermectin primer). They found no difference in production between non-breeding sheep with sustained anthelmintic treatment and those without, however, there was a significant advantage in favour of the sustained anthelmintic treatment in the breeding ewes with ewes on this treatment having higher bodyweights, higher fleece weights but with the disadvantage of higher fibre diameter. The estimated cost of treatment ranged from 8 to 62 cents per head but a cost-benefit analysis of treatment was not performed.

In South Australia, Brown *et al.* (1985) tested 3 levels of anthelmintic treatment on weaner sheep at two stocking rates (7.5/ha and 16/ha); Not-treated, Treated (every 3 weeks) and Planned (treated once in October, twice in summer and twice in winter). They observed that those treated tri-weekly were heavier and cut more wool and that the difference was greater at a higher stocking rate. The Planned program provided the same level of wool production as the tri-weekly treated sheep in 3 out of 5 years and similar bodyweights in all years. The difference between treated and non-treated sheep was more pronounced at the higher stocking rate. The nematode species involved included *Trichostrongylus* spp., *Teladorsagia* spp. and *H. contortus* at different times of the year, although none of the deaths in that study were attributed to *H. contortus* (Beveridge *et al.* 1985).

The results of these field studies show that the losses in bodyweight and wool production in a housed situation are of a similar magnitude to those observed in the field. All of the aforementioned experiments apart from that of Larsen et al. (1995) were conducted prior to the introduction of slow release and long acting anthelmintics and only one was conducted in a *H. controtus* dominant region with summer dominant rainfall. None of them involved the use of grazing management in their worm control programmes. On the Cicerone project there are major differences between treatments in WEC (Chapter 3) due primarily to differences in pasture

contamination (Chapter 5). It is of interest to determine the extent to which these differences are associated with differences in productivity between groups, and also the extent to which the residual worm burdens present in each treatment are causing production loss relative to "worm free" sheep. To investigate this, an experiment was designed to determine the impact of gastrointestinal nematode infection under the 3 different management systems by comparing the performance of conventionally managed sheep (CM) on each system with sheep maintained effectively worm free (WF) using long-acting and controlled release anthelmintics. The hypothesis was that the worm control measures on the HI and TYP management systems will not prevent significant production losses due to GIN while production losses will be minimal on IRG due to the low level WECs observed in Experiment 1 (Chapter 3).

7.2 Materials and Methods

7.2.1 Experimental design

Fourteen ewe hoggets from each of the three management systems were kept effectively worm-free using a combination of short-acting and long-acting anthelmintic treatments for a 12 month period between shearings (September 2004 to July 2005) and compared with CM contemporaries within each management system. The "worm-free" sheep were run in their normal management system mobs which were CM for worm control. Production traits were measured monthly and wool traits were measured at shearing for all experimental sheep. The main factors in the experiment were:

- Worm control treatment; worm-free (WF) and conventionally managed (CM)
- Management treatment; High input (HI), Typical (TYP) and Intensive rotational grazing (IRG) (section 2.2)
- Month (September 2004 to July 2005)

7.2.2 Animal selection and sampling

7.2.2.1 Worm-Free (WF) sheep

14 ewe hoggets (born Sept/Oct 2003) were randomly selected from each management treatment. A primer drench of albendazole, levamisole and naphthalophos (ABZ/LEV/NAP; COMBI®, 1ml/10kg of 34g/L albendazole oxide and 70g/L levamisole HCl, Novartis Animal Health Australasia Pty Ltd, Australia; Rametin®, 3ml/10kg of 800g/kg naphthalophos, Bayer Australia, Australia) was given orally (dose given was based on the heaviest animal) on 2nd September

2004 along with a slow release capsule (Extender 100, 3.85g of Albendazole, Merial Australia, Australia) and the sheep returned to their normal management treatment mobs.

Individual faecal worm egg counts (WEC) and bulked faecal cultures for larval differentiation were carried out monthly to monitor the effectiveness of the capsules as described in section 2.3.1. Positive egg counts were found in TYP WF sheep and larvae found in HI and IRG sheep cultures, in November 2004 after which the WF sheep on each treatment were dosed with Cydectin long acting for sheep® (1mg moxidectin/kg bodyweight, Merial Australia, Australia) as well as continuing with the Extender 100 capsule. Thus from December 2004 the worm-free sheep were given a primer drench of ABZ/LEV/RAM, an Extender 100 Capsule and an injection of Cydectin LA every 70-90 days. The anthelmintic treatment dates subsequent to September 2004 were 29th November 2004, 7th February 2005 and 23rd April 2005.

7.2.2.2 Conventionally managed (CM) sheep

These comprised 20 ewe hoggets born in Sept/Oct 2003 which had previously been randomly selected from each management system in November 2003 as part of the main longitudinal study (Expt 1, Chapter 3). The HI and TYP sheep were managed within their age group mobs per management system (HI: 126; B:74 sheep/mob) until June when they joined the main ewe mobs, IRG remained within ewe mobs for duration of the experimental period (HI: 410; TYP: 244; IRG: 250 sheep /mob). Anthelmintic treatment was given based on monthly WEC on advice from a consultant parasitologist on a management system basis as summarized in Table 7-1. Both CM sheep and WF sheep received a quarantine drench (moxidectin with levamisole and albendazole) as required by CSIRO prior to shearing on 30th July 2004. They thus started this experiment with low worm burdens.

Table 7-1: Experiment 5. Anthelmintic treatments given to conventionally managed sheep on each management system from July 2004 to July 2005. Moxidectin (MOX), albendazole (ABZ), levamisole (LEV), napthalophos (NAP). Asterisks denotes quarantine drench given at shearing prior to the start of this experiment.

	Anthelmintic treatment		
Date of treatment	High Input (HI)	Typical Management (TYP)	Intensive Rotational Grazing (IRG)
*30/07/2004	MOX + ABZ + LEV	MOX + ABZ + LEV	MOX + ABZ + LEV
15/11/2004		LEV	
5/01/2005	MOX		
24/01/2005	LEV	LEV	
24/02/2005	NAP/ABZ	NAP/ABZ	NAP/ABZ
23/04/2005	NAP/ABZ	NAP/ABZ	

7.2.3 Measurement schedule

For both WF and CM sheep monthly WEC were calculated from individual rectal faecal samples. Samples were bulked within management system and treatment and cultured for species identification of larvae as described in section 2.3.2.

Sheep were weighed monthly except for December 2004 (missed), May 2005 (not possible due to mating) and June 2005 (rainfall impeded weighing). Individual fat scores were recorded in February, March, April and July 2005. Pregnancy rates were determined by ultrasound scanning in June 2005. A mid-side sample of wool was taken on the 25th July 2005 (2 days prior to shearing) and a portion retained for mean fibre diameter profiling as outlined in section 7.2.4. The main part of the wool sample was sent for analysis to New England Fibre Testing, Walcha, NSW. The measurements made by this company included mean fibre diameter and comfort factor (OFDA 100, BSC Electronics Pty Ltd, Australia), yield (Goodwin's washing machine, J. Goodwin, Perth, Western Australia), staple length, staple strength, and point of break (Goodwin's Length and Strength, J. Goodwin, Perth, Western Australia). Fleece weights were recorded at shearing and included the fleece plus bellies and pieces.

7.2.4 Along Staple Fibre Diameter Measurement

Fibre diameter was measured at 5mm increments along the individual staples using an optical fibre diameter distribution analyser (OFDA 2000, BSC Electronics Pty Ltd, Australia) in the Animal Science Wool laboratory at UNE. As the staples differed in length the increments were converted to percentages of total staple length. A third order polynomial was fitted to the longitudinal fibre diameter distribution and then solved for 12 equal increments along the staple to create a derived mean fibre diameter for each animal for approximately each month of the year. The mean adjusted R^2 value for the individual third order polynomial curve fits was 0.83±0.01. This method enables staples from different animals to be compared at the same relative distance along the fibre, but the strict association with month of the year assumes wool growth is constant throughout the year, which it generally is not (Brown and Crook 2005). There are periods of faster growth leading to finer fibre diameter and periods of slower growth leading to broader diameter. Thus the derived mean fibre diameter may correspond to times that may be slightly to the left or right of where we have placed them, but the overall pattern is true.

7.2.5 Estimation of economic costs

The partial budget used to estimate potential economic costs of gastrointestinal nematode infections during this experiment were based on those described by Morris and Meek (1980). Each measurement can potentially be multiplied by its market value and its financial estimate included in the budget. The general formula used was:

Net return = (A + D) – (B + C)

Where, A = additional monetary returns achieved due to adoption of the WF procedure

B = returns no longer received

C = additional costs incurred due to control procedure

D = costs no longer incurred

Only two production measurements were used in the partial budget for this experiment, they were lambing percentage as determined at scanning (with replacement stock priced at $40/head) and actual fleece value achieved at sale (cents/kg based on a tensile strength of 33 N/kt and fleece weight). Fleeces from all management systems and worm control treatment were classed and sold as a single lot. Anthelmintic treatment and labour costs were factored into the parts C and D of the equation. The cost of anthelmintic treatment was removed for both WF and CM sheep to get an estimation of the cost of production differences only. The values were calculated for cost per head then transformed into cost per hectare by the stocking rate for each management system.

7.2.6 Statistical analysis

WEC data from the CM sheep were cubed-root transformed. This yielded back-transformed least squared means which were approximately a third to half that of the arithmetic means, which are shown in Figure 3-2b of Chapter 3. WEC were analysed using a repeated measures linear mixed model (REML in JMP IN 5.1, SAS Institute Inc., NC, USA) fitting management system (HI, TYP, IRG), worm control treatment (WF or CM) and month (August 2004 to July 2005) and their interactions as fixed effects and tag number as a random effect. Bodyweight and pregnancy status were included as covariates. Only significant interactions between effects were retained in the model. Significant differences between means were determined either using Tukey's HSD method, or by fitting specific contrasts within the linear model. WEC were also apportioned into *Trichostrongylus* spp WEC (*T*WEC) or *H. contortus* WEC (*Hc*WEC) based on the bulked faecal culture results. These WECs were submitted to the same analysis at total WEC.

Bodyweights and fat scores were analysed using the same model with initial bodyweight as a covariate. Pregnancy status (pregnant or dry) was subsequently fitted to determine its effect on bodyweight. Derived monthly MFD values were also initially analysed with the same model as total WEC with bodyweight and pregnancy status also subsequently fitted in the model to determine their impact on MFD. The remaining wool measurements were analysed using linear mixed models fitting management system and worm control treatment as fixed effects. Pregnancy scores (0 or 1) were analysed using a nominal logistic model with differences in means determined by chi-square tests. Backtransformed least square means (LSM) with 95% confidence intervals are presented for WEC and LSM±SEM will presented for all other data.

7.3 Results

7.3.1 Larval differentiation

The main nematode species and genera were *Haemonchus contortus*, *Trichostrongylus* spp., *Teladorsagia circumcincta* and *Oesophagostomum* spp. The raw proportion of nematode species varied between management treatments as shown in Table 7-2.

Table 7-2: Raw proportions of the three major parasitic nematodes found in faecal cultures of conventionally managed sheep under each management system from September 2004 to July 2005.

Nematode Species	High Input (HI) (%)	Typical Management (TYP) (%)	Intensive Rotational Grazing (IRG) (%)
Haemonchus contortus	67.8	82.8	66.7
Trichostrongylus spp.	25.7	2.4	22.8
Teladorsagia circumcincta	4.5	0.4	8.0
Oesophagostomum spp.	2.0	14.4	2.5

7.3.2 Faecal worm egg count

All WEC for the WF sheep were zero except for those on the TYP system in November 2004 (average 229, range 0 to 600 eggs/g, 100% *H. contortus*). This was 67 days after application of the slow release anthelmintic capsules that were administered in early September 2004. Although there were zero egg counts on the HI and IRG treatments larvae were recovered from faecal culture, these too were all *H. contortus*. After these breakthrough counts a long acting moxidectin injection was added to the anthelmintic treatment of the WF sheep and no subsequent eggs or larvae were recovered from faeces on this treatment.

In CM sheep on the HI treatment there were 2 major peaks of *Hc*WEC above 2000eggs/g and one above 1500 during the year, occurring in September, January and April (Figure 7-1). There were two instances of high counts of *Trichostrongylus* spp., one over 2000 eggs/g the other 1000eggs/g, in January and February. Intensive anthelmintic treatment in January and February kept egg counts down but they rose to a smaller peak in April when an anthelmintic treatment was given at joining. In CM sheep on the TYP treatment no high counts of *Trichostrongylus* spp. were observed, but there were 3 months in which *H. contortus* WEC was greater than 2000 eggs/g, reaching a peak of over 7000 eggs/g November. Frequent anthelmintic treatment controlled infections from January to July (Figure 7-1).

In CM sheep on the IRG treatment WEC was generally low throughout the year (<200-500 eggs/g), reaching over 1000 *H. contortus* eggs/g in October and November before declining without anthelmintic treatment to ~500 eggs/g in December and January. The anthelmintic treatment given on IRG in February did not seem warranted as *H. contortus* WEC were below 800 eggs/g and *Trichostrongylus* spp. WEC below 100 eggs/g (Figure 7-1).

Analysis of variance of cubed-root transformed total WEC of CM sheep revealed a significant effect of management system ($P<0.05$) with the HI and TYP treatments having significantly higher WEC than the IRG treatment (HI: 380, CI 250-520; TYP: 410, CI 270-560; IRG 200, CI 120-300 eggs/g, respectively, $P<0.01$, arithmetic means: HI: 1200; TYP: 1600; IRG: 600eggs/g). Arithmetic means are given alongside the LSM as the models returned values that were a fraction of the arithmetic means due to the highly skewed raw data. There was a highly significant effect of month ($P<0.0001$) and an interaction between management system and month ($P<0.0001$). The interaction between management system and month can be explained by differences in the timing of anthelmintic treatments on management systems and seasonal variation in larval availability. Pregnancy status and bodyweight were not significant when fitted as covariates ($P\sim0.09$ and $P\sim0.98$ respectively).

When CM sheep WEC was analysed as *H. contortus* WEC there was a significant effect of management system with TYP having higher *H. contortus* WEC than HI and IRG which did not differ (HI: 113; TYP: 313; and IRG: 100 eggs/g, respectively, $P<0.0001$, arithmetic means were HI: 750; TYP: 1500; IRG: 500eggs/g). Management system also had a significant effect on *Trichostrongylus* spp. WEC with HI having higher mean *Trichostrongylus* spp. WEC than IRG

which was higher than TYP (HI: 9; IRG: 0.6 and TYP: 0.003 eggs/g, P<0.0001, arithmetic means were HI: 340; IRG: 70; TYP: 27 eggs/g). Refer to Figure 7-1 for arithmetic means across the experimental period.

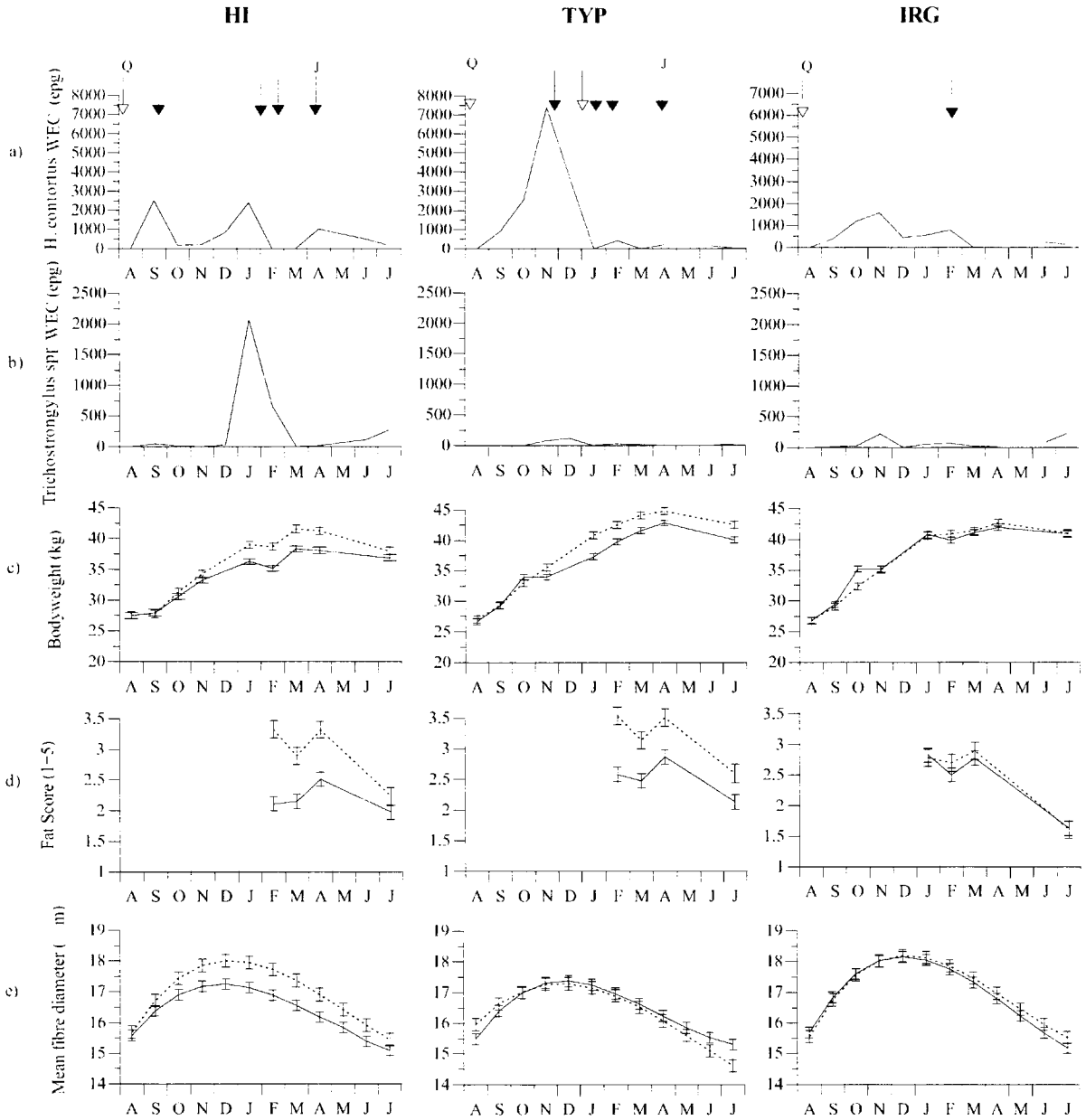


Figure 7-1: Experiment 5 data by management system from August 2004 to July 2005 for 'worm-free'-WF (⋯) and conventionally managed-CM (—) sheep; Arithmetic mean faecal worm egg count (for CM sheep only) expressed as a) *Haemonchus contortus* eggs/g, with anthelmintic treatments for CM sheep indicated by arrows: moxidectin (white), short-acting (black), quarantine (Q) and mating (J); or b) *Trichostrongylus* spp. eggs/g; c) LSM (±SEM) bodyweight of WF and CM sheep; d) LSM (±SEM) fat score; e) LSM (±SEM) fibre diameter with initial fibre diameter fitted as a covariate.

7.3.3 Bodyweight

Bodyweights for WF and CM sheep were similar on all management systems in the first 2 months of the experiment. The bodyweights of the two worm control treatments started to diverge on the HI treatment from January which coincides with high *Trichostrongylus* spp and *H. contortus* WEC. WF sheep remained significantly heavier than CM sheep on HI until July when bodyweights converged again. On the TYP management treatment WF sheep were heavier than CM sheep from November, coinciding with high *H. contortus* WEC, until the end of the experimental period. On the IRG management treatment the two worm control treatments did not differ in bodyweight throughout the experiment except in October (Figure 7-1).

Analysis of variance of bodyweight, with initial bodyweight (August 2004) fitted as a covariate, revealed a significant effect of all terms fitted in the model; management system ($P<0.0001$), month ($P<0.0001$), treatment ($P<0.001$), management system and month ($P<0.0001$), management system and treatment ($P\sim0.02$), month and treatment ($P<0.0001$), management system, month and treatment ($P<0.0001$) and August bodyweight ($P<0.0001$). Overall HI had significantly lower mean bodyweight than TYP and IRG (34.6±0.3, 37.0±0.3 and 36.7±0.3kg, respectively). The significant interaction between management system and worm control treatment was because there was an effect of worm control treatment on the HI and TYP but not the IRG management system. WF sheep on HI were significantly heavier than CM sheep (35.5±0.4 and 33.8±0.4kg, $P\sim0.001$), as was the case on the TYP system (37.8±0.4 and 36.2±0.4kg, $P<0.01$). However there was no difference between worm control treatments on the IRG system (36.8±0.4 and 36.6±0.4kg, $P\sim0.77$, Figure 7-1). There was no significant effect of WEC or scanned pregnancy status on bodyweights ($P\sim0.33$ and $P\sim0.08$).

7.3.4 Fat Score

Fat scores were higher in WF sheep on HI and TYP in all the months it was measured, converging slightly in July, thus following the same trend as bodyweight for these management systems. There was no difference between worm control treatments for fat score on IRG in any month (Figure 7-1).

Analysis of fat scores again showed a significant effect of all terms fitted in the model; management system ($P<0.001$), month ($P<0.0001$), treatment ($P<0.0001$), management system and treatment ($P<0.001$), management system and month ($P<0.001$), month and treatment

(P<0.01) and management system, month and treatment (P<0.05). The interaction between management system and treatment followed the same pattern as for bodyweight with WF sheep on HI and TYP having a higher mean fat score than CM sheep on their respective management systems (HI: 2.94±0.10 and 2.19±0.09, TYP: 3.20±0.10 and 2.51±0.10). On the IRG treatment WF and CM sheep did not differ in fat score (2.49±0.10 and 2.43±0.09 respectively).

7.3.5 Ultrasound scanned pregnancy status

Nominal logistic analysis of pregnancy status showed no overall effect of management system (P~0.7), treatment (P~0.2) nor interaction between the two (P~0.4). However, on the HI system WF sheep had pregnancy rate 18.8% higher than CM sheep (71.4 and 52.6%). The same pattern was observed on the TYP treatment with WF sheep having a 24% higher pregnancy rate than CM sheep (71.4 and 47.4%). The IRG treatment did not show this trend with only a 5% difference between treatments (WF: 50% and CM: 55% pregnant).

7.3.6 Greasy fleece weight

There was a significant effect of management system on fleece weight (P<0.0001) with mean fleece weights of sheep on the HI and TYP systems being significantly heavier than those on the IRG system (3.64±0.06, 3.64±0.06 and 3.12±0.06kg respectively). The effect of worm control treatment was almost significant (P~0.06) with WF sheep displaying a slightly heavier mean fleece weight then CM sheep (3.53±0.6 and 3.40±0.05kg). The interaction between management system and treatment was not significant (P~0.2), but there was a clear trend for WF sheep to have heavier fleece weights than CM sheep on the HI (3.76±0.09kg and 3.52±0.08kg P~0.06), and TYP (3.75±0.1kg and 3.54±0.08kg, P~0.1) systems but not the IRG system (3.10±0.1 and 3.14±0.08, P~0.75). There was no effect of pregnancy status on fleece weight (P~0.99).

7.3.7 Yield of clean wool

There was a significant effect of management system (P<0.001) and treatment (P<0.001) on wool yield with significant interaction between the two effects (P=0.05). There was no significant difference between worm control treatments on the HI system (77.8±0.8 and 78.0±0.7%, P~0.9), but on both the TYP and IRG systems WF sheep had lower yields than CM sheep (TYP:79.8±0.8 and 82.4±0.7%, P~0.02; IRG:78.4±0.8 and 82.2±0.7%, P<0.001, Figure 7-2). Overall the HI management treatment had significantly lower yields than the TYP and IRG treatments (77.9±0.5, 81.1±0.5 and 80.3±0.5%) and WF sheep had lower yields than CM sheep (78.6±0.5 and 80.9±0.4%).

7.3.8 Derived mean fibre diameter along the staple

On the HI management system mean fibre diameter along the staple (dMFD) was similar for WF and CM sheep for August and September after which they began to diverge with WF sheep showing higher MFD than CM sheep until July. On the TYP management system, dMFD did not differ between the two worm control treatments except in August, where WF sheep had a higher dMFD, and July, where WF sheep had lower dMFD than CM sheep. There was no difference in dMFD between WF and CM sheep in any month (Figure 7-1).

With initial dMFD fitted as a covariate to correct for differences in initial diameter, there was a significant effect of management system ($P<0.001$), month ($P<0.0001$) and significant interactions between management system and month ($P<0.0001$) and management system, treatment and month ($P<0.0001$, Figure 7-1b). There was no main effect of worm control treatment ($P\sim0.14$) or interaction between month and worm control treatment ($P\sim0.55$) but there was trend towards significant interaction between the effects of management system and treatment ($P\sim0.08$). Exploring this with specific contrasts revealed a significant difference between WF and CM sheep on the HI system with the WF sheep having a higher dMFD (16.96±0.14 and 16.34±0.17μm, $P\sim0.02$). There was no difference between worm-free and CM sheep on the TYP and IRG systems (TYP: 16.35±0.17 and 16.45±0.15μm, $P\sim0.98$, IRG: 17.04±0.17 and 16.94±0.15μm, $P\sim0.72$).

Fitting cubed-root transformed WEC as a covariate (with initial dMFD) weakened the trend towards interaction between management system and treatment ($P\sim0.19$) although the effect of cubed-root WEC was not significant ($P\sim0.22$). Neither were its interactions with management system or treatment ($P\sim0.30$). However there was still a significant contrast between WF and CM sheep on the HI management system ($P\sim0.03$) after these covariates were added. When pregnancy status was fitted with initial dMFD as a covariate it, too, weakened the trend towards interaction between management system and treatment ($P\sim0.17$), although pregnancy scan was not a significant covariate ($P\sim0.21$). However it also changed the effect of treatment from non-significant ($P\sim0.14$) to almost significant ($P\sim0.07$). Fitting initial bodyweight with initial dMFD as covariates had a similar effect as fitting cubed-root WEC, removing the interaction between treatment and management system ($P\sim0.22$) without having a significant effect of its own on dMFD ($P\sim0.30$).

7.3.9 Mean fibre diameter (cored mid-side sample, commercial lab)

When fitted as a covariate, the mean fibre diameter (cMFD) from the previous shearing (2004) was highly significant and thus included in the model ($P<0.0001$). Sheep on the IRG treatment had the highest cMFD with wool that was coarser than that on the TYP treatment with the HI treatment intermediate between the two (17.2±0.1, 16.6±0.2 and 16.9±0.1μm respectively, $P<0.05$). There was a main effect of worm control treatment with WF sheep having a higher cMFD than CM sheep (17.1±0.1 and 16.7±0.1, $P<0.05$). There was no significant interaction between worm control treatment and management system, but contrasts revealed a significant difference between WF sheep and CM sheep on the HI treatment (17.3±0.2 and 16.4±0.2, $P<0.05$), but not on the IRG or TYP treatments (IRG WF: 17.2±0.2; CM: 17.1±0.2, P~0.70; TYP WF: 16.8±0.2; CM: 16.4±0.2, P~0.28). Pregnancy status did not have a significant effect on cMFD (P~0.09) and was not retained in the model.

7.3.10 Staple strength

Analysis of variance of staple strength showed no effect of management system (P~0.80), treatment (P~0.30) or interaction between the two (P~0.40). Staple strength was relatively low for all management systems (HI:33±1, TYP:34±1 and IRG:33±1 N/kt). Pregnancy status had no effect on staple strength (P~0.99).

7.3.11 Staple length

Analysis of variance of staple length revealed an effect of management system ($P<0.0001$) but not worm control treatment (P~0.30) or interaction between management system and treatment (P~0.30, Figure 7-2). Sheep on the TYP system had the longest staple length which was significantly longer than that on the HI treatment which was significantly longer than that on the IRG treatment (90.0±1, 85.3±1 and 80.5±1mm). There was no effect of scanned pregnancy status on staple length (P~0.60).

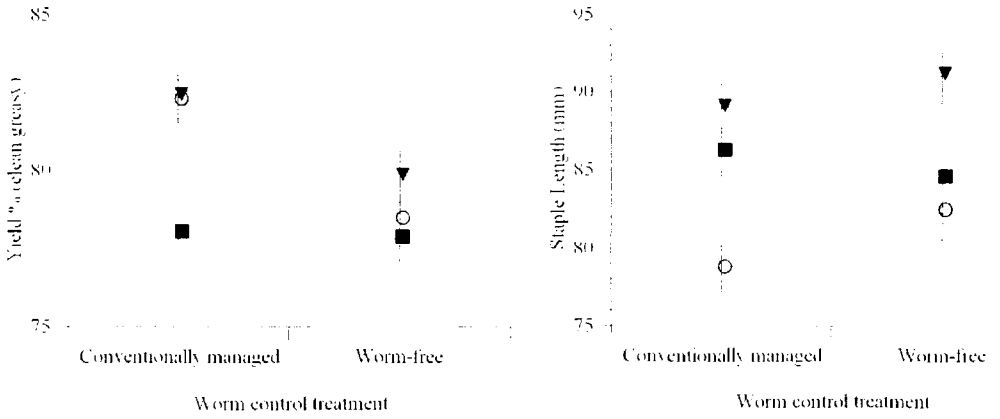


Figure 7-2: Experiment 5. Interaction plots (LSM ±SEM) showing the effects of management system and worm control treatment on wool yield (left) and staple length (right); HI (■), TYP (▼) and IRG (○).

7.3.12 Summary of the effect of gastrointestinal nematodiasis on animal production

Table 7-3 gives a summary of the differences in production between WF and CM sheep on each management system. GIN had the greatest effect on fat scores and pregnancy rates on HI and TYP, with a smaller effect on bodyweight and fleece weight. HI was the only management system in which GIN had a marked impact on mean fibre diameter (both dMFD and cMFD). There was a very small reduction fat score in CM IRG sheep but it did not translate into any other production losses and was not significant (see section 7.3.4).

7.3.13 Economic costs of production differences

A partial farm budget was calculated for each management system to compare the difference in profit associated with maintaining sheep 'worm-free' (Morris and Meek 1980). If the cost of anthelmintics is ignored, the additional profit associated with keeping sheep 'worm-free' was an additional $97, $58 or $-10/ha for the HI, TYP and IRG systems respectively. However if the costs of drench are included in the partial budget there are no financial benefits from keeping sheep worm-free using the anthelmintic method employed in this experiment with net returns of -$239, -$171 and -$245/ha for the HI, TYP and IRG systems respectively.

Table 7-3: Summary of least squared means ± SEM and percentage difference in production variables in worm-free and conventionally managed (CM) sheep on each management system. dMFD – derived mean fibre diameter, cMFD – cored mean fibre diameter.

Management system	Measurement	Worm-free (LSM±SEM)	CM (LSM±SEM)	% change (WF-CM)/WFx100)
HI	Bodyweight	35.5 ± 0.4	33.8 ± 0.4	4.8
	Fat score	2.94 ± 0.1	2.19 ± 0.1	25.5
	Fleece weight	3.76 ± 0.09	3.52 ± 0.08	6.4
	dMFD	16.96 ± 0.14	16.34 ± 0.02	3.7
	cMFD	17.3 ± 0.2	16.4 ± 0.2	5.2
	Pregnancy rate	71.4	52.6	26.3
TYP	Bodyweight	37.8 ± 0.4	36.2 ± 0.4	4.2
	Fat score	3.2 ± 0.1	2.5 ± 0.1	21.9
	Fleece weight	3.75 ± 0.1	3.54 ± 0.08	5.6
	dMFD	16.35 ± 0.17	16.45 ± 0.15	-0.6
	cMFD	16.8 ± 0.2	16.4 ± 0.2	2.3
	Pregnancy rate	71.4	47.4	33.6
IRG	Bodyweight	36.8 ± 0.4	36.6 ± 0.4	0.5
	Fat score	2.49 ± 0.1	2.43 ± 0.1	2.4
	Fleece weight	3.10 ± 0.1	3.14 ± 0.08	1.3
	dMFD	17.04 ± 0.17	16.94 ± 0.15	0.6
	cMFD	17.2 ± 0.2	17.1 ± 0.2	0.6
	Pregnancy rate	50	55	-10

7.4 Discussion

'Worm-free' sheep on the HI and TYP management systems had significantly higher bodyweights and fat scores than conventionally managed sheep on the same systems lending support the hypothesis that the worm control methods on these 2 management systems would not be sufficient to prevent production loss due to worms. The hypothesis that production loss due to worms would be minimal on the IRG system was also supported with no discernable production losses observed in conventionally managed sheep on this system. Bodyweights, fat scores, pregnancy rate and fleece weights were all comparable between WF and CM sheep on the IRG management system.

The bodyweights, fat scores, pregnancy rates, derived mean fibre diameter profiles (dMFD) and the cored mean fibre diameter (cMFD) on the HI and IRG systems all support the original hypothesis with no significant difference between WF and CM sheep on the IRG system. The derived mean fibre diameter of CM sheep on the HI system started to diverge as early as September 2004 coinciding with a rise in *H. contortus* WEC. The dMFD diverged even further

through the late spring and summer months concurrent with elevated WECs mainly *H. contortus*. However, bodyweight did differ between treatments until January 2005 which coincided with a significant infection of both *Trichostrongylus* spp and *H. contortus*. Fat score followed the same trend suggesting a significant impact of worms from February to April. The differences remained until the end of the study when the treatments began to converge again coinciding with mid-pregnancy and lower feed availability on HI (see Figure 2-3, Chapter 2). On the other hand, WF sheep on the IRG treatment had similar dMFD, bodyweights and fat scores to CM sheep throughout the whole experimental period. This is concurrent with generally low WEC with a maximum count in November of ~1500eggs/g (mostly *H. contortus*) which did not seem to affect bodyweights of the CM sheep. The dMFD profile on the TYP management treatment is unusual in that bodyweights started to diverge after moderate *H. contortus* infections in October and December and a large infection in November, but the dMFD did not. Fat scores were also divergent from February to April on TYP following a similar trend to the HI treatment. This difference in bodyweight and fat score between worm control treatments is reflected in the pregnancy rates on the TYP treatment but not in the dMFD profile which does not differ until July when it is lower in WF sheep. Differences in worm species is most likely responsible for the differences in dMFD profiles observed between the HI and TYP systems. There were higher proportions of *Trichostrongylus* spp. on HI and lower proportions on TYP which may be a result of stocking rate as Thamsborg *et al.* (1996) demonstrated increasing pasture infectivity of *Trichostrongylus vitrinus* with increasing stocking rate. This nematode species has been shown to be present alongside *Trichostrongylus colubriformis* in the New England and the increased levels of *Trichostrongylus* spp. in culture could be due to the presence of this worm species (Wooster 1997). Both *Trichostrongylus* spp. and *H. contortus* infections have been shown to significantly reduce fibre diameter (Albers *et al.* 1988; Larsen *et al.* 1995) although *Trichostrongylus* spp are thought to have a greater effect (Donald 1979). Indeed, there was no difference in cMFD between WF and CM sheep on the TYP treatment where *H. contortus* was the only worm to reach levels that caused bodyweight losses. As mentioned above, Brown *et al* (1985) found greater production losses due to nematodes at higher stocking rates. This may have contributed to observed treatment differences in this experiment with production loss seen on the HI (13.2 DSE/ha) relative to the TYP management treatment (9.2 DSE/ha). However there is conflicting evidence on the effect of stocking rate on worm burdens with some studies reporting no effect whilst others report a significant positive association which was dependant on worm species (Beveridge *et al.* 1985; Brown *et al.* 1985; Waller *et al.* 1987; Thamsborg *et al.* 1996).

The level at which WEC begins to have a negative effect on bodyweight is dependant upon species. Levels above 2000egg/g of an *H. contortus* infection begin to have an association with significant bodyweight loss in weaner sheep related to the blood loss associated with infections of this species (Albers *et al* 1990; Le Jambre, 1995; Le Jambre 2006). *Trichostrongylus* spp. cause bodyweight losses at much lower faecal worm egg counts with counts in the range of 200-500eggs/g causing significant production losses. Egg counts between 500 and 2000 eggs/g result in scouring and rapid bodyweight loss (Brightling 1988). Steel *et al* (1980) suggested that 950-3000eggs/g of *T. colubriformis* was the threshold level of exposure for reduced liveweight gain and wool growth. Thus, on the HI treatment there were 2 months where *H. contortus* and 2 months where *Trichostrongylus* spp. WEC were high enough to cause significant bodyweight, fat score and wool production losses. On the TYP treatment *H. contortus* WEC was high enough in 3 months to cause production losses whereas *Trichostrongylus* spp. did not reach levels above 100 eggs/g on this management system. On the IRG treatment there were no periods in which either *H. contortus* or *Trichostrongylus* spp. WEC were high enough to cause bodyweight losses which is reflected in the lack of difference between WF and CM sheep for most of the other measured traits.

The effect of nematode infections on pregnancy rates were quite marked on HI and TYP with a reduction of 26.3% and 33.6% respectively. Although the model showed no significant difference for any of the terms. Morris and Meek (1980) suggest that, due to its binary nature, data collected for measurement of reproductive performance should be from a sample size of at least 100 for ruminant animals. A figure of which this study clearly falls short. Although it was not statistically significant this magnitude of reduction has biological significance. The lower bodyweight and fat scores in CM sheep on HI and TYP, compared to WF sheep, would have driven the decline in pregnancy rates with both these traits inextricably linked to lower pregnancy and lambing rates (Kenyon *et al.* 2004). The effect of nematode infections on pregnancy rate is also supported by the lack of difference in bodyweights and fat scores between WF and CM sheep on IRG reflected in the lack of any real difference in pregnancy rate. Thus, where nematode infections significantly reduce bodyweights and fat scores, a subsequent reduction in reproductive potential results.

Overall greasy fleece weights were heavier from WF sheep compared to CM sheep. Whilst the interaction was not significant there was a strong trend for heavier fleece weights in WF sheep

on HI and TYP (240 and 210g heavier) but not on IRG on which there was only a 40g difference between worm control treatments. Again the experimental power may not have been strong enough to detect a significant difference in this trait but the differences should not be ignored. Johnstone *et al.* (1979), in a study near Armidale that where sheep had natural infections of *H. contortus* and *T. colubriformis*, found that greasy fleece weights increased with increasing anthelmintic treatment. Brown *et al.* (1985), too, showed that greasy fleece weight increased with increasing level of anthelmintic treatment, but also showed an effect of stocking rate on those changes. The difference in greasy fleece weight between sheep given 5 anthelmintics per annum and those given 17 was less on the lower stocking rate (130g, 7.5 sheep/ha) compared to the high (200g, 16 sheep/ha). A similar pattern occurred in this experiment with the CM sheep on HI losing an extra 40g of greasy fleece weight than the CM sheep on TYP. Staple strength was generally lower in CM sheep, but did not differ significantly between treatments or management systems. These results are in agreement with those of Brown *et al* (1985) who found no effect of treatment on staple strength, although a higher proportion of tender fleeces was observed on the higher stocking rate. Thompson and Callinan (1981) also observed a higher percentage of tender fleeces in the low drench frequency and zero treatment groups of their experiment suggesting sheep with a higher level of worm infection were more prone to produce tender fleeces ('tender' fleeces were determined subjectively by a professional wool classer). Lipson and Bacon-Hall (1976) studied the processing characteristics of the wool collected during the experiments reported by Johnstone *et al.* (1979) and also found an increase in tenderness and a higher degree of fibre breakage during carding and combing in the salvage treated sheep. The fact that peak worm infections coincided with periods of peak feed availability in the present experiment may explain the lack of an effect on worm control treatment on staple strength. Also the CM sheep had a relatively high level of worm control relative to some treatments in the study of Thompson and Callinan (1981) and Johnstone *et al* (1979). The yield of clean wool for WF sheep on the TYP and IRG management systems was lower than that of CM sheep. This suggests that wool growth is reduced by worm infection to a greater extent than wax and suint production. This is not unreasonable given the high and specific protein requirements for wool growth, and the fact that worm infection causes major disruption to protein metabolism. Thompson and Callinan (1981) and Johnstone (1979) did not find significant differences in yield between their worm control treatments. The absence of a worm treatment effect on staple length is puzzling, as there were treatment effects on fleece weight on HI and TYP one would expect an increase in staple length on those systems. This suggests that effects on fleece weight were mediated more

by effects on fibre diameter than length. Thus, during this experiment, where GIN was not sufficiently controlled, it had a significant impact on greasy fleece weight and fibre diameter which are the two main drivers of fleece value.

The economic analysis of the costs of worm infection was particularly revealing. It showed that, taking reproduction rates and fleece value into account, there was no benefit from keeping sheep "worm free" on the IRG system whereas there was a considerable benefit on the other two management systems. This is particularly noteworthy when it is taken into account that the sheep on the conventional IRG system only received a single anthelmintic treatment during the year-long experiment whereas those on the HI and TYP treatments received 5 treatments each. Thus, ewe hoggets on the IRG system, receiving a single anthelmintic treatment suffered no discernable effects of worm infection, whereas similar hoggets on the HI and TYP treatments continued to suffer considerable losses due to worms despite being administered 5 anthelmintic treatments during the experiment.

In conclusion the results of this experiment suggest that sheep in management systems conducive to higher worm infestations (eg. HI and TYP) continue to suffer significant production losses due to worm infection. On the other hand, sheep on systems such as IRG which maintain very low levels of WEC suffer no detectable production loss due to worms. The level at which WEC was associated with production loss in this study seemed to be above 2000 eggs/g for *H. contortus* and 250eggs/g for *Trichostrongylus* spp which is consistent with other reports. The mean WEC of IRG managed sheep did not rise above these levels. Large peaks in WEC (as observed on the HI and TYP treatments) seem to have a greater and more sustained effect on production than small, persistent infections. These findings support those of Experiments 1 and 3 by demonstrating that the IRG management system is a highly effective means of controlling GIN in this environment while, at the same time dramatically reducing the use of anthelmintics.

CHAPTER 8: General Discussion

The unifying hypothesis of this thesis was that intensive rotational grazing reduces faecal worm egg count in sheep and that this reduction is mediated through interruption of the nematode lifecycle in its free-living stages. The results from the experiments reported in this thesis provide strong evidence in favour of this hypothesis.

Lower total WEC was observed in IRG sheep during Experiment 1 (Chapter 3) and was achieved with only half the number of anthelmintic treatments given to sheep on the other two management systems. The subsequent experiments were designed to tease out the mechanisms behind this phenomenon. The fixed larval challenge study (Experiment 2, Chapter 4) showed that IRG sheep exhibited resistance to infection that was no better, and in two seasons much worse, than sheep on the HI and TYP treatments ruling out improved host resistance as a factor mediating the effects of IRG. In contrast the tracer experiment and pasture sampling (Expts 3 and 4, Chapters 5 and 6) demonstrated reduced pasture infectivity for all classes of stock on the IRG treatment for the 3 seasons of the year (winter, spring and summer) when the short graze periods and long rest periods were maintained. The tracer studies and fixed larval challenge both show that the dynamics of GIN epidemiology can change rapidly with small changes in the rotations on the IRG system which reinforces the hypothesis that GIN is reduced on IRG through interruption of the free-living stages of the parasitic lifecycle. The lower proportions of *H. contortus* contributing to WEC on the IRG treatment in Experiment 1 (Chapter 3) were also observed in the tracer experiment confirming a differential effect of IRG on the main worm species. Experiment 5 (Chapter 7) further confirms the high level of control of GIN achieved on IRG, with no production losses attributable to nematodes on IRG whilst bodyweights, fat score and fleece weights were higher in 'worm-free' sheep on the HI and TYP treatments. Faecal worm egg counts were used as a measure of GIN throughout this thesis and, although total worm counts were not performed, they proved to be a reliable indication. The results of Experiment 5 (Chapter 7) give strong support to the use of WEC as an indicator of GIN as higher WEC were strongly associated with higher production losses.

8.1 How does IRG work?

The reduction of GIN on IRG was not mediated by the host with a much lower level of resistance to infection observed on that management system compared to the HI and TYP treatments. The tracer study (Expt 3, Chapter 5) demonstrated that sheep on IRG had lower levels of larval challenge. Thus, the grazing management on IRG, consisting of short graze periods (~3 days) and long rest periods (>90 days), probably breaks the nematode lifecycle in its environmental stages in two ways. Firstly by preventing autoinfection from the current grazing period and secondly by allowing a rest period that is sufficient to prevent large numbers of larvae being present on pasture when sheep return to graze (as shown with the artificial plot contamination in Experiment 4, Chapter 6). A third factor reducing *H. contortus* infection levels is that there are a limited number of days in the year when conditions are conducive to development of eggs to L_3 for that species. In a set stocked system this results in the paddocks being permanently contaminated with *H. contortus* larvae despite sporadic episodes of development. In an intensive rotational grazing system it may mean that development only occurs in 20-50% of paddocks, with no effective contamination resulting from the majority of grazing episodes. Thus, for 50% or more of the time sheep are grazing pastures in which the previous contamination was effectively zero.

The first two criteria are the driving force behind the success of rapid rotational grazing in reducing GIN in the tropics (Barger *et al.* 1994; Chandrawathani *et al.* 1995; Sani *et al.* 1996; Chandrawathani 1997; Gray *et al.* 2000) and "progressional" grazing in northern Scotland (Robertson and Fraser 1933). Both Banks *et al.* (1990) and Barger *et al.* (1994) state that the rapid rotational grazing they employed in the tropics would not work in more temperate climates and they were probably right. The rigid time periods imposed on the rapid rotations work well in the tropics where temperatures are always warm and rainfall relatively constant resulting in constant development of eggs into infective larvae. The dynamics are quite different in temperate climates due to the large variability in rainfall and temperature throughout the year. These result in large fluctuations in development time and survival of the free-living stages which are not evident in the tropics. In the tropics a higher proportion of eggs deposited on pasture would hatch and develop into infective larvae than in this cool temperate climate, thus control of parasitic nematodes would hinge more on rate of larval mortality in the tropics than it would in cooler climates. This is assisted by higher larval mortality rates at high temperatures. On the other hand, in cooler drier climates cold and lack of moisture are often limiting for development of eggs to

L_3 and failure of development is likely to be the major mechanism operating in this environment. This lack of development for much of the time is best exploited by a rotational grazing system in which the lack of development results in complete failure of contamination of paddocks following many or most grazing episodes.

The IRG system is flexible with animal movements from paddock to paddock based on feed on offer, thus rotations speed up or slow down according to pasture growth and recovery rates. So when development of egg to L_3 and larval decay is rapid (i.e.: during summer and late spring) paddock rotations are also rapid. Conversely, when egg development and larval decay is slow (i.e.: autumn and winter) paddock rotations slow down. This flexibility allows for greater worm control in a cool climate than the set timed rotations of rapid rotational grazing. Based on the results of this study, during the warm, wet, summer months graze periods should be no longer than 3 days with rest periods no shorter than 60 to 80 days. In the cooler months graze periods can be lengthened to 7 days with rest periods lengthened also to 90 to 100 days.

8.2 Species differences in the effect of IRG

The effect of IRG on *Trichostrongylus* spp. was not as marked as its effect on *H. contortus*. This contrasts with the high efficacy of rapid rotational grazing against both genera in of the tropics (Barger *et al.* 1994). The reason that these authors did not see a differential effect of rapid rotational grazing on worm species was most likely due to the constant rainfall, warm temperatures and resulting high humidity prevailing in their tropical studies. Under these conditions *Trichostrongylus* spp. behave similarly to *H. contortus* with a high development rate as well as a rapid larval decay rate. There appeared to be a small effect of IRG on reducing *Trichostrongylus* spp. in the current study but results were difficult to interpret due to a generally low contribution to WEC from this genus during the experimental period and differences in anthelmintic treatment frequencies between treatments. There were definitely lower *Trichostrongylus* spp. WECs on the IRG than the HI management system in Experiment 1 (Chapter 3) but there was no difference between the TYP and IRG treatments. However, IRG had many fewer anthelmintic treatments than TYP so the IRG treatment had low *Trichostrongylus* spp WEC coupled with a low level of chemical intervention, suggestive of a beneficial effect of IRG. In experiment 3 (tracer study, Chapter 5) there was no overall effect of management treatment on *Trichostrongylus* spp. WEC although the IRG treatment had significantly higher *Trichostrongylus* spp. WEC than the other treatments in autumn when

infection levels were extremely low, and the TYP treatment had significantly higher *Trichostrongylus* spp. WEC than the HI treatment in spring. Again it could be argued that if IRG had no effect on *Trichostrongylus* spp., one would expect to see significantly higher *Trichstrongylus* spp. WEC on this treatment, given the low level of anthelmintic treatment used. The fact that the tracers used were not immunologically naïve to nematode infection prior to this experiment, and the relatively longer retention of resistance to *Trichostrongylus* spp. (Gibson *et al.* 1970; Barnes and Dobson 1993), may have biased the results concerning that nematode genera. Further work is required to provide a definitive conclusion about the effects of IRG on *Trichostrongylus* and *Teladorsagia*.

The differences in free-living ecology of *H. contortus* and *Trichostrongylus* spp. in this climate go some way to explain why IRG reduced *H. contortus* numbers more effectively. The eggs of *H. contortus* hatch between 3 and 5 days after deposition at 25-26°C and between 15 and 30 days after deposition at 10-11°C (Rose 1963) and require additional moisture within a short time of deposition (~5 days) to survive and develop (Donald 1973; Rossanigo and Gruner 1995). On the other hand the eggs and larvae of *Trichostrongylus* spp. and *Teladorsagia* spp. are significantly more resistant to the effects of desiccation and cold than those of *H. contortus* and thus survive for a much longer period (Waller and Donald 1970; Jasmer *et al.* 1986; Jasmer *et al.* 1987; Rossanigo and Gruner 1995). This superiority of *Trichostrongylus* spp. and *Teladorsagia* spp. eggs to survive until better conditions prevail for development suggests that, although IRG would have some effect on these genera by prevention of autoinfection, the eggs deposited are better placed to develop and survive the long rest periods including those rest periods that occur during the cold autumn and winter months. Indeed, *Trichostrongylus* spp. and *Teladorsagia* spp. were recovered in greater proportions from tracers on IRG in winter and spring, although these genera were also cultured in summer and were found in faecal cultures in all seasons of Experiment 4 (Chapter 6).

8.3 Possible differences in anthelmintic resistance between management systems

A faecal egg count reduction test was conducted on the IRG system only and was reported in section 2.4.3. Thus there was not the opportunity to compare anthelmintic resistance across the management systems. However differences between treatments cannot be ruled out. The management treatments imposed on sheep on the Cicerone Project commenced in 2000 and

caused major differences in faecal worm egg counts and anthelmintic treatment frequency. The HI and TYP treatments received a similar number of anthelmintic treatments over the experimental period but still had differences in the incidence of *Trichostrongylus* spp. with a higher incidence of this nematode genus on the HI system. On both of these management systems there was some evidence of resistance to the double doses of albendazole and levamisole given simultaneously during Experiment 2 (Chapter 4). There were low recoveries of *Trichostrongylus* spp. larvae from faecal culture on these treatments 7 days after the anthelmintic treatment and none from IRG sheep. This suggests that sheep on the HI and TYP treatments may have a similar resistance status for this anthelmintic combination. However, during experiment 5 (Chapter 7) on the TYP treatment there were breakthrough faecal egg counts 67 days after a slow release capsule containing albendazole was administered (mean 230epg, range 0-600epg, 100% *H. contortus*). Faeces from the other two management treatments yielded some larvae on faecal culture but no eggs were observed during WEC estimation. This implies that there are possible differences in benzimidazole resistance between the management systems.

The fact that IRG sheep have such low frequency of anthelmintic treatment may imply that worms on this type of system may not develop resistance to anthelmintic compounds as rapidly as a set stocked system where treatment is frequent. However, the low levels of refugia on pasture on an IRG system may provide greater selection pressure for development of resistance for any given anthelmintic treatment. This is because resistant worms that survive an anthelmintic treatment produce offspring that are diluted less by refugia than might be the case on a slow rotation or set stocked pasture. However there was no evidence in the present study of an inferior drench resistance status on the IRG system, suggesting that the low frequency of treatments more than compensated for the low level of refugia.

8.4 Comparison of the HI and TYP management systems

There were very few differences between the HI and TYP systems throughout all experiments although there were some differences in the incidence of *Trichostrongylus* spp. During Experiment 1 (Chapter 3) HI and TYP sheep had similar bodyweights, fleece weight, mean fibre diameter, haematocrit, eosinophil counts, *H. contortus* WEC and *Teladorsagia* spp. present in culture. They also had a similar number of anthelmintic treatments and similar intervals between anthelmintic treatments. This similarity of HI and TYP is also reflected in the timing and magnitude of peaks in WEC which often occurred simultaneously on the two management

systems. However there were differences in *Trichostrongylus* spp. WEC with HI having a higher proportion of this genus both in relative (larval differentiation) and absolute (larval diff applied to WEC) terms. HI and TYP sheep also differed in fat score with those on the HI treatment being significantly fatter than those on the TYP treatment. The IRG sheep were intermediate. Although there were also a lot of similarities between HI and TYP during the fixed challenge (Experiment 2, Chapter 4), TYP sheep had slightly lower resistance to infection than HI but it was not statistically significant.

There were also very few differences between the HI and TYP management treatments for the tracer study (Experiment 3, Chapter 5). Tracers on both systems had similar WECs in winter, spring and autumn and similar proportions of *H. contortus*, *Teladorsagia* spp. and *Trichostrongylus* spp. in faecal culture. Summer was the only season these two treatments differed with the TYP treatment having significantly higher WEC, but even then both treatments had similar proportions of the major worm species.

The comparison of HI and TYP for production losses due to worms (Experiment 5, Chapter 7) also showed similar results for bodyweight, fat score, fleece weight and pregnancy rate. However, there was no effect of nematode infection on fibre diameter in the TYP treatment whereas it significantly reduced fibre diameter in HI sheep. This difference is most likely to be due to the higher *Trichostrongylus* spp. infections in the HI treatment as this genus has a greater effect on production traits than *H. contortus* (Donald 1979). Stocking rate may also have contributed to this difference; however, the results from other studies on the effect of stocking rate on GIN are variable (Beveridge *et al.* 1985; Brown *et al.* 1985; Waller *et al.* 1987; Thamsborg *et al.* 1996).

8.5 Production differences between management systems

Although sheep on IRG had significantly better nematode control with lower WEC, a lower number anthelmintic treatments and no discernable production losses due to nematodes, it did not translate into a higher level of animal production. Sheep that were born prior to this experiment (older ewes and year 1 hoggets) had lower bodyweights compared to HI and TYP than those born during the experiment (year 1 and 2 lambs and year 2 hoggets). There were lower reproductive rates on IRG (as seen in Expt 5, Chapter 7) and lower fleece weights in all classes of sheep. Problems with bodyweight and wool production on IRG prior to 2003 were

most probably due to the rest periods being too long (up to 150-200 days) and the stocking rate not high enough resulting in grass becoming over-mature and losing its nutritional value. Lambs born prior to 2003 were generally lighter in bodyweight and produced less wool as hoggets and adults (Cicerone Project Inc. unpublished). As a result of poor performance of sheep on IRG, rest periods were shortened and, as can be seen from the results in Chapter 3, the lambs born in 2003 and 2004 have similar liveweights and growth rates to the HI and TYP lambs. The balance required to run a successful IRG system was simply not present prior to 2003 with too much emphasis on pasture recovery from grazing and not enough emphasis on animal production. This illustrates the nature of IRG systems where an equilibrium is necessary between requirements for pasture sustainability and animal requirements, which bring in the money. Discussion of the achievements and pitfalls of IRG systems is not within the scope of this thesis. However, the IRG treatment on the Cicerone Project reflects the need for proper understanding for implementation of this type of management system in order to achieve the benefits of both nematode control and better animal production.

8.6 Review of the experimental approach

There were many positive and a few negative aspects of working within a larger scale farming systems project such as the Cicerone Project. On a positive note, there was an enormous quantity of quality data available for use that had been collected contemporaneously with the experiments described in this thesis. These data not only permitted essential analyses (ie: graze and rest periods and wool measurements), but also allowed greater, more in depth, interpretation of the results of these experiments. As such the Cicerone Project was a unique environment in which to work. One downside was that this experimenter had no control over any management decisions made on the farmlets which were made by a panel of board members with little or no reference to these experiments. Rather decisions were made in reference to the Cicerone Project as a whole. As such these experiments are comparing populations of sheep in three systems which have been managed externally. However the consulting parasitologist generally recommended the use of short acting anthelmintics during the experimental period where possible to allow differences in parasite control due to other factors in the management systems to become apparent.

A concern of fellow scientists with the experimental work on the Cicerone project is the lack of farmlet replication. Replication on site, in this instance, would not only have been costly but it

may not have provided any further information as the geography of soil, slope and fertiliser history were accounted for in the planning stages of the Cicerone Project farmlets and the location of the management treatments was interspersed over a large geographical area. The confounding of the effects of treatment and location which is inherent in unreplicated designs, is therefore neutralized in this case by the careful planning, wide geographical spread and intermingling of paddocks under the different management systems. However there is no doubt that this study should be replicated at other geographic locations to broaden the inferences that can be drawn from it. The great care taken in establishing the Cicerone Project makes it difficult to ascribe the observed effects to anything other than the different management systems imposed. The reduction in GIN observed on the IRG treatment also makes strong biological sense and is consistent with our existing understanding of the ecology of gastrointestinal nematodes. These two points lend considerable credence to the collection of experiments in this thesis providing enough evidence between them to confidently link a cause (intensive rotational grazing) to the effect (reduced GIN).

8.7 Implications for the application of intensive rotational grazing for worm control

Intensive rotational grazing is usually implemented for improved pasture sustainability, animal performance and pasture performance rather than worm control. However, the worsening anthelmintic resistance problem, along with consumer concerns about animal welfare and chemical use in agriculture is putting pressure on sheep producers to run healthy sheep with minimal chemical intervention. Intensive rotational grazing may provide part of the solution to these issues where it can be applied. It is a good candidate for inclusion in integrated parasite management (IPM) strategies incorporating chemical and other non-chemical approaches to worm control. The greater diversity of plant and microbial species claimed to result from intensive rotational grazing could potentially nurture dung beetles and nematophagus fungi maintaining them in the farm ecosystem. Chandrawathani *et al* (2004) have already combined rapid rotational grazing with the use of nematophagus fungi with success. Combining worm control tools in this way reduces the reliance on a single form of control, as all worm control measures are susceptible to evolution of the parasite to overcome their effect.

The main barriers to the adoption of intensive rotational grazing are scepticism of producers about the outcomes of these management systems, and the costs associated with implementing

infrastructure such as fencing and watering points. There is also a limitation for running breeding ewes with disturbances during lambing due to paddock moves and the need to split up mobs for joining and lambing at times of the year when having the smallest number of mobs is desirable for nurturing pasture regrowth (i.e.: autumn and spring). As the Cicerone Project has shown, improved control of GIN using IRG has not necessarily translated into improved animal performance on this system. It is plausible that the theoretical benefits of IRG could be achieved with careful attention to implementation such that the worm control benefits are retained while the lower production levels are eliminated. Indeed this is the experience on the Cicerone project with the production penalty associated with the IRG treatment reducing markedly over the life of the project with experience and changes to the IRG system.

8.8 Main Conclusions

The results of this thesis indicate that substantial control of the sheep gastrointestinal parasite *Haemonchus contortus* can be achieved through intensive rotational grazing. Strong evidence is provided to suggest that IRG works by interrupting the nematode life-cycle in its free-living stages in two ways; i) prevention of autoinfection through short grazing periods and; ii) prevention of large scale re-infection of sheep by allowing resting of paddocks sufficient for significant decline in the population of infective larvae on pasture. Due to frequent rotation between paddocks and the narrow environmental criteria required for development of *H. contortus* eggs to L_3, successful development would only occur in a small number of paddocks and infections would not be perpetuated as they are on grazing systems with longer rotations or set-stocking. The ability to control one of the most devastating nematode parasites through non-anthelmintic means in a cool temperate environment is a breakthrough for cool climate sheep producers. The fact that intensive rotational grazing works on the environmental stages of the parasitic nematode means that a high proportion of the nematode population are susceptible and are likely to remain susceptible to this form of control. Intensive rotational grazing also adds another string to the bow of producers looking to integrate their worm control strategies. It will not eliminate the use of anthelmintic treatments but it will reduce their frequency and the dependence upon them and could possibly translate well to the control of cattle nematodes. There were some issues with animal production on intensive rotational grazing with lighter fleece weights and lighter bodyweights in sheep born prior to this experiment. However, sheep born during the experiment did not have lower bodyweights or bodyweight gains than those on

the other two systems and there was a trend for a reduced penalty on the IRG treatment over the life of the Cicerone Project.

The experimental work reported in this thesis suggests that there is a smaller impact of intensive rotational grazing on *Trichostrongylus* and very little, if any, on *Teladorsagia* spp. Further work is warranted to establish the effect of intensive rotational grazing on the latter worm species and would be best carried out in regions where they are the predominant pathogens. Further work is also needed to confirm the effect of intensive rotational grazing on *H. contortus* in different cool climates to determine the range of its efficacy. However, first principles suggest that IRG of the type reported in this thesis would have wide application in the control of *H. contortus* infection with some regional variation in precise IRG strategies.

References

Aalund O (1972) Immune response of sheep, goats, cattle and swine. In 'Immunity to Animal Parasites'. (Ed. EJL Soulsby) pp. 1-26. (Academic Press: New York)

Abbott EM, Holmes PH (1990) Influence of dietary protein on the immune responsiveness of sheep to *Haemonchus contortus*. *Research in Veterinary Science* **48**, 103-107.

Albers GAA, Gray GD, Le Jambre LF, Piper LR, Barger IA, Barker JSF (1988) The effects of *Haemonchus contortus* on liveweight gain and wool growth in young Merino sheep. *Australian Journal of Agricultural Research* **40**, 419.

Albers GAA, Gray GD, Piper LR, Barker JSF, Le Jambre LF, Barger IA (1987) The genetics of resistance and resilience to *Haemonchus contortus* infection in young merino sheep. *International Journal for Parasitology* **17**, 1355-63.

Allonby EW, Urquhart GM (1973) Self-cure of *Haemonchus contortus* infection under field conditions. *Parasitology* **66**, 43-53.

Amarante AFT, Barbosa MA (1998) Comparison between pasture sampling and tracer lambs to evaluate contamination of sheep pastures by nematode infective larvae. *Revista Brasileira de Parasitologia Veterinaria* **7**, 95-99.

Anderson FL, Boatman PA, Levine ND (1965) Development and survival of free-living stages of *Trichostrongylus colubriformis* on pasture. *Journal of Parasitology* **51**, 32.

Anderson FL, Levine ND (1968) Effect of desiccation on survival of the free-living stages of *Trichostrongylus colubriformis*. *Journal of Parasitology* **54**, 117-128.

Anderson FL, Levine ND, Boatman PA (1970) Survival of third-stage *Trichostrongylus colubriformis* larvae on pasture. *Journal of Parasitology* **56**, 209-232.

Anderson FL, Wang G, Levine ND (1966) Effect of temperature on survival of the free-living stages of *Trichostrongylus colubriformis*. *Journal of Parasitology* **52**, 712-721.

Anderson N (1972) Trichostrongyloid infections in sheep in a winter rainfall region. I. Epizootlogical studies in western districts of Victoria. *Australian Journal of Agricultural Research* **23**, 1113-1129.

Anonymous (1989) Report of the working party for the Animal Health Committee of the Standing Committee on Agriculture. SCA Technical Report Series 'Anthelmintic Resistance.' CSIRO, 28.

Anonymous (2004) Alternative de-wormers - Review by Aberdeen University 2004, http://www.abdn.ac.uk/organic/organic_14a.php.

Arundel JH, Hamilton D (1975) The effect of mixed grazing of sheep and cattle on worm burdens in lambs. *Australian Veterinary Journal* **51**, 436-439.

Aumont G, Gruner L (1989) Population evolution of the free-living stage of goat gastrointestinal nematodes on herbage under tropical conditions in Guadeloupe (French West Indies). *International Journal for Parasitology* **19**, 539-546.

Australian Bureau of Meteorology (2006) www.bom.gov.au/climate/averages/tables/cw-056002.shtml. In.

Baker RL, Nagda S, Rodriguez SL, Southey BR, Audho JO, Aduda EO, Thorpe W (2003) Resistance and resilience to gastro-intestinal nematode parasites and relationships with productivity of Red Maasai, Dorper and Red Maasai X Dorper crossbred lambs in the sub-humid tropics. *Animal Science* **76**, 119-136.

Banks DJD, Singh R, Barger IA, Pratap B, Le Jambre LF (1990) Development and survival of infective larvae of *Haemonchus contortus* and *Trichostrongylus colubriformis* on pasture in a tropical environment. *International Journal for Parasitology* **20**, 155-160.

Barger A, Le Jambre LF, Georgi JR, Davies HI (1985) Regulation of *Haemonchus contortus* populations in sheep exposed to continuous infection. *International Journal for Parasitology* **15**, 529-533.

Barger IA (1973) Feed digestibility and mineral absorption in infected sheep. *Australian Journal of Experimental Agriculture and Animal Husbandry* **13**, 42-47.

Barger IA (1989) Genetic resistance of hosts and its influence on epidemiology. *Veterinary Parasitology* **32**, 21-35.

Barger IA (1993) Influence of sex and reproductive status on susceptibility of ruminants to nematode parasitism. *International Journal for Parasitology* **23**, 463-469.

Barger IA (1997) Control by management. *Veterinary Parasitology* **72**, 493-506.

Barger IA (1999) The role of epidemiological knowledge and grazing management for helminth control in small ruminants. *International Journal for Parasitology* **29**, 41-47.

Barger IA, Beynon PR, Southcott WH (1972) Simulation of pasture larval populations of *Haemonchus contortus*. In 'Proceedings of the Australian Society of Animal Production' pp. 38-42.

Barger, IA, Lewis, RJ and Brown, GF (1984) Survival of infective larvae of nematode parasites of cattle during drought. *Veterinary Parasitology* **14**, 143-152.

Barger IA, Siale K, Banks DJD, Le Jambre LF (1994) Rotational grazing for control of gastrointestinal nematodes of goats in a wet tropical environment. *Veterinary Parasitology* **53**, 109-116.

Barger IA, Southcott WH (1975) Trichostrongylosis and wool growth. 3. The wool growth response of resistant grazing sheep to larval challenge. *Australian Journal of Experimental Agriculture and Animal Husbandry* **15**, 167-172.

Barger IA, Southcott WH (1978) Parasitism and production in weaner sheep grazing alternately with cattle. *Australian Journal of Experimental Agriculture and Animal Husbandry* **18**, 340-346.

Barnes EH, Dobson RJ (1990) Population dynamics of *Trichostrongylus colubriformis* in sheep: computer model to simulate grazing systems and the evolution of anthelmintic resistance. *International Journal for Parasitology* **20**, 823-831.

Barnes EH, Dobson RJ (1993) Persistence of acquired immunity to *Trichostrongylus colubriformis* in sheep after termination of infection. *International Journal for Parasitology* **23**, 1019-1026.

Barton NJ, Brimblecombe CJ (1983) The effects of anthelmintic treatment and season on the quantity and quality of wool grown by Merino sheep. *Australian Journal of Agricultural Research* **34**, 557-568.

Berbarian JF, Mizelle JD (1957) Developmental studies on *Haemonchus contortus* Rudolphi (1803). *American Midland Naturalist* **57**, 421-439.

Besier RB, Dunsmore JD (1993a) The ecology of *Haemonchus contortus* in a winter rainfall climate in Australia: the survival of infective larvae on pasture. *Veterinary Parasitology* **45**, 293-306.

Besier RB, Dunsmore JD (1993b) The ecology of *Haemonchus contortus* in a winter rainfall region in Australia: the development of eggs to infective larvae. *Veterinary Parasitology* **45**, 275-292.

Besier RB, Love SCJ (2003) Anthelmintic resistance in sheep nematodes in Australia: the need for new approaches. *Australian Journal of Experimental Agriculture* **43**, 1383-1391.

Beveridge I, Brown TH, Fitzsimons SM, Ford GE, Judson GJ, Martin RR, Miller DW (1985) Mortality in weaner sheep in South Australia under different regimes of anthelmintic treatment. *Australian Journal of Agricultural Research* **36**, 857-865.

Bisset SA, Morris CA (1996) Feasibility and implications of breeding sheep for resilience to nematode challenges. *International Journal for Parasitology* **26**, 857-868.

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

Bisset SA, Vlassoff A, Douch PGC, Jonas WE, West CJ, Green RS (1996) Nematode burdens and immunological responses following natural challenge in Romney lambs selectively bred for low or high faecal worm egg count. *Veterinary Parasitology* **61**, 249-263.

Bisset SA, Vlassoff A, West CJ, Morrison L (1997) Epidemiology of nematodosis in Romney lambs selectively bred for resistance or susceptibility to nematode infection. *Veterinary Parasitology* **70**, 255-269.

Boag B, Thomas RJ (1970) The development and survival of free-living stages of *Trichostrongylus colubriformis* and *Ostertagia circumcincta* on pasture. *Research in Veterinary Science* **11**, 380-381.

Boag B, Thomas RJ (1985) The effect of temperature on the survival of infective larvae of nematodes. *Journal of Parasitology* **71**, 383-384.

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

Bown MD, Poppi DP, Sykes AR (1991a) The effect of post-ruminal infusion of protein or energy on the pathophysiology of *Trichostrongylus colubriformis* infection and body composition in lambs. *Australian Journal of Agricultural Research* **42**, 253-267.

Bown MD, Poppi DP, Sykes AR (1991b) Nitrogen transactions along the digestive tract of lambs concurrently infected with *Trichostrongylus colubriformis* and *Ostertagia circumcincta*. *British Journal of Nutrition* **66**, 237-249.

Brightling A (1988) 'Sheep Diseases.' (Inkata Press: Melbourne)

Brown DJ, Crook BJ (2005) Environmental responsiveness to fibre diameter in grazing fine wool Merino sheep. *Australian Journal of Experimental Agriculture* **56**, 673-684.

Brown TH, Ford GE, Miller DW, Beveridge I (1985) Effect of anthelmintic dosing and stocking rate on the productivity of weaner sheep in a mediterranean climate environment. *Australian Journal of Agricultural Research* **36**, 845-855.

Brunsdon RV (1970) The spring-rise phenomenon: seasonal changes in the worm burdens of breeding ewes and in the availability of pasture infection. *New Zealand Veterinary Journal* **18**, 47-54.

Brunsdon RV, Vlassoff A (1982) Production and parasitological responses of lambs exposed to differing levels of trichostrongylid larvae on pasture. *New Zealand Journal of Experimental Agriculture* **10**, 391-394.

Buddle BM, Jowett G, Green RS, Douch PGC, Risdon PL (1992) Association of blood eosinophilia with the expression of resistance in Romney lambs to nematodes. *International Journal for Parasitology* **22**, 955-960.

Burr P (2006) Climate data. In. (University of New England: fehps.une.edu.au/x/weather/)

Callinan APL (1978) The ecology of the free-living stages if *Ostertagia circumcincta*. *International Journal for Parasitology* **8**, 233-237.

Callinan APL, Westcott JM (1986) Vertical distribution of trichostrongyloid larvae on herbage and in soil. *International Journal for Parasitology* **16**, 241-244.

Carter HB, Franklin MC, Gordon HM (1946) The effect on wool production of a mild infestation with *Trichostrongylus colubriformis*. *Journal of the Council for Scientific and Industrial Research* **10**, 313.

Chandrawathani P (1997) Control of gastrointestinal helminths in small ruminants the Malaysian perspective. In 'FAO Animal Production and Health Paper: Proceedings of a workshop organized by FAO for the Danish Centre for Experimental Parasitology'. Malaysia pp. 78-81. (Food and Agriculture Organisation of the United Nations)

Chandrawathani P, Jamnah O, Adnan M, Waller PJ, Larsen M, Gillespie AT (2004) Field studies on the biological control of nematode parasites of sheep in the tropics, using the microfungus *Duddingtonia flagrans*. *Veterinary Parasitology* **120**, 177-187.

Chandrawathani P, Jamnah O, Rajamanickham C (1995) Testing potential control programmes for helminthosis in sheep on open pasture. In '17th Malaysian Society of Animal Production Annual Conference'. Penang pp. 114-115

Ciordia H, Brizzell WE (1963) The effects of various constant temperatures on the development of the free-living stages of some nematode parasites of cattle. *Journal of Parasitology* **49**, 60-63.

Clunies Ross IC, Gordon HM (1933) Nutritional factors affecting resistance to Haemonchosis. *Australian Veterinary Journal* **9**, 100.

Cole VG (1986) 'Helminth parasites of sheep and cattle.' (Government Publishing Service: Canberra)

Coles GC, Bauer C, Borgsteede FHM, Geerts S, Klei TR, Taylor MA, Waller PJ (1992) World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) methods for the detection of anthelmintic resistance in nematodes of veterinary importance. *Veterinary Parasitology* **44**, 35-44.

Connan RM (1967) Observations on the post-parturient rise in the faecal nematode egg count of ewes. *The Veterinary Record* **80**, 401.

Coop RL, Angus KW (1975) The effect of continuous doses of *Trichostrongylus colubriformis* larvae on the intestinal mucosa of sheep and on liver vitamin A concentration. *Parasitology* **70**, 1-9.

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

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

Coop RL, Sykes AR, Angus KW (1982) The effect of three levels of intake of *Ostertagia circumcincta* larvae on growth rate, food intake and body composition of growing lambs. *Journal of Agricultural Science, Cambridge* **98**, 247-255.

Courtney CH, Parker CF, McClure KE, Herd RP (1983) Population dynamics of *Haemonchus contortus* and *Trichostrongylus spp.* in sheep. *International Journal for Parasitology* **13**, 557-560.

Coyne MJ, Smith G (1992a) The development and mortality of the free-living stages of *Haemonchus contortus* in laboratory culture. *International Journal for Parasitology* **22**, 641-650.

Coyne MJ, Smith G (1992b) The mortality and fecundity of *Haemonchus contortus* in parasite-naive and parasite-exposed sheep following single experimental infections. *International Journal for Parasitology* **22**, 315-325.

Coyne MJ, Smith G, Johnstone C (1991) Fecundity of gastro-intestinal trichostongyloid nematodes of sheep in the field. *American Journal of Veterinary Research* **52**, 1182-1188.

Crofton HD, Whitlock JH (1965a) Ecology and biological plasticity of sheep nematodes. V. The relationship between egg volume and hatching time. *Cornell Veterinarian* **55**, 274-279.

Crofton HD, Whitlock JH (1965b) Ecology and biological plasticity of sheep nematodes. III. Studies on *Ostertagia circumcincta* (Stadelmann 1894). *Cornell Veterinarian* **55**, 259.

Crowther B (1995) 'The wonders of the weather.' (Australian Bureau of Meteorology. Australian Government Printing Service.: Canberra)

Dinaburg AG (1944a) Development and survival under outdoor conditions of eggs and larvae of common ruminant stomach worm, *Haemonchus contortus*. *Journal of Agricultural Research* **69**, 421-433.

Dinaburg AG (1944b) The survival of the infective larvae of the common ruminant stomach worm, *Haemonchus contortus*, on outdoor grass plots. *American Journal of Veterinary Research* **5**, 32-37.

Dinaburg AG (1945) The effect of low outdoor temperatures on the free-living stages of some common nematode parasites of sheep. *Journal of Veterinary Research* **6**.

Dobson RJ, Donald AD, Barnes EH, Waller PJ (1990a) Population dynamics of *Trichostrongylus colubriformis* in sheep: model to predict the worm population over time as a function of infection rate and host age. *International Journal for Parasitology* **20**, 365-373.

Dobson RJ, Le Jambre LF, Gill JH (1996) Management of anthelmintic resistance: Inheritance of resistance and selection with persistent drugs. *International Journal for Parasitology* **26**, 993-1000.

Dobson RJ, Waller PJ, Donald AD (1990b) Population dynamics of *Trichostrongylus colubriformis* in sheep: the effect of infection rate on loss of adult parasites. *International Journal for Parasitology* **20**, 359-363.

Dobson RJ, Waller PJ, Donald AD (1990c) Population dynamics of *Trichostrongylus colubriformis* in sheep: the effect of infection rate on the establishment of infective larvae and parasite fecundity. *International Journal for Parasitology* **20**, 347-352.

Donald AD (1967) Populations of strongyloid infective larvae in pastures after sheep are removed from grazing. *Australian Veterinary Journal* **43**, 122-128.

Donald AD (1968) Ecology of the free-living stages of nematode parasites of sheep. *Australian Veterinary Journal* **44**, 139-144.

Donald AD (1973) Bionomics of the free-living stages of the gastro-intestinal nematode parasites of sheep in relation to epidemiology. In 'University of Sydney Post-graduate Committee in Veterinary Science.'

Donald AD (1979) Effects of parasites and disease on wool growth. In 'Physiological and environmental limitations to wool growth (Proceedings of a National Workshop)'. Leura, Australia. (Eds JL Black and PJ Reis) p. 99. (University of New England)

Donald AD, Morley FHW, Axelsen A, Donnelly JR, Waller PJ (1987) Integration of grazing management and anthelmintic treatment for the control of nematode infections in young sheep. In 'Temperate pastures, their use and management.' (Eds W JL, P CJ and R GE) pp. 567-569. (Australian Wool Corporation/CSIRO Technical Publication: Melbourne)

Donald AD, Morley FHW, Waller PJ, Axelsen A, Donnelly JR (1978a) Availability to grazing sheep of gastrointestinal nematode infection arising from summer contamination of pastures. *Australian Journal of Agricultural Research* **29**, 189-204.

Donald AD, Southcott WH, Dineen JK (1978b) 'The epidemiology and control of gastrointestinal parasites of sheep in Australia.' (CSIRO: Australia)

Donald AD, Waller PJ (1973) Gastro-intestinal nematode parasite populations in ewes and lambs and the origin and time course of infective larval availability in pastures. *International Journal for Parasitology* **3**, 219-233.

Dunn AM (1978) 'Veterinary Helminthology.' (William Heinemann Medical Books Ltd: London)

Dynes RA, Ankersmit AEL, Poppi DP, Barrell GK, Sykes AR (1990) Studies on the physiological basis of appetite depression in nematode infection in sheep. In 'Proceedings of the New Zealand Society of Animal Production' p. 249

Eady SJ (1995) Implications of non-normal distribution of faecal egg count for measuring worm resistance in merino sire evaluation schemes. In 'Proceedings of the Australian Association of Animal Breeding and Genetics' pp. 79-83

Eady SJ, Smith JL (2001) Production costs in sheep bred for worm resistance during Haemonchus challenge and infection. In 'Proceedings of the Fourteenth Conference of the Association for the Advancement of Animal Breeding and Genetics'. Queenstown, New Zealand pp. 191-194

Eady SJ, Woolaston RR, Mortimer SI (1994) Internal parasite resistance of merino flocks selected for production. In '5th World Congress of Genetics Applied in Livestock Production.' pp. 289-292

Earl JM, Jones CE (1996) The need for a new approach to grazing management- is cell grazing the answer? *Rangeland Journal* **18**, 327-350.

Eysker M, Bakker N, Kooyman FNJ, Ploeger HW (2005) The possibilities and limitations of evasive grazing as a control measure for parasitic gastroenteritis on small ruminants in temperate environments. *Veterinary Parasitology* **129**, 95-104.

Fincher GT (1973) Dung beetles as biological control agents for gastrointestinal parasites of livestock. *Journal of Parasitology* **59**, 396-399.

Fincher GT (1975) Effects of dung beetle activity on the number of parasites acquired by grazing cattle. *Journal of Parasitology* **61**, 759-762.

Finkelman FD, Pearce EJ, Urban JF, Sher A (1991) 'Regulation and biological function of helminth induced cytokine responses.' (Elservier Trends Journal: Cambridge)

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

Furman DP (1944) Effects of environment upon free-living stages of *Ostertagia circumcincta* (Stadelmann) Trichostrongylidae: I. Laboratory experiments. *American Journal of Veterinary Research* **5**, 79-86.

Gibbs HC, Barger IA (1986) *Haemonchus contortus* and other trichostrongylid infections in parturient, lactating and dry ewes. *Veterinary Parasitology* **22**, 57-66.

Gibson TE (1965) Helminthiasis in sheep. *Veterinary Record* **77**, 1034.

Gibson TE, Everett G (1967) The ecology of the free-living stages of *Trichostrongylus colubriformis*. *Parasitology* **57**, 533-547.

Gibson TE, Everett G (1968) A comparison of set stocking and rotational grazing for the control of Trichostrongylosis in sheep. *British Veterinary Journal* **124**, 287.

Gibson TE, Everett G (1972) The ecology of the free-living stages of *Ostertagia circumcincta*. *Parasitology* **64**, 451-460.

Gibson TE, Everett G (1976) The ecology of the free-living stages of *Haemonchus contortus*. *British Veterinary Journal* **132**, 50-59.

Gibson TE, Parfitt JW, Everett G (1970) The effect of anthelmintic treatment on the development of resistance to *Trichostrongylus colubriformis* in sheep. *Research in Veterinary Science* **11**, 138-145.

Gill HS, Gray GD, Watson DL, Husband AJ (1993) Isotype specific antibody responses to *Haemonchus contortus* in genetically resistant sheep. *Parasitic Immunology* **15**, 61-67.

Githigia SM, Thamsborg SM, Larsen M (2001) Effectiveness of grazing management on controlling gastrointestinal nematodes in weaner lambs on pasture in Denmark. *Veterinary Parasitology* **99**, 15-27.

Githiori JB, Hoglund J, Waller PJ, Leyden Baker R (2003) Evaluation of anthelmintic properties of extracts from some plants used as livestock dewormers by pastoralist and smallholder farmers in Kenya against *Heligmosomoides polygrus* infections in mice. *Veterinary Parasitology* **118**, 215-226.

Gordon HM (1948) The epidemiology of parasitic diseases, with special reference to studies with nematode parasites of sheep. *Australian Veterinary Journal* **26**.

Gray GD, Anchetta PA, Barcelo PM, Casis C, Cerbito WA, Dobson RJ, Dumilon R, Cruz EM, Knox MR, Le Jambre LF, Mateo KS, Suba MS, Villar EC (2000) Better worm control for goats and sheep in the Philippines. *Asian-Australian Journal of Animal Science* **13**, 548.

Healey AF, Hall E, Gaden CA, Scott JM, Walkden-Brown SW (2004) Intensive rotational grazing reduces nematode faecal egg counts in sheep on the Cicerone Project. In 'Animal Production in Australia'. Melbourne pp. 85-88. (CSIRO Publishing)

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

Holst P, White A (2001) Fat scoring sheep and lambs. In 'Agnote DAI 258 (1st Ed)'. (New South Wales Department of Primary Industries)

Hsu CK, Levine ND (1977) Degree-day concept in development of infective larvae of Haemonchus contortus and Trichostrongylus colubriformis under constant and cyclic conditions. *American Journal of Veterinary Research* **38**, 1115-1119.

Jackson F, Coop RL (2000) The development of anthelmintic resistance in sheep nematodes. *Parasitology* **120**, S95-S107.

Jasmer DP, Wescott RB, Crane JW (1986) Influence of cold temperatures upon development and survival of eggs of Washington isolates of *Haemonchus contortus* and *Ostertagia circumcincta*. *Proceedings of the Helminthological Society of Washington* **53**, 244-247.

Jasmer DP, Wescott RB, Crane JW (1987) Survival of third-stage larvae of Washington isolates of *Haemonchus contortus* and *Ostertagia circumcincta* exposed to cold temperatures. *Proceedings of the Helminthological Society of Washington* **54**, 48-52.

Johnstone IL, Darvill FM, Bowen FL, Butler RW, Smart KE, Pearson IG (1979) The effect of four schemes of parasite control on production on Merino wether weaners on two environments. *Australian Journal of Experimental Agriculture and Animal Husbandry* **19**, 303-311.

Jones WO, Symons LEA (1982) Protein synthesis in the whole body, liver, skeletal muscle and kidney cortex of lambs infected by the nematode *Trichostrongylus colubriformis*. *International Journal for Parasitology* **12**, 295-301.

Jordan HE, Phillips WA, Morrison RD, Doyle JJ, McKenzie K (1988) A 3-year study of continuous mixed grazing of cattle and sheep: parasitism of offspring. *International Journal for Parasitology* **18**, 779-784.

Kahn LP, Diaz-Hernandez A (2000) Tannins with anthelmintic properties. In 'Tannins in Livestock and Human Nutrition: Proceedings of and International Workshop'. Adelaide, Australia. (Ed. JD Brooker) pp. 130-139. (Australian Centre for International Agricultural Research)

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

Kahn LP, Knox MR, Walkden-Brown SW, Lea JM (2003b) Regulation of the resistance to nematode parasites of single- and twin-bearing Merino ewes through nutrition and genetic selection. *Veterinary Parasitology* **114**, 15-31.

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

Kahn LP, Walkden-Brown SW, Lea JM (2000b) Dietary enhancement of resistance to *Trichostrongylus colubriformis* in merino weaners. *Asian Journal of Animal Sciences* **13**, 68-71.

Kates KC (1950) Survival on pasture of free-living stages of some common gastrointestinal nematodes of sheep. *Proceedings of the Helminthological Society of Washington* **17**, 39-58.

Kauzal GP (1941) Examination of grass and soil to determine the population of infective larval nematodes on pastures. *Australian Veterinary Journal* **17**, 211-282.

Kemper K, Walkden-Brown SW (2004) Methods for Detecting Anthelmintic Resistance: A Case Study on the New England Tablelands. In 'Proceedings of the Australian Sheep Veterinary Society AVA Conference'. Canberra. (Australian Sheep Veterinary Society)

Kenyon PR, Morel PCH, Morris ST (2004) The effect of individual liveweight and condition scores of ewes at mating on reproductive and scanning performance. *New Zealand Veterinary Journal* **52**, 230-235.

Kimambo AE, MacRae JC, Walker A, Watt CF, Coop RL (1988) Effect of prolonged clinical infection with *Trichostrongylus colubriformis* on the performance and nitrogen metabolism of growing lambs. *Veterinary Parasitology* **28**, 191-203.

Knapp SE (1964) Relationship of different species of forage to the survival and infectivity of *Haemonchus contortus* in lambs. *Journal of Parasitology* **50**, 144-148.

Knox MR (2003) Impact of non-protein nitrogen supplements on nematode infected sheep. *Australian Journal of Experimental Agriculture* **43**, 1463-1468.

Knox MR, Steel JW (1999) The effects of urea supplementation on production and parasitological responses of sheep infected with *Haemonchus contortus* and *Trichostrongylus colubriformis*. *Veterinary Parasitology* **83**, 125-137.

Krecek RC, Groeneveld HT, Maritz JI (1992) A preliminary study of the effect of microclimate on third-stage larvae of *Haemonchus contortus* and *Haemonchus placei* on irrigated pasture. *International Journal for Parasitology* **22**, 747-752.

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

Larsen JWA, Vizard AL, Anderson N (1995) Production losses in Merino ewes and financial penalties caused by trichostrongylid infections during winter and spring. *Australian Veterinary Journal* **72**, 58-63.

Larsen M, Wolstrup S, Henrikson SA, Gronvold J, Nansen P (1992) *In vivo* passage through calves of nematophagus fungi selected for biocontrol of parasitic nematodes. *Journal of Helminthology* **66**, 137-141.

Le Jambre LF (2006) Turning the worm 'Haemonchus and weight gain.' NSW Department of Primary Industries, Armidale.

Leathwick DM, Barlow ND, Vlassoff A (1992) A model for nematodiasis in New Zealand lambs. *International Journal for Parasitology* **22**, 789-799.

Levine ND, Anderson FL (1973) Development and survival of *Trichostrongylus colubriformis* on pasture. *Journal of Parasitology* **59**, 147-165.

Levine ND, Todd KSJ (1975) Micrometeorological factors involved in development and survival of the free-living stages of the sheep nematodes *Haemonchus contortus* and *Trichostrongylus colubriformis*. A Review. *International Journal of Biometeorology* **19**, 174-183.

Levine ND, Todd KSJ, Boatman PA (1974) Development and Survival of *Haemonchus contortus* on pasture. *American Journal of Veterinary Research* **35**, 1413-1422.

Leyva V, Henderson AE, Sykes AR (1982) Effect of daily infections with *Ostertagia circumcincta* larvae on food intake, milk production and wool growth. *Journal of Agricultural Science* **99**, 249-259.

Lipson M, Bacon-Hall RE (1976) Some effects of various parasite populations in sheep on the processing performance of wool. *Wool Technology and Sheep Breeding* **23**, 18-20.

Lloyd S (1983) Effect of pregnancy and lactation upon infection. *Veterinary Immunology and Immunopathology* **4**, 153-176.

Love SCJ, Coles GC (2002) Anthelmintic resistance in sheep worms in New South Wales, Australia. *Veterinary Record* **150**, 87.

Love SCJ, Lloyd JB, Davis EO (1998) The New England Closantel resistance survey. In 'Proceedings of the Australian Sheep Veterinary Society AVA Conference'. Sydney. (Ed. TJ Watts) pp. 42-47. (Australian Sheep Veterinary Society)

MacRae JC (1993) Metabolic consequences of intestinal parasitism. In 'Proceedings of the Nutrition Society' pp. 121-130

Martin PJ, Le Jambre LF, Claxton JH (1981) The impact of refugia on the development of thibendazole resistance in *Haemonchus contortus*. *International Journal for Parasitology* **11**, 35-41.

Martin RR, Beveridge I, Pullman AL, Brown TH (1990) A modified technique for the estimation of the number of infective nematode larvae present on pasture, and its application in the field under South Australian conditions. *Veterinary Parasitology* **37**, 133-143.

McClure SJ, Emery DL, Bendixsen T, Davey RJ (1998) Attempts to generate immunity against *Trichostrongylus colubriformis* and *Haemonchus contortus* in young lambs by vaccination with viable parasites. *International Journal for Parasitology* **28**, 739-746.

McCosker T (2000) Cell Grazing- The first 10 years in Australia. *Tropical Grasslands* **34**, 207-218.

McEwan JC, Dodds KG, Greer GJ, Bain WE, Duncan SJ, Wheeler R, Knowler KJ, Green RS, Douch PGC (1995) Genetic estimates for parasite resistance traits in sheep and their correlations with production traits (abstract). *New Zealand Journal of Zoology* **22**, 177.

McLeod MK (2002) Soil water dynamics under grazed pastures in the Northern Tablelands of New South Wales Australia. PhD thesis, University of New England.

McLeod RS (1995) Costs of major parasites to the Australian livestock industries. *International Journal for Parasitology* **25**, 1363-1367.

Michel JF (1985) Strategies for the use of anthelmintics in livestock and their implications for the development of drug resistance. *Parasitology* **90**.

Ministry of Agriculture Fisheries and Food (1986) 'Manual of Veterinary Parasitological Laboratory Techniques.' (Her Majesty's Stationery Office: London)

Monnig HO (1930) Studies on the bionomics of the free-living stages of *Trichostrongylus spp.* and other parasitic nematodes. *Report of the Director of Veterinary Services and Animal Industry, Union of South Africa* **16**, 175-198.

Morgan DO (1933) The effect of heavy stocking on the worm burden under a system of rotational grazing. *Journal of Helminthology* **11**, 169-180.

Morgan DO, Oldham JN (1934) Further observations on the effect of heavy stocking on the worm burden under a system of rotational grazing. *Journal of Helminthology* **12**, 177-182.

Morris CA, Bisset SA, Vlassoff A, Baker RL, Watson TG, Leathwick DM (1997) Correlated responses in fleece weight to selection for divergence in faecal nematode egg count in New

Zealand Romneys and Perendales. *Proceedings of the New Zealand Society of Animal Production* **57**, 26-8.

Morris CA, Bisset SA, Vlassoff A, Mackay A, Betteridge K, Alderton MJ, West CJ, Devantier BP (2001) Genetic studies of resilience of Romney sheep to nematode challenge in New Zealand. In 'Proceedings of the New Zealand Society of Animal Production' pp. 92-95

Morris CA, Watson TG, Bisset SA, Vlassoff A, Douch PGC (1995) 'Breeding sheep in New Zealand for resistance or resilience to nematode parasites.' (Australian Centre for International Agricultural Research: Canberra)

Morris RS, Meek AH (1980) Measurement and evaluation of the economic effects of parasitic disease. *Veterinary Parasitology* **6**, 165-184.

Moss RA, Vlassoff A (1993) Effect of herbage species on gastro-intestinal roundworm populations and their distribution. *New Zealand Journal of Agricultural Research* **36**, 371-375.

Mpiti-Shakhane LM (2006) Investigations of pasture and grazing management within farmlet systems on the Northern Tablelands of New South Wales. PhD Thesis, University of New England.

Mulligan W, Gordon HM, Stewart DF, Wagland BM (1961) The use of irradiated larvae as immunizing agents in *Haemonchus contortus* and *Trichostrongylus colubriformis* infections if sheep. *Australian Journal of Agricultural Research* **12**, 1175.

Munroe M, Scott JM (2005). Personal communication (Armidale)

Niezen JH, Charleston WAG, Hodgson J, Miller CM, Waghorn TS, Robertson HA (1998a) Effect of plant species on the larvae of gastrointestinal nematodes which parasitise sheep. *International Journal for Parasitology* **28**, 791-803.

Niezen JH, Miller CM, Robertson HA, Wilson SR, Mackay AD (1998b) Effect of topographical aspect and farm system on the population dynamics of *Trichostrongylus* larvae on a hill pasture. *Veterinary Parasitology* **78**, 37-48.

Niezen JH, Robertson HA, Sidey A, Wilson SR (2002) The effect of pasture species on parasitism and performance of lambs grazing one of three grass-white clover pasture swards. *Veterinary Parasitology* **105**, 303-315.

Niven P, Anderson N, Vizard AL (2002) The integration of grazing management and summer treatments for the control of trichostrongylid infections in Merino weaners. *Australian Veterinary Journal* **80**, 559-566.

Noad B (2003) 'PROGRAZE: Profitable, Sustainable grazing.' New South Wales Agriculture., Orange, New South Wales.

O'Callaghan MG, Martin RR, McFarland IJ (1992) A natural infection of sheep with *Ostertagia ostertagi*. *Australian Veterinary Journal* **69**, 19-20.

O'Connor L, Kahn LP, Walkden-Brown SW, Crampton A (2005) The effect of timing and volume of rainfall on the development of *Haemonchus contortus*. In '20th International Conference of the World Association for the Advancement of Veterinary Parasitology'. Christchurch p. 177

Okon DE, Enyenihi UK (1977) Development and survival of *Haemonchus contortus* larvae on pastures in Ibadan. *Tropical Animal Health and Production* **9**, 7-10.

Olsen RG, Krakowka S (1979) 'Immunology and immunopathology of domestic animals.' (Charles C Thomas Publisher: Springfield)

Pandey VS (1972) Effect of temperature on survival of the free-living stages of *Ostertagia ostertagi*. *Journal of Parasitology* **58**, 1042-1046.

Parkins JJ, Holmes PH (1989) Effects of gastrointestinal helminth parasites on ruminant nutrition. *Nutrition Research Reviews* **2**, 227-246.

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

Poppi DP, MacRae JC, Brewer A, Dewey PJS, Walker A (1985) Calcium and phosphorus absorption in lambs exposed to *Trichostrongylus colubriformis*. *Journal of Comparative Pathology* **95**, 453-464.

Radostits OM, Gay CC, Blood DC, Hinchcliff KW (2000) 'Veterinary Medicine: A textbook of the diseases of cattle, sheep, pigs, goats and horses.' (W.B. Saunders Company Ltd.: London)

Ransom BH (1906) The life history of the twisted wireworm (*Haemonchus contortus*) of sheep and other ruminants. *Circulation of the United States Bureaux of Animal Industries* **93**, 7.

Rees G (1950) Observations on the vertical migration of third stage larvae of *Haemonchus contortus* (Rud) on experimental plots of *Lolium perenne* S24, in relation to meteorological and micrometeorological factors. *Parasitology* **40**, 127-143.

Reeve I, Thompson L (2005) '2004 Benchmark Survey- Report to IPM-sheep.' Australian Wool Innovation, Sydney.

Roberts JA, Adams DB (1990) The effect of level of nutrition on the development of resistance to *Haemonchus contortus* in sheep. *Australian Veterinary Journal* **67**, 89-91.

Roberts JL, Swan RA (1982) Quantitative studies of ovine haemonchosis. 2. Relationship between total worm counts of *Haemonchus contortus*, haemoglobin values and bodyweight. *Veterinary Parasitology* **9**, 201-209.

Robertson D, Fraser AHH (1933) On the incidence of stomach worms in lambs in the North of Scotland and their control by progressive sectional grazing. *Journal of Helminthology* **11**, 187-194.

Robinson GG (1983) 'The Native Grasses of the Northern Tablelands.' (N.S.W. Department of Agriculture)

Roe R, Southcott WH, Turner HN (1959) Grazing management of native pastures in the New England region of New South Wales. 1. Pasture and sheep production with special reference to systems of grazing and internal parasites. *Australian Journal of Agricultural Research* **10**, 530-554.

Rogers WP (1940) The effects of environmental conditions on the accessibility of third stage trichostrongyle larvae to grazing animals. *Parasitology* **32**, 209-223.

Rose JH (1963) Observations on the free-living stages of the stomach worm *Haemonchus contortus*. *Parasitology* **53**, 469-481.

Rose JH (1964) Relationship between environment and development and migration of the free-living stages of Haemonchus contortus. *Journal of Comparative Pathology* **74**, 163-172.

Roseby FB (1973) Effects of *Trichostrongylus colubriformis* (nematoda) on the nutrition and metabolism of sheep 1.Feed intake, digestion and utilization. *Australian Journal of Agricultural Research* **24**, 947-953.

Roseby FB (1977) Effects of *Trichostrongylus colubriformis* (Nematoda) on the nutrition and metabolism of sheep. III. Digesta flow and fermentation. *Australian Journal of Agricultural Research* **28**, 155-164.

Roseby FB, Leng RA (1974) Effects of *Trichostrongylus colubriformis* (nematoda) on the nutrition and metabolism of sheep. II. Metabolism of urea. *Australian Journal of Agricultural Research* **25**.

Rossanigo CE, Gruner L (1995) Moisture and temperature requirements in faeces for the development of free-living stages of gastrointestinal nematodes of sheep, cattle and deer. *Journal of Helminthology* **69**, 363-366.

Salisbury JR, Arundel JH (1970) The relationship between lactation and post-parturient rise in faecal nematode egg counts of ewes. *Australian Veterinary Journal* **46**, 267.

Sani RA, Mazila M, Zaki M, Chong DT, Tajuddin I (1996) Grazing management for worm control in a sheep-rubber integrated system. In 'Silver-Jubilee Malaysian Society of Animal Production Conference'. Sarawak pp. 301-302

Sanyal PK (2000) Screening for Indian isolates of predacious fungi for use in biological control against nematode parasites of ruminants. *Veterinary Research Communications* **24**, 55-62.

Savory A, Parsons SD (1980) The Savory grazing method. *Rangelands* **2**, 234-237.

Schallig HDFH (2000) Immunological responses of sheep to *Haemonchus contortus*. *Parasitology* **120**, S63-S72.

Scott JM, Gaden CA, Shakhane L, Healey AF (2004) Holding a measuring stick up to the Cicerone farmlets: how are they shaping up? In 'Proceedings of the 19th Grasslands Society of NSW Conference'. Gunnedah pp. 63-68

Shaw RJ, Morris CA, Green RS, Wheeler M, Bisset SA, Vlassoff A, Douch PGC (1999) Genetic and phenoypic relationships among *Trichostrongylus colubriformis*-specific immunoglobulin E, anti-*Trichostrongylus colubriformis* antibody, immunoglobulin G1, faecal egg count and bodyweight traits in grazing Romney lambs. *Livestock Production Science* **58**, 25-32.

Shorb DA (1944) Survival on grass plots of eggs and larvae of the stomach worm, *Haemonchus contortus*. *Journal of Agricultural Research* **68**, 317-324.

Silangwa SM, Todd AC (1964) Vertical migration of trichostrongyloid larvae on grasses. *Journal of Parasitology* **50**, 278-284.

Silverman PH, Campbell JA (1959) Studies on parasitic worms of sheep in Scotland. I. Embryonic and larval development of *Haemonchus contortus* at constant conditions. *Parasitology* **49**, 23-38.

Singh DR, McIntyre KH, Mua A (1972) The effects of drenching and rotational grazing on gastrointestinal parasites of sheep. *Fiji Agricultural Journal* **34**, 11-14.

Skinner WD, Todd KSJ (1980) Lateral migration of *Haemonchus contortus* larvae on pasture. *American Journal of Veterinary Research* **41**, 395-398.

Soulsby EJL (1965) 'Textbook of veterinary clinical parasitology. Volume 1 Helminths.' (Blackwell Scientific Publications: Oxford)

Southcott WH, Barger IA (1975) Control of nematode parasites by grazing management. 2. Decontamination of sheep and cattle pastures by varying periods of grazing with the alternate host. *International Journal for Parasitology* **5**, 45.

Southcott WH, Major GW, Barger IA (1976) Seasonal pasture contamination and availability of nematodes for grazing sheep. *Australian Journal of Agricultural Research* **27**, 277-286.

Sparke R (2000) Cell Grazing- A producer's perspective. *Tropical Grasslands* **34**, 219-222.

Stear MJ, Bairden K, *et al.* (2000) The influence of relative resistance and urea-supplementation on deliberate infection with *Teladorsagia circumcincta* during winter. *Veterinary Parasitology* **94**, 45-54.

Steel JW (1974) Pathophysiology of gastrointestinal nematode infections in the ruminant. In 'Proceedings of the Australian Society of Animal Production' p. 139

Steel JW (Ed.) (1978) 'Inter-relationships between gastrointestinal helminth infection, nutrition and impaired productivity in the ruminant.' Recent Advances in Animal Nutrition in Australia

Steel JW (2003) Effects of protein supplementation of young sheep on resistance development and resilience to parasitic nematodes. *Australian Journal of Experimental Agriculture.*

Steel JW, Jones WO, Symons LEA (1982) Effects of a concurrent infection of *Trichostrongylus colubriformis* on the productivity and physiology and metabolic responses of lambs infected with *Ostertagia circumcincta*. *Australian Journal of Agricultural Research* **33**, 131-140.

Stewart DF (1953) Studies on resistance of sheep to infestation with *Haemonchus contorts* and *Trichostrongylus* spp. and on the immunological reactions of sheep exposed to infestation. V. The nature of the "self-cure" phenomenon. *Australian Journal of Agricultural Research* **4**, 100-117.

Stewart DF (1955) "Self-cure" in nematode infestations of sheep. *Nature* **176**, 1273-1274.

Stoll NR (1929) Studies with the strongyloid nematode *Haemonchus contortus*. 1. Acquired resistance of hosts under natural reinfection conditions out of doors. *American Journal of Hygiene* **10**, 384-418.

Swan RA (1970) *Australian Veterinary Journal* **46**, 485.

Sykes AR (1983) Effects of parasitism on metabolism in the sheep. In 'Sheep Production. Proceedings of the 35th Nottingham University Easter School'. (Ed. W Haresign) pp. 317-333. (Butterworths, London)

Sykes AR, Coop RL (1977) Intake and utilisation of food by growing sheep with abomasal damage caused by daily dosing with *Ostertagia circumcincta* larvae. *Journal of Agricultural Science* **88**, 671-677.

Taylor EL (1939) Technique for the estimation of pasture infestation by stongyloid larvae. 473-478.

Thamsborg SM, Jorgenson RJ, Waller PJ, Nanson P (1996) The influence of stocking rate on gastrointestinal nematode infections of sheep over a 2-year grazing period. *Veterinary Parasitology* **67**, 207-224.

Thomas RJ, Ali DA (1983) The effect of *Haemonchus contortus* infection on the pregnant and lactating ewe. *International Journal for Parasitology* **13**, 393-398.

Thomas RJ, Boag B (1972) Epidemiological studies on gastro-intestinal nematode parasites of sheep. *Research in Veterinary Science* **13**, 61-69.

Thompson RL, Callinan APL (1981) The effects of nematodiasis on weaner sheep in Western Victoria. *Australian Journal of Agricultural Research* **32**, 975-985.

Thompson RL, Egan JK, McLaughlin JW (1978) The influence of internal parasites upon growth and productivity of weaner sheep. In 'Proceedings of the Australian Society of Animal Production'. Melbourne p. 274

Thrusfield M (1995) 'Veterinary Epidemiology.' (Blackwell Science: Oxford)

Tizard IR (1996) 'Veterinary Immunology: An Introduction.' (W.B. Saunders Company: Philadelphia)

Todd KSJ, Levine ND, Boatman PA (1976) Effect of temperature on survival of free-living stages of *Haemonchus contortus*. *American Journal of Veterinary Research* **37**, 991-992.

Todd KSJ, Levine ND, Whiteside CC (1970) Moisture stress effects on survival of infective *Haemonchus contortus* larvae. *Journal of Nematology* **2**, 330-333.

Topps JH (1983) 'Nutrition and gastrointestinal parasitism.' (Longman: London)

Torgerson PR, Lloyd S (1993) The same fractions of *Haemonchus contortus* soluble antigen induce lymphocyte responses in naive lambs and immune sheep. *Research in Veterinary Science* **54**, 224-246.

University A (2004) Alternative dewormers- Review by Aberdeen University 2004. In. (Aberdeen University http://www.abdn.ac.uk/organic/organic_14a.php: Aberdeen)

van Houtert MFJ, Barger IA, Steel JW, Windon RG, Emery DL (1995) Effects of dietary protein intake on responses of young sheep to infection with *Trichostrongylus colubriformis*. *Veterinary Parasitology* **56**, 163-180.

van Wyk JA (2001) Refugia-overlooked as perhaps the most potent factor concerning development of anthelmintic resistance. *Onderstepoort Journal of Veterinary Research* **68**, 55-67.

Veglia F (1915) The anatomy and life-history of the *Haemonchus contortus* (Rud.). *Report of Director of Veterinary Research South Africa* **3**, 349-500.

Waghorn TS, Leathwick DM, Chen L-Y, Gray RAJ, Skipp RA (2002) Influence of nematophagus fungi, earthworms and dung burial on development of the free-living stages of Ostertagia (Teladorsagia) circumcincta in New Zealand. *Veterinary Parasitology* **104**, 119-129.

Waghorn TS, Leathwick DM, Chen L-Y, Skipp RA (2003) Efficacy of the nematode-trapping fungus *Duddingtonia flagrans* against three species of gastro-intestinal nematodes in laboratory faecal cultures from sheep and goats. *Veterinary Parasitology* **118**, 227-234.

Wagland BM, Emery DL, McClure SJ (1996) Studies on the host-parasite relationship between *Trichostrongylus colubriformis* and susceptible and resistant sheep. *International Journal for Parasitology* **26**, 1279-1286.

Wagland BM, Steel JW, Windon RG, Dineen JK (1984) The response of lambs to vaccination and challenge with *Trichostrongylus colubriformis*: Effect of plane of nutrition on, and the interaction between, immunological responsiveness and resistance. *International Journal for Parasitology* **14**, 39.

Walkden-Brown SW, Eady SJ (2003) Nutritional influences on the expression of genotypic resistance to gastrointestinal nematode infection in sheep. *Australian Journal of Experimental Agriculture* **43**, 1445-1454.

Walkden-Brown SW, Kahn LP (2002) Nutritional modulation of resistance and resilience to gastro-intestinal nematode infection-A Review. *Asian-Australian Journal of Animal Science* **15**, 912-924.

Walkden-Brown SW, Maslen AJ (2002) Specificity of antibody responses of goats to infections with three species of gastrointestinal nematode. In 'Proceedings of the Australian Soceity of Animal Production' p. 367

Wallace DS, Bairden K, *et al.* (1998) The influence of dietary supplementation with urea on resilience and resistance to infection with *Haemonchus contortus*. *Parasitology* **116**, 67-72.

Waller PJ (1997) Anthelmintic Resistance. *Veterinary Parasitology* **72**, 391-412.

Waller PJ, Axelsen A, Donald AD, Morley FHW, Dobson RJ, Donnelly JR (1987) Effects of helminth infection on the pre-weaning production of ewes and lambs: comparison between safe and contaminated pasture. *Australian Veterinary Journal* **64**, 357-362.

Waller PJ, Dobson RJ, Donald AD, Thomas RJ (1981) Populations of strongyloid nematode infective stages in sheep pastures: comparison between direct pasture sampling and tracer lambs as estimators of larval abundance. *International Journal for Parasitology* **11**, 359-367.

Waller PJ, Donald AD (1970) The response to desiccation of eggs of *Trichostrongylus colubriformis* and *Haemonchus contortus* (Nematoda: Trichostrongylidae). *Parasitology* **61**, 195-204.

Waller PJ, Larsen M (1996) Workshop summary: Biological control of nematode parasites of livestock. *Veterinary Parasitology* **64**, 135-137.

Waller PJ, Rudby-Martin L, Ljungstrom BL, Rydzik A (2004) The epidemiology of abomasal nematodes of sheep in Sweden, with particular reference to over-winter survival strategies. *Veterinary Parasitology* **122**, 207-220.

Waller PJ, Thomas RJ (1975) Field studies on inhibition of *Haemonchus contortus* in sheep. *Parasitology* **71**, 285-291.

Watson DL, Gill HS (1991) Effect of weaning on antibody responses and nematode parasitism in Merino lambs. *Research in Veterinary Science* **51**, 128-132.

Woolaston RR, Eady SJ (1995) Australian research on genetic resistance to nematodes. In 'Breeding for disease resistance to infectious diseases in small ruminants'. (Eds GD Gray, RR Woolaston and BT Eaton) pp. 53-75. (Australian Centre for International Agricultural Research (ACIAR): Canberra)

Woolaston RR, Manueli P, Eady SJ, Barger IA, Le Jambre LF, Banks DJD, Windon RG (1996) The value of circulating eosinophil count as a selection criteria for resistance of sheep to Trichostrongyle parasites. *International Journal for Parasitology* **26**, 123-126.

Woolaston RR, Piper LR (1996) Selection of merino sheep for resistance to *Haemonchus contortus*: Genetic variation. *Animal Science* **62**, 451-460.

Woolaston RR, Windon RG, Gray GD (1991) Genetic variation in resistance to internal parasites in Armidale experimental flocks. In 'Breeding for Disease Resistance in Sheep' pp. 1-9: Melbourne)

Wooster MJ (1997) Interactions between *Trichostrongylus colubriformis* and *Trichostrongylus vitrinus*. PhD thesis, University of New England.