THE EFFECT OF HOST IMMUNITY ON THE DEVELOPMENT AND SURVIVAL OF THE FREE-LIVING STAGES OF COMMON TRICHOSTRONGYLID PARASITES OF SHEEP

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ABSTRACT

The effect of host immunity on the free-living stages of common trichostrongylid parasites was studied in a series of experiments, involving both artificially infected housed animals and naturally infected animals in the field.

In Perendale ewes, bred for either enhanced or lowered resistance to nematodes, reduced developmental success of eggs to infective larvae was found in the resistant animals at some times of the year ($p<0.01$). This was consistent with the hypothesis of an adverse effect of increased host immunity on the development of the free-living stages of gastrointestinal nematodes. In lambs, this effect had been demonstrated previously and again in 1998, whereas results from 1997 were inconsistent.

In fleece–weight selected and control lines of Romney lambs, exposed to the same level of pasture larval challenge, developmental success decreased with time ($p<0.001$), although the two lines did not differ. This was consistent with an increasing level of host immunity in both lines and provided strong support for the hypothesis of host immunity having an adverse effect on larval development.

Nematode eggs from lambs in the field treated orally with either ivermectin or albendazole, did not differ in developmental success, providing no evidence that host immunity was influenced by the type of anthelmintic used.

A lower developmental success of *O. circumcincta* in an LDA ($p<0.001$) was found in animals relatively immune to this parasite compared to control animals. In faecal cultures a significant difference was not demonstrated, but group sizes were very small.

An effect of host immunity on the developmental success and infectivity of larvae of *T. colubriformis* could not be demonstrated in trickle-infected groups of lambs that differed in their immunity to this parasite, one group being immunosuppressed with cortico-steroids.

An adverse effect of small intestinal mucus and contents on larval development was demonstrated. This was more potent in intestinal contents than mucus. Although source animals differed greatly in their immunity to *T. colubriformis*, differences between immune and immunosuppressed animals in the magnitude of the effect of intestinal mucus and contents on larval development were not found. The results suggested that the effect of intestinal mucus and contents was not immunological but rather caused by some physical and non-specific properties.

Overall, the results reported in this thesis further support the hypothesis of host immunity having an adverse effect on the development of the free-living stages of

gastrointestinal nematodes of sheep. This was most evident in animals with naturally acquired infections and in housed animals infected with *O. circumcincta*. It is suggested that the failure to demonstrate this in experimental infections with *T. colubriformis* may have been due to the use of cortico-steroids to suppress immune responses.

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I would like to dedicate this thesis to my late grandmother, Laura, whom I miss dearly.

The Road to Wisdom

The road to wisdom? – Well it's plain
and simple to express:
Err
and err
and err again
but less
and less
and less

(Piet Hein)

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CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

The research described in this thesis arose from some preliminary observations that detected an effect of host immunity on the developmental success of the free-living stages of gastrointestinal nematodes in sheep. The literature review that follows is, therefore, primarily concerned with aspects of the development and survival of the free-living stages of gastrointestinal nematodes of ruminants and the basis of, and factors affecting the development and expression of host immunity to these parasites. In considering the mass of published literature on these topics, the review is necessarily selective in its coverage. But first to set the scene, some general background on gastrointestinal parasitism in New Zealand sheep.

New Zealand's export income from the sheep and beef meat was in 1999 estimated at NZ$ 2.8 billion and wool brought in NZ$ 797 million. Overall sheep production forms a major part of the agricultural sector in New Zealand (New Zealand Yearbook 1999).

In contrast to most sheep production in Northern Europe, the New Zealand production system is an extensive one, involving all-year grazing and no housing of animals at any time. Lambing takes place from July (mid-winter) to October, with September and October (spring) being the months where most lambings occur. Lambs are generally weaned at about 12 weeks of age (usually in late November or early December) and graze pastures until about 4-6 months old and/or when ready to go to the meat works.

Gastrointestinal parasitism is one of the major production-limiting factors for New Zealand sheep producers, and parasite control is therefore an important issue on all New Zealand sheep farms. In 1999, nearly NZ$ 50 million was spent on anthelmintic treatment of sheep (Rochester, pers.comm.), with potential losses due to parasitism having been estimated at NZ$ 270 million per year (Leathwick and Vlassoff, 1996).

Currently the most common control strategy on New Zealand farms used against gastrointestinal nematode infections in sheep are regular anthelmintic treatments of lambs at intervals close to the prepatent period of common species, beginning at or just before weaning. The objective is to minimise pasture contamination over the main summer-autumn grazing period. The current minimum recommendation is to give five drenches at intervals of 21-28 days, with the first being given at weaning. Rotational grazing, pasture resting and alternate grazing by hosts of different species or age groups, and 'dose and move' strategies where animals are dosed with anthelmintics and moved to a clean pasture, are used as additional or alternative measures (Brunsdon and Vlassoff, 1982; Bruére and West, 1993). Increasing problems with anthelmintic resistance and a desire to reduce

chemical inputs into livestock farming has led to a search for ways of reducing the reliance on anthelmintics for parasite control. Breeding for increased resistance to parasitism and the development of 'organic' systems of animal production are examples of this.

When European settlers brought livestock into New Zealand mainly in the late 19th and early 20th century, gastrointestinal nematodes were introduced with them. There may have been a certain degree of adaptation to the local climate since then, but otherwise the parasites' requirements for development and survival appear to be as for the geographical region from which they originated.

A considerable number of species of nematodes have been recorded from New Zealand ruminants (Brunsdon, 1960; McKenna, 1997). The gastrointestinal nematodes that occur most commonly in sheep and their relative importance, are shown in Table 1.1.1.1.

	Major importance	Secondary importance
Abomasum	*Haemonchus contortus*	
	*Ostertagia circumcincta**	
	O. trifurcata	
	Trichostrongylus axei	
Small intestine	*T. colubriformis*	*Cooperia curticei*
	T. vitrinus	*Strongyloides papillosus*
	Nematodirus filicollis	*Bunostomum trigonocephalum*
	N. spathiger	
Large intestine		*Oesophagostomum venulosum*
		Chabertia ovina

Table 1.1.1.1 Important gastrointestinal nematodes in New Zealand sheep (Adapted from Charleston, 1982). *= *Teladorsagia circumcincta*

Recent classifications of the genera in the subfamily Ostertagiinae, according to certain anatomical features of the adult worms, have indicated that the name *Teladorsagia circumcinta* should be used rather than *Ostertagia circumcincta* (Lichtenfels *et al.*, 1988; Durette-Desset, 1989; Anderson, 2000). However, since most of the literature, reviewed in this thesis, refers to the parasite as *Ostertagia circumcincta*, this name was decided on to avoid confusion in the text.

1.2 Epidemiological and seasonal pattern of larval development and survival in New Zealand

Due to a temperate climate and adequate rainfall, the free-living stages of trichostrongylid nematodes generally have good conditions for developing on pastures in New Zealand for much of the year. The relatively mild winters ensure that a considerable number of 3rd stage larvae can survive until the following spring. Development is mainly

confined to the months of the year with a mean air temperature above 10°C, although development of eggs to 3rd stage larvae of *O. circumcincta* and *T. colubriformis*, at air temperatures below 8°C, has been reported in a plot trial carried out on the South Island (Familton and McAnulty, 1994). This means that development can take place in all months of spring, summer and autumn, with particularly good conditions present for periods in the spring and especially the autumn. The percentage of deposited eggs developing to 3rd stage larvae (developmental success) is very variable during the year and reflects climatic conditions. The maximum developmental success observed on New Zealand pastures is around 20 – 25 %, occurring in late summer/autumn. In most months, however, the developmental success is well below 1 % (Vlassoff, 1982).

The seasonal pattern of nematode infections of young sheep and the availability of infective larvae on pasture has been well established (Tetley, 1949; Brunsdon, 1963 and 1970; Vlassoff, 1973). Vlassoff (1973) furthermore found that many of the genera overwintered on pasture. He showed there to be a small peak in larval recoveries in the spring (September to November) and a larger one in autumn. But whereas the spring peak was dominated by *Nematodirus filicollis*, *Ostertagia* sp. and small numbers of *Trichostrongylus* sp., the autumn peak was dominated by *Trichostrongylus* sp., followed by *N. filicollis* and *N. spathiger*. This pattern applies over most of New Zealand, in that only very few geographical variations exist. *Haemonchus* is one such exception. *Haemonchus* requires a higher range of temperatures for larval development and therefore larger worm populations of this species are found in the North Island than in the South Island. It tends to be more numerous in mid to late summer.

Due to their larger production of faeces and their presence on pasture year-round, ewes have been considered to be the major contributors to pasture larval contamination (West, 1982; Familton, 1991; Stafford *et al.*, 1994). The contribution of the adult ewe to pasture larval contamination has been estimated largely by summing faecal egg counts (FEC) over time (West, 1982; Familton, 1991) - an approach that makes no allowance for a seasonal variation in developmental success. In addition, it should also be borne in mind that adult ewes effectively remove a much larger proportion of infective larvae from pasture than lambs, due to their higher feed intake. If developmental success is indeed lower in nematode eggs from more immune animals, as indicated by recent findings (Jorgensen *et al.*, 1998), the contribution of the adult ewe to pasture larval contamination may well have been overestimated in the past.

Vlassoff (1982) summarised the overall seasonal pattern of larval availability on New Zealand pastures (excluding *Nematodirus* sp.) (Figure 1.2.1.1). In general, there are two peaks in the larval availability, a small one in spring and a larger one in autumn. Few larvae are available on the pasture during summer (if dry) and in late winter. In the figure below the interactions between infection levels in lambs and ewes and the resulting pasture larval populations are shown and may be explained by the following sequence of events, the numbers of which relate to the number given in Figure 1.2.1.1 below (Vlassoff, 1982):

1. The peri-parturient rise (PPR) in faecal egg count of the breeding ewe is the main source of contamination contributing to the spring peak of larvae on pasture
2. Larvae from the PPR and any that have overwintered result in the first generation of worms that accumulate in the lambs in summer.
3. Eggs deposited by lambs in late February and early March are the source of the large autumn peak of infective larvae on pasture.
4. Larvae from the autumn peak produce the second generation of worms in lambs – that which causes clinical disease in autumn and winter. A proportion of these larvae overwinters on pasture to provide a source of infection for ewes and lambs in the following spring.
5. Most of the eggs deposited in the autumn – from the second generation of worms – fail to develop because of progressively declining temperatures and excessively wet conditions.

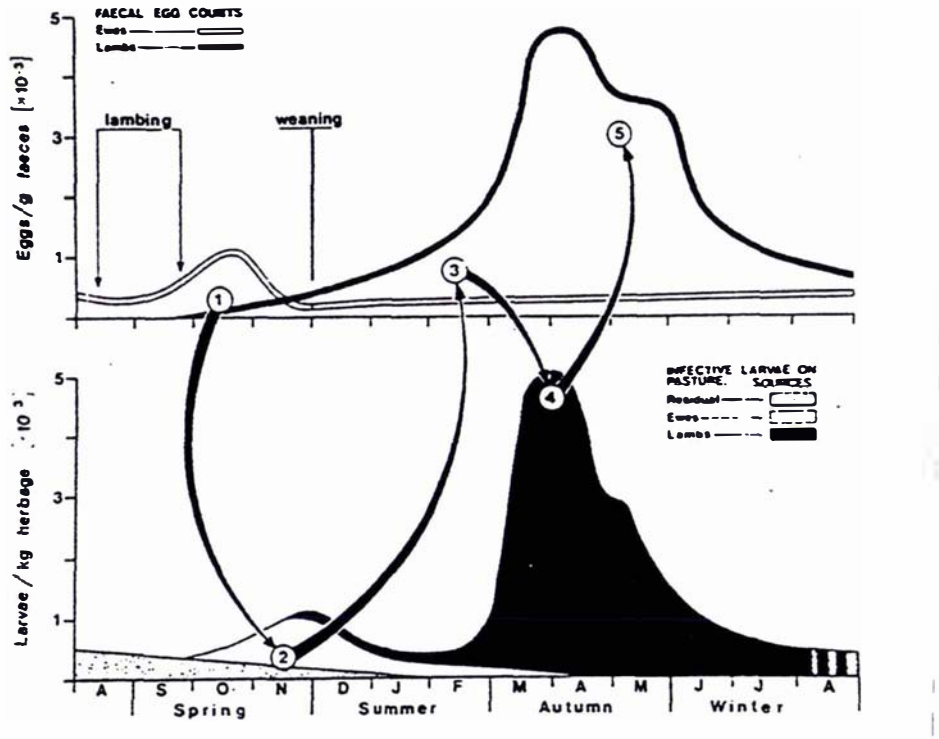

Figure 1.2.1.1 The sequential interrelationship between pasture contamination by ewes and lambs and the availability of infective larvae on pasture (Vlassoff, 1982).

1.3 The Development and Survival of the free-living stages of Trichostrongylid Parasites

1.3.1 General Lifecycle

The life cycle of trichostrongylid nematodes consists of 6 stages; the egg, 4 larval stages (L1, L2, L3, L4) and the adult stage (See Figure 1.3.1.1). The egg, L1, L2 and L3 are also known as the free-living or pre-parasitic stages, as they develop outside the host animal. The trichostrongylid nematodes all have a direct lifecycle.

In most species of nematodes, hatching occurs when the first larval stage (L1) has developed inside the egg. Larvae of *H. contortus* and *C. curticei* have been found to emerge from the egg tail end first (Silverman and Campbell, 1959; Ahluwalia and Charleston 1974)

although larvae of the former species have also been found to emerge headfirst (Rogers and Brooks, 1977).

The 3rd larval stage (L3) is the infective stage. The stages L4 and adult are parasitic stages that spend their entire lifetime within the alimentary tract of the host animal. Between each of the larval stages a moulting event takes place. The 3rd larval stage (L3), however, retains the cuticle of the second larval stage (L2) until it is ingested by the host animal and reaches the gastro-intestinal system of the host animal, where it exsheathes. The shedding of the sheath (L2's cuticle) takes place either in the rumen, abomasum or small intestine, depending on the genus and species of nematode. Exsheathment is a rapid procedure that can take place in less than 10 minutes (Reviewed by Wharton, 1986). It is initiated by CO_2 and is associated with a decrease in water content in larvae of *H. contortus* (Davey and Rogers, 1982).

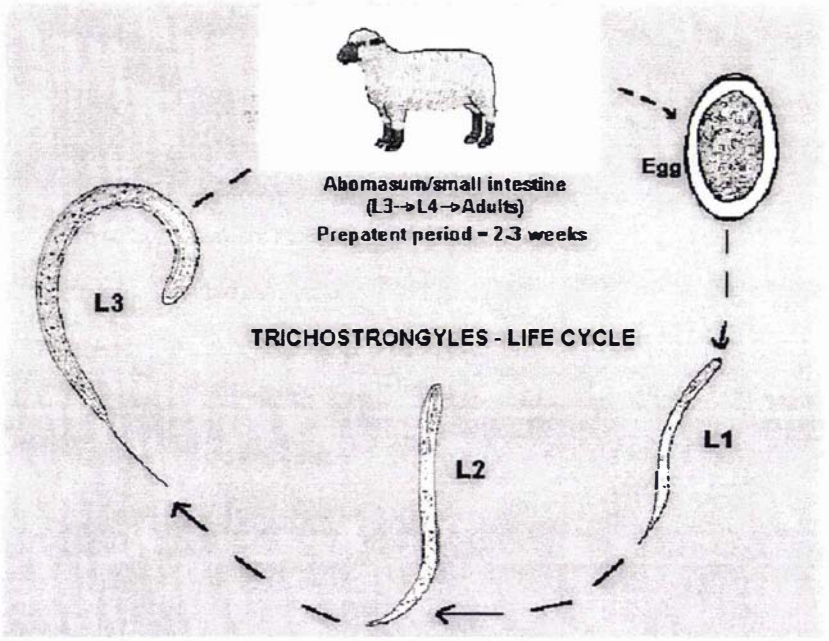

Figure 1.3.1.1 General life cycle for trichostrongyle parasites in ruminants. (adapted from image on the home page of University of Pennsylvania School of Veterinary Medicine, USA, 1995).

1.3.2 Development of the free-living stages

Numerous factors can influence the development of the free-living stages of gastrointestinal nematodes. Most of these are environmental and are the conditions the developing stages would naturally encounter in their microhabitat in and around the faecal pellets or dung pat, but some factors are apparently also intrinsic. The majority of published literature does not consider embryonation and development to hatching

separately from hatching to completion of L3 development. These will therefore be considered together in the following.

1.3.2.1 The effect of temperature

To investigate the relationship between temperature and hatching time, Crofton (1965) observed eggs from a number of commonly occurring trichostrongylid parasites hatching at different temperatures. Based on these results he defined lower and upper temperature limits for hatching (Table 1.3.2.1). Crofton's results for *H. contortus* were in accordance with those of Silverman and Campbell (1959) who, in addition, found eggs to be much more resistant to lower temperatures if they had developed beyond the morula stage before being exposed to temperatures as low as –2 °C to 1 °C, at which they survived up to 2 months. With regard to other common species (essentially those listed in Table 1.1.1.1), Silverman and Campbell found that these took considerably longer to embryonate and hatch than *H. contortus*, particularly at temperatures below 21 – 22 °C.

In contrast to Crofton's (1965) findings, later studies on the development of free-living stages of *C. curticei* showed that the development of this species from egg to 3rd stage larva was possible at temperatures as low as 10°C (Ahluwalia and Charleston, 1974; Ahluwalia, 1975). These studies were carried out in New Zealand, but as gastrointestinal nematodes in New Zealand probably all originated from Britain and Northern Europe, it is surprising that the results are not more in accordance with each other. However, this might reflect adaptation to the New Zealand climate.

Species	Minimum temperature for egg hatch, (°C)	Time to hatch at minimum temperature, (days)	Maximum temperature for Egg hatch, (°C)	Time to hatch at maximum temperature (hours)
Haemonchus contortus	9	7	36	13
Ostertagia circumcincta	4	7	34	17
Trichostrongylus axei	8 – 9	7	36	19
Cooperia curticei	16 (10*)	7	38	15
Chabertia ovina	6	7	36	17

Table 1.3.2.1 Upper and lower temperature limits for egg hatch and time to hatch in common gastrointestinal nematodes (Crofton, 1965) *Ahluwalia and Charleston, 1974.

Significant differences in hatching times have been found for different strains of *H. contortus* obtained from different geographical regions indicating an ecological selection between phenotypes (Crofton *et al.*, 1965; LeJambre and Whitlock, 1973). Similar observations have been made for geographically different strains of *O. circumcincta* (Crofton and Whitlock, 1965a; Young *et al.*, 1980a). The hatching times given in Table 1.3.2.1 should therefore be regarded as guidelines only.

In diagnostic work and in laboratory experiments concerned with culturing trichostrongylid eggs to 3rd stage larvae, faecal samples are often stored for several days in a refrigerator before being processed. McKenna (1998) found that when exposing faeces to 4 °C for increasing periods of time (up to 12 days) a decreasing number of 3rd stage larvae was recovered after culturing. There was a significant change in the generic composition in mixed infection cultures, with eggs of *Cooperia* sp. and *Haemonchus* sp. being particularly susceptible to low temperatures after 1-3 days. After more than 12 days of exposure to 4 °C, eggs of *Trichostrongylus* sp. and *Ostertagia* sp. were also affected.

With increasing temperature, there is an exponential decrease in the time needed for development of the free-living stages of the trichostrongylid parasites. This is true within a certain range of temperatures, above and below which there is a compromised development (Gibbs and Gibbs, 1959; Rose, 1961 and 1963; Pandey, 1972; Young *et al*, 1980b; Pandey *et al.*, 1989).

Below are summarised lower, upper and optimum temperatures for the development from eggs to infective larvae of common trichostrongylid parasites. The optimum temperature refers to the temperature at which the highest % development from egg to infective larva takes place.

Species	Experiment	Lower	Upper	Optimum	Country	Reference
Haemonchus contortus	plot trial	-5°C	33°C	18 – 28°C	USA	Dinaburg, 1944
Haemonchus contortus	Laboratory	7.2°C	?	?	Britain	Silverman & Campbell, 1959
Haemonchus contortus	plot trial	10°C	?	15 – 25°C	Britain	Gibson & Everett, 1976
Ostertagia circumcincta	Laboratory	>4°C	~45°C	16	British strain	Pandey *et al.*, 1989
Ostertagia ostertagi	Laboratory	10°C	35°C	25°C	India	Pandey, 1972
Trichostrongylus colubriformis	Laboratory	6°C	35°C	25°C	USA/USA	Ciordia & Bizzell, 1963 Wang, 1967
T. colubriformis	plot trial	10°C	?	Summer	England	Gibson & Everett, 1967 Boag & Thomas, 1970
T. colubriformis	plot trial	4.8°C	31.1°C	?	USA	Levine & Andersen, 1973
T. axei	Laboratory	10°C	35°C	27°C	Iran	Mirzayans, 1969
T. retortaeformis	Laboratory	5°C	30°C	20 – 30°C	Canada	Gupta, 1961
Mixed infection in cattle	Laboratory	6°C	35°C	25°C	USA	Ciordia & Bizzell, 1963
Cooperia curticei	Laboratory	10°C	37°C	27°C	N.Z.	Ahluwalia, 1975
Uncinaria stenocephala	Laboratory	7.5°C	37°C	20°C	Canada	Gibbs & Gibbs, 1959

Table 1.3.2.2 Lower, Upper and Optimum temperatures for the development of the free-living stages of common trichostrongylid parasites. ? = not recorded.

1.3.2.2 The effect of moisture level

In one of the early studies of nematode ecology, Dinaburg (1944) pointed out the importance of weather conditions and particularly available moisture, for the development of the free-living stages of *H. contortus*. However, moisture is usually considered to be of secondary importance compared with temperature (Levine and Andersen, 1973; Gibson and Everett, 1976). The normal moisture content in freshly deposited faeces from sheep is 60-70 % (Silverman and Campbell, 1959). It may be affected by, for instance, an alteration in diet and by the rate of water loss, which in turn depends on the size of the pellet, the ambient temperature and the ambient relative humidity (Silverman and Campbell, 1959). Development of the free-living stages of *T. colubriformis*, have been shown to require relative humidities of >76% (Wharton, 1982).

Water in excess inhibits the further development of eggs (Silverman and Campbell, 1959; Young *et al.*, 1980b; Gruner and Suryahadi, 1993) most probably due to a reduction in available oxygen. In contrast, lowering the faecal moisture content of or drying out cultures (desiccation) has generally been found to destroy unembryonated eggs rapidly (Shorb, 1944; Rose, 1961, 1962 and 1963; Gibson and Everett, 1967; Wharton, 1982) and inhibit further development of the larval stages (Hsu and Levine, 1977; Wharton, 1982; Rossanigo and Gruner, 1994 and 1995).

In cattle faeces, artificially spread in thin layers over grass plots, no infective larvae of *O. ostertagi* could be recovered subsequently (Rose, 1962). If the grass plots were watered, development to infective larvae was possible. However, Rose observed that cattle faecal pats took about a month to dry out completely and that from days 4 to 5 after deposition a dry crust formed on the surface of the pat. This crust served to keep the moisture higher inside the dung pat and ensured that development of the free-living stages of the parasite could take place.

In areas with long dry periods, moisture becomes a primary limiting factor for the development of the free-living stages. In winter rainfall areas of Australia, dry spells during summer prevent larval development from taking place with numbers of larvae recovered from plots being highest in either autumn-spring (*T. axei*), autumn-winter (*T. vitrinus*) or spring (*H. contortus)* (Anderson, 1972 and 1973; Callinan, 1978b and 1979; Beveridge *et al.*, 1989; Besier and Dunsmore, 1993a). In a summer rainfall region of Australia the picture is quite different. The spring contamination with mainly *H. contortus* and *O. circumcincta* was found to be rapidly translated to pasture but this infection was fairly short-lived. However, the above mentioned species and *T. colubriformis* were all able to overwinter (Southcott *et al.*, 1976).

For the trichostrongyloid nematode *N. battus*, the presence of free water is a requirement for the development from morula-stage to vermiform embryo (egg containing L1) and hatching (Parkin, 1976). Exposing *N. battus* eggs to moisture stress decreases the hatching rate. However, it was found that if the moisture level is excessively high, there is

a reduced supply of oxygen to the eggs and this also resulted in a lower hatching rate (Parkin, 1975).

1.3.2.3 The effect of oxygen availability

Eggs do not develop beyond the morula stage during their passage through the host's gastrointestinal tract. Oxygen inadequacy is thought to be the inhibiting factor (Silverman and Campbell, 1959). For nematodes, where hatching and the development to 3rd stage larvae taking place outside the host animal, oxygen is a most important requirement. A significantly higher rate of inhibited development of *H. contortus* and *O. ostertagi* has been shown in non-aerated egg-suspensions as compared to aerated egg suspensions (Shorb, 1944; Silverman and Campbell, 1959; Rose, 1961).

In the more central areas of cattle dung pats, a delay in the development of eggs of *O. ostertagi* due to a lack of aeration, has been found (Rose, 1961; Young *et al.*, 1980b) whereas faster development takes place near the surface (Rose, 1961). In pelleted sheep faeces, the situation is quite different. Lack of aeration is not thought to be a major problem for developing free-living stages on pasture. Firstly, because of the small size of a faecal pellet compared to a cattle dung pat, secondly, as sheep faeces on pasture usually disintegrate rapidly, particularly in periods with rain such as in autumn, where sheep dung has been found to disappear in as little as six days from deposition (Christie, 1963). However, if there is little or no rain this process will take longer (> 30 days). On New Zealand hill country pasture, sheep faeces took 28 days to decompose during winter, but more than 75 days during summer (Rowarth *et al.*, 1985). In addition to climatic factors, the disappearance of sheep dung from pastures may be part due to earthworm activity and appear to vary with the plant species, with the fastest disappearance on white clover and browntop grass pastures (Niezen *et al.*, 1998).

1.3.2.4 Host effect

That a host animal may have a direct effect on how successfully trichostrongylid eggs develop to 3rd stage larvae is a fairly recent observation. Studies in New Zealand were the first to demonstrate an effect of host animal on the developmental success of eggs to 3rd stage infective larvae of common trichostrongylid parasites in sheep (Jorgensen *et al.*, 1998). Initial observations from an indoor experiment suggested that the host animal was a significant factor for developmental success. A field study was carried out to confirm this result. Ewes and lambs from selection lines of sheep that had been bred for either enhanced resistance (Low-FEC) or lowered resistance (High-FEC) were used for this experiment. A significantly lower developmental success was found in eggs from ewes than from lambs and from animals in the Low-FEC group compared with those from the High FEC group, suggesting that a higher level of host immunity might account for this phenomenon. A possible explanation is that there was a direct effect of host immunity on the egg and developing stages, exerting its effect on the eggs while they are still inside the host animal and/or on the eggs and larval stages while developing in the faeces. This

could be mediated by the presence and direct effect, in faeces, of various products of the immune response to gastrointestinal nematodes.

Although significantly higher levels of IgG_1 and IgA have been found in faecal extracts from genetically resistant sheep than in faecal extracts from random-bred sheep infected with *H. contortus*, these have not been tested on the development of the free-living stages of the parasite (Gill *et al.*, 1993b). In rats, Wedrychowicz *et al.*, (1983) detected increased levels of IgA, IgM and IgG in faeces after infection with *Nippostrongylus brasiliensis*. A good correlation was found between antibody levels in mucosa and in faeces, but a poor correlation between both of the former and antibody levels in serum. Similar findings were made in faeces from rabbits infected with the stomach parasite *Obeliscoides cuniculi* (Wedrychowicz and Kowalczyk, 1991). Infection was associated with significant increases in proteins excreted in the faeces. These proteins included the immunoglobulins IgA and IgG and compounds from the complement cascade. When eggs were cultured in media containing fractions of the faecal proteins, a blocking of egg development took place in the presence of all protein fractions from infected animals. The effects of proteins in fractions from infected animals were thought to be due to specific antibodies. The faecal proteins from infected animals were also able to inflict damage on adult *O. cuniculi* (Wedrychowicz and Kowalczyk, 1991).

1.3.2.5 Intrinsic effects on the development of the free-living stages

A positive correlation between egg volume and time required for development to the hatching stage has been shown for eggs of *H. contortus* (Crofton and Whitlock, 1965b and c). It was suggested that this relationship would also apply to other common species of trichostrongylid parasites. LeJambre *et al.* (1970) found significant differences between the volumes of eggs from four phenotypes of *H. contortus* and concluded that this reflected an ecological selection in response to variations in temperature. The volume of *T. colubriformis* eggs has also been shown to be positively correlated with time to hatch (Waller and Donald, 1970). The same authors, in addition, found that the smaller eggs of *T. colubriformis* had an enhanced ability to survive in sub-optimal moisture conditions, possibly due to their larger surface area relative to volume, which would enhance the exchange or uptake of gases, such as oxygen.

Hatching is preceded and dependent on at least two important steps initiated by intrinsic factors. These include a change in the permeability of the eggshell and the effect of various enzymes found in the perivitelline fluid, on the eggshell. A breakdown of the lipid layer in the eggshell and a subsequent increase in the permeability of the eggshell took place only shortly before hatching and was associated with decreased activity of the larva in the egg of *T. retortaeformis*[1] (Wilson, 1958). Based on his findings, Wilson (1958) proposed

[1] *Trichostrongylus retortaeformis* is a nematode that infects rabbits.

a hatching mechanism for *T. retortaeformis*. This firstly involves a weakening and then breakdown of the inner lipoid layer of the egg caused by the larva agitating the egg fluid. Secondly, a chemical weakening of the protein shell and an increased hydrostatic pressure (created in the first step of hatching) enabling the larva to escape from the egg. For eggs of the human nematode *Ascaris lumbricoides*[2], one of the first steps in the hatching process was also shown to be associated with an increased permeability of the vitelline membrane (Fairbairn, 1961). The fully developed nematode larva in the egg produces a hatching fluid when the appropriate stimuli/conditions for hatching are present. The hatching fluid of eggs of *H. contortus* has been found to contain enzymes important for breaking down the layers in the eggshell, thus allowing the larva to escape from the egg, and appears to share some properties with exsheating fluid from 3rd stage larvae of the same parasite (Rogers, 1965 and 1982; Rogers and Brooks, 1977).

1.3.2.6 Anthelmintic effects on larval development

It is well known that benzimidazole anthelmintics are ovicidal as well as lethal to larval and adult stages of trichostrongylid parasites. This has been an added advantage of their use for parasite control on sheep farms and in the laboratory, when developing and using egg hatch assays to test for anthelmintic resistance (LeJambre, 1976; Coles and Simpkin, 1977; Smith Buijs and Borgsteede, 1986; Kerboeuf and Hubert, 1987; Borgsteede and Couwenberg, 1987).

Anthelmintics from the groups of macrocyclic lactones, imidothiazol and tetrahydropyrimidines are not ovicidal, but do instead act against and kill larval and adult stages of trichostrongylid nematodes including the free-living stages. These effects on larval stages have been used in the development of larval development assays (LDA) to test for anthelmintic resistance in trichostrongylid parasites in sheep (Coles *et al.*, 1988; Giordano *et al.*, 1988; Taylor, 1990; Hubert and Kerboeuf, 1992; Gill *et al.*, 1995; Amarante *et al.*, 1997; Sangster *et al.*, 1998; Várady and Corba, 1999; Gopal *et al.*, 1999; Kotze *et al.*, 1999).

Macrocyclic lactones are excreted almost exclusively in the faeces, where they bind tightly to digesta and have a prolonged half-life (reviewed by Herd, 1995). Subcutaneous injections with ivermectin in cattle have resulted in the drug being excreted in faeces for up to 14 days (Cook *et al.*, 1996) and having an effect on the dung pat fauna (such as dung beetles and dung dwelling Diptera) for up to 30 days (Madsen *et al.*, 1990). In sheep, however, where oral formulations of ivermectin are used, the clearance of the drug, at least from plasma, happens faster (Steel, 1993). Nevertheless, residues of ivermectin have been found in bile for up to 21 days after intra-ruminal treatment of cattle (Steel, 1993). Lesser effects on Diptera have been recorded following oral treatment of sheep (Steel, 1993).

[2] In *Ascaris lumbricoides* hatching is suppressed until a suitable host ingests the egg.

However, it is possible that the development of nematode larvae is more sensitive than that of larval Diptera. It is evident from larval development assay data, that concentrations of the order of ng/ml adversely affect larval development (Hoza, 1998; Gopal *et al.*, 1999; Jorgensen, unpublished results). For a combination anthelmintic containing levamisole and oxfendazole the clearance from faeces took place within the first 48 hours after oral treatment (Wardhaugh *et al.*, 1993).

Whether the residual concentrations of ivermectin in faeces from orally treated sheep are sufficiently high to adversely affect the development of the free-living stages of trichostrongylid parasites, for up to 3-4 weeks after treatment, has not been investigated.

1.3.3 Factors Affecting Survival and Fitness of Third Stage Larvae

The fitness of an infective trichostrongylid larva comprises its ability to survive in the environment (longevity), its migratory activity and its ability to infect a host animal (infectivity). The infectivity and activity of infective larvae depend on the amount of energy reserves stored, in the form of lipid (Lee and Atkinson, 1976). The ability to survive appears to be positively correlated with high levels of saturated fatty acids relative to unsaturated fatty acids, in the soil nematode *Heterorhabditis bacteriophora* (Selvan *et al.*, 1993b). A minimum amount of oxygen has also been shown to be crucial for the survival of larval stages of *T. colubriformis* (Sharpe and Lee, 1981).

In a field trial, a benzimidazole resistant strain of *H. contortus* was found to be fitter than a susceptible strain, with respect to both survival and infectivity (Kelly *et al.*, 1978). However, the fitness (egg production, larval development, larval survival, infectivity) of *O. circumcincta* did not differ between benzimidazole resistant (*rr*) and susceptible (*rS*, *SS*) genotypes (Elard *et al.*, 1998). Under field conditions lack of reversion from resistance, when anthelmintic treatment was discontinued, has been reported for both species mentioned above (Martin *et al.*, 1988; Borgsteede and Duyn, 1989), implying that resistant nematodes are no less fit than susceptible ones.

1.3.3.1 Effect of desiccation on larval survival

How well the free-living stages of trichostrongylid parasites cope with desiccation is important for the geographical distribution of the different species. The ability to withstand desiccation explained the geographical distribution of three species of *Trichostrongylus* in Australia, with *T. rugatus* being the most resistant (Beveridge *et al.*, 1989). It has been shown also that embryonated eggs (containing 1st stage larvae) and 3rd stage infective larvae of *T. colubriformis* are more resistant to desiccation than hatched 1st stage and 2nd stage larvae (Anderson and Levine, 1968; Wharton, 1982). Desiccation of 3rd stage larvae even proved beneficial to survival at temperatures below freezing and between 35-50°C, whereas there was no such effect at temperatures in between (Anderson and Levine, 1968). However, repeated desiccation of larvae of *T. colubriformis* has been found to be much more lethal than a single desiccation (Schmidt *et al.*, 1974). Exposing 3rd stage

infective larvae of *H. contortus* to UV-radiation alone and in combination with desiccation was shown to have an untoward effect on the survival of the larvae (Conder, 1978).

1.3.3.2 Effect of temperature on larval survival under controlled conditions

Infective larvae of trichostrongylid nematodes have been shown to survive well in water over a wide temperature range (Andersen *et al.*, 1966; Andersen and Levine, 1968; Beveridge *et al.*, 1989). Numerous laboratory experiments have aimed at determining upper, lower and optimum temperatures for survival in water, in order to exclude effects of humidity. The findings from several of these experiments are summarised in Table 1.3.3.1.

Species	Minimum temperature for survival and time of survival	Temperature range tested for survival and survival time	Optimum temperature for survival and days of survival	Reference
Trichostrongylus colubriformis	-95°C / 16 days	- 95°C to 45°C	4°C/ >425 days 95% alive after 312 days	Andersen et al., 1966
T. colubriformis	-95°C/~50 days	-95°C to 50°C	4°C / >680 days 92% alive after 128 days	Andersen and Levine, 1968
T. colubriformis		20–30°C/65 days *		Beveridge et al., 1989
T. retortaeformis	5°C/450 days	5°C to 40°C	5°C/450 days ~100% survival for the first 300 days	Gupta, 1961
T. vitrinus		20°C/210 days 95% survival after 60-90 days		Gruner and Suryahadi, 1993
T. vitrinus		20-30°C/ 85 days *		Beveridge et al., 1989
T. rugatus		20-30°C / 36 days *		Beveridge et al., 1989
Ostertagia ostertagi	6°C /938 days	6°C to 25°C	6°C/938 days	Rose, 1961
O. circumcincta		20°C /280 days 95% survival after 120-150 days		Gruner and Suryahadi, 1993
Haemonchus contortus		20°C/300 days 95% survival after 150-180 days		Gruner and Suryahadi, 1993
Cooperia curticei	-5°C/5% survival for 6 days	-5°C – 52°C	10 – 15 °C/311-299 days 75% alive after 118-106 days	Ahluwalia, 1974
C. oncophora		6 – 25°C	6-10°C/>730 days	Rose, 1963
Oesophagostomum columbianum		30°C to 45°C	30°C /105 days maximum survival for 25 days	Premvati and Lal,1961

Table 1.3.3.1 Upper, lower and optimum temperatures for survival in water of common trichostrongylid nematodes (* = average S_{50} for temperature range; S_{50} = 50% survival time).

Not surprisingly, the best survival occurs at low temperatures, such as 4 – 6 °C for most species. This also holds true for the red grouse pathogen *T. tenuis*, the infective larvae of which can survive at temperatures as low as –10°C and still remain infective (Connan and Wise, 1994). The long survival at low temperatures ensures that some infective larvae survive the winter, even when temperatures fall to below 0°C and may be an adaptation to a local climate. Long survival at low temperatures can be attributed to reduced activity and conservation of energy stores. The relationship between energy levels and the life span and/or activity level of 3rd stage infective larvae has been described by Selvan *et al.* (1993a) who also found that nematodes adapted to warmer climates tended to initially have higher levels of saturated fatty acids.

1.3.3.3 The effect of temperature on larval survival on pasture

As for the development of the free-living stages, larval survival also varies between species and between geographic regions. Findings regarding the survival of 3rd stage infective larvae on pasture are summarised below in Table 1.3.3.2. Some important findings from other plot trials carried out in Australia were not easily summarised in a table format, due to fundamental differences in climate, and are instead dealt with in the following. In a summer rainfall region (Northern New South Wales), infective larvae of common trichostrongylid parasites were able to overwinter and survive for up to 12 months (Southcott *et al.*, 1976). In a winter rainfall area (Western Victoria), *H. contortus* was not found in tracer lambs at any time, but for other common trichostrongylid parasites a marked seasonal pattern of availability on pasture was demonstrated, with *Ostertagia* and *Trichostrongylus* being virtually absent from pastures during summer months (Anderson, 1972 and 1973). This was in contrast to other findings in a winter rainfall area (Western Victoria), where low numbers of infective larvae of *T. vitrinus* and *O. circumcincta* survived over at least part of the summer (Callinan, 1978a and 1979). This difference in results might be explained by variations in rainfall and ambient temperature between the years in question and/or possibly by regional differences.

1.3.3.4 Nematophagous Fungi

Over the last few years a great deal of work has focused on using and developing biological control agents for various organisms. Promising candidates for biological control of parasites have been the endoparasitic and predatory fungi that attack the free-living stages. Endoparasitic fungi infect nematodes via spores. These adhere to the surface of the nematode and, following germination, fill the body with hyphae (reviewed by Nicholas, 1984 and Wharton, 1986). Predatory fungi, on the other hand, trap the nematodes with their invading hyphae. Once the nematode is trapped, proteins on the hyphae interact with the cuticle of the nematode to dissolve it, and the fungus is then able to invade and grow in the nematode (Skipp, pers.comm.). The outcomes and prospects of this research has recently been reviewed by a number of authors (Waller and Larsen, 1996; Gronvold *et al.*, 1996; Larsen *et al.*, 1997; Larsen, 1999). In a New Zealand study, nematophagous fungi were shown to enter sheep dung within a few days of deposition on pasture. In late summer, 71% of sheep dung samples on grass plots contained

nematophagous fungi, whereas in early autumn this number dropped to 57% (Hay *et al.*, 1997a and 1997b). Recent studies on nematophagous fungi have focused on the species that seem most promising for commercial development for controlling trichostrongylid parasites, namely *Duddingtonia flagrans* (Gronvold *et al.*, 1993; Larsen *et al.*, 1994; Githigia *et al.*, 1997; Mendoza de Gives *et al.*, 1998; Fernandéz *et al.*, 1999), *Arthrobotrys oligospora* (Gronvold *et al.*, 1993; Larsen *et al.*, 1994) and *Harposporium anguillulae* (Charles *et al.*, 1996).

Species	Winter survival; temperatures and survival time	Summer survival; temperatures and survival time	Location	Reference
Trichostrongylus colubriformis (larvae developed on plots)	< 10°C /<42 days	15-20°C/280 days ~100% survival for 6 weeks	Southern England	Gibson and Everett, 1967
T. colubriformis (larvae developed on plots)	°C ?/0 – 150 days	°C ?/280-300 days	Northern England	Boag and Thomas, 1970
T. colubriformis (larvae developed in lab)	-9 – 4°C/69-130 days	18-27°C / 14-63 days	Central US	Andersen *et al.*, 1970
T. colubriformis (larvae developed on plots)	1 – 12°C */1-8 days	35-40°C */24-61 days	Central US	Levine and Andersen, 1973
T. colubriformis (larvae developed on plots)	1 – 8°C / max. 152 days		France	Mallet and Kerboeuf, 1986
T. axei (larvae developed on plots)	6 -16°C */42-140 days	16-26°C */0 days	South Australia	Callinan, 1978b
T. vitrinus (larvae developed on plots)	7 – 15°C */142-174 days	13-31°C */0 –14 days	South Australia	Callinan, 1979
Ostertagia ostertagi (larvae developed in lab)	-7-13°C/182 days	2-38°C/365 days	Southern England	Rose, 1961
O. circumcincta larvae developed on plots)	°C ?/0 – 90 days	°C ?/330-365 days	Southern England	Boag and Thomas, 1970
O. circumcincta (larvae developed in lab)	6 – 13°C */89-183 days	11 – 29°C */1-15 days	South Australia	Callinan, 1978a
Haemonchus contortus (larvae developed in lab)	up to 140 days	up to 120 days on green pasture	Western Australia	Besier and Dunsmore, 1993b
H. contortus (larvae developed on plots)		up to 35 days under dry conditions	Western Australia	Besier and Dunsmore, 1993b
H. contortus (larvae developed on plots)	1-7°C */no survival	14-20°C */60-330 days	Southern England	Gibson and Everett, 1976
Cooperia curticei (larvae developed on grass in pots)	<10°C/90% after 70 days; max. 170 days	18-25°C/max. 63-112 days	Mid New Zealand	Ahluwalia, 1970

Table 1.3.3.2 Upper and lower and optimum temperatures for larval survival on pasture (* = Temperature measured at soil surface). Survival time describes the time from deposition of either eggs or larvae on plots (starting during winter or summer).

1.3.3.5 Infectivity and length of larvae

Since nematode species and strains within species differ in their developmental requirements and optima, it might be expected that development under conditions that are sub-optimal could affect the size, infectivity and survival of infective larvae. While there have been a number of investigations of this, or aspects of it, the results have been inconsistent.

For example, infective larvae of *T. axei* had a significantly higher infectivity for rabbits when cultured at 10°C than at 25°C or 32°C. In contrast, infective larvae of *T. colubriformis* had a significantly higher infectivity when cultured at 25°C than at 10°C or at 32°C (Ciordia *et al.*, 1966). The authors did not provide a clear explanation for this inconsistency. Infective larvae of *O. circumcincta* were not only longer when grown at between 18 and 23°C than when cultured at temperatures above and below this, but also had a higher establishment rate in sheep. Furthermore, a positive correlation was found between faecal moisture content and the length of infective larvae. However, adult worms developing from 'short' and 'long' infective larvae differed neither in length nor in fecundity (Rossanigo and Gruner, 1996).

A number of other authors have shown that infective larvae reach their maximum length at particular temperatures, but without examining infectivity. For example, infective larvae of *Oesophagostomum columbianum* were found to be longer when cultured at 34°C than at temperatures above and below this (Premvati and Lal, 1961). Infective larvae of *T. colubriformis* were significantly longer when cultured at 20°C than at 25°C or 30°C (Wang, 1967). Pandey (1972) made similar observations for infective larvae of *O. ostertagi*, and also found that larval length decreased at culture temperatures above and below 20°C. These quite specific temperatures for maximum length may reflect an adaptation of the species in question to the local climate.

A reduction in faecal water content (from 59 to 53 %) in cultures incubated at 20°C, resulted in smaller 3rd stage infective larvae of *O. circumcincta* and *T. vitrinus* (Gruner and Suryahadi, 1993). These 'small' infective larvae had a decreased survival time probably because they had reduced energy stores compared with normal sized larvae. Contrasting results were found for infective larvae of *O. circumcincta*, where a lower faecal moisture content in cultures produced smaller infective larvae that survived as well in water as 'normal' or 'long' infective larvae (Rossanigo and Gruner, 1996).

The effect of storing 3rd stage larvae for a shorter or longer period of time on the subsequent infectivity of the larvae has been investigated by several authors. Infective larvae of *C. oncophora* that had been stored at 6 - 10°C in water retained their infectivity even after 22 and 25 months of storage, although there was a tendency for it to be lower after 25 months (Rose, 1963). In contrast, infective larvae of *T. colubriformis* had maximum

infectivity during the first 6 weeks of storage at 24°C, after which the infectivity gradually declined (Mallet and Kerboeuf, 1985). Female worms which developed from larvae that had been stored for more than 6 weeks had a higher fecundity than those stored for a shorter time (Mallet and Kerboeuf, 1985). On pasture, the same authors again found an inverse relationship between infectivity of larvae of *T. colubriformis* and the later fecundity of female adult worms (Mallet and Kerboeuf, 1986). Surprisingly, overwintering larvae were found to have a higher infectivity than 'fresh' 3rd stage infective larvae. The observations on fecundity in both of the above studies could not be explained by an effect of population density in the host animals.

1.4 The immune response to gastro-intestinal nematodes

1.4.1 The immune response in the intestine

The gastrointestinal system is the major route of entry for antigens into the body and contains a major portion of the lymphoid tissue in a mammal. The epithelial layer of the mucosa consists of enterocytes, which are specialised cells that provide a physical barrier, but at the same time allow antigens to be taken up readily. The permeability of this layer is greatly enhanced during inflammation. Enterocytes may themselves act as antigen-presenting cells (APC). Antigen uptake also occurs via specialized cells (M-cells) overlying Peyer's patches, found primarily in the jejunum. In the Peyer's patches, which are elevated patches of closely packed lymph follicles, important components for an immune response, such as T-cells, T-helper cells, B-cells and macrophages are found, and these play a major role in the intestinal defence against antigens. The M-cells overlying the Peyer's patches are very efficient in taking up antigen, which they then present to lymphocytes. This in turn results in IgA-production by plasma cells (B-lymphocytes that have undergone cell division following antigen stimulation) found in the walls of the intestine (reviewed by Wakelin, 1984 and Tizard, 1992).

1.4.1.1 Innate and acquired immunity

In mammals, the immune response to infectious agents consists of innate (non-specific) immunity and acquired (specific) immunity. The innate immunity is important at the initial exposure to an infectious agent and dictates the effector responses from the acquired immune response. However, acquired immunity is more important in continuing and secondary infections. The innate immunity features several kinds of defence barriers; anatomic (intestinal epithelium and mucus), physiologic (intestinal microflora, peristalsis, biliary secretions), inflammatory (complement, phagocytes) and phagocytic (uptake and destruction of antigen) (reviewed by Tizard, 1992; McFarlane, 1997). The acquired immune response, comprising cellular and humoral immunity, is described in more detail in the following sections.

1.4.2 Components important in an acquired immune response towards parasites

The following descriptions of the different components and phases of a typical response to infection with nematodes/helminths are related to the overview given in Figure 1.4.2.1.

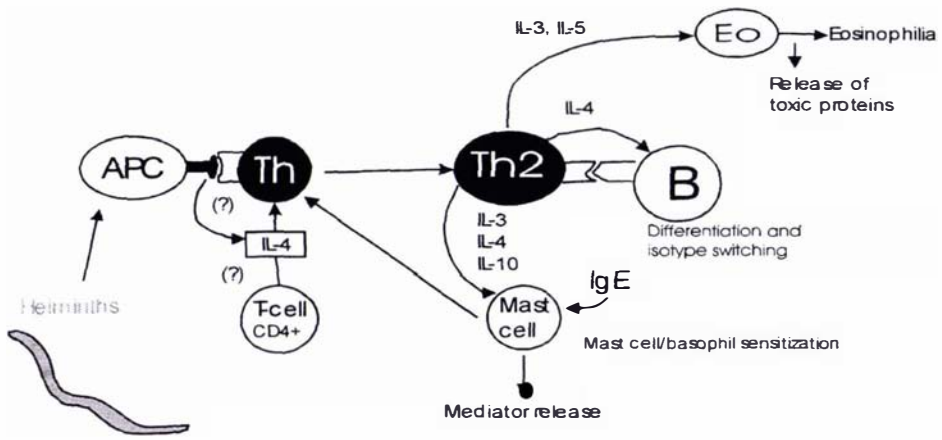

Figure 1.4.2.1 Overview of the Th-2 polarised immune response to helminth infection (adapted from Romagnani, 1996). APC = antigen presenting cell; Th = T-helper cell; Th2 = type 2 T-helper cell; B = B-cell; Eo = eosinophil

1.4.2.1 Parasite antigens

The cuticle covering nematodes is in itself antigenic (somatic antigen), but excretory/secretory (E/S) products released via the nematode's excretory pores are more likely the major antigens for stimulating an immune response (Wakelin, 1984).

1.4.2.2 Induction phase

Antigen is presented to T-helper cells by antigen presenting cells (APC). Apart from the enterocytes and M-cells, mentioned above, these include macrophages, dendritic cells[3] and B-cells. APC carry MHC II[4] molecules on their surface. These are recognized by T-cells of the $CD4^+$ type which, because of specific T-cell receptors (TCR) and possibly because of their production of IL-4, an interleukin[5], aid in directing the T-helper cell response towards a type 2 response. As well as presenting and processing antigen, APC

[3] Dendritic cells are macrophage-like cells with long filamentous processes located in the cortex of lymph nodes and the skin. They are important in antigen trapping.

[4] MHC = Major Histocompatibility Complex. MHC II: A cluster of loci on one autosomal chromosome containing the genes that determine the antigens present on the cell membranes of nucleated cells of most tissues

[5] Interleukins are polypeptides that carry signals between cells in the immune system. They are produced by macrophages, T- and B-lymphocytes and bind to specific receptors on the surface of appropriate target cells

are a source of the cytokine IL-1 which, along with IL-4, is essential for initiating the Th-2 type response. This is typical for infections with extra-cellular organisms and associated with nematode infections in mice and in ruminants, although there is a less clear distinction between T-helper cell responses in the latter host animals (reviewed by Tizard, 1992; Else and Finkelman, 1998). When IL-1 reacts with the corresponding receptors on the T-helper cells, these respond by initiating/inducing an immune response of the Th-2 type, which involves the production of a number of cytokines specific for this cell type (reviewed by Else and Finkelman, 1998). The immune response now enters the effector phase.

Antigen recognition is highly specific and requires the formation of a complex interaction between the TCR on the T-cell surface and processed antigen in the APC with MHC II expressed on their cell surface. The T-cells respond to this interaction by undergoing repeated division to produce clones with the specific TCR. These act as future memory cells or T-helper cells secreting cytokines (reviewed by Wakelin, 1984).

1.4.2.3 Effector phase

As mentioned above, Type 2 T-helper cells are important for immune responses to nematode infections. These secrete a range of cytokines in response to stimulation, including IL-4, IL-5, IL-6, IL-9, IL10 and IL-13. Many of these cytokines are growth factors, which ensure the production of cell types important for the effector response. The effector response of type 2 is typically associated with the production of IgE and IgG_1, eosinophilia and mastocytosis. Although the exact mechanisms of these, in resistance to nematode infection, have yet to be determined, it is likely to be that of a non-specific inflammatory response in the gut through the secretion of inflammatory mediators such as proteases and leukotrienes (reviewed by Hamblin, 1993; Else and Finkelman, 1998).

Immunoglobulins produced as part of a type 2 response include some IgG subclasses (IgG_1 and IgG_2), IgM, IgA and IgE. IgE and IgG are capable of antibody-dependent cell-mediated cytotoxicity (ADCC) and may trigger the release of mucus from goblet cells. IgE is associated with eosinophils and mast cells and cause degranulation of these. IgA has primarily an anti-inflammatory function and may reduce the absorption of antigens across the epithelial barrier by 'immune exclusion' (reviewed by Tizard, 1992).

1.4.2.4 Intestinal mucus

Mucus is produced by goblet cells found in the epithelial layer of mucous membranes, including the abomasal and intestinal mucosae. Although evidence for a positive correlation between number of goblet cells and mucus production has been found for intestinal nematodiasis in rodents, this relationship is less clear in ruminants (reviewed by Miller, 1987). For instance, Douch *et al.* (1986) did not find the number of goblet cells in the intestinal mucosa of sheep to be correlated with resistance to *T. colubriformis*.

Adult parasites live close to the epithelial layer of the mucosa and are therefore likely to be in close contact with the mucus layer lining the mucosal surface. Adult worms

have been shown to ingest mucus. Whether any of the immune mediators or antibodies present in the mucus have a direct effect in the intestinal tract of the worms is not known (Miller, 1987).

In infections with *Trichinella spiralis* in rats, intestinal mucus was not considered to be the main mechanism for rapid expulsion, although it was shown to be trapping the nematodes (Carlisle *et al.*, 1990). However, a reduction in mucus in infections with *Nippostrongylus brasiliensis* in mice interfered with the spontaneous cure and mucus therefore appeared to be of importance for this process (Khan *et al.*, 1995). Mucus trapping of larvae has been suggested to be an important part of the immune response to gastrointestinal nematodes in sheep (Miller, 1987).

1.4.3 *Ostertagia circumcincta* and development of immunity

Results from a number of studies on *O. circumcincta* infections in sheep are summarised in Appendix 1a. The most important results and those of relevance to this thesis are dealt with in the following.

1.4.3.1 The effect of age and infection dose

Lambs are capable of developing an effective immune response to *O. circumcincta* from when they are 4-6 months old (Hong *et al.*, 1986 and 1987; Seaton *et al.*, 1989; Wedrychowicz *et al.*, 1992). It is generally agreed that adult sheep are effectively resistant to nematode parasites. However, pregnant field-reared ewes have been found to be as susceptible to a challenge infection as naïve animals, suggesting some effect of pregnancy on the immune response (Jackson *et al.*, 1988). In contrast, a later study has showed that lactating field-reared ewes were able to effectively prevent establishment of ingested larvae (Leathwick *et al.*, 1999). Whether the ewes in the two trials had been exposed to similar levels of larval challenge with *O. circumcincta* before being housed is not known. This might have contributed to the differences observed.

The rate of development of immunity to *O. circumcincta* is faster the larger the dose of larvae given. After a single infection with either 3000, 10000 or 30000 infective *O. circumcincta* larvae, it took 7, 5 or 4 weeks, respectively, for the worm burden to start declining (Hong *et al.*, 1986). When lambs were trickle infected daily with either 250, 500 or 1000 *O. circumcincta* infective larvae, it took approximately 11, 11 or 9 weeks before worm burdens started to decline (Hong *et al.*, 1987).

1.4.3.2 Effects on the parasites

The first sign of developing immunity to *O. circumcincta* is the stunting of adult worms (Seaton *et al.*, 1989; Coop *et al.*, 1995; Sutherland *et al.*, 1999b). This has been found to happen after the first four weeks of trickle infection of lambs given 1000 L3/day (Seaton *et al.*, 1989). In the same experiment, resistance to establishment of incoming larvae developed from weeks 4-8, including the occurrence of a rapid turnover of the adult worms and arrested development of larvae as L4. Both the rapid turnover of adult worms

(Hong *et al.*, 1987; Seaton *et al.*, 1989) and the inhibition of larvae at the L4 stage (Callinan and Arundel, 1982; Coop *et al.*, 1985; Stear *et al.*, 1995a and b) are characteristic of developing immunity to *O. circumcincta* infections. Adult worms lost due to rapid turnover are replaced by arrested L4s that have resumed their development (Hong *et al.*, 1987). The half-life of adult *Ostertagia* has been estimated to be approximately 10-12 days after single infections of lambs (Charleston, pers.comm.) and approximately five days in continuous infections of lactating ewes (Leathwick *et al.*, 1997). The size of the worm burden appears to reflect the intake of larvae at least until animals are highly immune to *O. circumcincta* (Callinan and Arundel, 1982; Hong *et al.*, 1987; Seaton et al., 1989). As larval intake increases and immunity develops, the proportion of arrested L4s also increases (Coop *et al.*, 1985), resulting in a net loss of adult worms and a reduction in the adult worm burden. A reduction in *in utero* egg counts has been associated with development of immunity and was correlated positively with the length of female worms (Stear *et al.*, 1995b; Sutherland *et al.*, 1999b).

FEC has been reported to be a poor indicator of *O. circumcincta* worm burdens and/or immunity levels (Jackson and Christie, 1979; Coop et al., 1985) as it did not differ at any time between groups of lambs trickle infected with high or low level doses of *O. circumcincta* L3.

1.4.3.3 Histopathological changes in the intestinal mucosa

Immunity to infections with *O. circumcincta* has been associated with increases in mucosal mast cells (MMC) in the abomasal mucosa of sheep (Coop *et al.*, 1985 and 1995; Huntley *et al.*, 1995; Stear *et al.*, 1995b). Globule leukocytes (GL) are considered to be MMC in which the granules have been altered in response to infection (Huntley *et al.*, 1984). Higher levels of GL have been found in the abomasal mucosa of lambs that had developed resistance to *O. circumcincta* (Seaton *et al.*, 1989; Stear *et al.*, 1995b). Increases in the number of tissue eosinophils have also been found in lambs that had been rendered relatively immune to *O. circumcincta* (Stear *et al.*, 1995b).

1.4.4 *Trichostrongylus colubriformis* and development of immunity.

The results from a large number of studies are summarised in Appendix 1b. Many factors have been found to influence the development of immunity to *T. colubriformis*. The more important ones are dealt with in the following.

1.4.4.1 The effect of age

A number of studies involving trickle-infected lambs, have shown that at an age of 5-6 months, they start to express resistance to *T. colubriformis* (Gibson and Parfitt, 1972 and 1973; Chiejina and Sewell, 1974b; Steel *et al.*, 1980; Dobson *et al.*, 1990a, b and c). Until the lambs reach this age, their worm burdens and FEC tend to increase exponentially (Gibson and Parfitt, 1973; Chiejina and Sewell, 1974a and b). When sheep are older than 9-10 months a highly effective immune response to infection can be elicited, eliminating more than 90% of a challenge infection (Gregg *et al.*, 1978; Gregg and Dineen, 1978;

Kimambo *et al.*, 1988b; Emery *et al.*, 1993; Leathwick *et al.*, 1999). The rate of development of resistance to infection in worm-free animals has been found to increase with age. In 12-weeks-old lambs, a trickle infection period of nine weeks was needed before resistance was expressed, whereas in 20-weeks-old lambs, 6 weeks or less was needed Dobson *et al.*, 1990b).

1.4.4.2 The effect of a threshold worm burden

In five-months-old lambs, that had been reared worm-free, an establishment rate of 65% was achieved (Dobson *et al.*, 1990a and b). However, low establishment rates were found when the lambs were trickle-infected for a minimum of 7 weeks. This finding indicated that although the lambs were old enough to express resistance to *T. colubriformis*, a certain threshold of infection dose/worm burden first had to be reached. The threshold worm burden was estimated at 3000-3500 worms, although earlier studies had suggested higher levels (Chiejina and Sewell, 1974a; Windon *et al.*, 1984). Dobson *et al.* (1990b) went on to conclude that once the threshold worm burden had been reached, the rate of development of resistance to *T. colubriformis* appeared to be dependent on age only.

1.4.4.3 The effect of genetic factors

The presence of genetic variation in the response to infection with *T. colubriformis* has been utilised in a number of breeding programmes. Some of these are described elsewhere in this chapter (see section 1.5.4.2). 'High responders' to infection with *T. colubriformis* generally are more immune responsive, as measured by a lower FEC, smaller worm burdens, higher numbers of GL's in the intestinal mucosa and higher levels of specific IgG_1 antibody, than 'non-responders' (Douch, 1988; Douch *et al.*, 1988; Hohenhaus *et al.*, 1995).

1.4.4.4 Effects on the parasites

In infections with *T. colubriformis*, there is no evidence of a rapid turnover of the adult worms (Chiejina and Sewell, 1974a) as is the case with *O. circumcincta* (see above). Instead, developing immunity to *T. colubriformis* is characterised by a rejection of incoming larvae (Dobson *et al.*, 1990a; Steel *et al.*, 1990; McClure *et al.*, 1992; Emery *et al.*, 1992a) along with a loss of adult worms, resulting in a reduction in the worm burden (Chiejina and Sewell, 1974b; Dobson *et al.*, 1990c; Emery *et al.*, 1992b). Highly immune sheep can expel most of the larvae in a challenge infection within one day (McClure *et al.*, 1992). A recent study has even shown that in some immune animals, a challenge infection can be expelled within two hours (Harrison *et al.*, 1999).

Reductions in fecundity and worm length are well-established indicators of immunity to *T. colubriformis* (Gibson and Parfitt, 1973; Chiejina and Sewell, 1974b; Wagland *et al.*, 1984; Douch *et al.*, 1988; Stankiewicz *et al.*, 1993). It has been suggested that a reduction in fecundity precedes the expulsion of adult worms (Chiejina and Sewell, 1974b) whereas others have proposed that these two events take place at approximately the same time (Dobson *et al.*, 1990c). In goats, trickle infected with *T. colubriformis*, immunity was

associated with a reduction in both fecundity as well as a reduction in the male/female worm ratio (Pomroy and Charleston, 1989).

1.4.4.5 Histopathological changes in the intestinal mucosa

Various cellular changes take place in the intestinal mucosa in response to infection with *T. colubriformis*. A clear inverse relationship between the worm burden and numbers of globule leukocytes (GL) in the epithelial layer of the intestinal mucosa has been shown by a number of authors (O'Sullivan and Donald, 1973; Gregg *et al.*, 1978; Douch *et al.*, 1986; Douch *et al.*, 1988; Douch, 1988 and 1989; McClure *et al.*, 1992; Harrison *et al.*, 1999). The appearance of high numbers of GL's is generally taken as a reliable indicator of immunity to *T. colubriformis*. An increase in the numbers of mucosal mast cells (MMC) has, in some instances, also been associated with increasing levels of host immunity to *T. colubriformis* (O'Sullivan and Donald, 1973; Douch, 1989; Harrison *et al.*, 1999) whereas in other studies there was no clear relationship with immunity (Gregg *et al.*, 1978; Douch *et al.*, 1986). Although increased numbers of eosinophils have been reported to be associated with an increased level of immunity to *T. colubriformis* (Douch, 1989), others have found no such relationship (Gregg *et al.*, 1978). For other species of nematodes, there is some indication of a positive correlation between host immunity and the numbers of eosinophils in the mucosa. For example, eosinophilic infiltration in the abomasal mucosa was associated with a higher level of resistance to *H. contortus* in Florida Native lambs (Bradley *et al.*, 1973).

1.4.4.6 Changes in intestinal mucus

Higher Larval Migration Inhibition (LMI) activity has been found in mucus from 'high responder' and immune sheep (Douch *et al.*, 1983; Douch *et al.*, 1986; Douch *et al.*, 1988; Kimambo and MacRae, 1988; Douch, 1989; Stankiewicz *et al.*, 1993; Douch *et al.*, 1996; Harrison *et al.*, 1999), suggesting that paralysing incoming larvae is an important part of an effective immune response to *T. colubriformis* and important for the rejection of incoming larvae. Rapid rejection (within the first day) of a challenge infection of *T. colubriformis* in immune sheep has also been found to be associated with increases in IgG_1 and IgG_2 in mucus, whereas the rejection of the remaining worms, over the following days, was associated with increases in IgA and IgG_2 (McClure *et al.*, 1992). In a more recent study, rapid rejection of a *T. colubriformis* challenge infection was found to be associated with increases in IgG_1, IgA and histamine in the intestinal mucus (Harrison *et al.*, 1999).

1.4.5 Other factors that may affect the development and expression of immunity

1.4.5.1 The effect of nutrition

An inadequate diet may interfere with the development and expression of immunity. Coop *et al.* (1995) found indications that age-dependent immunity to *O. circumcincta* may in fact be due to a relative protein deficiency in young, growing lambs.

Similar findings have been made for *T. colubriformis* where animals, in order to rapidly develop an immune response to the parasite, needed a diet adequate in protein (Wagland *et al.*, 1984; Houtert *et al.*, 1995; Kambara and McFarlane, 1996). However, others have found the development of acquired immunity to *T. colubriformis* to be unaffected by the nutritional treatment before the challenge infection (Kyriazakis *et al.*, 1996). Recently, it has even been reported that nutritional requirement for immune functions appear to have priority over those of growth, and that various indicators of immunity (FEC, worm burden and fecundity) were unaffected by a decrease in the supply of metabolisable protein (Kahn *et al.*, 2000).

1.4.5.2 Sex of host animal

Differences in host immunity, due to gender, have been shown in both ruminants and rodents. The mechanism for this difference is thought to be related to a better ability of females to sustain high levels of antibody and that their cell-mediated immune response is more active (reviewed by Barger, 1993). In sheep, it is generally accepted that post-pubertal ewes are more resistant to parasitic infection than post-pubertal rams (reviewed by Barger, 1993). However such a difference has also been shown in lambs prior to their reaching puberty. Five to six months old ewe lambs were shown to be more responsive to vaccination with irradiated larvae of *T. colubriformis* than ram lambs of the same age (Windon and Dineen, 1981; Windon *et al.*, 1984). The authors suggested that this be due to some effect of non-specific components as well as specific components of resistance.

1.4.5.3 Cortico-steroids

Systemic treatment with dexamethasone effectively abrogates resistance to infections with trichostrongylid parasites (Douch *et al.*, 1986 and 1994; Presson *et al.*, 1988; Emery and McClure, 1995), although the effect is reversible (Douch *et al.*, 1988; Buddle *et al.*, 1992). Dexamethasone specifically suppresses T-lymphocyte function, inhibits monocyte-macrophage activities and suppresses antibody production (reviewed by Jenkins, 1992); all important parts of an effective immune response to infections with nematodes. As a consequence, the administration of cortico-steroids is commonly used in experimental studies of gastrointestinal parasites. It is not known whether cortico-steroids or metabolites thereof have a direct effect on the development of the free-living stages. However, in *T. colubriformis* infected animals that were immune-suppressed with cortico-steroids, LD_{50} values for ivermectin in an LDA, were shown to decrease and remain at a low level in contrast to in infected animals that were not immune-suppressed, where LD_{50} values were consistently higher and rose to a peak 70 days after infection (Hoza, 1998). These results indicated that cortico-steroids may in some way increase the sensitivity of *T. colubriformis* eggs to ivermectin and perhaps, in general, lower the viability of the eggs.

1.4.5.4 Anthelmintics

An effect of anthelmintic treatment on the immune response has been reported recently. Oxfendazole, fenbendazole and ivermectin were all found to have an adverse effect on lymphocyte blastogenesis *in vitro* and on antibody production to human

erythrocytes and ovalbumin after two drenches, in lambs that had been reared worm free before being infected and drenched (Stankiewicz *et al.*, 1994; Cabaj *et al.*, 1994; Stankiewicz *et al.*, 1995). However, in later field studies, oxfendazole was found to have a beneficial effect on the development of resistance to *T. colubriformis* and *O. circumcincta* (Stankiewicz *et al.*, 1996b; Stankiewicz and Hadas, 1996). The authors did not comment upon why their later results for oxfendazole did not agree with what they had reported earlier (Stankiewicz *et al.*, 1994). In a more recent study on the effect of oxfendazole on the development of immunity to *H. contortus*, no adverse effects of the anthelmintic could be demonstrated (Schallig *et al.*, 2000). Ivermectin-abbreviated infections were, in a field study, found to prevent the development of an effective immune response in lambs to infections with *T. colubriformis* and *O. circumcincta* (Stankiewicz *et al.*, 1996b). However, a later study, following the same experimental protocol, did not demonstrate this effect of ivermectin-abbreviated infections on the development of immunity to *T. colubriformis* and *O. circumcincta* (Vlassoff *et al.*, 1999).

A possible way in which anthelmintics could reduce the development of immunity is simply due to their removal of antigenic stimulation. When lambs trickle infected with *T. colubriformis* were drenched weekly, development of resistance to infection was prevented (Gibson *et al.*, 1970). A reduction in protective immune response following continuous anthelmintic treatment has also been demonstrated in the field in sheep treated with capsules (CRC) releasing albendazole (Sutherland *et al.*, 1999a; Schallig *et al.*, 2000) and in cattle treated with ivermectin CRC (Claerebout *et al.*, 1999).

1.4.6 How the immune system may affect the development of the free-living stages of gastrointestinal nematodes

Assuming that there is an effect of the immune response on the development of the free-living stages, it is not known how and where it manifests itself. One possibility could be that the formation of immune complexes (between antigenic excretory and secretory products from the parasites and immunoglobulins (Tizard, 1992)), at the parasites' secretory and excretory pores, interferes with the uptake of nutrients and the excretion of waste products. This may in turn have an effect on their reproductive capability, rendering them less fertile and could as a consequence result in less viable or sterile eggs. Another possibility is that when adult worms ingest mucus (Miller, 1987), any immune mediators and/or immune products that might be present in this, could exert an inflammatory reaction directly in the parasites' digestive system and/or act on smooth muscle herein, subsequently interfering with the uptake and breakdown of nutrients, with the same consequences as mentioned above. Finally, there is the possibility of a direct effect of immune mediators and/or immune products on the eggs as they pass through the gastro-intestinal tract.

1.5 Breeding for resistance to trichostrongylid parasites

The main incentive for selective breeding of sheep that are more resistant to gastro-intestinal nematodes has been increasing problems with anthelmintic resistance. It is no

coincidence that the initiation of such selection lines was to take place in Australia and New Zealand as these are two countries where the problem of anthelmintic resistance is particularly grave. Most breeding programmes started in the early 80's and many authors have since then reviewed and discussed the mechanisms involved, as well as the strategies for, and the results of, breeding for resistance to parasites in sheep (Albers and Gray, 1987; Bisset *et al.*, 1991; Windon 1991; Gruner, 1991; Gray, 1991; Gray, 1995; Woolaston and Eady, 1995; Morris *et al.*, 1995; Windon *et al.*, 1996; Woolaston, 1996; Baker, 1996; Callaghan and Beh, 1996; McEwan *et al.*, 1997).

Today, selection for host resistance to internal parasites is widely practised by New Zealand ram breeders as part of their overall genetic improvement strategy (McEwan *et al.*, 1997).

Although the breeding programs in New Zealand and Australia have involved sheep, elsewhere in the world there have been studies carried out using other ruminants, such as cattle (Gasbarre *et al.*, 1990). Selection for enhanced disease resistance has also been carried out with pigs, in order to study inflammatory responses to bacterial infection (Magnusson *et al.*, 1999).

1.5.1 Resistance and Resilience

When categorising an animal's response to gastrointestinal parasites there are several ways of doing so. One possibility is to consider 'high responders' and 'low responders', determined by the speed and magnitude of an animal's immune response to parasitic infection. However, if one is mainly concerned with how well the animals deal with an infection and whether their production level is affected to any great extent because of the infection, the distinction between 'resistance' and 'resilience' put forward by Albers and Gray (1987) may be of more use. They defined **resistance** as 'the ability to suppress establishment and/or subsequent development of infection', and **resilience** as 'the ability to maintain a relatively undepressed production level when infected'.

Albers and Gray (1987) reported a fairly strong and positive correlation between resistance and resilience. This positive correlation has since been questioned by a number of authors who have found a negative correlation between resistance and resilience, as measured by various production parameters (McEwan *et al.*, 1992; Howse *et al.*, 1992; Williamson *et al.*, 1995a). In the light of this, one may argue that resilience would be the preferred trait to breed for, as grazing animals are always likely to encounter parasites on pasture and as highly resilient animals are more able to cope with an infection. On the other hand, one must not forget the benefits of breeding for resistance in sheep. Resistant animals carry smaller worm burdens and consequently pass fewer eggs out onto the pasture, i.e. they can provide valuable means of reducing pasture contamination. This in turn provides a lower challenge to the animals' immune system. Among the consequences of selective breeding for resilience is that levels of pasture contamination with infective larvae could increase because of higher FEC in host animals. It is not known how this

may affect animal production under farming conditions. Certainly, with some parasites such as the blood-sucking parasite *H. contortus*, adverse effects would be expected.

Most selection work in sheep in New Zealand and Australia has focused on selection for enhanced resistance rather than resilience.

1.5.2 Genetics of host resistance

Albers *et al.* (1987) suggested a polygenic selection approach for breeding programmes. This was discussed and supported by Beh and Maddox (1996), who suggested that 'resistance to gastrointestinal nematodes in sheep is likely to be a polygenic trait with a small number of genes encoding products affecting functions of the immune system accounting for a significant proportion of the population variation.

Genetic research is at present directed at locating microsatellite[6] markers with a close proximity to major genes involved, and at identifying genetic markers linked to genes with large effects (QTL[7]) on host resistance to internal parasites (reviewed by Stear and Murray, 1994 and McEwan *et al.*, 1997)

1.5.2.1 Experiments involving rodents

The effect of genotype has been studied in several studies on gastrointestinal parasitic infections in rodents. Some important findings from these studies are summarised below.

In random-bred mice infected with *Trichuris muris* it was found that there was a bimodal variation in their ability to effect an immune expulsion of the parasite. This variation could only be ascribed to genetic variation and was independent of the size of the infection. It was furthermore found that the ability to effect worm expulsion was inherited as a dominant characteristic. It was suggested that the genetic control of resistance to *T. muris* involved only a small number of genes (Wakelin, 1975). A later study confirmed the marked host strain variation in resistance of mice to *T. muris*. It went on to show that genes both within and outside the mouse major histocompatibility complex (H-2) were involved in determining the host response genotype and that susceptibility or resistance to *T. muris* could at least partly be ascribed to different haplotypes of H-2 (Else and Wakelin, 1988).

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[6] Microsatellites are randomly distributed throughout the mammalian genome and consist of tandem repeats of di-, tri-, tetra- or penta-nucleotide sequences.

[7] QTL = Quantitative Trait Loci. These are genes that have a major effect on the trait of interest.

Genetically determined differences in the immune response of mice have also been shown to infection with *Trichinella spiralis*[8]. These manifested themselves as differences in the mice's ability to produce early and high level responses to the antigens of *T. spiralis* and the further expression of intestinal effector mechanisms (Robinson *et al.*, 1995).

In guinea pigs, high and low responder lines to infection with *T. colubriformis* have been established and their immune responses studied (Rothwell *et al.*, 1978; Manjili *et al.*, 1999). Amongst other things, these selection lines differ in IgG_1 responses to *T. colubriformis*.

1.5.2.2 Immune mechanism for genetic resistance in sheep

In an attempt to confirm earlier findings that there was an immunological basis for differences in resistance to infections with gastrointestinal nematodes in sheep as shown by numerous workers (Dineen and Windon, 1980a and b; Windon *et al.*, 1980; Windon and Dineen, 1981; Albers *et al.*, 1987), Presson *et al.* (1988) immunosuppressed genetically parasite resistant 12 months-old Merino sheep. The immunosuppressive treatment abolished differences between resistant and susceptible animals with respect to FEC, worm weights, thymus weights and infiltration with globule leukocytes in response to infections with *H. contortus*.

It appears that animals selected for resistance do not always express this when first infected. In response to a primary infection with *H. contortus*, lambs that were genetically resistant to *H. contortus* had significantly higher FEC and total worm burdens than random-bred lambs (Gill, 1991). Since there were no differences between the two groups of lambs with respect to various acquired immune responses, it was suggested that this difference was due to an innate characteristic, yet to be defined. However, after a secondary infection with *H. contortus*, the resistant lambs had significantly lower FEC and worm burdens than the random-bred lambs. As acquired immune responses, such as levels of mucosal mast cells (MMC), anti-*Haemonchus* antibodies and mucosal tissue eosinophils, correlated positively with the resistance status of the host, it was concluded that the genetic resistance to *H. contortus* in lambs results from the expression of an acquired immune response.

Having established the requirement for an acquired immune response, the importance of a T lymphocyte response in lambs genetically resistant to *H. contortus* was soon demonstrated (Gill *et al.*, 1993c). It appeared that only after a secondary infection were such differences significant – as shown in previous work (Gill, 1991; Gill *et al.*, 1993b). Gill *et al.* (1993a) then elegantly demonstrated how the $CD4^+$ subset of T-cells played a major and crucial role in mediating genetic resistance to *H. contortus*. By

[8] *Trichinella spiralis* is an intestinal nematode in the mouse and other mammals

selectively depleting $CD4^+$ cells in six months old genetically resistant lambs, the expression of genetic resistance was abrogated, i.e. FEC and worm burdens increased and numbers of MMC, tissue eosinophils and specific antibodies decreased. In contrast, depletion of $CD8^+$ T-cells had no effect on genetic resistance and the associated parameters.

Peripheral blood mononuclear cells (PBMC) from 6 months old genetically resistant Merino lambs showed consistently higher blastogenic responses to both larval and adult antigens of *H. contortus* than PBMC from random-bred lambs (Gill, 1994). Responding cells were mainly of the T-helper cell type and it was suggested that resistant lambs have a greater ability to mount a parasite-specific cell-mediated immune response than random-bred lambs.

The thymus is at its maximum capacity during puberty and is essential to the development of cell-mediated immunity/T-cell dependent immune response. Some workers have measured thymus weights, but inconsistent results have been reported. Presson *et al.* (1988) found thymus weights to be higher in 12 months old sheep genetically resistant to *H. contortus*, whereas Williamson (1994) found thymus weights to be higher in six months old high greasy fleece-weight selected sheep, shown to be more susceptible to infections with *H. contortus* and *O. circumcincta*. A possible explanation for this discrepancy might be differences between breeds with respect to the acquired immune response or perhaps the age difference of the animals in the two trials.

1.5.3 Breed differences

Numerous studies have demonstrated that there are breed differences in susceptibility to gastrointestinal nematodes. Some hair sheep breeds from tropical climates and some breeds bred primarily for their wool have been shown to be the most resistant (Bradley *et al.*, 1973; Yazwinski *et al.*, 1979; Courtney *et al.*, 1984, 1985a and 1985b; Baker, 1996; Stear *et al.*, 1996, 1997 and 1999; Amarante *et al.*, 1999). These include the Florida Native, Barbados Blackbelly, St. Croix, Red Maasai and Scottish Blackface. European breeds were generally less resistant than the breeds mentioned above (Altaif and Dargie, 1978; Bouix *et al.*, 1998) but more resistant than other breeds that are primarily for fine-wool production, such as the Merino and Rambouillet breeds (Bradley *et al.*, 1973; Courtney *et al.*, 1985b; Amarante *et al.*, 1999).

In New Zealand, sheep of the Perendale breed and Texel breed crosses have been shown to be more resistant to parasite infection than sheep of the Romney breed (McSporran and Andrewes, 1988; Watson *et al.*, 1992b; McEwan *et al.*,1997).

Generally, FEC was used an indicator of the resistance level in all of the above listed references and the mechanism responsible for differences in resistance was not further described. There are some indications that between-breed variations may be caused by differences in the acquired immune response, such as elevated levels of circulating eosinophils (Bradley *et al.*, 1973; Amarante *et al.*, 1999). However, some authors

have found no differences in other immune characteristics, such as specific serum antibody levels (Yazwinski *et al.*, 1979).

In a tropical climate there is a rapid development of nematode eggs to larvae and a rapid build-up of contamination on pastures. It therefore seems likely that the local sheep breeds would be under a strong selection pressure to select those individuals that early on in life exhibit a strong resistance particularly to *H. contortus*. Given that this 'natural' selection has taken place over a long period of time, it would account for the observed differences between some of the breeds (except for Scottish Blackface sheep, which are predominant in a cold temperate climate zone).

1.5.4 Within breed differences

As noted earlier, most sheep selection programmes have aimed at improving resistance rather than resilience to gastrointestinal nematodes. In Australia and New Zealand where most of these breeding programmes have taken place, the Merino and Romney breeds have been the preferred breeds to use. In New Zealand some genetic improvement in resistance to parasites has also been achieved with the Perendale breed (Watson *et al.*, 1992a and b). Several of the selection programmes have been based on differentiating responses to single species infections, whereas others have used responses to naturally acquired infections as a basis for selecting for increased resistance to gastrointestinal nematodes.

1.5.4.1 Breeding flocks selected for resistance to Haemonchus contortus

In New Zealand a Perendale breeding flock, initially selected for resistance to infections with *H. contortus*, was established in 1986 (Watson *et al.*, 1992a). This flock is described in greater detail in a later section (see 1.5.8.1).

Several breeding flocks with enhanced resistance to *H. contortus* exist in Australia (Albers *et al.*, 1987; Woolaston *et al.*, 1990). Before challenge infection with *H. contortus* and selection for enhanced resistance on the basis of responses to this infection, lambs in these breeding flocks had experienced natural challenge. Albers *et al.* (1987) investigated resistance and resilience to *H. contortus* in a Merino flock, which consisted of descendants of the so-called 'Golden Ram', a ram whose progeny had showed well above average resistance to *H. contortus*. High genetic correlations between FEC and haematocrit (PCV) were found and, interestingly, heritabilities (h^2) of the traits mentioned, were higher at 4 than at 5 weeks after infection, suggesting that the pre-adult stages of the parasites were also important to the stimulation of resistance. Overall, breeding for resistance to *H. contortus* in Merino lambs did not decrease production (as measured by liveweight gain and wool production) and it was concluded that it was worthwhile to include production parameters in a selection programme for enhanced parasite resistance.

A large divergence in resistance has been obtained with the Merino flocks. Highly significant differences between lines in FEC were obtained after a few generations of

selection (Woolaston *et al.*, 1990). This difference was also present in adult sheep, at least until the age of 5 years (Thamsborg *et al.*, 1999) and, at the time of the PPR, where High Responder ewes maintained a lower FEC than control ewes (Woolaston, 1992).

There is some evidence that Merinos selected for enhanced resistance to *H. contortus* also show higher resistance to infections with *T. colubriformis* (Woolaston *et al.*, 1990; Gray *et al.*, 1992; Sréter *et al.*, 1994). A similar observation has been made for Romneys (Pernthaner *et al.*, 1996).

1.5.4.2 Breeding flocks selected for resistance to Trichostrongylus colubriformis

Numerous studies have been carried out in Australia using animals from flocks selected for enhanced resistance to *T. colubriformis* (Dineen and Windon, 1980 a and b; Windon *et al.*, 1980; Windon and Dineen, 1981; Outteridge *et al.*, 1985, 1986 and 1988; Dawkins *et al.*, 1989; Jones *et al.*, 1990; Rothwell *et al.*, 1993; Larsen *et al.*, 1999). The testing for responsiveness to 'vaccination' with *T. colubriformis* was, in all studies, as follows: lambs were reared worm free, weaned at 11 weeks of age, vaccinated with irradiated larvae of *T. colubriformis* at 8 and 12 weeks of age, drenched at 16 weeks of age and challenged with normal larvae of *T. colubriformis* at 17 weeks of age (Dineen and Windon, 1980b). Lambs were tested for FEC after challenge and divided into high responders (HR) and low responders (LR). Since then, significant differences in FEC has been confirmed in several studies (Windon *et al.*, 1980; Windon and Dineen, 1981; Outteridge *et al.*, 1986 and 1988).

Significantly lower worm burdens were found in HR 5-month-old wethers (Dineen and Windon, 1980a and b) and a positive correlation was found between worm burdens and worm lengths, eggs *in utero* and male/female worm ratio. The authors suggested that immune mediators were responsible for these adverse effects on the adult worms in the gastrointestinal system.

A Romney breeding flock, initially selected by screening a large population of random bred ewes for responsiveness to natural mixed infections containing a large component of *T. colubriformis* and now selected for low FEC following natural mixed larval challenge on pasture, was initiated in New Zealand in 1979 (Bisset *et al.*, 1991). This flock has since been employed in a large number of studies (Buddle *et al.*, 1992; Pernthaner *et al.*, 1995 and 1996; Douch *et al.*, 1995; Bisset *et al.*, 1996 and 1997) and is described in further detail in section 1.5.8.2.

1.5.5 Age dependence of resistance

From a number of studies it appears that increased resistance to gastrointestinal nematodes is expressed more strongly at certain stages of a sheep's life. The response also appears to differ between the different species of parasites used for the initial infection or challenge. For example, in Merino flocks selectively bred for resistance to *H. contortus*, it was found that selection based on FEC from when the lambs were five months old, predicted the future resistance level of the animals well, at least until they were 5 years old

(Albers *et al.*, 1987; Gray, 1991; Thamsborg *et al.*, 1999). Although Barger (1989) found highly significant differences between selection lines in establishment rate of *H. contortus* in 4-5 months old Merino lambs, these differences could not be demonstrated when the lambs from the same flock were 8-9 months old. In 8-9 months old Romney lambs from a flock selected for responsiveness natural mixed infections, there were highly significant differences between High and Low FEC lines in FEC and worm burdens after natural challenge (Bisset *et al.*, 1996). Results from the WormFEC™ breeding programme in New Zealand suggests that the biggest differences in FEC in response to mixed natural challenge may be found when the lambs are approximately 7-9 months old (McEwan *et al.*, 1997).

1.5.6 Selection criteria

When selecting for resistance to parasites after either natural challenge or 'vaccination', a number of criteria may be employed. These are listed and commented upon in the following.

1.5.6.1 FEC

Using FEC as the sole criterion for the selection of animals that are more resistant to nematode parasites, has been a common procedure for most breeding flocks. FEC is easily and cheaply carried out, and has generally proved to be a relatively good measure of an animal's worm burden, at least in animals younger than 12 months (Dineen and Windon, 1980a; McKenna, 1981; Bisset *et al.*, 1996). FEC is likely to remain the most popular criterion until another equally simple and cost-effective method is available.

1.5.6.2 Specific Antibody levels - IgG

Windon and Dineen (1981) found serum antibody levels to larval antigen of *T. colubriformis* to be inversely related to FEC, suggesting that this parameter might be a good indicator of resistance to parasites. Although this favourable relationship between FEC, resistance level and specific antibody levels (IgG_1) has since been confirmed by a number of workers (Gill, 1991; Gill *et al.*, 1993a and b; Douch *et al.*, 1995; Bisset *et al.*, 1996) there are also reports that suggest no such relationship (Williamson, 1994; Williamson *et al.*, 1995a and b; Larsen *et al.*, 1999). When testing immune responsiveness in three months old lambs, Kassai *et al.* (1990) failed to demonstrate that development of immunity was associated with an increase in specific antibody levels to *H. contortus*. It was concluded that this parameter was of no predictive value when identifying lambs that are genetically resistant to *H. contortus*.

1.5.6.3 Packed Cell Volume (PCV) / Haematocrit (cell volume/plasma volume)

There is some evidence of PCV being lower and less prone to decrease in tropical sheep breeds (Bradley *et al.*, 1973; Courtney *et al.*, 1985b; Baker, 1996), Merinos (Albers *et al.*, 1987; Woolaston *et al.*, 1990) and in High Greasy Fleece-weight selected Romneys (Williamson, 1994, Williamson *et al.*, 1995b), indicating that these animals experience a lower degree of anaemia in response to infection with *H. contortus*. However, other authors have found this parameter to be of no value when selecting for resistance in Merino sheep

(Kassai *et al.*, 1990; Gray *et al.*, 1992). Generally this parameter has not been of value and is rarely used in studies of genetically resistant sheep.

1.5.6.4 Haemoglobin type (Hb type)

There have been some reports of a correlation between Hb type AA or AB and low FEC and worm burdens in infections with *H. contortus* (Altaif and Dargie, 1978; Courtney *et al.*, 1985a), whereas others have failed to demonstrate such a relationship (Riffkin and Dobson, 1979; Yazwinski *et al.*, 1979; Windon *et al.*, 1980; Kassai *et al.*, 1990). Given these discrepancies, screening for Hb type is of limited value to breeding programmes.

1.5.6.5 Eosinophil counts

Numbers of circulating eosinophils were higher at some times after infection with *T. colubriformis* in high responder Romney and Merino lambs, and furthermore a negative correlation with FEC was demonstrated (Dawkins *et al.*, 1989; Buddle *et al.*, 1992; Rothwell *et al.*, 1993; Woolaston *et al.*, 1996; Larsen *et al.*, 1999). Other workers have failed to demonstrate this relationship and indeed found no differences between selection lines (Dineen and Windon, 1980b; Topper *et al.*, 1992; Pernthaner *et al.*, 1995; Bisset *et al.*, 1996). In addition, eosinophil counts prior to challenge infection were not indicative of peak eosinophil counts (7 – 10 weeks PI) or development of resistance to *T. colubriformis* (Buddle *et al.*, 1992).

Woolaston *et al.* (1996) concluded that since eosinophil counts are less heritable than FEC and are not simpler to measure, they offer no advantage over FEC as a selection criterion for resistance.

1.5.6.6 Ovine lymphocyte antigen (OLA)

Antigen of the type SY1 on the OLA in Merino sheep has been shown to be associated with high responsiveness to infection with *T. colubriformis* and to low FEC (Outteridge *et al.*, 1985, 1986 and 1988; Douch and Outteridge, 1989). In Scottish Blackface lambs presence of the DRB1 allele on the ovine major histocompatibility complex (MHC or OLA) was associated with low FEC in response to natural infections consisting mainly of *O. circumcincta* (Schwaiger *et al.*, 1995; Buitkamp and Epplen, 1996). OLA testing may be of some merit to breeding programmes but is not cost-effective at this stage.

1.5.7 Effects of selecting for resistance on production

Favourable responses in production (such as weight gain and wool production) to selection for enhanced resistance to parasites have been reported for several breeds of sheep (Bradley *et al.*, 1973; Windon *et al.*, 1980; Outteridge *et al.*, 1988; McEwan *et al.*, 1992; Bisset *et al.*, 1997). Other workers have found no effects of selecting for resistance on production parameters (Albers *et al.*, 1987; Leathwick, pers.comm.).

In contrast, there are a large number of publications reporting unfavourable associations between genetic resistance (as judged by low FEC) and production parameters such as liveweight gain (Watson *et al.*, 1986; Howse *et al.*, 1992; Eady *et al.*, 1998), wool production (Howse *et al.*, 1992; Williamson, 1994; Williamson *et al.*, 1995a; Eady *et al.*, 1998) and dag score (Watson *et al.*, 1986; Douch *et al.*, 1995; Bisset *et al.*, 1997; Larsen *et al.*, 1999).

Since a majority of publications report unfavourable production responses to selection, it would appear that we can't 'have it all' and, as Riffkin and Dobson (1979) conclude, having selected sheep for production traits appears to have rendered them more susceptible to infections with parasites (*H. contortus*). One should, however, bear in mind the main positive feature of genetically resistant sheep, namely their ability to lower pasture contamination. This in itself has an effect on the size of infections sheep will encounter on pasture and should subsequently reduce production losses due to parasitism. In general, there have been very few studies of how resistant animals' production behaves if grazed under low larval challenge, and separate from unselected or susceptible animals, over a longer period. However, some studies in New Zealand have indicated that production advantages in resistant animals (weight gain over autumn winter) were small (Bisset *et al.*, 1997) or negligible (Leathwick, pers.comm.).

1.5.8 Selection lines in New Zealand

1.5.8.1 Perendale flock

This flock was established at Ruakura Agricultural Centre in Hamilton in 1984-5 when Perendale lambs were screened for either extreme high or low FEC. Single-trait selection (FEC) was then carried out on the lambs after an artificial infection with *H. contortus* (infected at 12 and 16 weeks of age, drenched 63 days later and challenged 14 days after being drenched. Selection of ram and ewe replacements has been based only on FEC ranking in samples taken 5 – 7 weeks after each infection (Watson *et al.*, 1992a; Morris *et al.*, 1995). In 1992, the Perendale flock was moved to Flock House Research Station near Bulls, where the two selection lines (High FEC line and Low FEC line) were grazing separate farmlets. No further selection took place after the shift to Flock House (Leathwick, pers.comm.).

Both ewes and lambs in these lines have been shown to be very divergent in FEC (Watson *et al.*, 1992, Morris *et al.*, 1995, Jorgensen *et al.*, 1998; Leathwick, pers.comm.). The difference in egg output resulted in significantly different parasite larval infestations on pasture. Although Low FEC line ewes tended to be smaller than High FEC line ewes, this was shown not to have a significant effect on the performance of their lambs (Leathwick, pers.comm.). Recently a variation in developmental success of eggs to 3rd stage larvae in samples from Perendale ewes and lambs in both lines has been demonstrated (Jorgensen *et al.*, 1998). It was shown that the developmental success of eggs was significantly lower in more resistant animals (Low FEC line ewes and lambs) than in susceptible animals (High

FEC line ewes and lambs). These findings prompted further studies which form an important part of the study reported in this thesis (see Chapter 2).

1.5.8.2 Romney selection lines at Wallaceville

At Wallaceville Animal Research Centre in Upper Hutt, Romney selection lines were started in 1979 and merged with a second flock in 1988. This flock was single trait selected on the basis of FEC after naturally acquired infections on pasture and consists of a High FEC line and a Low FEC line which are very divergent in FEC (Morris *et al.*, 1995; Bisset *et al.*, 1996).

Grazing the two lines separately resulted in a significant growth rate advantage for the Low FEC line (resistant) lambs. However the Low FEC line lambs have also been shown to be more prone to breech soiling, the reason for this probably being a hypersensitivity reaction due to their 'high responder'/resistant status (Bisset *et al.*, 1997). Overall, there are not large advantages in animal performance in this flock, but potential epidemiological benefits due to lower egg output from Low FEC line animals (Bisset *et al.*, 1997).

1.5.8.3 PT-flock at Massey

This selection flock was established in 1956 when sheep for two lines were randomly drawn from a common base population of New Zealand Romneys. In one of the lines selection for yearling high greasy fleece-weight (HFW line) took place until in 1958 the lines were closed and further selection was carried out on progeny from the HFW line. The other line was a control line (C Line). Replacement selection in the C line was and is random, whereas in the HFW line animals were and are selected on the basis of high greasy fleece-weight at hogget shearing. The heritability of high greasy fleece-weight was estimated at 0.10 – 0.17 and after selection for 24 years each ewe in the HFW line was producing 4 – 5 kg more greasy wool than a ewe in the C line over a five year period (Blair *et al.*, 1985).

An unfavourable correlation between high greasy fleece-weight and FEC in the two selection lines was first reported by Howse *et al.* (1992). Although not significantly different, both ewes and lambs in the HFW line had consistently higher FEC than ewes and lambs in the C line. The dominant species in larval cultures was found to be *H. contortus*. FEC was found to be consistently higher in 15 months old male sheep in the HFW line, although not always significantly so (Williamson, 1994; Williamson *et al.*, 1995a and b). Generally, HFW line sheep were more susceptible to establishment of challenge infections of *H. contortus* and *O. circumcincta* but not *T. colubriformis* (Williamson, 1994).

Although different with respect to FEC, the two selection lines did not differ in levels of specific antibody (IgG_1) to *H. contortus* and *T. colubriformis* (Williamson, 1994; Williamson *et al.*, 1995a and b).

The PCV (Packed Cell Volume) was found to be lower in HFW line animals than in C line animals (Williamson, 1994, Williamson *et al.*, 1995b). It turned out that a larger proportion of animals in the C line had type A haemoglobin (Williamson *et al.*, 1995b), a type associated in some studies with greater resistance to parasites (see also section 1.5.6.4).

A significant negative correlation was found between numbers of MMC in the abomasal mucosa and numbers of *H. contortus* and *O. circumcincta* and numbers of MMC in the intestinal mucosa and numbers of *T. colubriformis* in 15 months old HFW line sheep, but not in C line sheep of the same age (Williamson *et al.*, 1995b).

It was concluded that the HFW line animals appeared to be more resilient to nematode infections as they maintained a high production level in spite of carrying a larger worm burden than C line sheep (Williamson, 1994). One possible explanation for this was that HFW line sheep had lower levels of blood gastrin than C line sheep in response to infections with *H. contortus* and *T. colubriformis*. It was suggested that the HFW line animals suffered less gastric dysfunction and their feed intake was subsequently less affected by the larger worm burden (Williamson *et al.*, 1995a).

1.6 Background for this study - Pilot Trial; January 1996

The research carried out for this thesis was prompted by the results of experiments carried out at AgResearch at the end of 1995 and the beginning of 1996 (Jorgensen *et al.*, 1998). The scientific paper describing these results is presented in full in Appendix 1c. FEC and developmental success of eggs to 3rd stage larvae were examined in two experiments. In one indoor trial where ewes and lambs had been infected with *O. circumcincta*, and in a field trial using ewes and lambs from a breeding flock selected for either Low or High FEC (resistance and susceptibility, respectively), comparisons were made with respect to developmental success, FEC, generic composition in larval cultures and faecal dry matter percentage. In both trials, the developmental success was lower in samples from adult ewes than from lambs, and in the field trial, also from animals bred for Low FEC. These differences could not be accounted for by variations in generic composition or faecal dry matter percentage. It was hypothesised, on the basis of these results, that there is an adverse effect of host immunity on the development of the free-living stages of trichostrongylid parasites.

1.6.1 Objectives

The research described in this thesis was designed to test and explore further the hypothesis that there is an adverse effect of host immunity on the development of the free-living stages of common trichostrongylid parasites of sheep. Several objectives were developed, the overall aim of which were to again demonstrate and thus confirm this phenomenon both in the field and in housed animals and to investigate some aspects of the effects of this on parasite biology and possible mechanisms involved.

The objectives and the chapters in which they are dealt with are summarised below:

1. To study possible differences and changes in the developmental success of trichostrongylid parasites in mixed infections in the field. Relate this to variations in host immunity due to seasonal, genetic or age-related effects (Chapters 2, 3 and 4).

2. To study the effect of host immunity in single-species infections with *T. colubriformis* or *O. circumcincta* in housed animals, on the developmental success of the free-living stages and the survival of infective larvae (Chapters 5, 6 and 7).

3. To study the effect of host immunity on the infectivity of infective larvae of *T. colubriformis* (Chapter 6).

4. To study the effect of small intestinal mucus on the development of the free-living stages of *T. colubriformis* (Chapter 7).

CHAPTER TWO

THE PERENDALE TRIAL - AUGUST 1996 - MARCH 1998

2.1 Introduction

An apparent effect of host immunity on the free-living stages of common gastrointestinal nematodes was recently reported by our research groups at AgResearch, Grasslands and at Massey University (Jorgensen *et al.*, 1998). This work was carried out in 1995 and 1996 and showed that eggs from relatively more immune animals (different age classes and lines of sheep genetically divergent in their immunity to parasites) were adversely affected in their developmental success (%eggs developed to 3rd stage larvae). The findings contradicted the general assumption that nematode eggs shed from different host animals in principle all have the same ability to develop, although subject to climatic influences.

The aim of the experiments described in this chapter was to investigate and provide further evidence for the effect of host animal on the developmental success of the free-living stages of nematodes and to do this over a longer period in order to establish possible seasonal variations. Furthermore, a comparison between susceptible (High FEC Line) and resistant (Low FEC Line) ewes and lambs was also of interest, to look for further evidence that the effect may be linked to host immunity.

2.2 Materials and methods

2.2.1 Experimental animals and farmlets

Ewes and lambs from two Perendale selection lines grazed at AgResearch, Flock House Research Station near Bulls, were used for this experiment. The flock originated from Ruakura (near Hamilton on the North Island) where, in 1984-85, the first selections took place after initial infections with *H. contortus*. This breeding flock is described in further detail in section 1.5.8.1 of Chapter 1. The present experiment was part of a larger 3-year experiment to investigate the effect of breeding for resistance to parasites on the level of pasture larval contamination and production parameters such as wool weights and live weight gains (Leathwick, pers.comm.).

All experimental animals were grazing ryegrass-clover pastures and were carrying naturally acquired parasite infections. The selection lines were grazing separate pastures. Each line of animals was replicated three times (i.e. three farmlets). After weaning the High FEC Line farmlets would be grazed first by the lambs and then by the ewes of that line, and the same for the Low FEC Line animals.

2.2.2 Experimental Design

The experiment was designed to have two treatments (High or Low FEC line) and two age classes (ewes and lambs). Ewes were sampled 12 times and lambs six times and

for the most part analysed separately. The main effects (or factors) were 'sampling time', 'line' and 'farmlet' and the nested effect, the animal within line variation. For the ewes, the effect of farmlet could not be analysed for, as all ewes sampled within a line were only grazing one of the farmlets available per line (see also below). For the lambs, however, this factor could be analysed for, as all lambs sampled within a line were distributed on all three of the farmlets available per line.

2.2.3 Sampling procedures

The sampling schedule is outlined in Table 2.2.3.1 below. On the day of sampling the animals were brought to a set of yards in close proximity of the farmlets they were grazing. Due to their physical size and because animals had to be sampled more than once to get sufficient faeces, it was only possible to have one line group of ewes in the yards at a time and therefore only one farmlet-group per line was sampled at each sampling. For the lambs, only wether lambs were used (see explanation below). It was possible to include lambs from all three farmlet-groups per line and keep these simultaneously in the yards for sampling, due to the smaller body size of the lambs. The ewes were sampled at regular intervals throughout a 15-month period from August 1996 to November 1997. The lambs were sampled at regular intervals throughout a 7-month period from after weaning in November 1996 until May 1997 after which the majority of lambs were removed from the trial. In April 1997, 10 ewe lambs from each of the selection lines were sampled to allow a comparison between ewe and ram lambs with respect to FEC and developmental success. In addition, ram lambs born in 1997 were sampled in March the following year (1998) to compare samples taken in late summer/early autumn in the different years.

Sampling Time & Events	Perendale Ewes Faecal Samples	Perendale Ewes Blood Samples	Perendale Lambs Faecal Samples	Perendale Lambs Blood Samples
1996				
*July : **Lambing***				
August	Yes			
September	Yes			
October	Yes	Yes		
*November : **Weaning***	Yes	Yes	Yes D	Yes
December			Yes	
1997				
January	Yes		Yes D	Yes
*February : **Tupping***	Yes	Yes		
March			Yes D	Yes
April	Yes		Yes D	Yes
*May : **Lambs sold***	Yes	Yes	Yes D	Yes
June	Yes	Yes		
*July : **Lambing***				
*August : **Lambing***				
September	Yes	Yes		
October	Yes	Yes		
*November : **Weaning***	Yes	Yes		
1998				
March			Yes	

Table 2.2.3.1 Sampling schedule for Perendale Experiment, including important events during the years 1996 to 1998. D = drench

Approximately 25 animals from the High FEC Line and 25 from the Low FEC Line were sampled on each sampling occasion. The same animals were sampled throughout the sampling period, except for the March 1998 sampling of lambs where lambs born in 1997 were used.

All lambs were treated with anthelmintic when any one of the groups within the lines reached a mean FEC of 1500 epg (eggs per gram faeces). The timing of the anthelmintic treatments was based on FEC in samples collected and processed by other workers working with the same flock of animals. Samples were then taken for the present experiment and within one week, except after the December 1996 sampling, the lambs were treated orally with Ivomec®[1] at a dose rate of ≥ 0.2 mg kg^{-1}. The ewes were not drenched at any time during this experiment.

Faecal samples were collected per rectum from the ewes. As at least 45 – 50 g of faeces were needed, it was usually necessary to sample the same animals more than once on the day of sampling. To collect enough faeces from the lambs, linen bags lined with plastic bags were attached to the hindquarters by means of cotton tape. These bags were left on for a maximum of four hours. Only wether lambs were used for the lamb samples in order to avoid urine contamination, which is unavoidable when using bags for faecal sampling of ewe lambs. As soon as possible after sampling (within 2 – 3 hours) the samples were taken to a cool storage room (approximately 10°C), from where they were transported back to the laboratory for further processing.

Blood samples were collected from the jugular vein of ewes and lambs at the sampling times shown in Table 2.2.3.1 above. For the ewes, these sampling times were chosen to coincide with likely changes in immunity, i.e. around the time of lambing, early lactation, weaning and tupping. For the lambs, blood samples were taken at all sampling times except for the December 1996 sampling, when it was not practically possible to take blood samples as well as faecal samples. Blood samples were collected in heparinised Vacutainer tubes, so that they could also be analysed for number of circulating eosinophils by a researcher at AgResearch. Blood samples from animals where FEC was positive (and developmental success could be measured) were processed as described in Appendix 2h.

2.2.4 Faecal Egg Counts

Faecal egg counts were carried out using a modified McMaster method where each egg counted represented 50 eggs per gram of faeces (see Appendix 2a). Six counts per animal sample were carried out. Using an electric stirrer to mix 2 g faecal samples with saturated salt solution facilitated the processing of a much larger number of samples than would otherwise have been possible.

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[1] Ivomec liquid for sheep and goats; 0.08% v/w solution of ivermectin; Merial, New Zealand Ltd.

Various approaches to evaluating the faecal egg counting technique are presented in Appendix 2j. It was shown that at FEC below 500 epg, the actual number of eggs present in the faeces tends to be underestimated by approximately 50%. It was also shown that if faeces are stored at higher temperatures (25 and 43°C), there appears to be no significant drop in FEC for up to 20 hours at 43°C and for up to 30 hours at 25°C.

2.2.5 Faecal Dry Matter Percentage

The faecal dry matter percentage (%D.M.) was determined when enough faeces were left over after performing FEC and setting up faecal cultures. The standard procedure for the analysis used in this and later experiments is described in Appendix 2g.

2.2.6 Developmental Success and Generic Composition

Larval cultures were set up as soon as possible after obtaining the faecal samples, either on the same day of sampling or the next morning. At all sampling times except the September, October and November samplings, faeces were processed on the day of sampling, otherwise faeces were stored at 4°C until the next day, or in the case of the October sampling, for four days. The standard method used for the larval cultures in this and the following chapters is given in Appendix 2b. Essentially this involved incubating the faeces in petri dishes that were enclosed in larger petri dishes, containing water. The only modification was that in cultures from August, September and October 1996, tap water instead of distilled water was added to the base of the large petri dish. Third stage larvae were recovered, counted and identified as described in Appendices 2c, 2e and 2f.

Various modifications to the culturing and extraction methods were tried during the first months of the experiment, before the final method was decided on. These modifications are described in Appendix 2k. Cold storage was found to affect developmental success after more than one day of storage at 4 °C. A culture size of 10 g was chosen as 5 g cultures often yielded insufficient larvae for identification of genera present, particularly in samples from the Low FEC Line animals. Adding vermiculite to cultures was found not to increase the developmental success and they were therefore incubated without having vermiculite added. Faecal pellets were halved before being placed in the Baermann funnels as this was shown to improve larval recovery.

2.2.7 IgG_1 Levels

Specific antibody levels to larval and adult antigen of the most commonly occurring species of trichostrongylid parasites (as determined from faecal cultures) were measured using an ELISA method specific for the IgG_1 class of antibodies. All assays were carried out in collaboration with the technical staff of the Immuno-parasitology Laboratory at AgResearch, Wallaceville, Upper Hutt. The ELISA method used is described in Appendix 2i. Results are presented as 'Units of Optical Density'.

Plasma samples from ewes were assayed for IgG_1 levels against larval and adult antigen of *Ostertagia circumcincta* and *Cooperia curticei*. Plasma samples from lambs were

assayed for IgG_1 levels against larval and adult antigen of *O. circumcincta* and *Trichostrongylus colubriformis* and against larval antigen of *C. curticei*. The reason for not including adult antigen from *C. curticei* in the latter assay was that at the time of analysis of these samples it was not possible to obtain enough antigen material.

2.2.8 Statistical Analysis

Details of the statistical analysis are presented in Appendix 2m.

Ewe and lamb results from the analyses for Faecal Egg Counts, Faecal Dry Matter Percentage and Percentage Developmental Success were $\ln(x + 1)$ transformed to normalise the residuals and thus meet the requirements for the analysis of variance. These results are presented as geometric group means ± standard errors. Results from the analyses for Generic Composition and Specific Antibody Levels did not require transformation. These results are presented as least squares group means ± standard errors. All data were analysed using a generalised linear model (GLM) in the SAS version 6.12 statistical package. In all cases Type III sums of squares were used to estimate significance levels.

For the ewe data a factorial model was fitted where the main factors were **time** (sampling times) and **line** (High FEC Line versus Low FEC Line). As each animal was 'nested', within the line, this was also analysed for in the fitted model as was the interaction between time and line. Faecal Dry Matter Percentage was included as a covariate in the analysis of %Developmental Success.

For the lamb data a factorial model was fitted where the main factors were **time** (sampling times) and **line** (High FEC Line versus Low FEC Line) and **farmlet** (farmlet grazed). Animal within line (nested effect) and the interaction between time and line were also analysed for. Faecal Dry Matter Percentage was included as a covariate in the analysis of %Developmental Success. As **farmlet** was found not to have a significant effect on the measured response variables (FEC, faecal dry matter percentage, developmental success, generic composition and IgG levels), it was omitted from the subsequent analysis.

Where factors were significant, comparisons by time were made by means of a t-test.

It was, in addition, investigated whether the data would more suitably be analysed as a repeated measures design. However, the correlation matrices showed no consistent pattern that would indicate that variables measured at sampling times closer in time were more correlated than at sampling times further apart. A repeated measures analysis was therefore found not to be appropriate for the present data set.

2.3 Results – Perendale Ewes

All data are presented in Appendix 2l.

2.3.1 Faecal Egg Counts

There were obvious seasonal fluctuations during the 15-month period the Perendale ewes were sampled, as indicated by a significant difference between sampling times ($p<0.001$) (Figure 2.3.1.1). Overall, FEC was significantly higher in the High FEC Line than in the Low FEC Line ($p<0.001$). This difference was evident on most but not all sampling occasions. Furthermore, there were significant differences between animals within the lines ($p<0.001$), particularly in the High FEC Line, and a significant interaction between time and line ($p<0.01$), reflecting that the lines behaved differently over time. FEC was low in both selection lines soon after lambing but increased in the High FEC Line animals in October and November 1996 indicating a periparturient rise (PPR), whereas FEC remained low in the Low FEC Line animals. In mid-summer (January 1997) FEC was again low in both lines, but then increased in the High FEC Line during late summer and autumn, decreasing in early winter and then increasing after lambing, which took place during July and August. Thus a PPR was observed in the High FEC Line ewes in both 1996 and 1997, but not in the Low FEC Line.

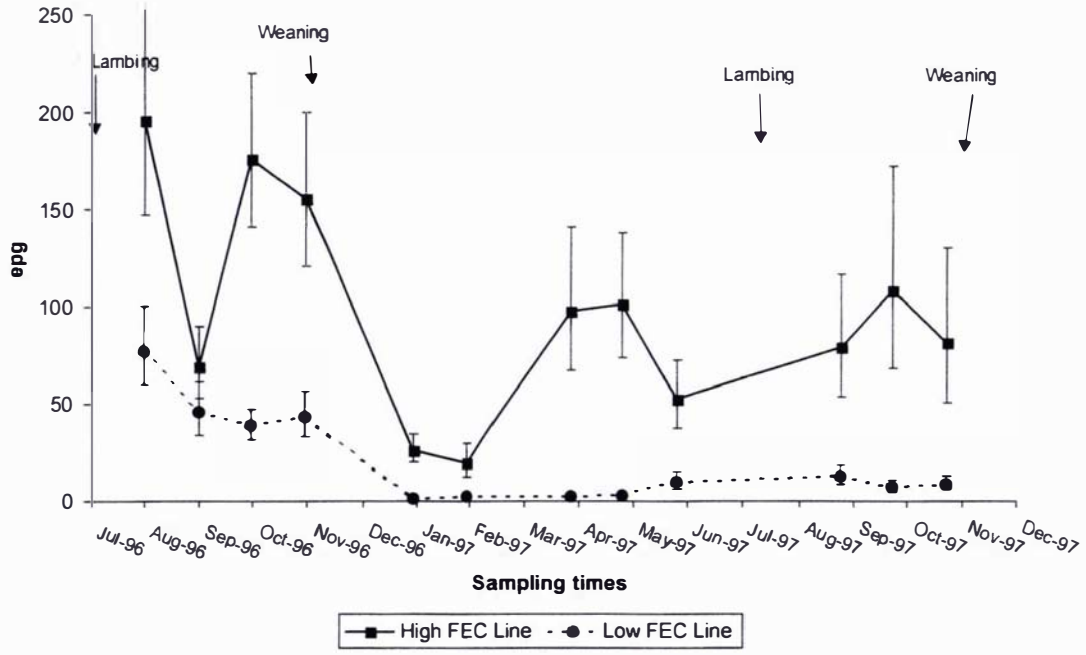

Figure 2.3.1.1 Perendale Ewes - Faecal Egg Counts (Geometric group means ± S.E.). High FEC Line = susceptible line; Low FEC Line = resistant line.

2.3.2 Faecal Dry Matter Percentage

There were no differences in faecal dry matter percentage between the two lines at any of the sampling times (Figure 2.3.2.1). However, there were differences between sampling times ($p<0.001$), with a higher faecal dry matter percentage during spring in both 1996 and 1997. There was significant variation between animals within the lines ($p<0.01$),

but no interaction between line and sampling time, reflecting the similarity of the two lines with respect to this variable.

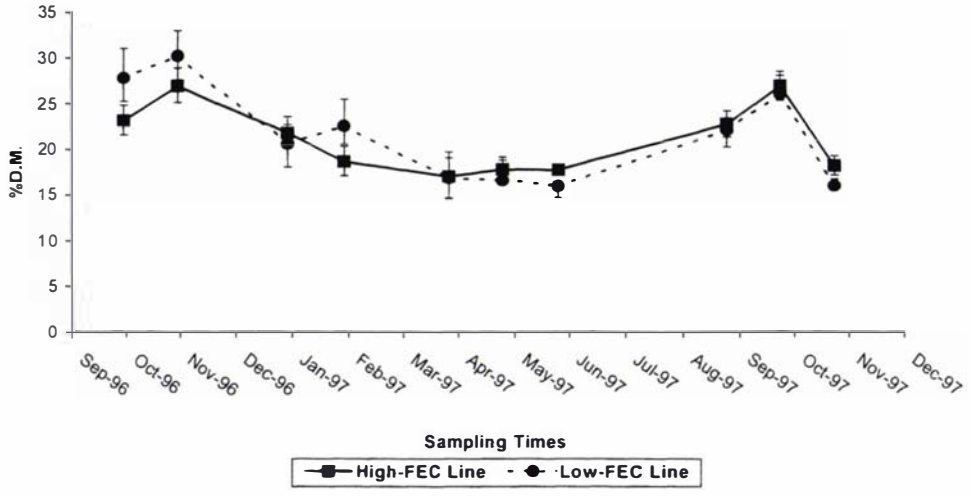

Figure 2.3.2.1 Perendale Ewes - Faecal Dry Matter Percentage (Geometric group means ± S.E). High FEC Line = susceptible line; Low FEC Line = resistant line.

2.3.3 Developmental Success

Overall, developmental success was significantly higher in samples from High FEC Line than from Low FEC Line ewes ($p<0.01$) (Figure 2.3.3.1). When faecal dry matter percentage was included as a covariate it was not significant and the lines remained different although at a somewhat lower level ($p<0.04$). The lines were not different on all sampling occasions, but particularly in September 1996 ($p<0.01$), at the time of the PPR in October 1997 ($p<0.01$) and in November 1997 ($p<0.05$). Significant differences between sampling times ($p<0.001$) indicated seasonal variations in the developmental success, in particular in the High FEC Line ewes. There were significant differences between ewes within both of the lines ($p<0.001$), but no interaction between line and sampling time, suggesting that the lines were largely following the same seasonal trend. Relatively high values for developmental success were recorded in samples from January to February 1997 in both lines. Particularly during this time FEC was low, well below 500 epg in both lines, and many ewes in the Low FEC Line had zero epg. As at counts lower than 500 epg FEC tends to be underestimated, developmental success may consequently have been overestimated. Another contributory factor may have been that the number of samples with positive FEC were reduced during the summer and early autumn months, particularly in the Low FEC Line ewes, effectively reducing the number of observations from that line available for analysis (Table 2.3.3.1).

Year	Sampling time	High FEC Line	Low FEC Line
1996	August	20	24
	September	20	21
	October	22	23
	November	28	26
1997	January	28	9
	February	23	13
	April	23	10
	May	23	11
	June	25	17
	September	22	19
	October	20	15
	November	20	18

Table 2.3.3.1 Perendale ewes – Group sizes at individual sampling times

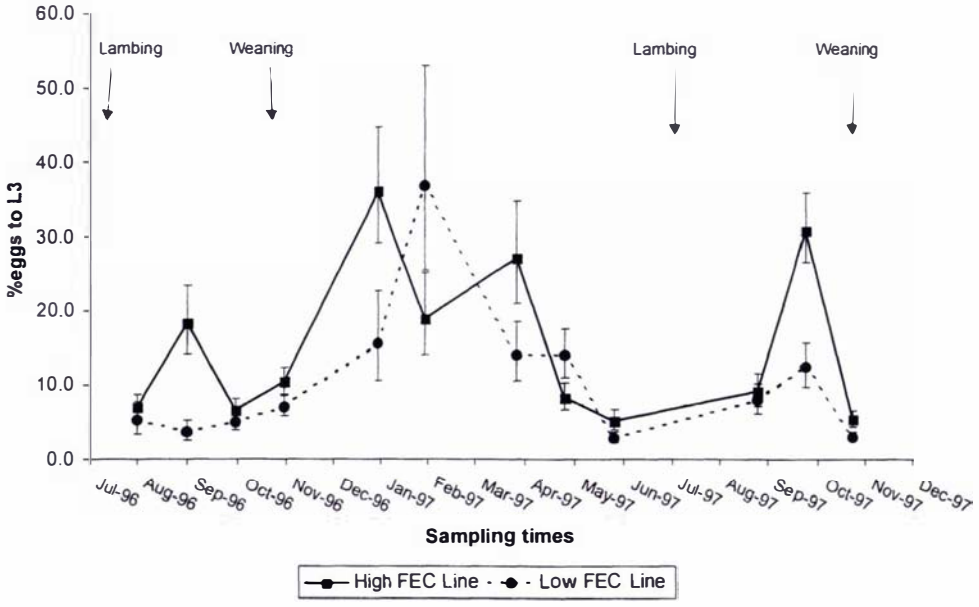

Figure 2.3.3.1 Perendale Ewes - Developmental Success of eggs to 3rd stage larvae (Geometric group means ± S.E.). High FEC Line = susceptible line; Low FEC Line = resistant line.

2.3.4 Generic Composition

Overall, there were no differences in generic composition between the two lines (Figure 2.3.4.1 and Figure 2.3.4.2), whereas there were significant differences between sampling times ($p<0.001$) for all genera recorded, indicating a seasonal variation in the generic composition of parasite infections. The variation between animals within the lines was significant for all genera ($p<0.01$-0.001) recorded. There was no interaction between lines and sampling times. The dominant genera were *Cooperia*, *Ostertagia* and *Chabertia/Oesophagostomum*. *Cooperia* was always present and dominated for much of the year with decreasing levels during summer (January-February). *Ostertagia* was also present

at all sampling times, but was particularly abundant during spring in both 1996 and 1997. *Chabertia/Oesophagostomum* (L.T.) dominated in summer (January-February) but were present in much lower numbers for the remainder of the year. *Haemonchus* and *Trichostrongylus* were present in low numbers at all sampling times.

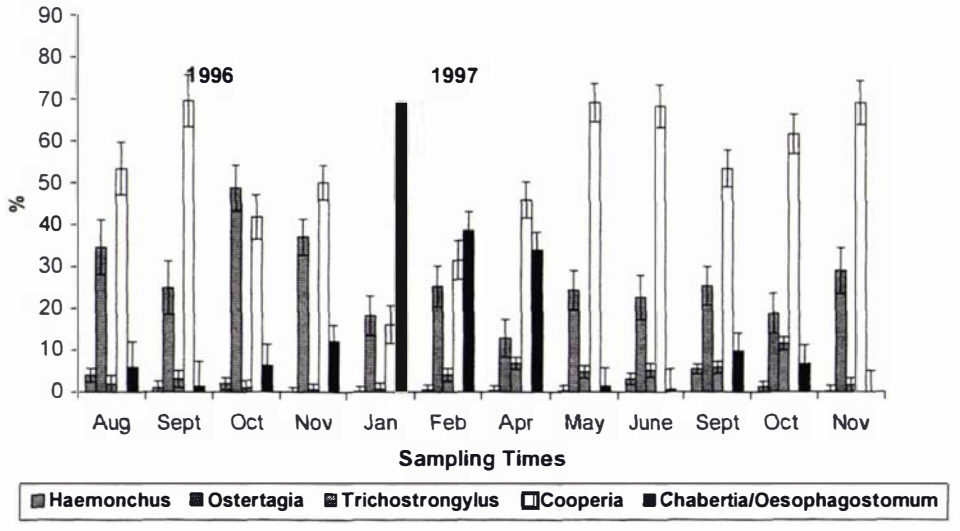

Figure 2.3.4.1 High FEC Line Ewes – Generic Composition (Least squares group means ± S.E.). High FEC Line = susceptible Line.

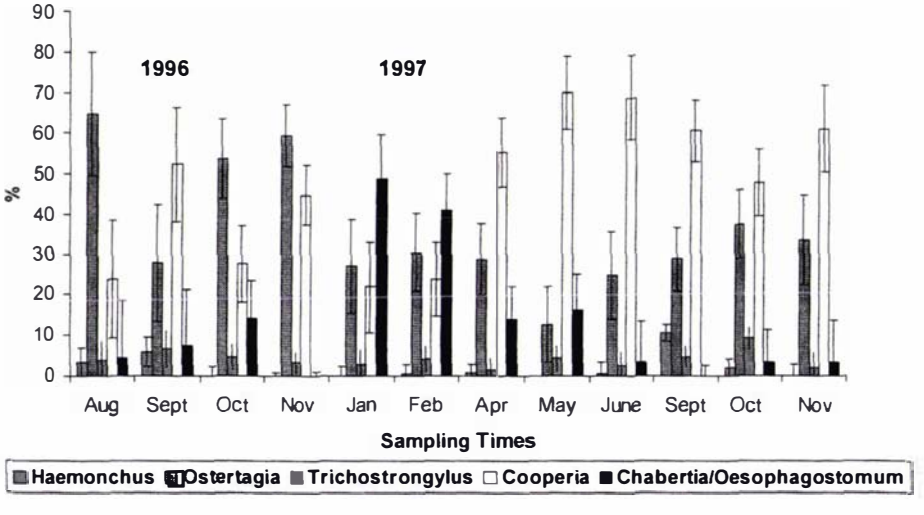

Figure 2.3.4.2 Low FEC Line Ewes – Generic Composition (Least squares group means ± S.E.). Low FEC Line = resistant Line.

2.3.5 IgG_1 Levels

As mentioned previously, only plasma from animals with a positive FEC were examined. This is likely to have created a downward bias in the IgG_1 group mean in the Low FEC Line as animals with zero FEC would be the more resistant within the line. The following results should be interpreted bearing this in mind. Overall, IgG_1 levels to both larval and adult antigens of *O. circumcincta* were significantly higher in the High FEC Line than in the Low FEC Line ($p<0.05$) (Figure 2.3.5.1). Significantly higher levels of IgG_1 to larval antigen in the High FEC Line ewes were found at all sampling times ($p<0.01$-0.05), except in October 1996 and June 1997, whereas significantly higher levels of IgG_1 to adult antigen in the same line of ewes were found in February, September and November 1997 ($p<0.01$-0.05). Overall, IgG_1 levels to larval and adult antigen of *Cooperia curticei* did not differ between the two lines (Figure 2.3.5.2), although significantly higher levels of IgG to larval antigen to *C. curticei* was found in the High FEC Line in October and November 1996 ($p<0.01$). Due to logistical problems, these two samplings included a low number of blood samples (<10 per line) and the observed differences may have been chance findings. Significantly higher levels of IgG_1 to adult antigen of *C. curticei* were found in February and September 1997 in the High FEC Line ($p<0.001$ and 0.01). For both genera, there were highly significant differences in IgG_1 levels between sampling times ($p<0.001$) and between animals within the lines ($p<0.001$). The interaction between lines and sampling times was significant for adult IgG to both *O. circumcincta* and *C. curticei* ($p<0.05$ and $p<0.001$, respectively) but not for larval IgG to either species.

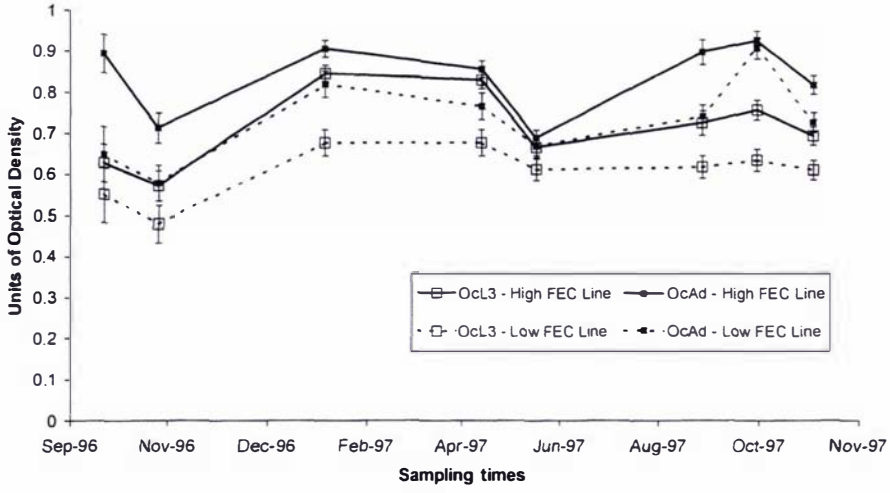

Figure 2.3.5.1 Perendale Ewes - Specific Antibody to *Ostertagia circumcincta* (Least Squares group means ± S.E). OcL3 = IgG_1 antibody to larval antigen of *O. circumcincta*; OcAd = IgG_1 antibody to adult antigen of *O. circumcincta*. High FEC Line = susceptible line; Low FEC Line = resistant line.

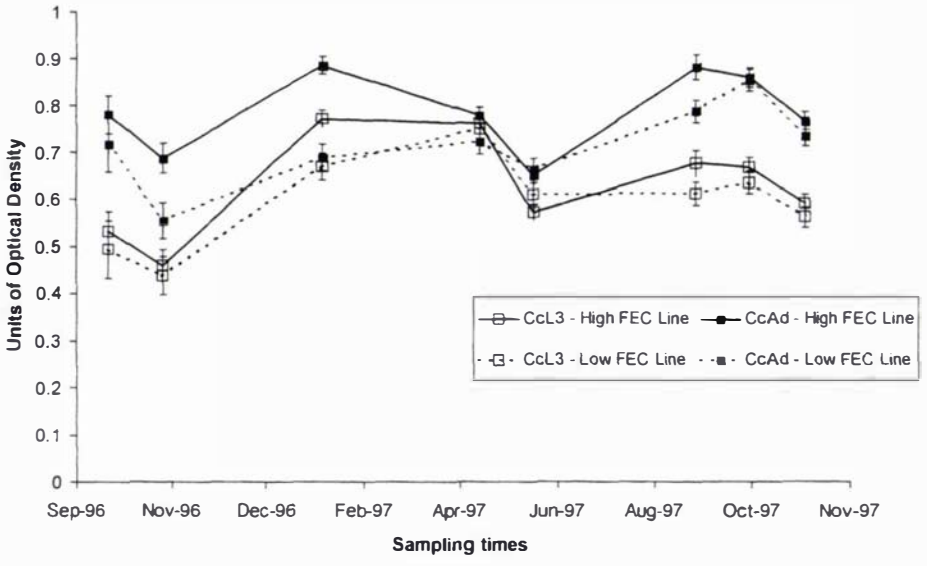

Figure 2.3.5.2 Perendale Ewes – Specific Antibody to *Cooperia curticei* (Least Squares group means ± S.E). CcL3 = IgG_1 antibody to larval antigen of *C. curticei*; CcAd = IgG_1 antibody to adult antigen of *C. curticei*. High FEC Line = susceptible line; Low FEC Line = resistant line.

2.4 Results – Perendale Lambs

All data are presented in Appendix 2l.

2.4.1 Faecal Egg Counts

FEC was always significantly higher in the High FEC Line than in the Low FEC Line lambs ($p<0.001$) (Figure 2.4.1.1). From midsummer to mid-autumn, there was a steady increase in FEC in the High FEC Line lambs, whereas FEC remained at a low level in the Low FEC Line lambs. In mid-autumn when FEC peaked in the High FEC Line, there was a 30-fold difference between the two lines. Consequently, differences between sampling times were significant ($p<0.001$) as was the interaction between lines and sampling times ($p<0.001$) (i.e. the High FEC line FEC increased while the Low FEC line FEC remained low throughout the sampling period). Furthermore there was significant variation between lambs within the lines ($p<0.001$).

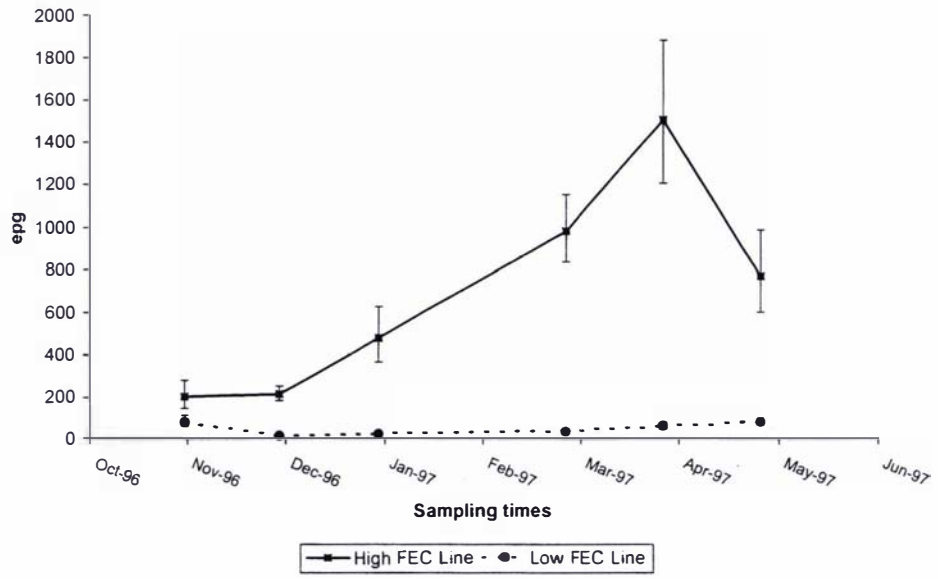

Figure 2.4.1.1 Perendale Lambs – Faecal Egg Counts (Geometric group means ± S.E.). High FEC Line = susceptible line; Low FEC Line = resistant line.

2.4.2 Faecal Dry Matter Percentage

The two lines did not differ with respect to faecal dry matter percentage in faecal samples (Figure 2.4.2.1). However, there were significant differences between sampling times ($p<0.001$), suggesting seasonal differences, and significant variation between animals within the lines ($p<0.05$). There was no interaction between lines and sampling times.

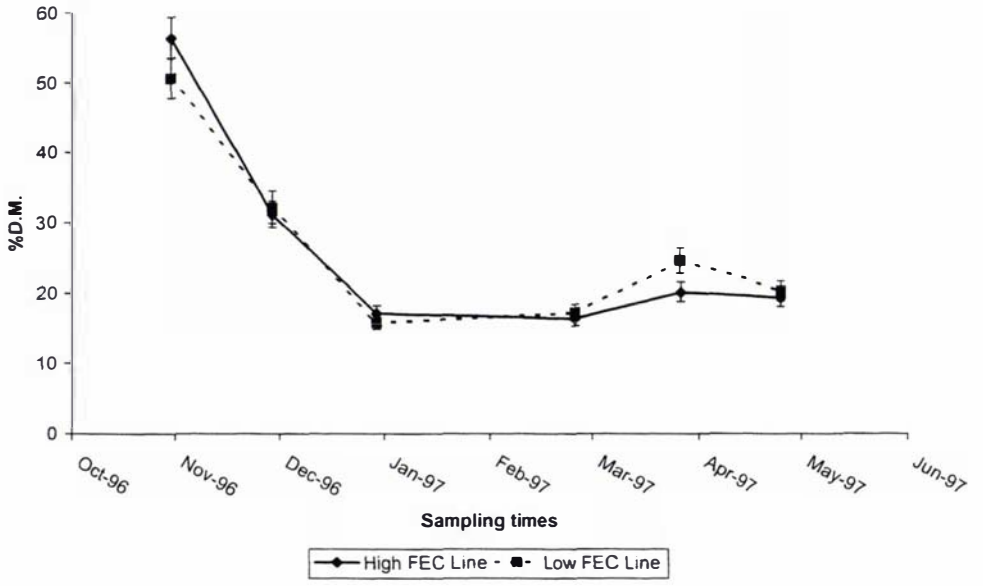

Figure 2.4.2.1 Perendale Lambs – Faecal Dry Matter Percentage (Least squares group means ± S.E.). High FEC Line = susceptible line; Low FEC Line = resistant line.

2.4.3 Developmental Success

Overall, developmental success was significantly higher in the Low FEC Line than in the High FEC Line lambs ($p<0.05$) (Figure 2.4.3.1). Specifically, developmental success was higher in the Low FEC Line lambs only in January and April 1997 (midsummer and mid-autumn) ($p<0.05$ on both occasions). There were significant differences between sampling times ($p<0.001$) and significant variation between lambs within the two lines ($p<0.001$) but no interaction between lines and sampling times, indicating that the two lines did not differ to any great extent. Farmlet was found not to be a significant factor in any of the two lines. Developmental success was relatively high in samples from December 1996. As with the ewes, many of the lamb samples had zero epg (particularly in the Low FEC Line) and these could therefore not be analysed for developmental success (in December 1996, N=20 in High FEC Line and N=14 in Low FEC Line).

The observation that developmental success was found to be higher in the Low FEC Line than in the High FEC Line lambs in some of the 1997 samplings, was in contrast to earlier findings (Jorgensen *et al.*, 1998). To investigate this inconsistency further, 15 lambs from each of the two Perendale selection lines were sampled in March 1998 (i.e. the following year's crop of lambs). The results of this sampling are presented as geometric means ± standard errors in Table 2.4.3.1 along with results from summer/early autumn from the two previous years (from Jorgensen *et al.*, 1998). In March 1998, developmental success was significantly higher in the High FEC Line than in the Low FEC Line lambs ($p<0.001$).

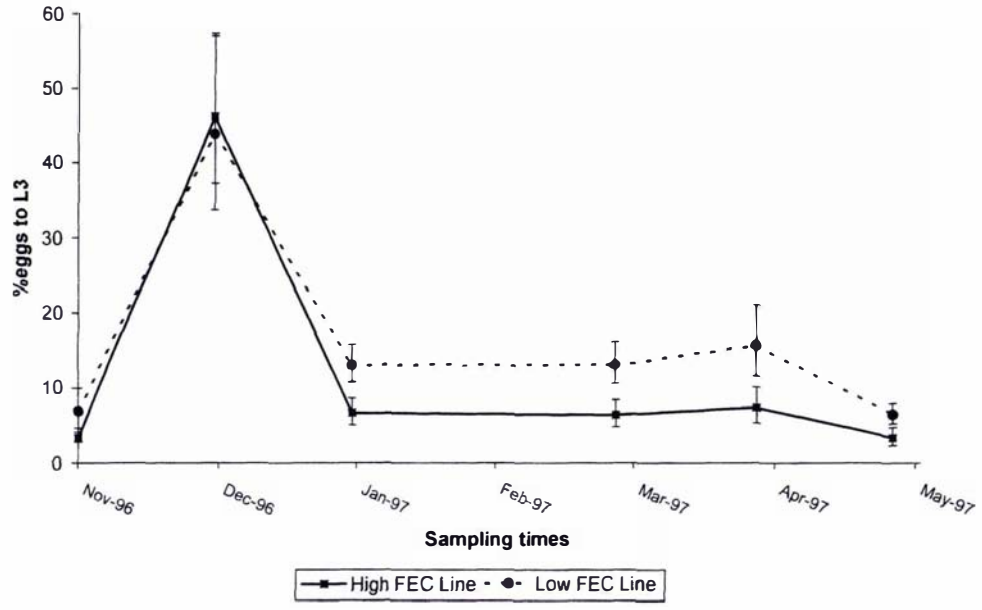

Figure 2.4.3.1 Perendale Lambs – Developmental Success (Geometric group means ± S.E.). High FEC Line = susceptible line; Low FEC Line = resistant line.

Sampling date	Line	FEC	Developmental Success
January 1996*	High FEC	848 ± 136 a	10.7 ± 2 a
	Low FEC	272 ± 56 b	5.9 ± 1 b
March 1997	High FEC	980 ± 170 a	6.4 ± 2 a
	Low FEC	35 ±7 b	13.0 ± 3 b
March 1998	High FEC	311 ± 26 a	9.6 ± 2 a
	Low FEC	38 ± 4 b	3.2 ± 1 b

Table 2.4.3.1 Perendale Lambs - Developmental Success during summer/early autumn in three consecutive years (Geometric group means ± S.E.). High FEC Line = susceptible line; Low FEC Line = resistant line. Column means (for individual sampling times) with the same letters are not significantly different. *Source: Jorgensen *et al.*, 1998.

2.4.4 FEC and Developmental success – comparing ewe and ram lambs

The results from the samples taken from ewe lambs in April 1997 are presented in Table 2.4.4.1 along with results obtained from ram lambs at the April 1997 sampling. There was a significant difference in FEC between ewe and ram lambs and between the two lines. There was no difference in developmental success between the ewe and ram lambs within one line and no difference between ewe lambs from the two lines.

Line	Sex	N	FEC (epg)	N	Developmental success (%)
High FEC	ewe	10	281 ± 78 a	10	6.7 ± 2.4 a
High FEC	ram	19	1573 ± 350 b	19	6.5 ± 2.3 a
Low FEC	ewe	10	21 ± 29 c	6	11.1 ± 10.3 a
Low FEC	ram	19	58 ± 14 d	19	16.7 ± 3.9 a

Table 2.4.4.1 Perendale lambs – FEC and Developmental Success in samples from ewe and ram lambs (Geometric group means ± S.E.). Column means with the same letters are not significantly different ($p<0.05$)

2.4.5 Generic Composition

Overall, there was a higher proportion of *Trichostrongylus* in the Low FEC Line (Figure 2.4.5.2) than in the High FEC Line lambs ($p<0.05$) (Figure 2.4.5.1), although this was largely attributable to the May sampling. In addition, a higher proportion of *Chabertia/Oesophagostomum* was present in the High FEC Line than in the Low FEC Line lambs ($p<0.05$). For the remaining genera, there were no differences between the two lines. There were significant differences between sampling times for all genera present ($p<0.001$), reflecting seasonal variations in the generic composition of infections. *Ostertagia* dominated the late spring and summer samplings (November to January), *Cooperia* increased dramatically over summer and was, along with *Ostertagia*, one of the two dominating species until the May sampling, where a large proportion of *Trichostrongylus* was present, particularly in samples from the Low FEC Line lambs. There was significant variation between animals within the lines with respect to the genera *Ostertagia* ($p<0.001$), *Trichostrongylus* ($p<0.05$) and *Cooperia* ($p<0.001$) and a significant interaction between lines and sampling times for the genera *Trichostrongylus* ($p<0.001$), *Cooperia* ($p<0.01$) and *Chabertia/Oesophagostomum* ($p<0.05$), reflecting some differences over time between the two lines.

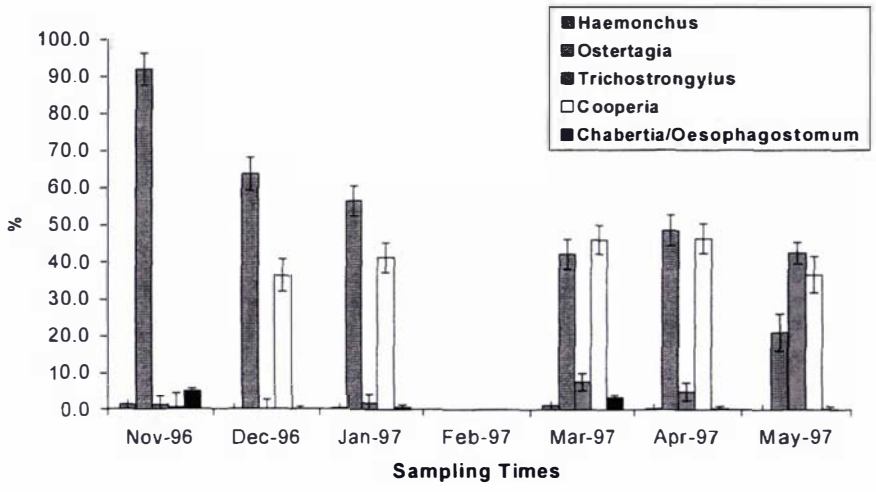

Figure 2.4.5.1 Perendale Lambs, High FEC Line – Generic Composition (Least squares group means ± S.E.). High FEC Line = susceptible line

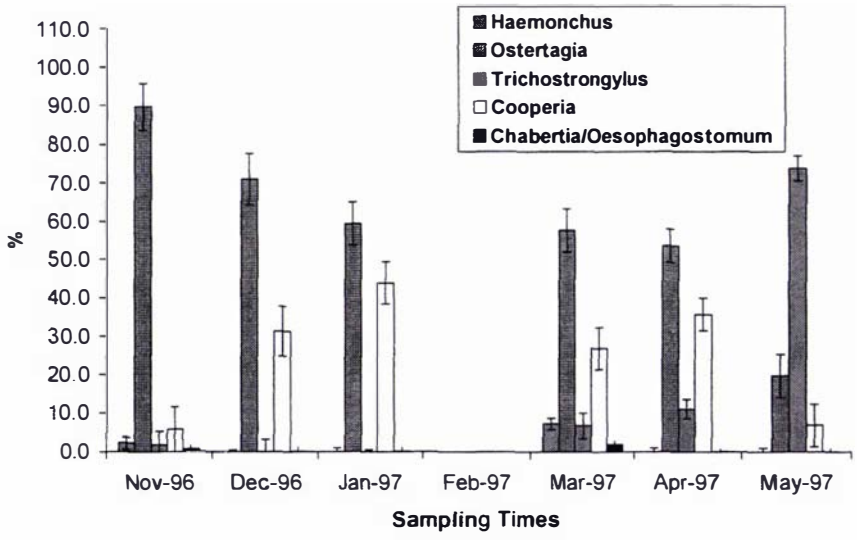

Figure 2.4.5.2 Perendale Lambs, Low FEC Line – Generic Composition (Least squares group means ± S.E.). Low FEC Line = resistant line.

2.4.6 IgG_1 Levels

As with the ewe samples, only plasma from animals with a positive FEC were examined and is likely to have created a downward bias in the IgG_1 group mean in the Low FEC Line. The results should be interpreted bearing this in mind. There were no significant differences between the two lines with respect to any of the antibodies measured (Figure 2.4.6.1, Figure 2.4.6.2 and Figure 2.4.6.3). However, higher levels of IgG_1 were

found at individual sampling times in the Low FEC Line animals. For IgG_1 to adult antigen of *O. circumcincta*, this was the case in March and May ($p<0.01$ and 0.05, respectively). For IgG_1 to larval antigen of *T. colubriformis*, this was the case in March, April and May ($p<0.001$, 0.01 and 0.001, respectively) and for IgG_1 to adult antigen of *T. colubriformis*, in March ($p<0.01$). For IgG_1 to larval antigen of *C. curticei*, a difference was found in March, April and May ($p<0.01$, 0.01 and 0.001, respectively). There were significant differences between sampling times ($p<0.001$) and a significant variation between animals within a line ($p<0.001$) for all types of antibody measured. There was also a significant interaction between lines and sampling times for all antibodies measured ($p<0.001$ to 0.05), reflecting differing antibody responses in the two lines. Generally, levels of antibody in both lines increased steadily from the first sampling in late spring (November) until the final sampling in late autumn (May).

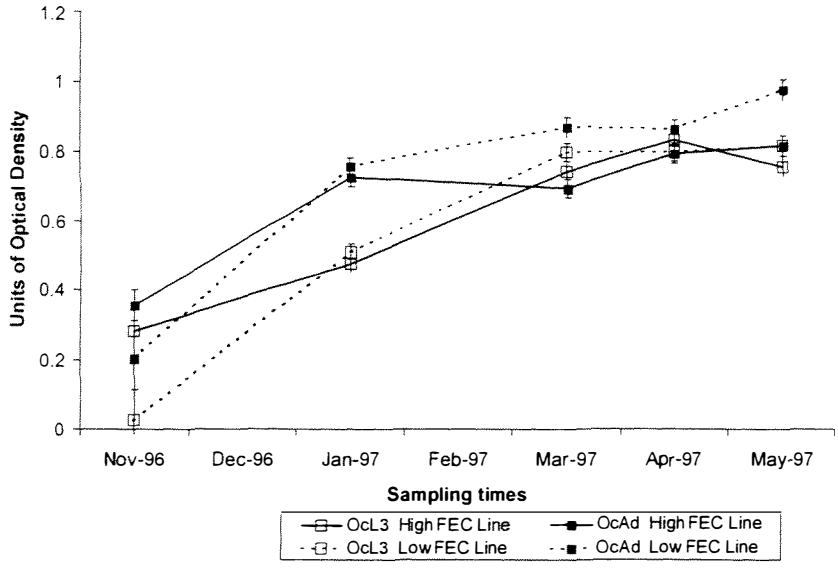

Figure 2.4.6.1 Perendale Lambs – Specific antibody to *Ostertagia circumcincta* (Least squares group means ± S.E.). OcL3 = IgG_1 antibody to larval antigen of *O. circumcincta*; OcAd = IgG_1 antibody to adult antigen of *O. circumcincta*; High FEC Line = susceptible line; Low FEC Line = resistant line.

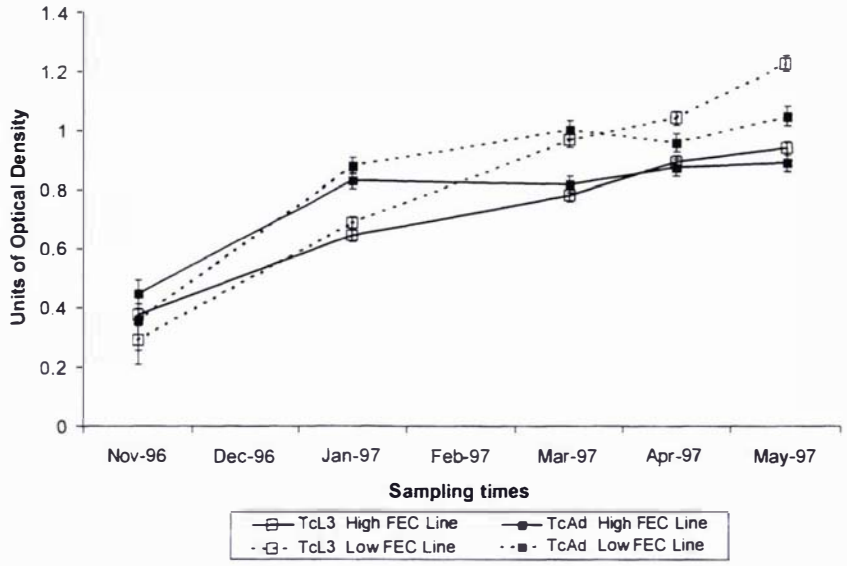

Figure 2.4.6.2 Perendale Lambs – Specific antibody to *Trichostrongylus colubriformis* (Least squares group means ± S.E.). TcL3 = IgG_1 antibody to larval antigen of *T. colubriformis*; TcAd = IgG_1 antibody to adult antigen of *T. colubriformis*; High FEC Line = susceptible line; Low FEC Line = resistant line.

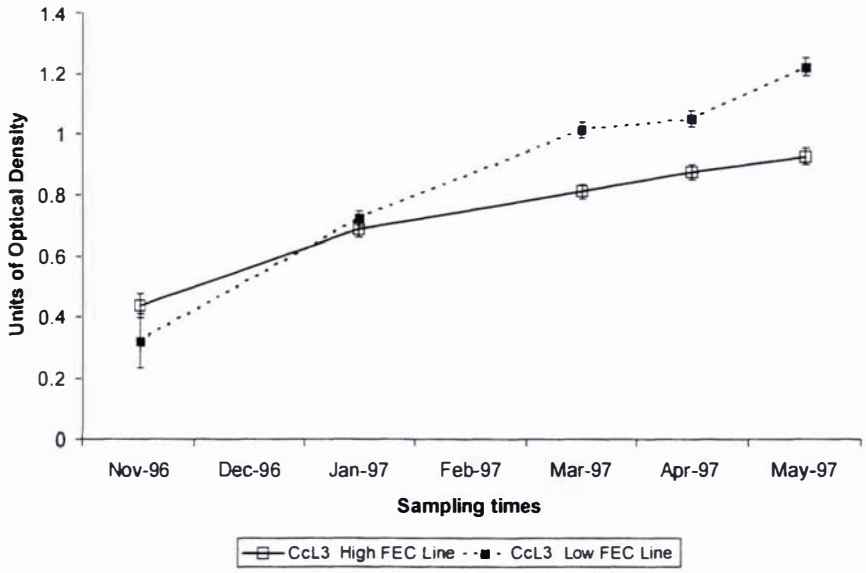

Figure 2.4.6.3 Perendale Lambs – Specific antibody to *Cooperia curticei* (Least squares group means ± S.E.). CcL3 = IgG_1 antibody to larval antigen of *C. curticei*; High FEC Line = susceptible line; Low FEC Line = resistant line.

2.5 Discussion

The present experiment set out to confirm findings from an earlier study where parasite eggs shed from relatively more immune host animals had a reduced developmental success/viability compared to eggs from less immune animals (Jorgensen *et al.*, 1998). Although the phenomenon was confirmed in Perendale ewes at some times of the year, results from Perendale lambs were less conclusive.

The overall seasonal pattern of FEC in the High FEC Line ewe was consistent with findings from other New Zealand studies, although the magnitude of the PPR reported in those studies (Brunsdon, 1963; Brunsdon and Vlassoff, 1982; Stafford *et al.*, 1994) was often greater than that seen during spring the present experiment. In the Low FEC Line, a PPR in FEC was suppressed - a finding previously made in lines of sheep selected for enhanced resistance to parasites (Courtney *et al.*, 1984 and 1985b; Woolaston, 1992; Watson *et al.*, 1992a).

Given the large difference in FEC between the two lines in the present study, it was, according to our hypothesis, expected that a lower developmental success would be found in eggs from the Low FEC Line ewes. This was indeed the case, although it was not consistent in several respects.

A peak in developmental success in the High FEC Line and differences between the two lines were expected over the PPR, when the ewe's immune system is thought to be relatively suppressed/relaxed (reviewed by Barger, 1993) possibly caused by nutritional stress particularly in early lactation (Donaldson, 1997; Huntley, pers.comm.). This was the case in 1997, whereas in 1996 a peak in developmental success did not coincide with a high FEC in the High FEC Line. It seems likely that the low developmental success recorded in October 1996, was due to storage of faecal samples from this sampling at 4°C for four days before being cultured. This was due to logistical problems and was unfortunate as it is likely to have lowered the developmental success in cultures by as much as 50% (McKenna, 1998; Appendix 2k).

After weaning in November 1996, when FEC decreased in both lines of ewes and High FEC Line ewes were no longer affected by the PPR, it was expected that there would be a decrease in developmental success in both lines. However, an increase in developmental success was observed over the January to April period. After April-May, the developmental success decreased in both lines and remained below 10% until September. This coincided with a steady increase in FEC in the High FEC Line in the late summer-autumn, contributing to the autumn peak of infective larvae on pasture, as is generally the case on New Zealand pastures (Brunsdon, 1963; Vlassoff, 1973).

The higher developmental success recorded over the summer period, may be attributable to several factors.

One may have been the lower number of samples with a positive FEC available at this time from the Low FEC Line, effectively reducing the number of samples that could be analysed for developmental success. This was the case in the sampling from January to May. It also seems likely that the ewes that did have a positive egg count would be the relatively less resistant ones within the lines. This would, according to our hypothesis of a higher developmental success in less immune animals, tend to create an upward bias in the line group means.

Another contributory factor is the likely underestimation of FEC when counts are below 500 epg (Appendix 2j) and the subsequent overestimation of developmental success. Although the line mean FEC were below 500 epg at all sampling times in both lines, there was a large and significant variation between ewes in the High FEC Line, reflecting the fact that some ewes in this line had FEC well above the 500 epg average. Thus, an overestimation of developmental success is likely to have been more pronounced and consistent over time in the Low FEC Line ewes and certainly during summer, when FEC were very low. One may argue that recovering eggs from the faeces and culturing them in a larval development assay (LDA), would have avoided any problems with estimating the exact developmental success. However, as FEC were often very low, particularly in samples from the Low FEC line animals, it was not feasible to obtain enough eggs for an LDA. The culturing method applied in the present study was, in spite of its shortcomings, the best option available. Culturing eggs in an LDA would also have meant removing them from any factor influencing their development in faeces.

A third factor may have been a difference in the antigenic stimulation the lines were exposed to, not allowing the Low FEC Line ewes to fully express their higher level of resistance to parasites if insufficient pasture larval challenge was present. Low FEC Line ewes were found to have significantly lower levels of specific antibody to *O. circumcincta* at most sampling times. As the Low FEC Line was expected to be more immune responsive, as observed in other resistant flocks (Presson *et al.*, 1988; Gill, 1991; Gill *et al.*, 1993b), and as IgG_1 levels are considered to be positively correlated with genetic resistance to parasites (Windon and Dineen, 1981; Gill, 1991; Gill *et al.*, 1993a and b; Douch *et al.*, 1994; Bisset *et al.*, 1996) this was an unexpected observation. Although this result was confounded by having samples only from animals with a positive FEC, a significantly lower pasture larval contamination on Low FEC Line farmlets than on High FEC Line farmlets (Leathwick, pers.comm.) may also have been a contributory factor.

The lambs differed in FEC at all sampling times from December onwards. In spite of regular drenching, FEC continued to increase in the High FEC Line until April. Whether ivermectin resistance was present is not known, but there had been no indications of resistance in the past although no formal efficay test had been carried out. The rapid increase in FEC between samplings may have been the result of high parasite contamination levels on pasture. Waiting for mean FEC in one of the groups to reach

1500 epg would have meant that high numbers of eggs were deposited on the pasture just before the anthel intic treatment was applied.

It was expected that there would be little or no difference in developmental success between the two lines early in the season as they had had little antigenic stimulation at this stage and that any differences would become apparent when the lambs reached an age of 6-9 months (10 months old in May) and were more immunologically capable. Although differences between the lines were observed, the finding that developmental success was higher in the Low FEC Line, was the reverse of what was expected. An underestimation of FEC and subsequent overestimation of developmental success may have complicated the picture, as FEC from individual animals were well below 500 epg in the Low FEC Line at all sampling times (except for two lambs in the May sampling). This was not thought to be a problem in the High FEC Line, certainly not from January and until the end of the sampling period, when mean FEC were higher than or equal to 500 epg. Although lambs were experiencing different levels of pasture larval contamination (High FEC Line>Low FEC Line), the IgG_1 levels did not differ between the two lines. This result was complicated by the fact that only plasma from animals with a positive FEC was analysed, but still suggests that the Low FEC Line could be more responsive to antigenic challenge than the High FEC Line.

When lambs from the two selection lines were sampled again in 1998, developmental success was shown to be higher in the High FEC Line than in the Low FEC Line lambs and thus consistent with earlier published results (Jorgensen *et al.*, 1998). The inconsistency between the three years posed the question as to the cause. One possibility is that the levels of larval challenge had differed between the three years and that a low larval challenge in 1997 might have meant insufficient antigenic sti ulation for the Low FEC Line lambs to maintain their level of resistance. This could be supported by the observation that IgG_1 levels did not differ between the two lines of lambs in 1997. The enhanced resistance expressed by genetically selected sheep is known to be an acquired type immune response and is therefore dependent on a certain level of antigenic stimulation (Presson *et al.*, 1988; Gill, 1991). Levels of pasture larvae on farmlets grazed by Low FEC Line lambs were lower in 1997 than in 1996 and an autumn peak in larval availability was absent in 1997 but present in 1996 and 1998 (Leathwick, pers.comm.). To further examine the effect of the same larval challenge on the expression of genetic resistance, lambs from High Fleece-weight selected (and parasite susceptible) and control (random-bred) lines of Romney sheep grazing together, and not separate as in the present experiment, were sampled. This experi ent is described in Chapter 3.

Another possible explanation for the unexpected results in 1997, was the type of anthelmintic used during that year and in 1996 and 1998. In 1997, ivermectin[2] was used to

[2] Ivomec liquid for sheep and goats; 0.08% v/w solution of ivermectin; Merial, New Zealand Ltd.

drench all lambs, whereas in 1996 and 1998, albendazole[3] was used. Treatment with ivermectin has been reported to adversely affect the blastogenic response in lymphocytes and the production of antibodies in lambs (Stankiewicz *et al.*, 1995). However, the same authors have reported benzimidazole anthelmintics, as well, have a similar effect on the acquired immune response in treated lambs (Stankiewicz *et al.*, 1994; Cabaj *et al.*, 1994). If ivermectin quantitatively had a greater suppressive effect on the immune response than a benzimidazole anthelmintic, then that might explain why there were no differences in IgG response between the two lines of Perendale lambs. However, one would expect a suppression of antibody response in both the High FEC Line and Low FEC Line lambs as all were treated at the same frequency and with the same dose. To exclude the possible effect of the anthelmintics used for treatment, on the lambs' immune response and subsequently on the developmental success of the free-living stages, an experiment was subsequently carried out where groups of lambs treated with either ivermectin or albendazole where sampled to compare FEC and developmental success. This experiment is described in Chapter 4.

Unexpectedly, developmental success was often found to be lower in the lambs than in the ewes. This is inconsistent with the hypothesis that eggs from less immune animals (lambs or Low FEC Line animals) show a higher developmental success. Although the reason for this is not known, one may consider the possibility of ivermectin residues in faeces exerting a direct effect on the developing free-living stages in cultures. Effects on the development of Diptera in the dung fauna for up to 30 days have been reported in the past (Madsen *et al.*, 1990) and ivermectin residues have been detected in the bile of cattle treated intraruminally, for up to 21 days (Steel, 1993). Lesser effects on Diptera have been reported following oral treatment of sheep (Steel, 1993). Nevertheless, it is a possibility that larval stages of nematodes are more sensitive that those of Diptera. From LDA data, it is known that ivermectin concentrations in the range of ng/ml adversely affect larval development (Hoza, 1998; Gopal *et al.*, 1999; Jorgensen, unpublished results).

Although there is some debate as to whether selecting for parasite resistance increases production in sheep, the ability of resistant animals to lower pasture contamination is well-known (Bisset *et al.*, 1997; Leathwick, pers.comm.). Results from the present experiment would suggest that there may in fact be an added benefit to grazing resistant lines of sheep; not only do these animals shed fewer eggs, but the eggs, at least from ewes, also appear to be less viable. This should result in a lower pasture contamination with infective larvae and could potentially have a great impact on our understanding of larval epidemiology and prediction of pasture larvae infestation. A strategic use of resistant animals as means of 'cleaning' pastures could reduce the need for

[3] Valbazen® 25g/L albendazole with added Cobalt, Copper, Zinc and Selenium; Pfizer Animal Health, NZ

preventative anthelmintic treatments and be a step in the direction of chemical free management and control of parasites in sheep.

Adult ewes have been proposed as the major contributors to pasture larval contamination, as they produce more faeces than lambs and as they are present on pastures all year round (Familton, 1991). However, as the contribution of the adult ewe to pasture larval contamination has mainly been estimated by summing FEC over time (West, 1982; Familton, 1991), the data presented here and those of an earlier study (Jorgensen *et al.*, 1998), suggest that this method would overestimate the actual number of larvae present as seasonal and host related influences are not taken into account.

In conclusion, the present experiment showed a measurable effect of host immunity on the development of free-living stages of mixed strongylid parasites infecting sheep, although not consistently through the year. The effect manifested itself as a reduction in developmental success of eggs to infective larvae in samples from relatively more immune animals. This was only shown in Perendale ewes selectively bred for enhanced resistance to parasites. In Perendale lambs, results from 1997 were harder to interpret, as they were inconsistent with results from the previous and following years.

Plate 2.4.6.1 Perendale ewes in the yards at Flock House

Plate 2.4.6.2 Farmlets grazed by Perendale ewes and lambs

Plate 2.4.6.3 Perendale ewes

CHAPTER THREE

HIGH FLEECEWEIGHT-SELECTED AND CONTROL LINES OF ROMNEY SHEEP EXPERIENCING THE SAME LEVEL OF LARVAL CHALLENGE ON PASTURE

3.1 Introduction

Results from previous work with Perendale lambs (Chapter 2) prompted further studies into why developmental success in faecal cultures was higher in samples from resistant than from susceptible lambs in 1997. This result was in contrast to lamb results from 1996 and 1998 and ewe results from 1996 and 1997. One possible explanation was that a difference in larval challenge between paddocks might have meant that resistant lambs did not experience a high enough level of infection for them to fully express their genetic resistance. This might in turn have rendered them relatively less immune than High FEC Line animals that experienced a higher level of larval challenge on pasture.

The aim of this experiment was to investigate the expression of host immunity (as measured by FEC and Larval Developmental Success) under the same level of larval challenge in Romney lambs from selection lines that differed in their susceptibility to parasitis. These lines of sheep from a Massey University farm had previously been shown to be divergent in their susceptibility to parasites (Howse *et al.*, 1992; Williamson *et al.*, 1995a and b). Progeny of both lines were grazing together at all times and therefore experienced the same level of larval challenge on pasture.

3.2 Materials and Methods

3.2.1 Experimental Animals

Lambs used for the experiment were from a flock that had been selected for high, low or normal greasy fleece weight at hogget shearing. Only the selection lines for high and normal greasy fleece weight are now maintained. Selection for high greasy fleece weight started in 1956 from a population of randomly chosen New Zealand Romneys (Blair *et al.*, 1985). High Fleece Weight selected sheep had previously been shown to have consistently higher faecal egg counts (FEC) than animals from the control line (Howse *et al.*, 1992; Williamson *et al.*, 1995a and b).

In the present experiment, ram lambs from the high fleece-weight-selected (HFW) line and the control line were used. They were born during September 1998 and were approximately four months old at the start of the experiment. All lambs were drenched at weaning (29/11/98), one month after weaning (26/12/98) and thereafter on the same day

of sampling, after all samples had been taken. On all drenching occasions, the lambs were drenched with Rycozole®[1]. No treatments were given between February and May.

3.2.2 Experimental Design and Sampling Schedule

The experiment was designed to have two treatments (HFW and Control lines) and three sampling times. Faecal and blood samples were taken from 20 ram lambs in each of the two lines. The 20 ram lambs per line were randomly picked on the first sampling occasion and the same lambs, identified by ear-tag numbers, were then sampled on the following two sampling occasions. The samplings took place on January 20, February 23 and May 12 1999.

3.2.3 Faecal Samples

The animals were faecal sampled using linen bags, which were attached to the animal's hindquarters with cotton tape and left on for a maximum of three hours at a time, to collect enough faeces for analysis.

Faecal Egg Counts (six replicates per animal sample) were estimated from the faecal samples (See Appendix 2a). Faecal Dry Matter Content (one per animal sample) (Appendix 2g) and Developmental Success of eggs (three replicates per animal sample) (Appendix 2b) were also estimated. Replicate samples were averaged to give a mean for each animal to be used for the statistical analysis. A minimum of 100 third stage larvae in cultures from each individual animal were identified to genus level as described in Appendix 2f.

3.2.4 Blood Samples

Blood samples were collected from the jugular vein in one 10 ml heparinised 'Vacutainer' tube per animal and further processed as described in Appendix 2h. All blood samples were analyzed for IgG_1 antibodies to larval and adult antigens of *Trichostrongylus colubriformis* and *Ostertagia circumcincta* using an ELISA method (see Appendix 2i).

3.2.5 Statistical Analysis of Data

Details of the statistical analysis are presented in Appendix 3b.

Data for the analyses for Faecal Egg Counts and Percentage Developmental Success were $\ln(x + 1)$ transformed to normalise the residuals and thus meet the requirements for the analysis of variance. These results are presented as geometric group means ± standard errors.

Data for the analyses for Faecal Dry Matter Percentage, Generic Composition and Specific Antibody Levels did not require transformation. These results are presented as either arithmetic or least squares group means ± standard errors.

[1] 40g/L Levamisole hydrochloride; Young's Animal Health, New Zealand

All data were analysed using a generalised linear model (GLM) using the SAS version 6.12 statistical package, and fitting a factorial model where the main factors were **time** (sampling times) and **line** (control line versus fleece weight selected line). As each animal was 'nested', within the line this was also catered for in the fitted model. Finally, as it was of interest to analyse for an interaction between time and line, this was also incorporated in the model. In all cases, Type III sums of squares were used to estimate significance levels. Faecal Dry Matter Percentage was included as a covariate in the analysis of FEC and %Developmental Success.

3.3 Results

All raw data and arithmetic group means are presented in Appendix 3a.

3.3.1 Faecal Egg Counts

FEC was low in both lines on the first two sampling occasions, and the two lines were not significantly different at any point in time (Figure 3.3.1.1). When including faecal dry matter percentage as a covariate, it was not significant. Time was a significant factor ($p<0.01$), reflecting the increase in FEC between mid-summer and May, whereas there was no significant interaction between time and line. The animal within line variation was significant, most likely mainly due to the May sampling ($p<0.05$).

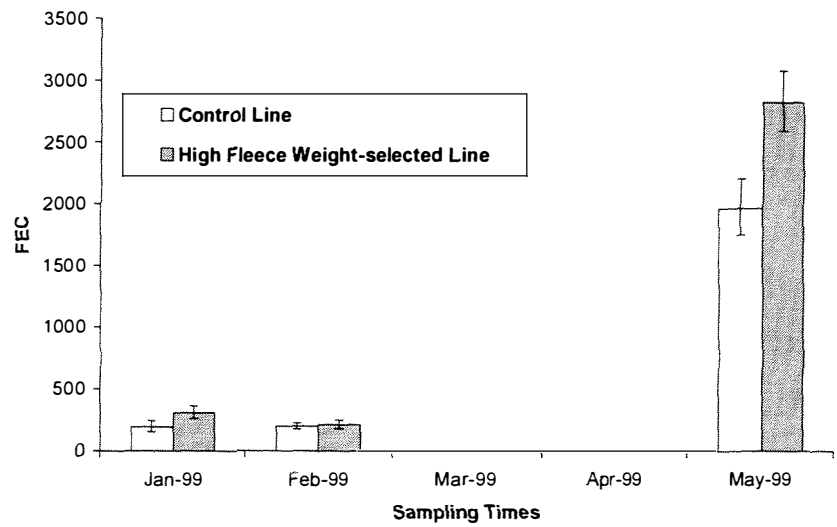

Figure 3.3.1.1 Fleece Weight Selected Romneys – Faecal Egg Counts (Geometric Group Means ± S.E.).

3.3.2 Faecal Dry Matter Percentage (%DM)

There were significant changes over time ($p<0.01$) whereas the lines did not differ (Figure 3.3.2.1). Faecal dry matter percentage was not significant as a covariate in the analysis of the FEC and developmental success results.

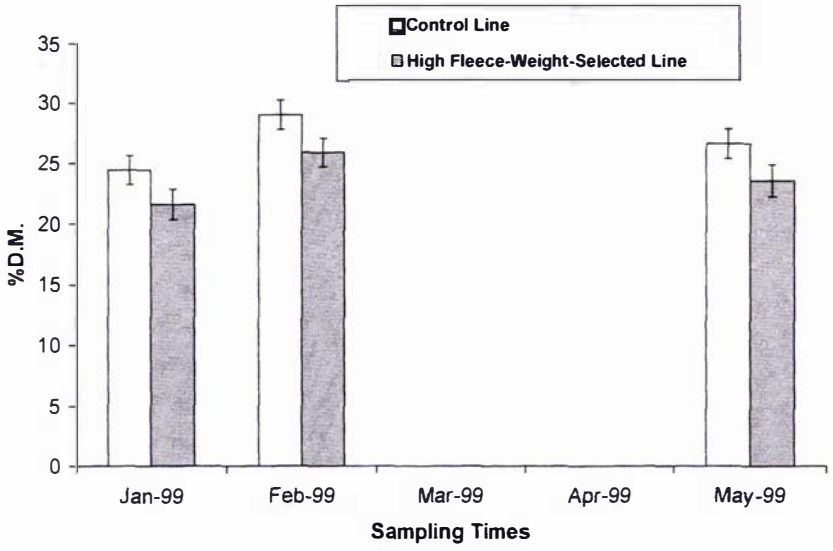

Figure 3.3.2.1 Fleece Weight Selected Romneys – Faecal Dry Matter Percentage
(Least Squares Group Means ± S.E.)

3.3.3 Generic Composition

Haemonchus, *Ostertagia*, *Trichostrongylus*, *Cooperia* and *Chabertia/Oesophagostomum* least squares group means for the Control line are presented in Figure 3.3.3.1 and for the High Fleece Weight Selected line in Figure 3.3.3.2. *Ostertagia* was the dominant genus in both lines. There were no differences between the lines with respect to any of the genera. However, there were significant changes over time for the *Haemonchus*, *Ostertagia*, *Cooperia* and *Chabertia/Oesophagostomum* genera (in all cases: $p<0.001$), but not for the *Trichostrongylus* genus. There were significant variations between animals within the lines for the two genera *Ostertagia* ($p<0.05$) and *Chabertia/Oesophagostomum* ($p<0.05$), and significant interactions between time and line for the genera *Haemonchus* ($p<0.05$) and *Cooperia* ($p<0.05$), indicating some differences between lines over time.

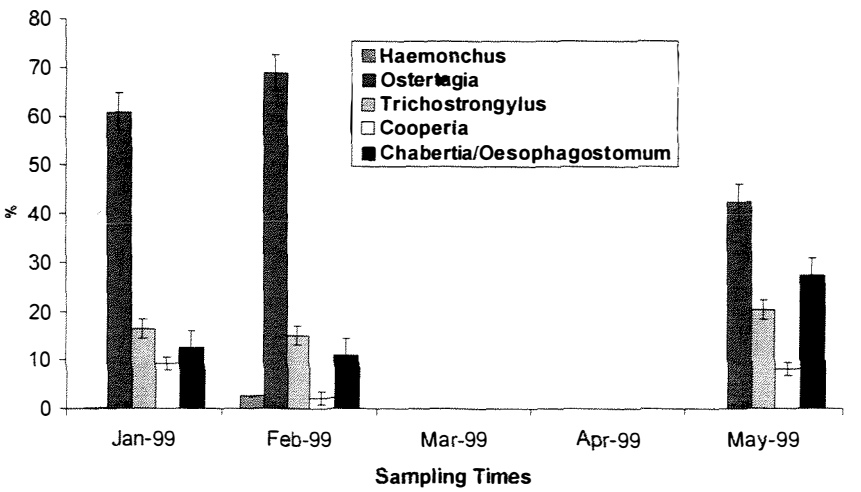

Figure 3.3.3.1 Control Line – Generic Composition (Least Squares Group Means ± S.E.)

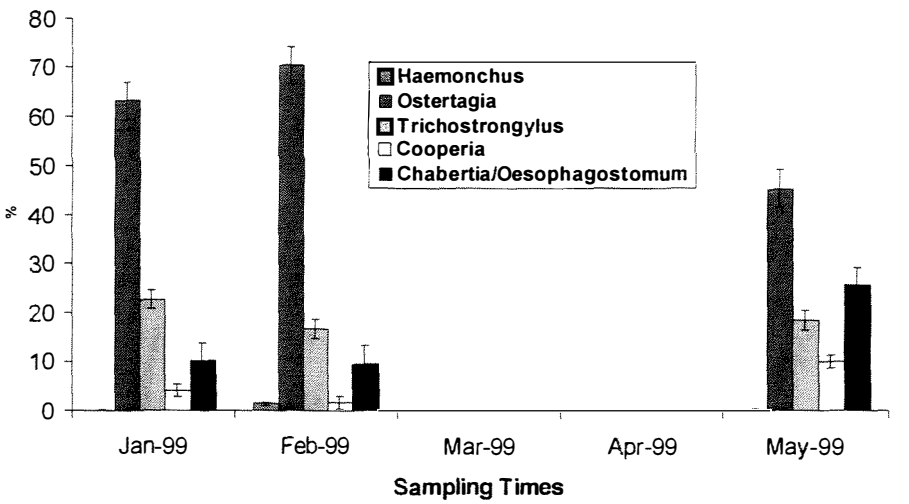

Figure 3.3.3.2 High Fleece Weight Selected Line – Generic Composition (Least Squares Group Means ± S.E.)

3.3.4 Developmental Success

Although the two lines did not differ in developmental success, there were significant differences between sampling times ($p<0.001$), in that there was a dramatic decrease in both lines in developmental success, between the February and the May sampling (from 86.6% to 12.0% in the Control line and from 95.1% to 9.0 % in the HFW

line) (Figure 3.3.4.1). The variation between animals within a line and the time and line interaction were also non-significant.

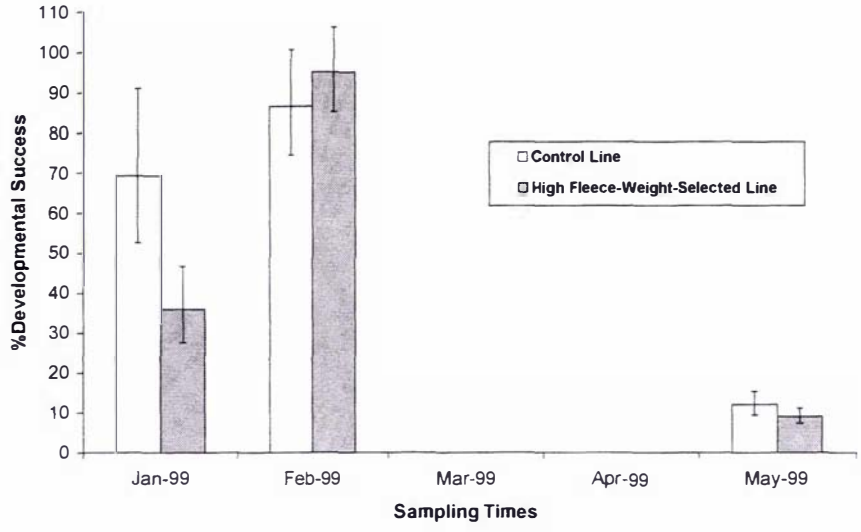

Figure 3.3.4.1 Fleece Weight Selected Romneys – Developmental Success (Geometric Group Means ± S.E.).

3.3.5 IgG_1 Levels

Specific Antibody to *Trichostrongylus colubriformis*

There were no differences in levels of specific antibody to TcL3 and TcAd between the two lines (Figure 3.3.5.1). There were significant increases over time with respect to antibodies to both TcL3 and TcAd in both lines ($p<0.01$). The variation between animals within the lines was always greater in the Control line than in the HFW line, as judged by the coefficient of variation (CV^2). When analysing the data by line, there were significant variations between Control line animals with respect to both types of antibodies measured ($p<0.001$), whereas there was no significant variation between HFW line animals.

Specific Antibody to *Ostertagia circumcincta*

There were no differences in levels of specific antibody to OcL3 and OcAd between the two lines at any of the three sampling times (Figure 3.3.5.2), but a significant increase over time in both lines with respect to antibodies to both OcL3 and OcAd ($p<0.001$). The time and line interaction was not significant for either type of antibody. The variation between animals within a line was significant in the Control Line for specific antibody to both OcL3 ($p<0.001$) and OcAd ($p<0.01$) and in the HFW line for specific antibody to OcAd ($p<0.05$).

[2] The coefficient of variation (CV) is calculated as the group variance divided by the group mean

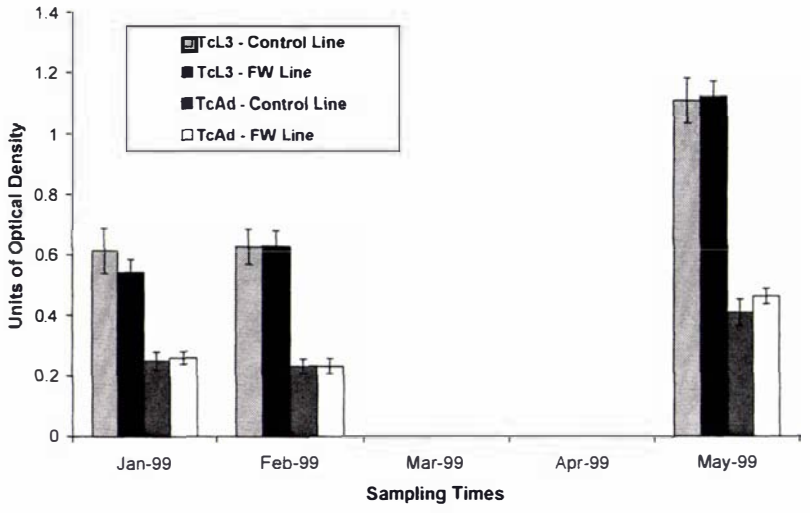

Figure 3.3.5.1 Fleece Weight Selected Romneys - Specific Antibody Levels to larval and adult antigen of *Trichostrongylus colubriformis* (Arithmetic Group Means ± S.E.). 'TcL3' = IgG_1 antibody to larval antigen of *T. colubriformis*; 'TcAd' = IgG_1 antibody to adult antigen of *T. colubriformis*. 'HFW Line' = High Fleece Weight Selected Line.

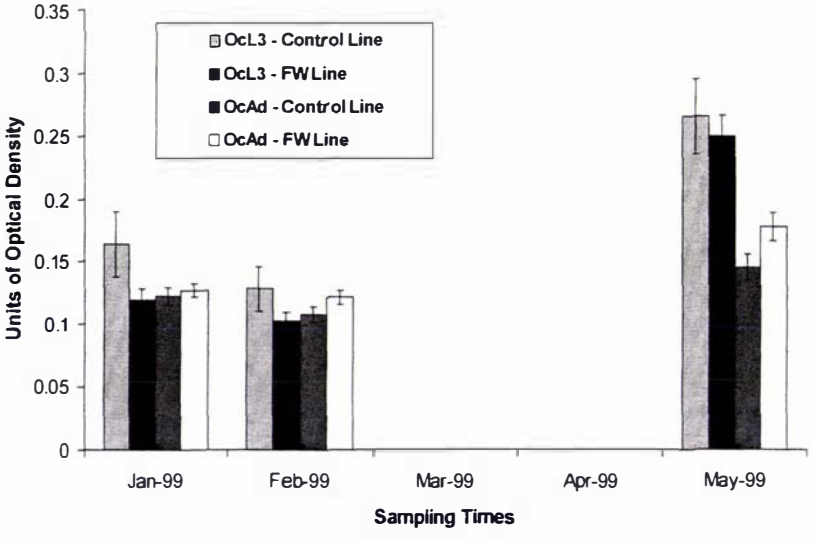

Figure 3.3.5.2 Fleece Weight Selected Romneys - Specific Antibody Levels to larval and adult antigen of *Ostertagia circumcincta* (Arithmetic Group Means ± S.E.). 'OcL3' = IgG_1 antibody to larval antigen of *O. circumcincta*; 'OcAd' = IgG_1 antibody to adult antigen of *O. circumcincta*. 'HFW Line' = Fleece Weight Selected Line.

3.4 Discussion

In the present experiment a decrease in developmental success over time, consistent with an increase in host immunity, was demonstrated. However, the fleece–weight selected (HFW) and control lines of 4 – 8 months old Romney lambs did not differ in FEC, developmental success of eggs to infective larvae, faecal dry matter percentage, generic composition in larval cultures and levels of specific antibody to *T. colubriformis* and *O. circumcincta.*

It was surprising to find that the HFW and Control lines did not differ at any of the three sampling times with respect to FEC. Differences in FEC had previously been demonstrated in progeny of this flock infected either naturally or artificially, although these were not always significant (Howse *et al.*, 1992; Williamson, 1994; Williamson 1995a and b; Simpson, pers.comm.). It was with the expectation of a difference that the animals were chosen for this experiment. The data indicate, however, that such a difference did not eventuate.

There were significant increases in FEC over time, in particular, between the February and May samplings, where counts rose 5-6 fold. A comparable increase during the same months of the year was also found by Williamson *et al.* (1995a) although in that experiment the lambs received an anthelmintic treatment in March. The dramatic increase in FEC in the present experiment was almost certainly a result of the drenching regime applied. The lambs had been treated with an anthelmintic at weaning and thereafter at 3-4 weekly intervals until the day after the February sampling. Between the February and May samplings the lambs received no further anthelmintic treatments. Often during early and mid-summer many eggs die on New Zealand pastures due to high temperatures and dry conditions (Vlassoff, 1973). The period from December 1998 to early March in 1999, had rainfall[3] well below the 10-year average and pastures were very dry and slow growing during this time. A low development rate of eggs to infective larvae, combined with regular anthelmintic treatment is likely to have ensured that the lambs were experiencing a low larval challenge on pasture over midsummer. Levels of pasture larval contamination were not measured during the course of this experiment, nevertheless, one may assume that pasture larval contamination was indeed low during January to early March. In contrast, the late summer/autumn period is considered optimal for larval development under New Zealand conditions (Vlassoff, 1973) and it seems likely that the lambs were experiencing a higher larval challenge on pasture from early April and onwards. Infections established from that time resulted in the high FEC recorded in both lines in the May sampling.

There was a highly significant decrease in developmental success between the February and May samplings, in both lines. This decrease was consistent with an increase

[3] Total rainfall from December 1998 to March 1999: 130.2 mm ; 10 year average for 1988-1998 for the same months: 311.0 (Source: Weather station at AgResearch, Palmerston North)

in host immunity in both lines, as judged by increases in IgG_1-levels and thus appears to strongly support the hypothesis of an adverse effect of host immunity on developmental success. Certainly, the observed decrease could not be accounted for by variations in faecal dry matter percentage or by significant changes over time in the generic composition of the parasite infections. However, the two lines did not differ in developmental success of eggs to 3rd stage larvae in faecal cultures at individual sampling times. No animals were killed during this experiment and it was therefore not possible to determine whether worm burdens were in fact starting to decrease at the time of the last sampling. In the past, however, 14-15 months old sheep from the HFW line have been shown to be more susceptible to *H. contortus* and *O. circumcincta* than the Control line. Recent indoor experiments using progeny, reared worm-free, from the same flock have confirmed the higher susceptibility of the HFW line to infection with *O. circumcincta* (Simpson, pers.comm.).

Some results in the January and February samplings showed a developmental success above 100%. This was probably due to an underestimation of the number of eggs present in the faeces which appears to be a particular problem at lower faecal egg counts (<500 epg), as mentioned in Chapter Two.

The developmental success in samples from January and February was high and in contrast to the low values found in samples from Perendale lambs during the same period of time (see Chapter Two). One possible explanation for this is the effect of age on the development of immunity to gastrointestinal nematodes. The Perendale lambs were winter born (July-August) and thus were approximately six months old whereas the Romney lambs were four months old at the January sampling. Indoor trickle-infections with *T. colubriformis* have shown that lambs are unable to develop resistance to this nematode when they are young (4 - 5 months of age) and that maximum resistance is developed at approximately eight months of age (Gibson and Parfitt, 1972; Dobson *et al.*, 1990b). In *O. circumcincta* infected lambs worm burdens started to decline at approximately six months of age (Hong *et al.*, 1987), indicating some development of resistance from this time. In May, the Romney lambs in the present experiment were eight months old and therefore, in principle, capable of expressing resistance to the two species mentioned above, which were the most prevalent ones. This may, therefore, explain the low developmental success of eggs to 3rd stage larvae recorded in samples from the May sampling in both groups although FEC were high. The fact that sheep of the Perendale breed are generally more resistant to parasites than sheep of the Romney breed (McEwan *et al.*, 1997) may also have contributed to the difference in developmental success between the two experiments.

Levels of specific antibody (IgG_1) to both larval and adult antigens of *T. colubriformis* and *O. circumcincta* were not found to differ between the lines. This is in accordance with previous findings in this flock for antibodies to *T. colubriformis* (Williamson *et al.*, 1995a) but in contrast to other studies on genetically resistant sheep where FEC was found to be

inversely correlated with specific antibody levels (Gill, 1991; Gill *et al.*, 1993a and b; Douch *et al.*, 1995; Bisset *et al.*, 1996). There are no previous reports on levels of specific antibody to *O. circumcincta* in this flock, but with respect to specific antibody to *H. contortus*, the lines have shown no differences in the past (Williamson *et al.*, 1995a and b). In the present experiment, there were large differences between sampling times, particularly for antibody to larval antigen of both species examined. This most likely reflected an increased level of larval challenge on pasture and a developing immune response. Significant variation between lambs in specific antibody to larval and adult antigen of both species examined was found mainly in the Control line, suggesting greater variability between animals in the acquired immune response in this line. In contrast, animals in the HFW line would appear to be genetically more homogenous in their immune responsiveness. A similar variability between animals within a group is reported in Chapter Six when lambs, trickle-infected with *T. colubriformis* and immune-suppressed, showed less variation in specific antibody within the group than lambs that were only trickle-infected. The HFW selected sheep have been shown to be immunologically less responsive to parasites, as indicated by lower FEC and fewer Mucosal Mast Cells and Globule Leukocytes in response to infection (Williamson *et al.*, 1995b). However, the differences in for instance FEC, between these lines, were not as dramatic as those reported for flocks where lines had been selected primarily for their resistance or susceptibility to parasites (Bissett *et al.*, 1997; Leathwick, pers.comm.; Chapter 2).

Haemonchus has been reported to be the most prevalent genus in late summer/early autumn in faecal cultures from this breeding flock (Howse *et al.*, 1992). Three genera, *Haemonchus, Ostertagia* and *Trichostrongylus*, were found in more recent experiments in samples from both spring and summer (Williamson, 1994; Williamson *et al.*, 1995a). However, these authors did not provide information on the exact generic composition. In the present experiment *Ostertagia* was the most prevalent genus, followed by *Trichostrongylus* and *Chabertia/Oesophagostomum*. Variations between years were most likely caused by year-to-year differences in rainfall and temperature.

In conclusion, the present experiment did not help to explain the observed differences in developmental success between the years, in samples from Perendale lambs. It is suggested that grazing selection lines together under a low level of larval challenge resulted in a failure to express differences in acquired resistance to gastrointestinal nematodes and a subsequent effect on the development of the free-living stages. A further contributing factor may have been that the Romney flock used for this experiment was bred primarily for wool production and that a difference in susceptibility to parasitism was only discovered later. FEC was therefore not as divergent between the lines as observed in the Perendale flock, which was specifically selected for resistance to parasites. Ideally, a comparison should have been made between flocks that had both been selected for resistance and susceptibility to parasites, primarily on the basis of FEC, but one was not available.

CHAPTER FOUR

THE EFFECT OF IVERMECTIN-TREATMENT ON HOST IMMUNITY

4.1 Introduction

Some of the results for developmental success of nematode eggs in samples from Perendale lambs (Chapter Two) were unexpected, in that in 1997 a higher developmental success was recorded in samples from resistant line lambs than from susceptible line lambs. This was in contrast to the hypothesis of host immunity adversely affecting developmental success and to results obtained for the Perendale ewes that same year and for Perendale lambs in the previous year (1996) and the following year (1998). The Perendale lambs had been treated with albendazole in 1996 and 1998 and ivermectin in 1997. Therefore the question arose, as to whether the routine use of ivermectin in 1997 had adversely affected the development of immunity; impairing the resistant line lambs from expressing their superiority in acquired resistance to gastrointestinal nematodes. An untoward effect of using anthelmintics on the acquired immune response has been reported in sheep (Stankiewicz *et al.*, 1994, 1995 and 1996b).

The aim of the present experiment was therefore to compare the host effect on larval developmental success in lambs treated with either albendazole or ivermectin. Ideally, a control group receiving no anthelmintic treatment should have been included, but as the experiment was part of an already running larger trial (see section 4.2.1), this was not possible.

4.2 Materials and Methods

4.2.1 Experimental Animals

The East Friesian (3/4) x Romney (1/4) ewe lambs used for this experiment belonged to a commercial farm close to Palmerston North. The animals were, at the time of this experiment, part of a larger trial to study the effects on production of a '3, 3, 4, 4 week interval' drenching schedule (Leathwick, pers.comm.). The ewe lambs were approximately six months old at the first sampling and had by then been treated three times, at three week intervals, since weaning with either albendazole (Valbazen®)[1] or ivermectin (Ivomec®)[2]. All lambs were grazed together throughout the experiment.

4.2.2 Experimental Design and Sampling Schedule

The experiment involved two treatments (albendazole- or ivermectin-treated) and two sampling times. Lambs had previously been randomly assigned to the treatments

[1] 25 g/L Albendazole, Pfizer Animal Health, New Zealand

[2] Ivomec liquid for sheep and goats; 0.08% v/w solution of Ivermectin; Merial, New Zealand

based on live weight and FEC. The same twenty lambs from each treatment group were sampled on two occasions; March 3 and April 1 1999. On the day of sampling, all lambs were treated with anthelmintic according to the drenching schedule, but not until all samples had been taken. Samples taken in December 1998 from 25 randomly chosen ewe lambs in the same flock (but not the same ones that were later used in the experiment), before any drench had been given, showed a mean faecal egg count (FEC) of 166 epg (two replicates per animal sample) and a mean developmental success of 5.6% (one replicate per animal sample).

4.2.3 Faecal Samples

Faecal samples were analysed for FEC as described in Appendix 2a. Six replicates were counted per animal sample and the mean value of these used in all analysis.

Developmental success was estimated in faecal cultures. Three replicates were carried out per animal sample and the mean value of these used for analysis. The procedures for culturing, extracting, counting and identifying 3rd stage larvae were as described in Appendices 2c, 2e and 2f, respectively.

Faecal dry matter percentage (%D.M.) was estimated when enough faeces were left over from the FEC and larval cultures (see Appendix 2g).

4.2.4 Statistical analysis

Details of the statistical analysis are given in Appendix 4b.

Data on FEC and developmental success were ln(x+1) transformed to normalise the residuals and meet the requirements for an analysis of variance. These results are presented as geometric group means ± standard errors.

Results from the analysis for faecal dry matter percentage (%D.M.) did not require transformation as the residuals were normally distributed and are presented as least squares means ± standard errors. The residuals of the *Haemonchus*, *Trichostrongylus* and *Chabertia/Oesophagostomum* genera were not normally distributed. None of the common transformations (ln, square root, and arcsine) normalised the residuals, and since these genera only had a low prevalence and the two main genera, *Ostertagia* and *Cooperia,* had normally distributed residuals, it was decided to present all results of the generic composition as arithmetic means ± standard errors.

All data were analysed using a generalised linear model (GLM) in the SAS version 6.12 statistical package. The main factors examined were **time** (sampling times) and **treatment** (ivermectin versus albendazole treated group). As each animal was 'nested' within the treatment this was also catered for in the fitted model. Finally, it was of interest to analyse for an interaction between time and treatment. In all cases Type III sums of

squares were used to estimate significance levels. Faecal dry matter percentage was included as a covariate in the analysis of FEC and developmental Success.

4.3 Results

All data are presented in Appendix 4a.

4.3.1 Faecal Egg Counts (FEC)

FEC did not differ significantly between the two treatment groups on either of the sampling occasions (Figure 4.3.1.1). Generally, FEC were low in both groups but there was a significant increase in FEC from the first to the second sampling time ($p<0.01$), most likely due to a longer interval after anthelmintic treatment on the second sampling time (four weeks instead of three). The pre-patent period for most gastrointestinal nematodes is approximately three weeks; hardly enough time for infective larvae to develop into egg laying adult worms between drenches. Therefore it was not surprising to find that at the first sampling time, 12 out of 20 faecal samples in the ivermectin treated group had zero epg, whereas only one sample out of 20 in the albendazole treated group had zero epg. On the second sampling time there were eight samples and one sample with zero epg in the ivermectin and albendazole treated groups, respectively. The difference between treatment groups in the number of samples with a positive egg count might indicate a reduced efficacy of albendazole, although this was not tested for. The variation between animals within a treatment group and time by treatment interactions were not significant. Inclusion of the faecal dry matter percentage as a covariate was not significant and did not affect the outcome of the analysis.

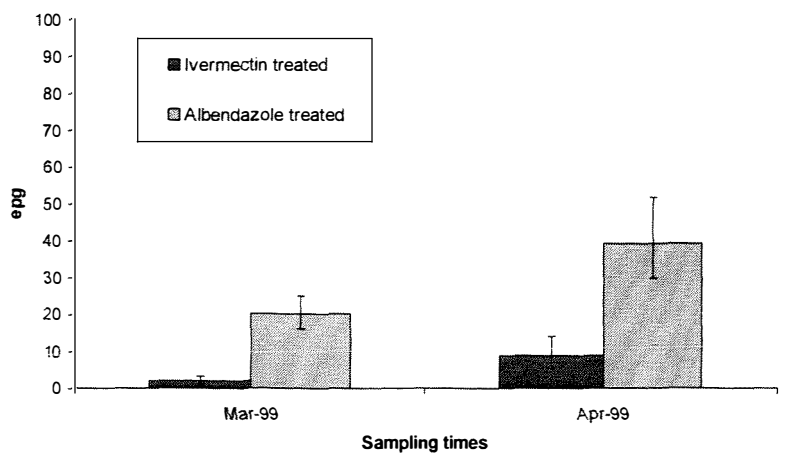

Figure 4.3.1.1 Faecal Egg Counts (FEC) (Geometric group means ± S.E.).

4.3.2 Developmental Success

Developmental success of eggs to 3rd stage larvae did not differ between the treatment groups at either of the two sampling times (Figure 4.3.2.1). However, there was

significant variation between animals within treatment groups ($p<0.01$), a difference that diminished somewhat when including %D.M. as a covariate ($p<0.05$), suggesting that this variable could account for at least some of the variation between animals. There was no significant time by treatment interaction.

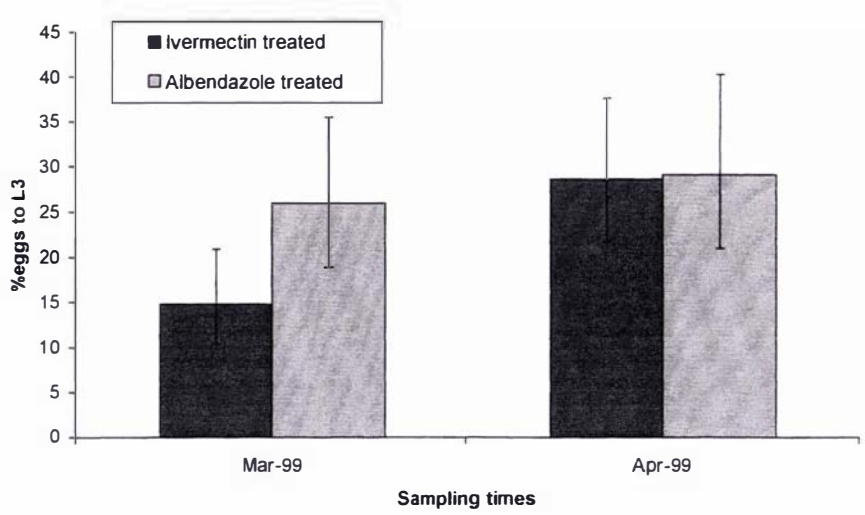

Figure 4.3.2.1 Developmental Success of eggs to 3rd stage larvae (Geometric group means ± S.E.)

4.3.3 Faecal Dry Matter Percentage (%D.M.)

The two treatment groups did not differ significantly with respect to faecal dry matter percentage (Figure 4.3.3.1). There was a significant difference between the two sampling times ($p<0.001$) but no significant between animal variation or time and treatment interaction.

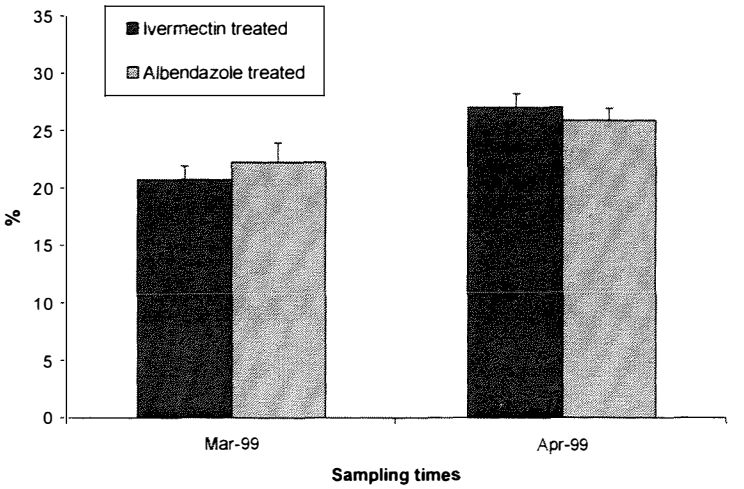

Figure 4.3.3.1 Faecal Dry Matter Percentage (%D.M.) (Least squares group means ± S.E.).

4.3.4 Generic composition

Haemonchus, *Ostertagia*, *Trichostrongylus*, *Cooperia* and *Chabertia/Oesophagostomum* arithmetic means for the ivermectin treated and the albendazole treated groups are presented in Figure 4.3.4.1 and Figure 4.3.4.2, respectively. There were no significant differences in generic composition between the two treatment groups. However, time, the time by treatment interaction and the animal within treatment variation were significant effects for the two main genera (*Ostertagia*: $p< 0.05$, 0.01 and 0.01, respectively; *Cooperia*: 0.01, 0.01 and 0.05, respectively). For *Haemonchus*, only time was significant ($p<0.01$), for *Chabertia/Oesophagostomum* only the animal within treatment variation was significant ($p<0.05$), whereas for *Trichostrongylus* no effects were significant.

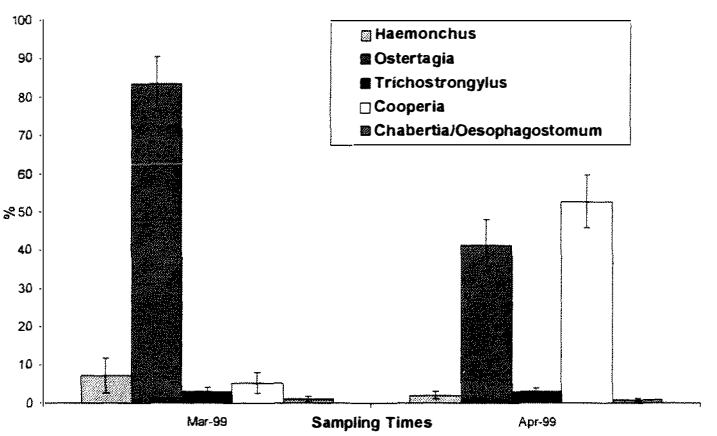

Figure 4.3.4.1 Ivermectin treated group – Generic composition (Arithmetic group means ± S.E.).

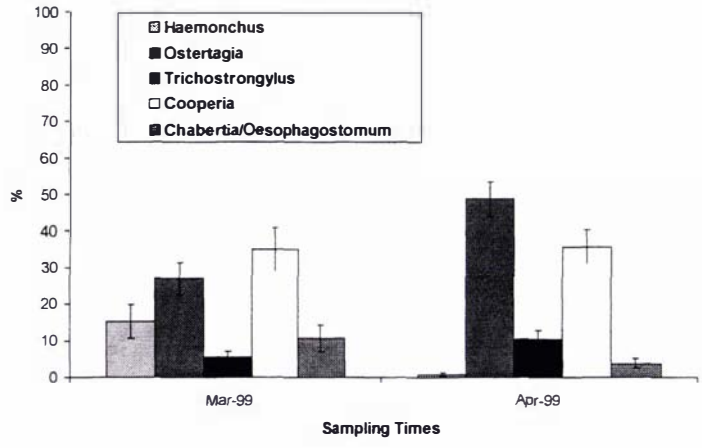

Figure 4.3.4.2 Albendazole treated group – Generic composition (Arithmetic group means ± S.E.).

4.4 Discussion

The two treatment groups did not differ with respect to FEC, developmental success, faecal dry matter percentage or generic composition, providing no evidence that any effect of host immunity on the free-living stages of gastrointestinal nematodes was influenced by the type of anthelmintic used for parasite control.

Variation in host immunity in sheep has previously been linked to variation in developmental success of eggs to 3rd stage larvae in mixed nematode infections (Jorgensen *et al.*, 1998; Chapter 2) and in single species infections with *O. circumcincta* (Sutherland *et al.*, 1999b and Chapter 5). On the basis of these findings, it was hypothesised that in relatively more immune animals the developmental success of eggs in faecal cultures would be reduced. In the present study there was no difference between the two groups with respect to developmental success of eggs to 3rd stage larvae. According to the hypothesis, this would suggest that the level of host immunity did not differ between the two groups.

It has been proposed that the use of certain anthelmintics may adversely affect an animal's development of an acquired immune response to nematode infections. Oral treatment of parasite-free lambs with ivermectin, prior to antigenic stimulation *in vitro* of peripheral blood lymphocytes with a mitogen[3], resulted in decreased blastogenic activity in cells from these animals compared with lymphocytes from control animals (Stankiewicz *et al.*, 1995). In a secondary response to the antigen ovalbumin, there was a significant reduction in antibody response in the ivermectin-treated group. In sheep immunised with ivermectin-abbreviated infections of *O. circumcincta* and *T. colubriformis*, there was no

[3] An agent that induces mitosis and lymphocyte blastogenesis

significant protection to a subsequent infection (Stankiewicz *et al.*, 1996b). However, a more recent study has shown no adverse effect of ivermectin on the immune response in sheep to the same two species of parasites (Vlassoff *et al.*, 1999). Conflicting results have also been reported concerning the effect of oxfendazole on immunity in sheep. In one study, two treatments of sheep with oxfendazole were shown to decrease the blastogenic activity of their lymphocytes and depress antibody responses (Stankiewicz *et al.*, 1994). However, in a later study, sheep receiving oxfendazole-abbreviated infections of *O. circumcincta* and *T. colubriformis* showed a very high level of protection against subsequent infections with the same nematodes, relative to sheep given ivermectin-abbreviated infections (Stankiewicz *et al.*, 1996b). The increased level of protection/immunity against subsequent infection was not associated with an increase in antibody levels, and the authors suggested that a cellular response might be more important for expression of immunity against the nematodes in question. The authors did not comment on their earlier work on oxfendazole and its possible effect on immunity, in which it appeared that the cellular component of the immune response was also adversely affected by the drug (as measured by decreased blastogenic activity of lymphocytes). A recent study using oxfendazole-abbreviated infections with *H. contortus* in sheep failed to demonstrate the high reduction in FEC that Stankiewicz *et al.* (1996a) had found in their study (Schallig *et al.*, 2000).

In the light of the earlier work described above, the question arises as to whether there is in fact an effect of ivermectin on host immunity in the field. A possible way for anthelmintics to adversely influence the development of immunity may simply be that they remove a substantial part of the antigenic stimulation necessary for the development of a protective immune response thus leaving the animals relatively less protected against subsequent infections. This has been demonstrated in sheep for *T. colubriformis* (Sutherland *et al.*, 1999a) and *H. contortus* (Schallig *et al.*, 2000) and suggested in mixed nematode infections in cattle (Claerebout *et al.*, 1999). The sheep in the present experiment were treated with anthelmintics at three and four week intervals, and given that the experiment took place during a dry summer/early autumn, the animals would have probably experienced low larval challenge. Although the ability to develop an effective immune response to parasitic infections is largely dependent on age (Gibson and Parfitt, 1972; Hong *et al.*, 1987; Dobson *et al.*, 1990b), it also appears that reaching a certain threshold level of infection is important, at least in infections with *T. colubriformis* (Dobson *et al.*, 1990a and b). The two treatment groups in the present experiment were grazing together so that any effect of a low larval challenge would have been experienced in both groups. It might be argued that the lambs did not experience sufficient challenge to develop an effective immune response to infections due to the probably low larval challenge present on pasture during the experimental period. As a result, it was not possible to discriminate between the two treatments. However, since the lambs were six to seven months old and grazing in the field, they would be able to and also be expected to be expressing some level of acquired immunity. If it is argued that the lambs did not experience sufficient challenge on pasture to develop an effective immune response, the results could be interpreted to mean that an effect of ivermectin on the immune response in lambs cannot be excluded.

Another factor that needs to be considered is the possibility that anthelmintic administration has a direct effect on larval development, as discussed in Chapter 2. Results of the present experiment suggest that, if there is such an effect, this is probably negligible, as samples taken before any anthelmintic was given showed very low developmental success. However, as these samples were smaller and no replication for cultures was possible, the results for developmental success may have been imprecise.

Whereas ram lambs were sampled in the Perendale experiment, ewe lambs were used in the present experiment. It is generally accepted and documented that post-pubertal ewes tend to be more resistant to parasites than post-pubertal rams, except during lactation (reviewed by Barger, 1993). The influence of sex on the resistance to parasites was not considered to be an important issue in the present experiment as the ewe lambs used were about seven months of age when the experiment ended and had probably not reached puberty yet.

This experiment did not show that the type of anthelmintic used for drenching sheep influenced the level of host immunity, as judged by the developmental success of eggs to 3rd stage larvae. While this experiment is not conclusive, its findings are consistent with a number of other recent studies which have failed to show any effect of anthelmintic-type on the development of immunity to parasites (Vlassoff et al., 1999; Leathwick, unpublished). It did not provide any support, therefore, for the hypothesis that the inconsistent results in the Perendale lambs in 1997 were attributable to the type of anthelmintic being used.

Plate 4.3.4.1 Ewe lambs being sampled in the yards

CHAPTER FIVE

THE EFFECT OF HOST IMMUNITY ON THE DEVELOPMENT AND SURVIVAL OF THE FREE-LIVING STAGES OF *OSTERTAGIA CIRCUMCINCTA*

5.1 Introduction

The present experiment provided an opportunity to investigate the possible effect of host immunity on the free-living stages of *O. circumcincta* from infections in housed animals. The aim of the experiment was to investigate whether host immunity affected developmental success and other physical and biological properties of the free-living stages, i.e. the size of eggs, length of infective larvae and survival of infective larvae. The opportunity was taken to utilise an experiment being conducted by AgResearch staff. Unfortunately, the experimental conditions were not ideal as group sizes were small and experimental animals older (>one year) than the ones used in other experiments described in this thesis.

5.2 Materials and Methods

5.2.1 Experimental Animals

Fourteen-month-old Romney rams were used for this experiment. The animals were housed indoors at AgResearch, Flock House Research Station near Bulls and were fed a diet consisting of chaffed hay and lucerne pellets with free access to water.

5.2.2 Parasites

The strain of *O. circumcincta* used for trickle- and challenge-infections in this experiment was originally obtained from Dr. W.E. Pomroy, IVABS, Massey University. This strain is susceptible to all common anthelmintics.

5.2.3 Experimental Design and Sampling Schedule

This experiment was part of a bigger project in which experiments to test the effect of host immunity to *O. circumcincta* on L3 survival at different temperatures and on L3 infectivity were being carried out by researchers at AgResearch. Some of these results have since been published (Sutherland *et al.*, 1999b). The treatment schedule was as follows.

Ten lambs were drenched with twice the manufacturer's recommended dose of the combination anthelmintic Arrest®[1], housed and divided into two groups of five based on liveweight. Eight weeks after housing the trickle infections began. One group (*O. circumcincta* group) was trickle infected with 5,000 L3 of *O. circumcincta* per week (given in

[1] 23.8 g/L Albendazole, 37.5 g/L Levamisole; Ancare N.Z. Ltd., New Zealand

two equal doses) for seven weeks and then drenched with Arrest according to live weight, whereas the second group (Control group) was not trickle infected but drenched on the same day as the trickle infected group. The timing of the drench was decided on the basis of declining FEC and increasing peripheral eosinophil numbers in the *O. circumcincta* group. This was taken as evidence of an increased level of host immunity to *O. circumcincta* in the infected group, a conclusion later supported by a consistent increase in anti-*O. circumcincta* larval IgG titre in the weeks before and after the challenge infection was given (Sutherland *et al.*, 1999b). One week after drenching, both groups were challenged with 15,000 L3 of *O. circumcincta*, administered as two doses of 7,500 L3 given on consecutive days. On days 25 and 31 after the challenge infection, faeces were collected from all animals in the two groups for use in the present experiment. Two weeks after patency of the challenge infection (day 35), all lambs were killed.

5.2.4 Faecal Samples

5.2.4.1 FEC

Faecal samples were collected on day 25 after challenge, by means of faecal bags attached to a harness fitted on the sheep. Bags were left on the sheep for approximately 12 hours.

Faecal samples were analysed for FEC as described in Appendix 2a. Three replicates were counted per animal sample and the mean value of these used for the statistical analysis.

5.2.4.2 Developmental Success

Developmental success was estimated in faecal cultures using the faeces collected on day 25 after challenge. Faeces were cultured as described in Appendix 2b, with the modification that two incubation temperatures were used. For each animal sample four cultures were set up. Two were incubated at 20°C for 14 days whereas the other two were incubated at 10°C for 21 days. The procedure for extracting, counting and identifying 3rd stage larvae was as described in Appendices 2c, 2e and 2f, respectively. The mean values for developmental success of the two replicates, per temperature, were used for the statistical analysis.

5.2.4.3 Developmental Success in a Larval Development Assay

Faeces were collected on day 31 after challenge. Eggs were recovered from faeces as described in Appendix 5a and cultured for seven days in 96-well microtitre plates to 3rd stage larvae and counted as described in Appendix 5b. A concentration of approximately 80 eggs per well was intended, but an actual concentration of 47 ± 8 eggs per well was achieved. For each animal sample, eggs were cultured in 20 wells. This mean was calculated from all wells counted in the Control group and was a total count including all stages from egg to 3rd stage larvae. This count did not include any eggs or larval stages that might have disintegrated within the seven days of incubation. In the *O. circumcincta*

group wells, unfortunately only third stage larvae were counted. In hindsight, this was regrettable as it made the comparison between groups difficult. The developmental success (%) of eggs to 3rd stage larvae was then calculated as:
(number of 3rd stage larvae found in well/47)*100 - based on the mean number of eggs estimated from the Control group wells.

5.2.4.4 Faecal Dry Matter Percentage

Faecal dry matter percentage was estimated when enough faeces were left over from the FEC and larval cultures in faecal samples from day 25 after challenge (see Appendix 2g).

5.2.4.5 Egg Size (Volume) and Larval Length

Egg size was determined for 50 eggs per animal sample and the volume calculated as described in Appendix 5c. Faeces for this analysis had been collected on day 31 after challenge.

Larval length was determined for 100 3rd stage larvae per animal sample as described in Appendix 5c. Larvae obtained from the faecal cultures, from faeces collected on day 25 after challenge, were used for this part of the experiment.

5.2.4.6 Larval Survival at 20°C and 30°C

Larvae recovered from the faecal cultures grown at 10°C and 20°C were used for the survival experiment. Cultures from only eight animals were available for the study of larval survival, as two animals in the *O. circumcincta* group had zero epg when faeces were collected for culturing on day 25 after challenge. As there were low numbers of larvae in some animal samples, the duplicate cultures from each animal were pooled to provide enough larvae. Survival was studied at two temperatures, 20°C and 30°C, and for each of these temperatures larvae cultured at either 10°C or 20°C were added to culture bottles in duplicate. This meant that a total of 32 culture bottles were set up per survival temperature (eight animals x two culturing temperatures x two replicates). To each culture flask 300 ± 100 3rd stage larvae were transferred. Distilled water was added to reach a volume of approximately 30 ml in all culture bottles. The culture flasks were stored lying down which meant that the depth of water during storage was approximately one cm. Caps were loosely fitted so that oxygen could still enter the bottles.

Approximately every three weeks, the proportion of dead versus live larvae was determined by counting all larvae in the flasks and identifying live and dead ones. Any 'missing' larvae in relation to the initial total count in Week 0 were assumed to be dead larvae that had disintegrated. For flasks stored at 30°C, the counting continued until less than 50% of the larvae were alive. For flasks stored at 20°C, the counting continued until week 24/25 after the start of storage, although at this time 50 % or more of the larvae were still alive in some treatments. The counting was at times complicated by the presence of condensation in the flasks and that, due to the surface tension of the water, some larvae in

small volumes of water would adhere to the sides of the culture flasks and not be visible for inspection. This did at times cause the number of larvae in the culture flasks to be underestimated. To minimise these problems, culture flasks were taken out of the incubators well in advance of being counted. This allowed the larvae to settle at the bottom of the flasks and be visible for counting, and to let the water containing the larvae adjust to the temperature in the laboratory and thereby reduce the presence of condensation.

5.2.5 Statistical Analysis of Data

All results of the statistical analysis are presented in Appendix 5e.

None of the measured variables required transformation before analysis, and the results are therefore presented as least squares group means ± standard errors. All data were analysed using a factorial generalised linear model (GLM) in the SAS version 6.12 statistical package. In all cases, type III sums of squares were used to estimate significance levels.

For the developmental success and larval length results, the main factors examined were **treatment** and **culturing temperature**. In addition, the nested effect (animal within treatment) and the interaction between treatment and culturing temperature were incorporated into the statistical model. For faecal dry matter, faecal egg counts and LDA larval development results, the main factor examined was **treatment.** For the egg volume, the main factor examined was **treatment** with the nested effect (animal within treatment) also taken into account in the statistical model. For the larval survival results, the main factors examined were **treatment, culturing temperature** and **storage temperature**. The nested effect (animal within treatment) and interactions between the main factors were also analysed.

5.3 Results

All data are presented in Appendix 5d.

5.3.1 Faecal Egg Counts (FEC) and Faecal Dry Matter Percentage (%D.M.)

Neither FEC nor %D.M. differed between the two groups at day 25 after the challenge infection (see Table 5.3.1.1). Two of the animals in the *O. circumcincta* group had zero FEC at this time, but at day 31 after challenge, when samples were taken for recovering eggs for the LDA, positive egg counts were found in all animals in both groups.

	O. circumcincta group	Control group
FEC	200 ± 48 a	170 ± 88 a
% D.M.	38.6 ± 5.7 a	43.9 ± 1.2 a

Table 5.3.1.1 FEC Arithmetic group means ± S.E. and Faecal Dry Matter Percentage Least squares group means ± S.E.. In both groups n=5. Row values with the same letters are not significantly different.

5.3.2 Developmental Success in Faecal Cultures

As two of the animals in the *O. circumcincta* group had zero FEC, these samples could not be used for the comparison of developmental success in faecal cultures. The results presented in Figure 5.3.2.1 below are therefore based on results from only three animals in the *O. circumcincta* group, but on all 5 animals in the Control group. Given these small group sizes and the large variation between animals in the *O. circumcincta* group, the power of the analysis was greatly reduced. The two groups did not differ in developmental success and there was no significant effect of temperature on the developmental success of eggs to 3rd stage larvae, when culturing faeces at the temperatures chosen for this experiment (10°C and 20°C).

Developmental success rates of over 100% in some samples was almost certainly due to an underestimation of FEC, as FEC was low (<500 epg) in both groups (see Chapter 2). The underestimation of FEC resulted in an overestimation of developmental success. As FEC did not differ between the two groups, it was assumed that any bias created by the underestimated FEC would have an equal impact on developmental success in faecal cultures in both groups. As eggs were also cultured in an LDA, these results could be compared to those of the faecal cultures. In the LDA where a known number of eggs were added to each well of a micro titre plate, a developmental success above 100% did not occur. The difference in developmental success between the two groups was of a comparable magnitude when considering least squares group means (see section 5.3.3 below).

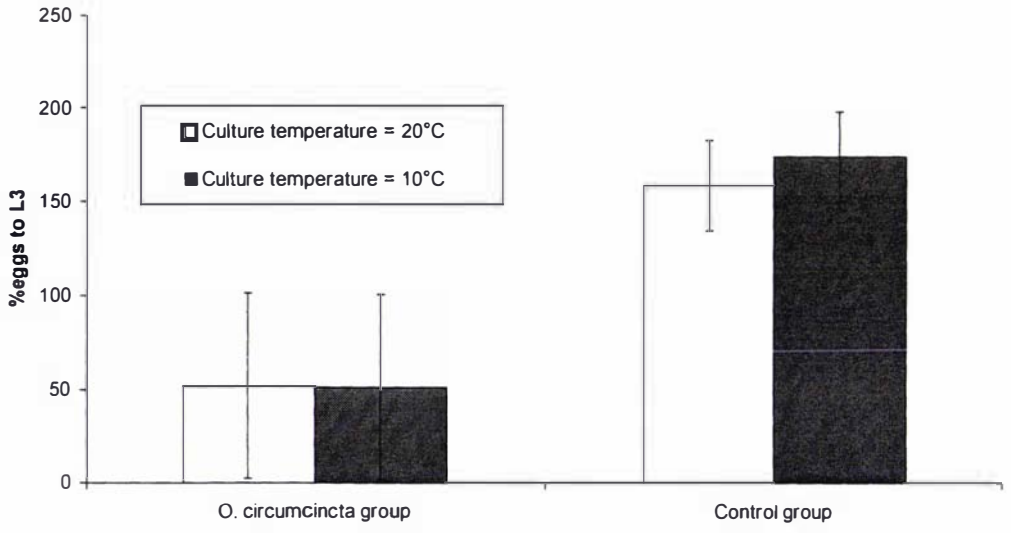

Figure 5.3.2.1 Developmental success in faecal cultures at two temperatures (Least squares group means ± S.E.). In the *O. circumcincta* group, n = 3, whereas in the Control group, n = 5.

5.3.3 Developmental Success in a Larval Development Assay (LDA)

Results from culturing eggs in an LDA (with no anthelmintic added) are presented in Table 5.3.3.1. Bearing in mind the limitations of these results (see section 5.2.4.3), it would appear that there were still differences between the two groups with respect to egg development to 3rd stage larvae ($p<0.001$), with a much higher developmental success in the Control group than in the *O. circumcincta* group.

	O. circumcincta group	Control group
%Developmental Success	26.5 ± 3.1 a***	70.8 ± 3.1 b

Table 5.3.3.1 Developmental Success in control wells of a Larval Development Assay (Least squares group means ± S.E.). Developmental Success = % eggs developed to 3rd stage larvae. Values with the same letter are not significantly different. *** = $p<0.001$

5.3.4 Egg Size and Larval Length

Egg size, measured as egg volume, did not differ between the treatment groups (Table 5.3.4.1), but there was significant variation between animals within both groups ($p<0.01$).

	O. circumcincta group	Control group
Egg Volume (μm^3)	104956 ± 943 a	110588 ± 889 a

Table 5.3.4.1 Egg Volume measured in μm^3 (Least squares means ± S.E.). Fifty eggs were measured in samples from each of five animals in both groups.

As can be seen in Table 5.3.4.2 below, the length of infective 3rd stage larvae did not differ between treatment groups. However, in both treatment groups, larvae cultured at 20°C were found to be significantly longer than larvae cultured at 10°C ($p<0.05$). Furthermore, there was a significant variation between animals in both groups ($p<0.01$).

O. circumcincta group Larvae cultured @ 20°C	*O. circumcincta* group Larvae cultured @ 10°C	Control group Larvae cultured @ 20°C	Control group Larvae cultured @ 10°C
845 ± 4 a	818 ± 4 b	863 ± 3 a	833 ± 3 b

Table 5.3.4.2 Length of infective larvae measured in μm (Least squares group means ± S.E.). In the *O. circumcincta* group 100 infective larvae from each of three animals were measured, whereas in the Control group, 100 infective larvae from each of five animals were measured. Row values with the same letters are not significantly different.

5.3.5 Larval Survival

Results are presented as least squares group means ± standard errors in Figure 5.3.5.1 for larvae cultured at 10°C and in Figure 5.3.5.2 for larvae cultured at 20°C.

There were no significant differences between the two groups of animals with respect to survival of third stage larvae at either of the two storage temperatures. There was a significant interaction between culturing temperature and storage temperature ($p<0.01$) indicating that larvae died faster when being cultured at the lower temperature (10°C) and/or being stored at the higher temperature (30°C).

When analysing the treatments separately, it was found that in the control group, larvae survived longer when having been cultured at 20°C ($p<0.05$) than at 10°C, and when having been stored at 20°C ($p<0.01$) than at 30°C. For the *O. circumcincta* group, larvae that had been cultured at 20°C tended to survive longer ($p<0.06$) than larvae that had been cultured at 10°C, whereas there was no difference in survival when comparing the two storage temperatures alone. However, in both treatment groups, there were significant differences between the number of weeks the larvae survived, with larvae (cultured at both temperatures) surviving longer at 20°C than at 30°C ($p<0.01$).

Generally, larvae that were stored at 30°C started to die after about five weeks of storage if they had been cultured at 10°C, and after about ten weeks of storage if they had been cultured at 20°C.

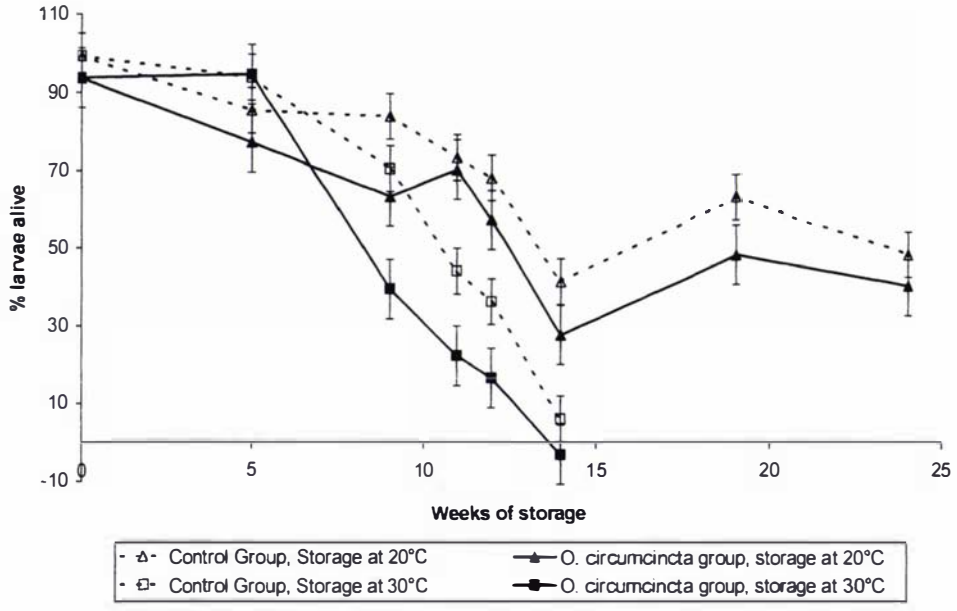

Figure 5.3.5.1 Survival at two different temperatures of larvae cultured at 10°C (Least squares group means ± S.E.). In the *O. circumcincta* group n=3 animals and in the Control group n=5 animals.

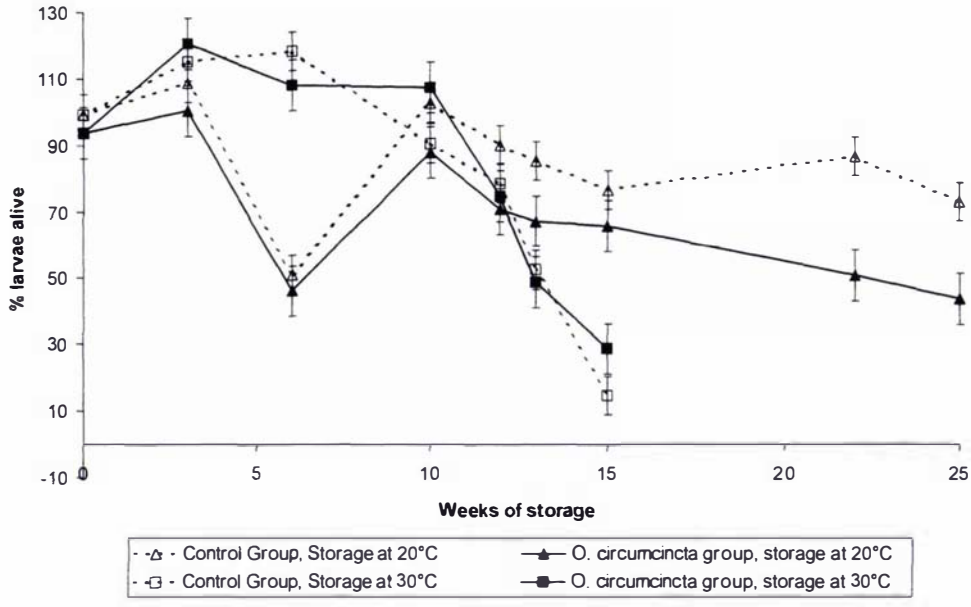

Figure 5.3.5.2 Survival at two different temperatures of larvae cultured at 20°C (Least squares group means ± S.E.). In the *O. circumcincta* group n=3 animals and in the Control group n=5 animals.

5.4 Discussion

A lower developmental success of *O. circumcincta* in an LDA was found in animals that had been rendered relative immune to this parasite compared to control animals. This finding appears to support the hypothesis of an adverse effect on larval development in relatively more immune animals, as reported previously (Jorgensen *et al.*, 1998; Chapter 2).

There were no differences in worm burdens at slaughter between the two groups but female worms were significantly shorter in the *O. circumcincta* group and also had fewer eggs *in utero* (Sutherland *et al.*, 1999b). These findings suggested a somewhat higher level of immunity to *O. circumcincta* in the trickle infected group than in the control group, although the difference between the groups was probably not great. These results are in accordance with previously published work (using animals reared worm-free) which reports that an early sign of onset of immunity to *O. circumcincta* is the retardation of developing worms and smaller size of adult female worms (Hong *et al.*, 1986; Seaton *et al.*, 1989). The stunting of worms is evident from four weeks after the start of infection (p.i.) and precedes a reduction in worm burden, which occurs between four and eight weeks p.i. (Seaton *et al.*, 1989). However, other authors have failed to demonstrate the retardation of worms even after 20 weeks of trickle infection with either 1000 or 10,000 infective larvae of *O. circumcincta* per week (Callinan and Arundel, 1982).

Establishment rates of the challenge infection were low in the present experiment (<13%; source: Sutherland *et al.*, 1999b) and may have been due to the age of the animals at

the beginning of the experiment (fourteen months old) and the fact that they had been field-reared and experienced mixed natural infections on pasture before being housed. In *O. circumcincta* trickle infected lambs, worm burdens have been shown to decline when the lambs are approximately six months of age (Hong *et al.*, 1987). In lambs trickle infected with *T. colubriformis* the ability to express resistance to infection is highly developed at approximately eight months of age (Gibson and Parfitt, 1972). Fourteen month old lambs would therefore be expected to be fully immune-capable and be able to develop resistance to incoming larvae very quickly.

Although any differences in host immunity after patency of the challenge infection were not reflected in the FEC, it appeared that there were differences in developmental success of the eggs the animals were shedding, with eggs shed from animals in the *O. circumcincta* group having a lower developmental success. This difference was not significant when culturing eggs in faecal cultures. However, significantly fewer of the eggs shed from animals in the *O. circumcincta* group developed to 3rd stage larvae in an LDA. Although based on a small data set, these findings may provide some support for the hypothesis that an increased level of host immunity adversely affects the developmental success of eggs. This finding was first made in Perendale sheep carrying mixed natural infections (Chapter 2) and in housed animals infected with *O. circumcincta*. The present experiment provides further evidence that the phenomenon exists for a single species infection with *O. circumcincta*. If larger treatment groups had been available, a difference in developmental success in faecal cultures might have been shown, as there were some indications in that direction, possibly obscured by a large animal within group variation. If not, the results would suggest that the effect is not in the faeces.

A larger egg volume has been linked to a longer time required for eggs of *H. contortus* to hatch (Crofton and Whitlock, 1965b). In the present experiment egg volume did not differ significantly between the two groups and it may therefore be that variations in host immunity does not have an effect on the volume of eggs and the subsequent time to hatching.

It is well established that culturing temperature has an effect on larval size/length, in that larvae cultured closer to their optimum temperature are longer than larvae cultured at a sub-optimum temperature (Premvati and Lal, 1961; Wang, 1967; Pandey, 1972; Rossanigo and Gruner, 1996). In the present experiment, the effect of culturing temperature on larval length was confirmed, as larvae cultured at 20°C were significantly longer than larvae cultured at 10°C. However, there was no evidence for an effect of host immunity on larval length.

Nor was there evidence for an effect of host immunity on larval survival, but it was affected by both the temperature at which the larvae had been cultured and the temperature at which they were stored. Larvae cultured at 20°C survived longer than larvae cultured at 10°C and did so at storage temperatures of both 30°C and 20°C. Small

infective larvae of *O. circumcincta* have been shown to survive a shorter time in water than normal length larvae (Gruner and Suryahadi, 1993). This is likely to be related to reduced energy stores in the smaller larvae. The finding in the present experiment, that larvae of *O. circumcincta* survived a shorter time at a high temperature (30°C) than at approximately room temperature (20°C) agrees with the findings of many authors not only for this species but also other trichostrongylid parasites (see table 1.3.3.1). A shorter survival at high temperatures is likely to be associated with a higher activity level and therefore higher energy consumption by larvae. At times, the proportion of live larvae was unexpectedly low for instance at week 6 for larvae cultured at 20°C and stored at 20°C (see figure 5.3.5.2). Although this might have been due to occurrence of condensation in the culture flasks (and consequently not being able to count all larvae), there is also the possibility that a lower oxygen availability caused live larvae to appear lifeless on occasion and that they were therefore counted as dead.

Although based on a small number of experimental units and given that the difference in host immunity between the two groups was probably not great, results from the present experiment suggest that a relatively modest increase in host immunity to *O. circumcincta* adversely affected developmental success of larvae in an LDA. Developmental success in faecal cultures, FEC, egg size, larval length, faecal dry matter percentage or larval survival were not affected to any extent that could be measured in this experiment.

CHAPTER SIX

THE EFFECT OF HOST IMMUNITY ON *TRICHOSTRONGYLUS COLUBRIFORMIS*

6.1 Introduction

Having demonstrated an effect of host immunity on the developmental success in a mixed infection with trichostrongylid parasites in animals in the field and found evidence for the same phenomenon in housed animals infected with *O. circumcincta*, it was of interest to see if the effect could be demonstrated in housed animals, infected with the common sheep parasite *T. colubriformis*. The objectives of the present experiment were to investigate two issues, firstly, the effect of host immunity in sheep on the development of *T. colubriformis* eggs to infective larvae and on adult worms in the small intestine, and secondly, the possible effect of host immunity in sheep on the infectivity of the 3rd infective larval stage.

6.2 Materials and Methods

The experiment consisted of two parts. Experiment 1 addressed the question of the effect of host immunity on development of the free-living stages and on parasitic stages of *T. colubriformis* whereas Experiment 2 specifically addressed the question of the effect of host immunity on the infectivity of 3rd stage larvae of *T. colubriformis*.

6.2.1 Experiment 1

Eighteen three-month-old Romney ram lambs were purchased from AgResearch Ballantrae Research Station near Woodville and transported to Massey University where they were housed and fed a diet of lucerne pellets and hay. Prior to housing, the lambs had been grazing parasite contaminated pasture and at weaning had received one treatment with a combination drench (Leviben®[1]). At housing, all lambs were drenched with twice the manufacturer's recommended dose of combination drench (see above). Faecal samples taken ten days later detected no eggs in the faeces of any of the lambs.

The lambs were randomly allocated to two groups of nine on the basis of live weight and each treatment group was housed in a separate pen. From two weeks after housing, both groups were trickle-infected with an anthelmintic susceptible strain of *T. colubriformis* (5000 L3/week administered as two equal doses) obtained from Massey University. This infection was given orally by means of a syringe. The trickle infection continued for a period of 9 weeks. From one week prior to the start of the trickle infection, one group was given Opticortenol[2] twice weekly for the duration of the

[1] 20 g/L ricobendazole, 37.5 g/L levamisole hydrochloride; Young's Animal Health, NZ

[2] 0.5% dexamethasone trimethylacetate, Ciba Animal Health Division, Switzerland; Opticortenol was used at a dose rate of 0.25 mg/kg bodyweight

experiment to suppress the immune response to parasitic infection (steroid-treated group) whereas the other was trickle infected only (non-steroid-treated group). Three weeks after the start of the trickle-infection, the infections in both groups were patent and faecal samples were collected weekly from then on. All lambs were weighed and blood sampled weekly.

After eight weeks, when animals in the non-steroid-treated group showed signs of immunity to *T. colubriformis*, as judged by FEC being consistently lower than in the steroid-treated group, all animals in both groups were drenched with twice the manufacturer's recommended dose of Leviben®. One week later, all animals were challenged with 20,000 L3 of the same susceptible strain of *T. colubriformis* that had been used for the trickle infection. From day 21 after challenge, when infections were patent in all lambs, faecal samples were collected and faecal cultures were carried out to culture infective 3rd stage larvae to be used for the challenge infections in Experiment 2 (Appendix 6i). Faeces from individual animals were cultured separately. At day 28 after infection, all animals were humanely killed, small intestines recovered, and worm counts were performed (see Appendices 6b, 6c and 6d) to determine the establishment rate of *T. colubriformis* after the challenge infection. The sex ratio (male/female) of adult worms, the lengths of adult female and male worms and *in utero* egg counts in adult female worms were also determined (see Appendix 6e). During necropsy, sections of small intestine were excised at 1.5 and 3 m distal to the pylorus for histological examination. Sections to be stained with Luna's stain were fixed in 10% neutral buffered formalin (FA) until processed as described in Appendix 6h. Sections to be stained with Toluidine Blue were fixed in an iso-osmotic solution of 0.6% formalin + 0.5% acetic acid (IFAA) for 12 hours and were then transferred to 70% alcohol before being processed as described in Appendix 6g. Sections fixed in FA were also stained with Gill's haematoxylin and eosin (Appendix 6f), in case cells were not easily counted using the other staining procedures.

6.2.2 Experiment 2

Forty spring-born lambs were purchased from AgResearch Ballantrae Research Station. These had been weaned and drenched at the same time as the lambs used in Experiment 1. When the lambs for Experiment 1 were housed, the 40 lambs for Experiment 2 were each given an Extender 100®[3] capsule in an attempt to minimise nematode antigenic stimulation. These lambs remained on pasture for the next 100 days, after which they were housed and drenched with twice the manufacturers recommended dose of Leviben®. The lambs were at that time approximately seven months old. All lambs were weighed and restrictively randomised into ten groups of four on the basis of live-weight. All lambs had zero epg in faecal samples taken on day 15 after drenching.

From the post-challenge larval cultures generated in Experiment 1, larvae from five lambs randomly selected from each of the two groups were used. For each of the resulting

[3] 3.85g albendazole/capsule; Nufarm Ltd., New Zealand

ten collections of larvae, four lambs in Experiment 2 were challenged with an oral dose of 20 000 L3, giving a total of 40 lambs infected. The challenge dose was administered three weeks after housing. At day 28 after the challenge infection, all animals were humanely killed and the small intestines recovered (see Appendix 6b). Worm counts were carried out to determine the establishment rate (or infectivity) of the larvae used for the infection and, in addition, the sex-ratio (male/female) of worms was also determined (see Appendices 6c and 6d).

6.2.3 Faecal samples – Experiment 1

Faecal samples were collected weekly by means of canvas bags attached to harnesses fitted on the sheep. Bags were left on for a maximum of eight hours. Two Faecal Egg Counts (FEC) were carried out per animal sample, using a modified McMaster method (see Appendix 2a), and the mean count per animal used for the analysis. Developmental success of eggs to infective 3rd stage larvae was measured in faecal cultures (see Appendix 2b). Two faecal cultures per animal sample were carried out and the resulting larvae extracted and counted (Appendices 2c and 2e), and the mean count used for the analysis.

6.2.4 Blood samples – Experiment 1

Blood samples were collected in heparinised Vacutainer tubes and examined for circulating eosinophils (Appendix 6a) and circulating specific anti-*T. colubriformis* antibodies using an ELISA method (Appendix 2i).

6.2.5 Histology – Experiment 1

Mucosal mast cells (MMC) and globule leukocytes (GL) were counted in sections stained with Toluidine Blue and eosinophils in sections stained with Luna's method. Cells were counted at a magnification of 400x, using a graticule eyepiece that covered an area of 0.0625 mm^2. Cells in 20 fields were included in each count and three counts were made per tissue section. Counts were made systematically from the submucosa to the lumen and back, moving along the length of the section. Only cells in the mucosa were counted.

6.2.6 Statistical analysis

Details of the statistical analysis are presented in Appendix 6k. Apart from circulating eosinophils and numbers of globule leukocytes, all measured variables did not require transformation and are presented as either arithmetic or least squares group means ± standard errors. Results for circulating eosinophils and numbers of globule leukocytes were 'ln(x+1)' transformed to normalise the residuals and meet the requirements for the analysis of variance, and are presented as geometric group means ± standard errors.

All data in Experiment 1 were analysed using a factorial generalised linear model (GLM) in the SAS version 6.12 statistical package. For the FEC, developmental success, circulating eosinophils and specific antibody levels results, the main factors examined were **treatment** and **week** (after start of trickle infection). In addition, variation between

animals within treatment and the interaction between week and treatment were analysed for. Group means for individual weeks were compared by t-test. For the histology results (MMC, GL and eosinophils) the main factors examined were **treatment** and **section** as well as the animal within treatment variation. For the worm count, male/female-ratio, female and male worm length and *in utero* egg count results the main factor examined was **treatment**. Type III sums of squares were used to estimate significance levels.

Data in Experiment 2 were analysed as a two-factor completely nested design, using a factorial generalised linear model. The main factor examined was **treatment**, as well as the nested effect (larvae within treatment) and the completely nested effect (animals within larvae within treatment). Again, Type III sums of squares were used to estimate significance levels.

6.3 Results – Experiment 1

All data from the experiment are presented in Appendix 6j.

6.3.1 Faecal Egg Counts

Soon after patency (Week 3 and onwards) of the trickle-infection the two treatment groups started to diverge in FEC (Figure 6.3.1.1). Lambs in the steroid-treated group generally had a significantly higher FEC than lambs in the non-steroid-treated group during the trickle infection period at weeks 4, 6 and 8 ($p<0.05$, 0.01 and 0.001, respectively), when immunity to *T. colubriformis* started to develop in the latter group of animals. There were significant differences between weeks ($p<0.001$), variation between animals within the groups ($p<0.01$) and a significant interaction between treatment groups and weeks ($p<0.001$), reflecting that the groups behaved differently over time with respect to FEC.

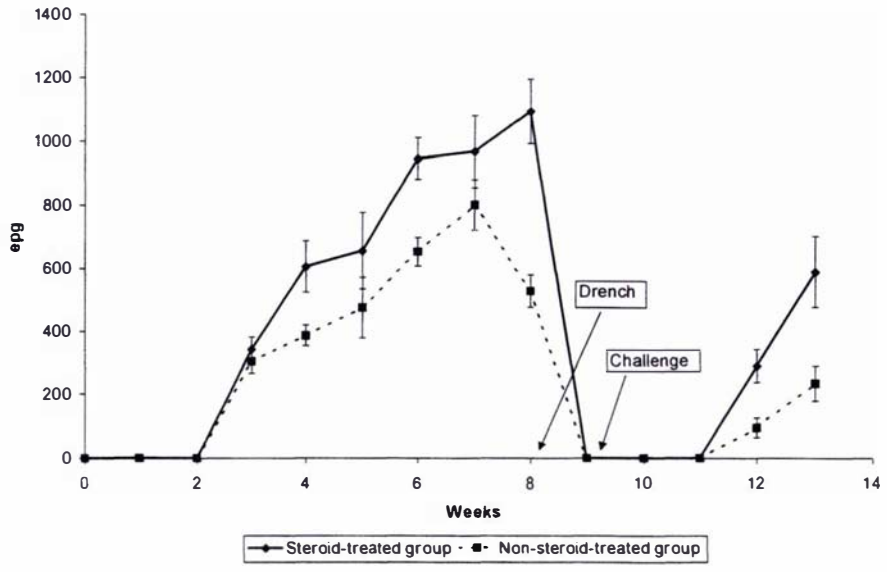

Figure 6.3.1.1 Experiment 1 - Faecal Egg Counts (Arithmetic group means ± S.E.).

At week 8, the FEC in the steroid-treated group was still increasing, whereas the FEC in the non-steroid-treated group had started to decrease, indicating development of immunity to *T. colubriformis* in the latter group. It was decided to drench all animals in week 8 and re-infect them 10 days later with a challenge dose of 20 000 L3 of *T. colubriformis*. Thus the worm burden at slaughter (day 28 after challenge infection) would be of the same age in both groups. Any effect on the worms and the eggs shed by them would then originate from a host effect and could not be attributed to variations in the age of the worm burden the different animals were harbouring. After patency of the challenge infection (Week 12), the animals in the steroid-treated group continued to have a significantly higher FEC than animals in the non-steroid-treated group ($p<0.01$). At slaughter this difference was 3-fold ($p<0.05$).

6.3.2 Developmental Success of eggs to 3rd stage infective larvae.

Developmental success of eggs to infective 3rd stage larvae appeared higher in samples from the immune-suppressed group in Week 8, although the difference was not significant ($p<0.10$) (Figure 6.3.2.1). The finding coincided with the greatest divergence between the two groups in FEC. At the remaining sampling times, including after patency of the challenge infection, there was no difference between the two groups. There was a significant difference between weeks ($p<0.05$), whereas neither the variation between animals within the groups nor the interaction between treatment and week were significant. No consistent trend was found for individual animals, i.e. no animals within a group showed consistently high or low developmental success over time.

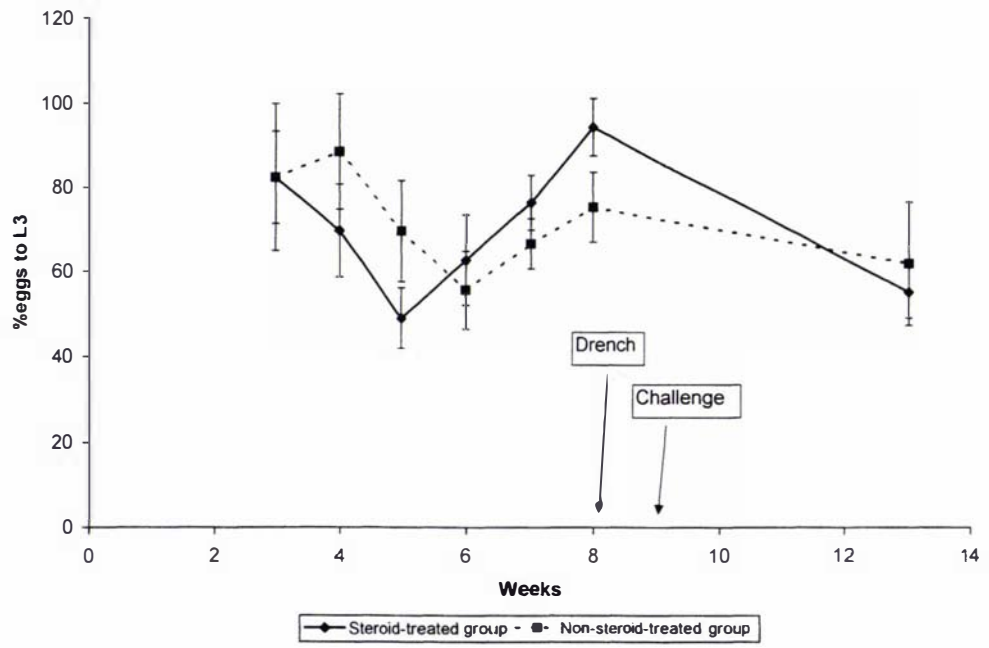

Figure 6.3.2.1 Experiment 1 - Developmental success of eggs to 3rd stage larvae (Arithmetic group means ± S.E.).

6.3.3 Circulating Eosinophils

Overall, there was a significant difference between the treatment groups ($p<0.001$). More specifically, numbers of circulating eosinophils were significantly higher in the non-steroid-treated group in week 0 and weeks 4 to 13 (Figure 6.3.3.1). There were significant differences between weeks ($p<0.001$), a significant variation between animals within the non-steroid-treated group ($p<0.001$) and a significant interaction between treatment and week ($p<0.001$). In the non-steroid-treated group there was a steady increase in the numbers of circulating eosinophils from week 4 to week 11 and then a steady decline. In the steroid-treated group circulating eosinophil numbers remained low and slightly decreasing throughout the trial period.

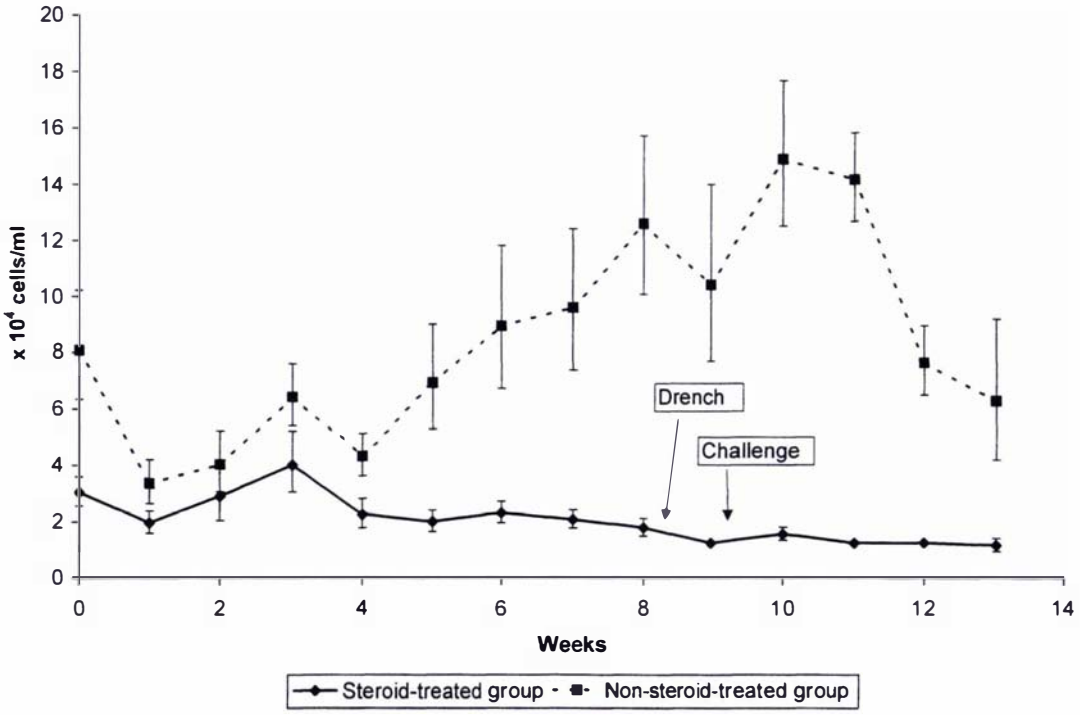

Figure 6.3.3.1 Experiment 1 Circulating Eosinophils (Geometric group means ± S.E.)

6.3.4 IgG_1 levels.

In all samplings, the animals in the non-steroid-treated group were found to have significantly higher levels of specific antibody to both larval and adult antigen of *T. colubriformis* ($p<0.001$ and $p<0.001$, respectively) (Figure 6.3.4.1). All animals had been field reared and were expected to have developed antibodies to natural nematode challenge before being housed. As the immune-suppressive treatment in the steroid-treated group had commenced a week before starting the trickle infection, this may explain the difference between the two groups in antibody response already when taking the first blood sample in Week 0.

After drenching the lambs (and thereby eliminating the worm burden) in Week 8, there was a decrease in antibody to adult antigen in the non-steroid-treated group. In response to the challenge infection, however, there was an increase in antibody to larval antigen of *T. colubriformis* in the non-steroid-treated group in weeks 10 to 11. Throughout the sampling period there was a low and steadily declining level of antibodies to both larval and adult antigen of *T. colubriformis* in the steroid-treated group.

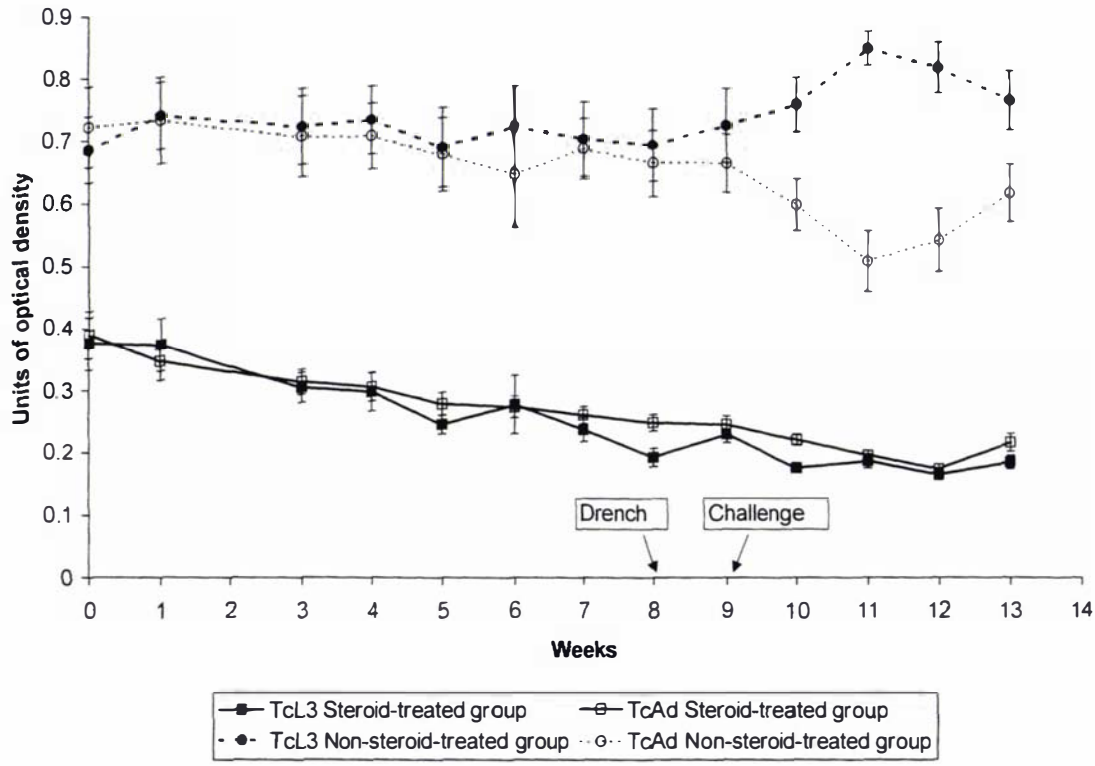

Figure 6.3.4.1 Experiment 1 - Specific Antibody to *Trichostrongylus colubriformis* (Arithmetic group means ± S.E.). TcL3 = IgG_1 antibody to larval antigen of *T. colubriformis*; TcAd = IgG_1 antibody to adult antigen of *T. colubriformis*.

6.3.5 Worm burdens, Establishment rates, Worm lengths, Sex ratios and *In utero* egg counts

The results are presented as arithmetic group means in Table 6.3.5.1. Worm burdens and establishment rates were found to be significantly higher in the steroid-treated group ($p<0.001$). There was no difference in sex ratios (Female/Male ratio) between the two groups. However, the female and male adult worms were longer and *in utero* egg counts higher ($p<0.001$, $p<0.01$ and $p<0.001$, respectively) in animals from the steroid-treated group than from the non-steroid-treated group.

	Steroid-treated group	Non-steroid-treated group
Worm burden	10722 ± 554 a	4932 ± 601 b
Establishment (%)	53.6 ± 2.8 a	24.7 ± 3.0 b
Male/Female-ratio	0.39 ± 0.06 a	0.30 ± 0.04 a
Female worm length (mm)	7.4 ± 0.1 a	5.8 ± 0.1 b
Male worm length (mm)	4.7 ± 0.1 a	4.2 ± 0.1 b
In utero egg counts	24 ± 1 a	13 ± 1 b

Table 6.3.5.1 Experiment 1 - Worm burdens, Establishment rates, Sex ratios, Worm lengths and *In utero* egg counts (Arithmetic group means ± S.E.). Row results with the same letters are not significantly different ($p<0.05$).

6.3.6 Histopathological changes in the mucosa of the small intestine

Results of the histological examination are presented in Table 6.3.6.1 as arithmetic group means ± standard errors, for numbers of mucosal mast cells (MMC) and eosinophils (EOS), and geometric group means ± standard errors, for numbers of globule leukocytes (GL). In the sections taken 1.5 m from the pylorus, there were significantly more MMC ($p<0.001$) and GL ($p<0.001$) in animals from the non-steroid-treated group than from the steroid-treated group. There were also more eosinophils in animals from non-steroid-treated group than in the steroid-treated group in sections taken 1.5 m from the pylorus, although this difference was not quite significant ($p<0.07$). In sections taken 3 m from the pylorus, there were again significantly more MMC's ($p<0.001$) and GL's ($p<0.001$) in animals from the non-steroid-treated group, whereas there was no difference between the two groups with respect to eosinophils.

Location (distal to pylorus)	Cell type	Steroid-treated group	Non-steroid-treated group
1.5 m	MMC (cells/mm^2)$^{\$}$	58 ± 6 a	180 ± 18 b
	GL (cells/mm^2)$^{\#}$	0 a	22 ± 10 b
	EOS (cells/mm^2)$^{\$}$	93 ± 9 a	150 ± 28 a
3 m	MMC (cells/mm^2)$^{\$}$	67 ± 9 a	147 ± 14 b
	GL (cells/mm^2)$^{\#}$	0 a	5 ± 2 b
	EOS (cells/mm^2)$^{\$}$	145 ± 18 a	216 ± 47 a

Table 6.3.6.1 Experiment 1 - Mucosal mast cells (MMC), globule leukocytes (GL) and eosinophils (EOS) in the mucosa of the small intestine ($^{\$}$Arithmetic group means ± S.E.; $^{\#}$Geometric group means ± S.E.). Row means with the same letters are not significantly different ($p<0.05$)

6.4 Results – Experiment 2

6.4.1 Infectivity of 3rd stage larvae (Experiment 2)

There were no detectable differences in the infectivity (establishment) of larvae cultured in samples from either steroid-treated or non-steroid-treated animals and no difference between the infectivity of larvae within the groups (Table 6.4.1.1). In addition, there was no difference in the M/F-ratio of adult worms developed, neither from the larvae obtained from either steroid-treated or non-steroid-treated animals nor between animals within the challenged groups.

Larvae from:	Steroid-treated group	Non-steroid-treated group
Worm burden	8446 ± 361 a	8607 ± 619 a
Establishment (%)	42.2 ± 1.8 a	43.0 ± 3.1 a
M/F-ratio	0.29 ± 0.03 a	0.28 ± 0.03 a

Table 6.4.1.1 Experiment 2 - Establishment rates and Sex ratios (Arithmetic group means ± S.E.). Row means with the same letters are not significantly different.

6.5 Discussion

In experiment 1, increased levels of host immunity in a trickle infected non-steroid-treated group of animals were demonstrated by significantly lower FEC, significantly higher levels of circulating eosinophils and IgG_1 antibodies to larval and adult antigen of *T. colubriformis* and significantly lower worm burdens following a challenge infection. However, a significant effect of host immunity on the development of eggs to infective 3rd stage larvae and on the infectivity of infective larvae of *T. colubriformis* could not be demonstrated.

A decline in FEC was used as an indicator of when the lambs in the non-steroid-treated group were starting to express immunity to *T. colubriformis*. Nevertheless, the timing of the drench, to remove the worm burdens resulting from the trickle infection, was not easy to decide on. On the one hand, it was desirable that the animals in the non-steroid-treated group had achieved a high level of immunity to *T. colubriformis* but on the other hand, it was important that FEC in this group did not become so low that insufficient numbers of cultured larvae would be available for Experiment 2. As a result, it may be that when they were drenched, the animals in the non-steroid-treated group were only just starting to express their immunity to *T. colubriformis* and were not yet effectively expelling their worm burdens.

Until the development of an effective immune response to *T. colubriformis*, FEC is an indicator of larval intake, in trickle infected animals (Steel *et al.*, 1980). In the present experiment this was the case until approximately weeks 7 to 8. An effect of steroid-treatment on FEC was first evident in samples in week 8 and after patency of the challenge

infection, when FEC was significantly higher in steroid-treated animals. A difference in FEC between steroid-treated and non-steroid treated animals was demonstrated already at day 35 (week 5) after the start of trickle infection with *T. colubriformis* in eight to nine months old lambs that had been reared worm-free (Douch *et al.*, 1994). The reason for not demonstrating a difference in FEC already at week 5 in the present experiment was most likely that the lambs that were used were younger. They were only three and a half months old at the start of the trickle infection and five and a half months old when FEC was first found to be significantly higher in the steroid-treated group. The effect of age on the development of immunity to *T. colubriformis* is well known (Gibson and Parfitt, 1972 and 1973; Chiejina and Sewell, 1974a and b; Dobson *et al.*, 1990b). Generally, lambs are capable of expressing immunity to *T. colubriformis*, measured as a reduction in worm burden, *in utero* egg counts and in FEC, from when they are five and a half months old, but worm burdens are not effectively expelled until they are six months of age or older (Gibson and Parfitt, 1972; Chiejina and Sewell, 1974b). Another factor to be considered, is that, irrespective of an animal's age, a certain threshold worm burden, which has been estimated to be between 3500 and 5000 worms, must be reached before an effective host immune response to *T. colubriformis* is expressed (Windon *et al.*, 1984; Dobson *et al.*, 1990a and b). Once the threshold worm burden has been reached, the rate of development of resistance to *T. colubriformis* is age-dependent only (Dobson *et al.*, 1990b). The worm burdens at week 8 in both groups of lambs were likely to have been above 5000 worms. This is supported by the fact that about 5000 worms established from the challenge infection in animals in the non-steroid-treated group and more than 10000 worms established in animals in the steroid-treated group. Therefore, the age of the animals is likely to have been the most important factor, for the rate of development of immunity to *T. colubriformis* in the present experiment.

Assuming that the two treatment groups were differing in their immunity to *T. colubriformis* from week 8 onwards, a significantly lower developmental success was expected in the non-steroid-treated group from that time. This could not be demonstrated, although there was an indication of it being higher in week 8 coinciding with the greatest divergence in FEC between the two groups. After patency of the challenge infection, however, no difference was found although samples from only one week were available for testing.

It is well-established that the hallmark of an effective immune response to *T. colubriformis*, is the ability of host animals to expel a major part of their worm burden (Gibson and Parfitt, 1972 and 1973; Chiejina and Sewell, 1974a and b; Dineen *et al.*, 1977; Gregg and Dineen, 1978; Steel *et al.*, 1980; Windon *et al.*, 1984; Kimambo *et al.*, 1988b; Dobson *et al.*, 1990a, b and c; Steel *et al.*, 1990; McClure *et al.*, 1992; Emery *et al.*, 1993; Barnes and Dobson, 1993; Harrison *et al.*, 1999). The rate of development of immunity is dependent on age and on reaching a certain threshold worm burden, as discussed earlier. In experiment 1, significantly lower worm burdens were found in animals from the non-steroid-treated group where the establishment was approximately 25%. This would

indicate that these animals were not highly immune to *T. colubriformis*. In highly immune animals the establishment would be expected to be less than 10% (Emery *et al.*, 1993; Stankiewicz *et al.*, 1996b) and has even been reported to be as low as 1% (Leathwick *et al.*, 1999). Female and male worms were significantly shorter and *in utero* egg counts significantly lower in animals in the non-steroid-treated group. The inverse relationship between these parameters and immunity to *T. colubriformis* is well known (Gibson and Parfitt, 1973; Chiejina and Sewell, 1974b; Douch *et al.*, 1988; Kahn *et al.*, 2000).

Levels of circulating eosinophils have been found to reflect responsiveness to infection with *T. colubriformis* and not the size of the worm burden the animals are harbouring (Dawkins *et al.*, 1989; Rothwell *et al.*, 1993; Kyriazakis *et al.*, 1996). In experiment 1, levels of circulating eosinophils increased in the non-steroid-treated group soon after patency of the trickle infection and continued to do so until the animals were drenched. After patency of the challenge infection, there was first an increase in the same group after which the levels of circulating eosinophils started to decrease, most likely reflecting the absence of a continued larval challenge. Dexamethasone-treatment was shown to effectively decrease the level of circulating eosinophils in the steroid-treated group in which levels of circulating eosinophils remained low and slightly decreasing throughout the experiment, a finding similar to that reported by other workers (Buddle *et al.*, 1992). Buddle *et al.* (1992) found a marked rise in circulating eosinophils to coincide with the start of decline in FEC and overall found a significant negative correlation between circulating eosinophils and FEC. This was also the case in experiment 1 where the pre-drench peak in FEC in the non-steroid-treated group coincided with a peak in the level of circulating eosinophils.

Steroid-treatment was also found to effectively reduce the production of IgG_1 to both larval and adult antigen in the steroid-treated group in experiment 1. This is in accordance with previously published results (Douch *et al.*, 1994). In the non-steroid-treated group of animals, IgG_1 levels to larval and adult antigen appeared to reflect larval intake, with comparable levels throughout the trickle infection period, a peak in IgG_1 to larval antigen after the challenge infection, a decrease in IgG_1 to adult antigen after drenching and removal of the adult worm burden and an increase in IgG_1 to adult antigen as adult worms developed from the challenge infection.

Higher levels of mucosal mast cells (MMC) were found in the intestinal mucosa of animals in the non-steroid-treated group, agreeing with already published work (O'Sullivan and Donald, 1973; Harrison *et al.*, 1999). However, others have failed to demonstrate a relationship between immunity and numbers of MMC in animals infected with *T. colubriformis* (Gregg *et al.*, 1978; Douch *et al.*, 1986). In contrast, numerous workers have found a clear positive correlation between the numbers of globule leukocytes (GL) in the epithelial layer of the mucosa and the level of host immunity to *T. colubriformis* (O'Sullivan and Donald, 1973; Gregg *et al.*, 1978; Douch *et al.*, 1986 and 1988; Douch, 1988; McClure *et al.*, 1992; Stankiewicz *et al.*, 1993; Harrison *et al.*, 1999). This relationship was confirmed in

the present study, where significantly higher numbers of GL's were found in both of the two locations examined in the small intestine. Numbers of eosinophils did not differ between groups. Although a positive correlation between numbers of eosinophils and host immunity to *T. colubriformis* has been reported (Douch *et al.*, 1986), others have failed to demonstrate such a relationship (Gregg *et al.*, 1978).

Animals infected in Experiment 2 were grazing contaminated pasture until they were seven months old. As they had been treated with a capsule, that released anthelmintic throughout the grazing period, they had mainly experienced larval antigenic stimulation before being housed. This may explain why the establishment rate was relatively high in all animals, as larval antigenic stimulation alone has been shown to provide incomplete protection against challenge with *T. colubriformis* (Sutherland *et al.*, 1999a).

No difference in infectivity of *T. colubriformis* 3rd stage larvae was found between larvae originating from either steroid-treated or non-steroid-treated animals. This result would suggest that the infectivity of larvae is not affected by host immunity. However, as the animals in the non-steroid-treated group, from which some of the larvae originated, were not highly immune to *T. colubriformis*, the possibility of an effect of higher levels of host immunity on the infectivity of the larvae cannot be ruled out.

All the evidence in the present study (differences in FEC, circulating eosinophils, IgG_1 levels, worm burdens, worm length, in utero egg counts and histological findings) seemed to indicate that the two groups were indeed differing in their immunity to *T. colubriformis*. However, this did not, as expected, result in differences in developmental success or infectivity of the free-living stages of *T. colubriformis*. One may speculate whether using cortico-steroids to suppress host immunity interfered with the host effect on developmental success. Certainly, the experiments, where the effect could be demonstrated, did not involve the use of steroids to create different levels of host immunity (Chapters 2 and 5). This issue is discussed further in the general discussion (Chapter 8).

Plate 6.4.1.1 ***T. colubriformis*** **infected lambs wearing harnesses and canvas bags**

Plate 6.4.1.2 A ***T. colubriformis*** **infected lamb and the author**

Plate 6.4.1.3 *T. colubriformis* infected lambs

Plate 6.4.1.4 Lambs housed at Haurongo (Experiment 2)

CHAPTER SEVEN

THE EFFECT OF INTESTINAL MUCUS AND CONTENTS FROM IMMUNE AND IMMUNE-SUPPRESSED LAMBS ON THE DEVELOPMENT OF THE FREE-LIVING STAGES OF *TRICHOSTRONGYLUS COLUBRIFORMIS*

7.1 Introduction

To try to identify the source of the effect of host immunity on developmental success, an experiment was set up to study the direct effects of intestinal mucus and contents on the development of eggs of *T. colubriformis* to infective larvae. Intestinal mucus forms a protective layer at the surface of the mucosa, in which, amongst other things, immunoglobulins, lysozyme and plasma proteins are present (reviewed by Miller, 1987), and has been shown to inhibit larval migration *in vitro* (Pomroy, 1994; Douch *et al.*, 1996). When looking for the origin of the host effect on the developmental success of nematode eggs, it seemed possible, that it might be found in the mucus. Some intestinal mucus forms part of the intestinal contents and later faeces. When eggs pass out through the gut of the host animal a prolonged contact between intestinal mucus and the developing eggs (and later larvae) will occur.

A further aim of this experiment was to relate the development of eggs to infective larvae in faecal cultures and in a Larval Development Assay (LDA), to the effect of host immunity on the adult stages of *T. colubriformis* and to pathological changes in the small intestine.

7.2 Materials and Methods

7.2.1 Experimental Animals

Twenty 5-month-old field-reared Romney ram lambs were purchased from AgResearch Ballantrae Research Station near Woodville and transported to Massey University where they were housed and fed a diet of lucerne pellets and hay. The lambs had been weaned and drenched with a combination drench (Leviben®[1]) six weeks before being housed and had subsequently been exposed to a natural challenge on pasture. At housing, all 20 lambs were drenched with double the manufacturer's recommended dose of Leviben®. Faecal samples taken ten days later showed that no eggs were present in the faeces of any of the lambs.

7.2.2 Parasites

An anthelmintic susceptible strain of *T. colubriformis*, obtained from Massey University, was used to infect a Romney ram lamb. After patency, faeces were collected

[1] 20 g/L ricobendazole, 37.5 g/L levamisole hydrochloride; Young's Animal Health, NZ

for culturing (Appendix 6i) and the resulting 3rd stage larvae were used for the trickle infections in the present experiment.

7.2.3 Experimental Design and Sampling Schedule

The experiment was designed as a factorial experiment, with three treatments and up to 15 weekly samplings of the same animals after the start of the trickle infection.

The lambs were allocated to three groups on the basis of liveweight. The three treatment groups were as follows:

Steroid-treated group (n=7):	trickle-infected with 5600 L3/week of *T. colubriformis* AND treated twice weekly with Opticortenol®[2]
Non-steroid-treated group (n=7):	trickle-infected with 5600 L3/week of *T.colubriformis*
Control group (n=6):	uninfected control group

Two lambs from the steroid-treated group died during the experiment (weeks 2 and 4 after the start of trickle infection) due to reasons unrelated to the parasitic infections (necropsies revealed acute pleuropneumonia probably due to infection with *Pasteurella* sp.). To prevent further deaths among the animals, all were treated intramuscularly with long acting oxytetracyclin during week 4.

From one week after housing and one week prior to the start of the trickle-infection, animals in the steroid-treated group were injected with Opticortenol® intramuscularly twice a week to suppress their immune response to parasitic infection. Larvae were administered orally in a small volume of water, twice a week, by means of a syringe. The trickle-infection continued for a maximum of 15 weeks. After the first three weeks of trickle-infection, the infections in the steroid-treated group and the non-steroid-treated group were patent and faecal samples were collected weekly from then on. Faecal samples were also taken weekly from the Control group. All lambs were weighed and blood sampled weekly.

When FEC in the non-steroid-treated group became significantly lower than that of the steroid-treated group, indicating that these lambs were becoming immune to *T. colubriformis*, three sheep from each of the three groups were humanely killed. This took place at week 12 after the start of the trickle infection. One day before killing the lambs, faecal samples were collected from those in the steroid-treated group and non-steroid-treated group for the recovery of eggs for the modified LDA. At necropsy, small intestines were recovered, and worm counts performed (see Appendices 6c, 6d and 6e).

[2] 0.5% dexamethasone trimethylacetate, Ciba Animal Health Division, Switzerland; Opticortenol was used at a dose rate of 0.25 mg/kg bodyweight

Intestinal mucus and contents were recovered from the small intestine as described in Appendices 7b and 7c. These were used to test developmental success of eggs (collected one day before the slaughter of the lambs) to infective larvae in a modified LDA (Appendix 7d). The development of eggs obtained from each individual animal in the two infected groups was tested with mucus and contents from individual animals from all three groups, in a crossover experiment. Plates were laid out as described in Appendix 7d, with 24 serial dilutions in duplicate of each test substance (intestinal mucus or contents from individual animals).

Furthermore, the lengths of adult female and male worms and the sex ratio (male/female-ratio) of adult worms were determined (Appendix 6e). During necropsy, sections of small intestine were excised at 0.3 m and 1.5 m distal to the pylorus for histological examination. Sections to be stained with Luna's stain were fixed in 10% neutral buffered formalin until processed as described in Appendix 6h. Sections to be stained with Toluidine Blue were fixed in an iso-osmotic solution of 0.6% formalin + 0.5% acetic acid (IFAA) for 12 hours and were then transferred to 70% alcohol before being processed as described in Appendix 6g.

The remaining lambs in the steroid-treated and non-steroid-treated groups were trickle-infected for three more weeks and then the lambs in all three groups were humanely killed (at 15 weeks after the start of trickle infection). One day before killing, faecal samples were collected from the two lambs in the steroid-treated group and from the one animal from the non-steroid-treated group that had a positive egg count, for the recovery of eggs for the modified LDA. After killing, small intestines were recovered and processed as above. Mucus and contents were recovered from the small intestine to test, once again, the developmental success of eggs to infective larvae in the modified LDA in the presence of intestinal mucus or contents from the three groups of lambs. In the animal from the non-steroid-treated group, *Haemonchus* 3rd stage larvae were detected when examining the modified LDA for larval development. In the abomasum of that animal, four adult male *Haemonchus* worms and two adult female *Ostertagia* worms were found in the 10 % aliquot counted. The results for the modified LDA from this animal were excluded from the analysis.

7.2.4 Faecal Samples

Faecal samples were collected by means of canvas bags attached to harnesses fitted on the sheep. Bags were left on for a maximum of eight hours. Faecal samples were collected per rectum from animals in the Control group. Three faecal egg counts (FEC) were carried out per animal sample, using a modified McMaster method (see Appendix 2a) and the mean value per animal used for the analysis.

Developmental success of eggs to infective 3rd stage larvae was measured in three faecal cultures per animal sample (Appendix 2b) and the resulting larvae extracted and

counted as described in Appendices 2c and 2e. The mean value per animal sample was used for the further analysis.

Faecal samples were also individually assayed for larval development in control wells of an LDA, using approximately 50 eggs per well (see Appendices 5b and 7a).

7.2.5 Blood Samples

Blood samples were collected from the jugular vein into heparinised Vacutainer tubes and examined for circulating specific anti-*T. colubriformis* antibodies using an ELISA method (Appendix 2i).

7.2.6 Histology

Mucosal mast cells (MMC) and globule leukocytes (GL) were counted in sections stained with Toluidine Blue and eosinophils (EOS) in sections stained with Luna's method. Cells were counted at a magnification of 400x, using a graticule eyepiece that covered an area of 0.0625 mm^2. Cells in 20 fields were included in each count and three counts were made per tissue section. Counts were made systematically from the submucosa to the lumen and back, moving along the length of the section. Only cells in the mucosa were counted.

7.2.7 Statistical Analysis of Data

Details of the statistical analysis are presented in Appendix 7f. Apart from developmental success in faecal cultures and IgG_1 levels to larval and adult antigen, all measured variables did not require transformation and are presented as either arithmetic or least squares group means ± standard errors. Results for developmental success in faecal cultures and IgG_1 levels to larval and adult antigen were 'ln(x+1)' transformed to normalise the residuals and are presented as geometric means ± standard errors.

All data were analysed using a factorial generalised linear model (GLM) in the SAS version 6.12 statistical package. For the FEC, developmental success in faecal cultures, developmental success in an LDA and specific antibody levels results, the main factors examined were **treatment** and **week** (after start of trickle infection). In addition, the nested effect (animal within treatment) and the interaction between week and treatment were included. Comparisons at individual weeks were made by t-test. For the results of the modified LDA with either mucus or contents, the main factors examined were **test group** (treatment group from which the test substance originated), **egg treatment** (treatment group from which the eggs added to the modified LDA originated) and the nested effects ('animal within test group' and 'animal within egg treatment'). **Week** was not analysed for as a factor, as the groups were unbalanced and no comparison for egg treatment was possible in week 15. For the histology results (MMC, GL and eosinophils), the main factors examined were **treatment**, **week** (either week 12 or 15 after start of trickle infection) and **section**. Within each of the two weeks, treatment groups were compared with Tukey's multiple comparison test. For the worm count, male/female-ratio, female

worm length, male worm length and *in utero* egg count results, the main factors examined were **treatment** and **week** (either week 12 or 15 after start of trickle infection).

7.3 Results

All data are presented in Appendix 7e.

7.3.1 Faecal Egg Counts

In the steroid-treated group, FEC was significantly higher than in the non-steroid-treated group ($p<0.05$ to 0.01) from Week 9 onwards after start of the trickle-infection (Figure 7.3.1.1). When half of the animals were killed in Week 12, the FEC appeared to have peaked in the steroid-treated group. However, FEC increased further in the remaining animals.

In the non-steroid-treated group, FEC reached a plateau from Week 5 to Week 9 whereafter it decreased. By Week 15, the remaining lambs in the non-steroid-treated group had a mean FEC of less than 100 epg.

In the Control group, the mean FEC remained at 0 epg throughout the experiment, although a positive FEC (50 epg) was found in one animal in week 3 and in another animal in week 5 after start of trickle infection. Cultures set up from the faecal samples revealed the presence of *Haemonchus* larvae only. In both instances, the animals were drenched with a double dose of combination drench on the same day as the positive FEC was discovered. The two animals had zero epg at all the following samplings in the experiment.

Overall, there were significant variations between animals within the groups and significant interaction between week and treatment, reflecting that the groups were behaving differently over time.

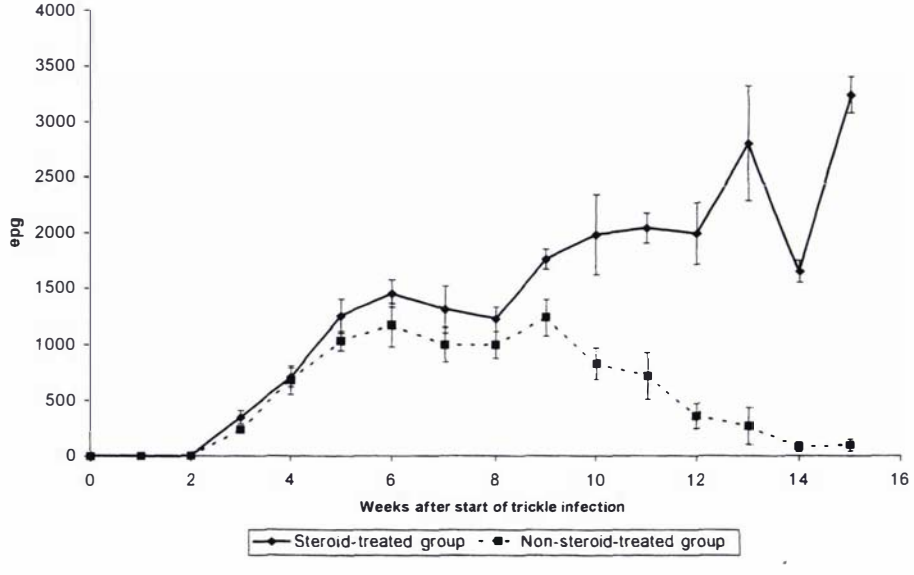

Figure 7.3.1.1 Faecal Egg Counts (Arithmetic group means ± S.E.)

7.3.2 Developmental Success of eggs to 3rd stage infective larvae in faecal cultures.

The results are presented as geometric group means ± standard errors in Figure 7.3.2.1. Only at one time was there a significant difference between the groups. This occurred at Week 8 after the start of the trickle-infection, when the developmental success was significantly higher in the steroid-treated group ($p<0.05$) than in the non-steroid-treated group. Overall, there was a significant difference between weeks ($p<0.01$) and a significant variation between animals within the groups ($p<0.05$). The interaction between week and treatment was not significant. Generally the developmental success remained high in both groups until Weeks 9 to 10, after which it decreased to a low level in Week 14.

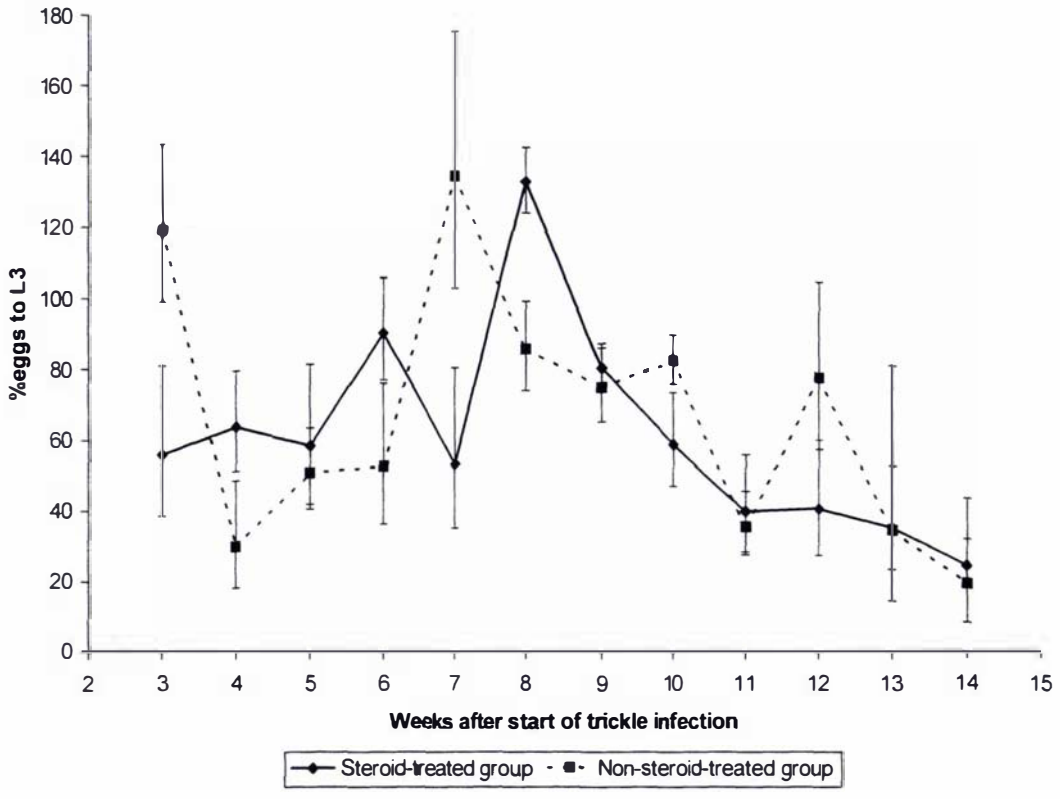

Figure 7.3.2.1 Developmental Success in faecal cultures (Geometric group means ± S.E.)

7.3.3 Larval Development Assay - Control wells only

The results are presented as arithmetic group means ± standard errors in Figure 7.3.3.1. Overall, there was a higher developmental success in the steroid-treated group than in the non-steroid-treated group, although the difference only approached significance ($p<0.06$) and was largely attributable to one data point (week 5). There was a significant difference between weeks ($p<0.001$), a significant variation between animals within the groups ($p<0.001$) and a significant interaction between week and treatment ($p<0.001$). At most sampling times, the developmental success was close to 100% in both groups throughout the experiment (until Week 11).

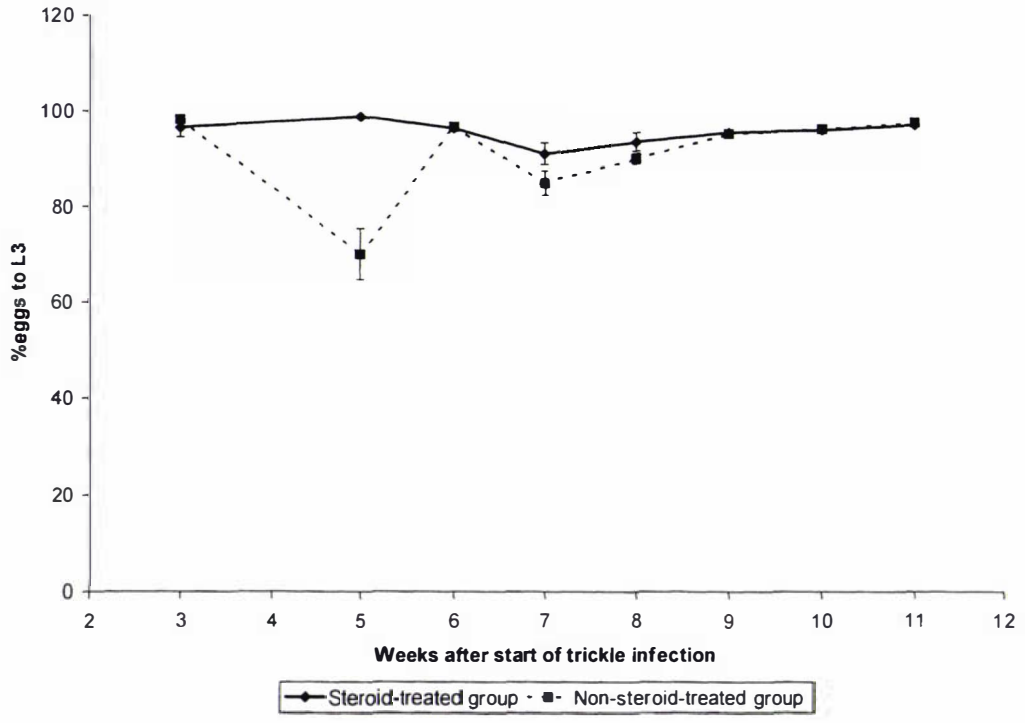

Figure 7.3.3.1 Developmental Success in a Larval Development Assay (Arithmetic group means ± S.E.).

7.3.4 Modified Larval Development Assay - with mucus and contents

Observations regarding the volume and consistency of recovered mucus from the three treatment groups are presented in Table 7.3.4.1. Generally, somewhat more mucus could be recovered from animals in the non-steroid-treated group. With respect to consistency, there were some minor changes over time in the steroid-treated group and in the non-steroid-treated group where mucus recovered in week 15 tended to be thicker than mucus recovered in week 12. In the control group the consistency of the mucus remained the same.

Group	Number of animals	Weeks after start of trickle infection	Volume recovered (ml) (from 2 m of intestine)	Consistency
Steroid-treated	3	12	7 – 12	thin – medium thin
Non-steroid-treated	3	12	12 – 15	medium-thin – thick
Control	3	12	6 – 14	thin – thick
Steroid-treated	2	15	7.5 – 11	medium-thick – thick
Non-steroid-treated	4	15	12 – 17	medium-thick – thick
Control	3	15	8 – 11	thin – medium-thick

Table 7.3.4.1 Mucus characteristics – qualitative observations

The results from the modified LDA are presented as arithmetic group means in Figure 7.3.4.1 for results when mucus was added to the LDA and in Figure 7.3.4.2 for results when contents were added to the LDA.

Close to 100 % of eggs developed to 3rd stage infective larvae in control wells, where no mucus or contents had been added. When mucus or contents were added to wells in the LDA, larval development was reduced. A dose response could be established and was measured as an LC_{50} value representing the concentration of the test substance at which 50 % of the eggs develop successfully to 3rd stage larvae. This means that the lower the LC_{50} the more concentrated and/or more potent the effect of the test substance.

Overall, lower LC_{50} values were found with contents as compared to mucus ($p<0.001$) indicating that the effect on eggs was generally more concentrated or potent in the contents. There was no difference in LC_{50} with respect to the origin of the mucus or eggs and only one of the nested effects, 'animal within test group' was significant ($p<0.01$).

For mucus in both weeks 12 and 15, there was no difference in LC_{50} with respect to the origin of the mucus or eggs but the variation between animals within the test substance group (nested effect) was significant ($p<0.001$ and 0.05, respectively). For contents, there were in both weeks 12 and 15 no differences in LC_{50} values with respect to the origin of the contents or the eggs, but both nested effects were significant (week 12: $p<0.001$; week 15: $p<0.01$ and $p<0.05$, respectively).

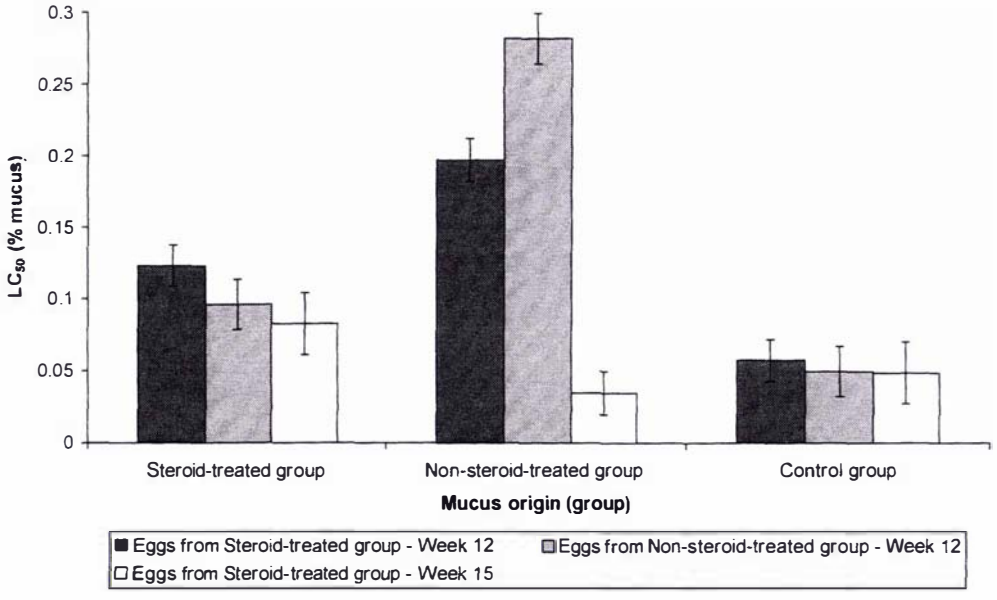

Figure 7.3.4.1 LC_{50} values for intestinal mucus (Least squares group means ± S.E.)

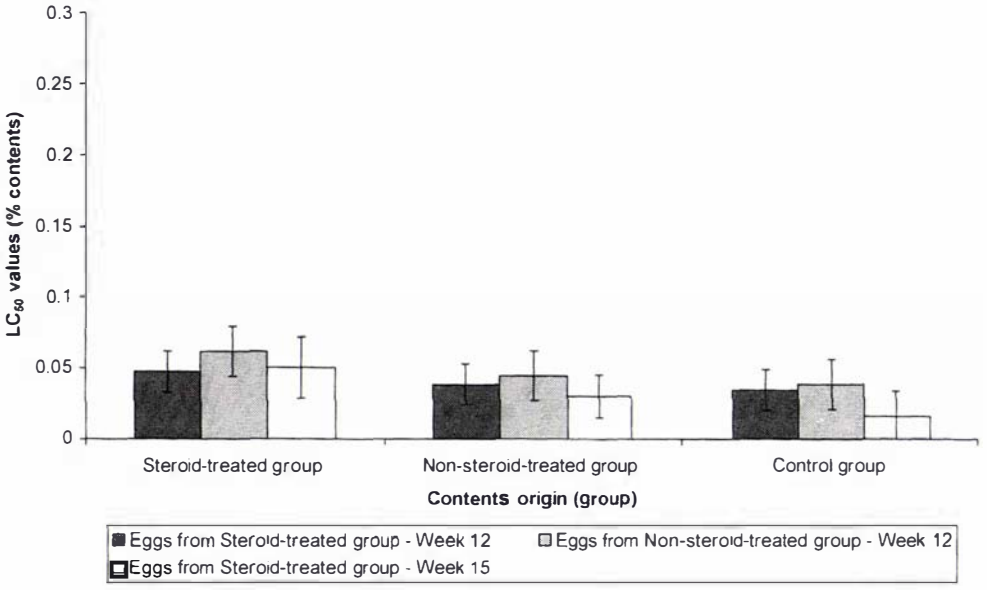

Figure 7.3.4.2 LC_{50} values for intestinal contents (Least squares group means ± S.E.)

7.3.5 IgG_1 levels

Throughout the sampling period, there were low and steadily declining levels of antibody to larval antigen of *T. colubriformis* in the steroid-treated group and in the Control group (Figure 7.3.5.1). In all weekly samplings from week 4 to 15, the animals in the non-steroid-treated group were found to have significantly higher levels of antibody to larval antigen of *T. colubriformis* ($p<0.05 – 0.01$) than animals in the two other groups, and these continued to increase throughout the experiment. Overall, there were significant differences between weeks ($p<0.001$), significant variation between animals, in particular in the non-steroid-treated group ($p<0.001$), and a significant interaction between treatments and weeks ($p<0.001$), reflecting the increasing difference between the steroid-treated and control groups and the non-steroid-treated group over time.

In both the steroid-treated and the uninfected groups there were, generally, low and slightly decreasing levels of antibody to adult antigen of *T. colubriformis* (Figure 7.3.5.2). In the non-steroid-treated group, levels of antibody were significantly higher than in the two other groups from week 4 to 12 ($p<0.05 – 0.01$). After week 12, levels declined so that, for weeks 13 to 15, antibody levels in the non-steroid-treated group were not significantly different from those in the steroid-treated group. Overall, there were significant differences between weeks ($p<0.001$), a significant variation between animals ($p<0.001$), in particular in the non-steroid-treated group, and a significant interaction between treatment groups and weeks ($p<0.001$), as the difference between groups increased with time.

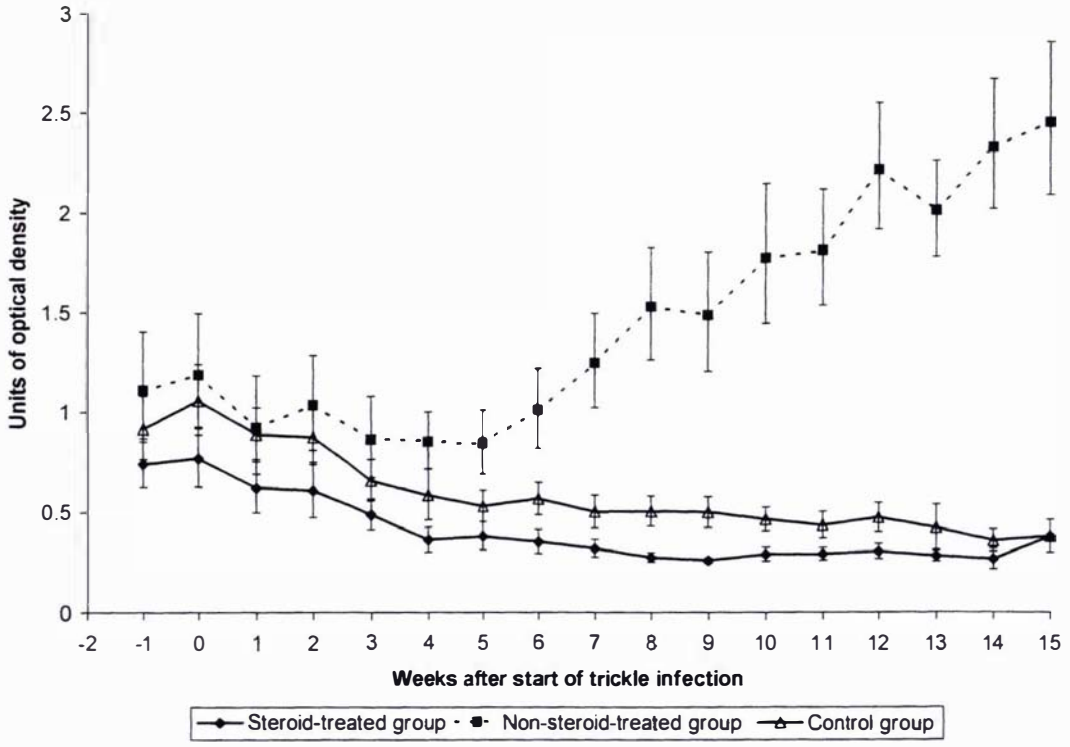

Figure 7.3.5.1 Specific antibody (IgG_1) to larval antigen of *Trichostrongylus colubriformis* (Geometric group means ± S.E.)

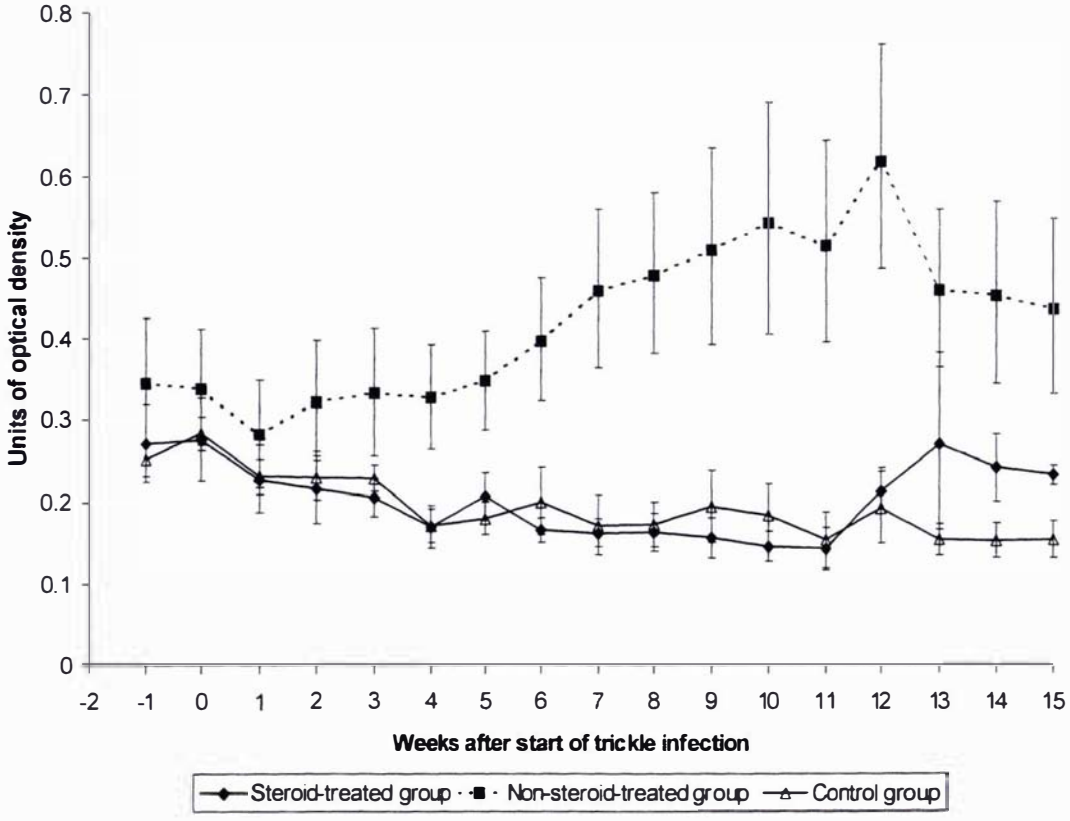

Figure 7.3.5.2 Specific antibody (IgG_1) to adult antigen of *Trichostrongylus colubriformis* (Geometric group means ± S.E.)

7.3.6 Worm burdens, Worm lengths, Sex ratios and *in utero* egg counts

There were significant differences in worm burden between weeks 12 and 15 ($p<0.05$) and a significant interaction between week and treatment group, reflecting that the two groups became more different with time ($p<0.05$). At week 12 the two trickle infected groups did not differ with respect to worm burden (Table 7.3.6.1). At week 15, however, animals in the steroid-treated group had significantly larger worm burdens than animals in the non-steroid-treated group ($p<0.01$). Uninfected control animals had no worm burdens (except for the one animal mentioned in section 7.2.3).

Female worms were significantly longer (week 12: $p<0.001$; week 15: $p<0.01$) and *in utero* egg counts higher (week 12: $p<0.01$; week 15: $p<0.001$) in animals from the steroid-treated group than in animals from the non-steroid-treated group (Table 7.3.6.1). Male/Female-ratios and male worm lengths did not differ between the two trickle infected groups (Table 7.3.6.1).

Weeks after start of trickle infection	Steroid-treated group	Non-steroid-treated group	Control group
Week 12			
Worm burden	20560 ± 1359 a	13860 ± 2619 a	0*
M/F-ratio	0.46 ± 0.09 a	0.59 ± 0.04 a	
Female Worm length (mm)	6.80 ± 0.05 a	5.70 ± 0.10 b	
Male Worm length (mm)	5.46 ± 0.14 a	5.12 ± 0.13 a	
In utero egg counts	18 ± 1 a	5 ± 1 b	
Week 15			
Worm burden	19765 ± 1045 a	2580 ± 1769 b	0
M/F-ratio	0.68 ± 0.11 a	0.65 ± 0.07 a	
Female Worm length (mm)	6.49 ± 0.17 a	5.59 ± 0.07 b	
Male Worm length (mm)	5.51 ± 0.23 a	5.16 ± 0.16 a	
In utero egg counts	20 ± 1 a	2 ± 1 b	

Table 7.3.6.1 Worm burdens, Male/Female-ratios, Female worm lengths, Male worm lengths and *In utero* egg counts (Arithmetic group means ± S.E.). Row means with the same letters are not significantly different ($p<0.05$). * In one animal a total of 40 worms was recovered. N=3 for each group in week 12. N=2, 4 and 3, respectively, in the steroid-treated group, the non-steroid-treated group and the control group in week 15.

7.3.7 Pathological changes in the mucosa

With respect to all three cell types examined, there were, overall, significant differences between treatments ($p<0.001$) but no differences between weeks and sections. As there were no differences between sections, the results from these have been combined in Table 7.3.7.1 below.

In week 12, there were significant differences between the groups with respect to all three cell types examined (MMC: $p<0.001$, GL: $p<0.001$ and EOS: $p<0.01$). In week 15, there were again significant differences between the groups for all cell types (MMC: $p<0.001$, GL: $p<0.001$ and EOS: $p<0.01$).

Tukey's multiple comparison test showed differences ($p<0.05$) between the groups as outlined in Table 7.3.7.1. There were significantly more MMC, GL and EOS in the non-steroid-treated group than in the steroid-treated group in both weeks. With respect to MMC, the non-steroid-treated group and the control group did not differ at either time. In both weeks, the non-steroid-treated group had significantly higher numbers of GL than any of the two other groups, which in turn did not differ from each other. The steroid-treated group had fewer EOS than the non-steroid-treated group which in turn did not differ from the control group.

Cell type	Steroid-treated group	Non-steroid-treated group	Control group
Week 12			
MMC (cells/mm^2)	29 ± 6 a	148 ± 15 b	144 ± 24 b
GL (cells/mm^2)	0 a	109 ± 37 b	4 ± 2 a
EOS (cells/mm^2)	27 ± 6 a	150 ± 41 b	116 ± 21 b
Week 15			
MMC (cells/mm^2)	47 ± 9 a	174 ± 20 b	129 ± 17 b
GL (cells/mm^2)	0 a	163 ± 47 b	5 ± 3 a
EOS (cells/mm^2)	28 ± 2 a	112 ± 31 b	59 ± 15 b

Table 7.3.7.1 Mucosal mast cells (MMC), globule leukocytes (GL) and eosinophils (EOS) in the mucosa of the small intestine (Arithmetic group means ± S.E.). Row means with the same letters are not significantly different ($p<0.05$). N=3 for each group in week 12. N=2, 4 and 3, respectively, in the steroid-treated group, the non-steroid-treated group and the control group in week 15.

7.4 Discussion

An effect of intestinal mucus and contents on larval development was demonstrated in the present experiment with intestinal contents being more potent than mucus. However, although host animals were shown to differ in their immunity to *T. colubriformis*, this did not cause measurable differences between immune and immune-suppressed animals in the magnitude of the effect of intestinal mucus and contents on larval development. Thus, no evidence for an immune-mediated factor adversely affecting developmental success was found.

Many of the findings in the present experiment indicated that the two infected groups differed in their immunity to *T. colubriformis*, particularly towards the end of the experiment. For example, FEC were lower from week 9 onwards and levels of specific IgG_1 to larval and adult antigen to *T. colubriformis*, higher from week 4 onwards, in the non-steroid-treated group, suggesting a higher level of host immunity to the infection in this group. Given these differences in host immunity, according to the hypothesis that increased levels of host immunity have an adverse effect on larval development, it was expected that developmental success of *T. colubriformis* eggs to 3rd stage larvae would be lower in the non-steroid-treated group towards the end of the experiment. However, a difference between the groups could not be shown in faecal cultures or in an LDA, although the latter was only carried out until week 11.

The decline in developmental success observed in faecal cultures after week 9 was expected in the non-steroid-treated group only. Why this was also observed in the steroid-treated group is not known, but there is the possibility of an effect of the chronic steroid-treatment on the viability of eggs. Certainly, an increased sensitivity of eggs to ivermectin has been shown in lambs receiving long-term cortico-steroid treatment (Hoza, 1998). Nevertheless, eggs obtained from either the steroid-treated group or the non-steroid-treated group and used for the modified LDA did not differ in their contents or mucus LC_{50} values. But as discussed below, mucus and contents may have had a uniform and non-immune-mediated effect on all eggs, regardless of their origin. Alternatively, a residual effect of some of the metabolites of cortico-steroids (dexamethasone[3]) may have had some adverse effect on the developing eggs in the faeces, although this effect would have been expected to be more consistent throughout the experimental period. A third possibility is that cortico-steroids do not abrogate the effect of host immunity on larval development. A decrease in developmental success after week 8 was also present to some extent in the steroid-treated group in Chapter 6, providing some support for the possibility of long-term treatment with cortico-steroids affecting egg viability and larval development. The fluctuations over time in both groups are likely to have been influenced, to some extent, by the significant variation between animals in both of the trickle infected groups.

[3] Dexamethasone metabolites are excreted via the bile and via urine. The metabolites are compounds such as glucuronides, sulfates and unconjugated compounds (Jenkins, 1992)

Recovery rates higher than 100% in some faecal cultures may in part have been due to an underestimation of FEC and overestimation of developmental success, although this may be mainly a problem in FEC below 500 epg.

As developmental success of eggs in control wells of an LDA was close to 100% at least until week 11, this suggested that the substance (-s) that caused the decrease in developmental success after week 8 may have been found primarily in the faecal environment in the cultures. However, results in Chapter 4 indicated that the developmental success of *O. circumcincta* eggs isolated from faeces was lower in animals that were relatively more immune to the parasite than in control animals. Why such a difference was not found in the control wells of the LDA in the present experiment is not known, but there may be a difference in susceptibility between trichostrongylid nematode species.

In weeks 12 and 15, when the lambs were killed, worm counts, worm lengths, in utero egg counts and histopathological findings all provided further evidence that the infected groups differed substantially in their immunity to *T. colubriformis*. Differences between the non-steroid-treated group and the steroid-treated group were even more pronounced in week 15 than in week 12, as expected. The discovery of adult worms in one of the animals in the control group was unfortunate. These worms probably originated from the hay fed to the animals.

Given the evidence for a difference in host immunity, it was anticipated that an effect of intestinal mucus and contents on the development of *T. colubriformis* eggs to 3rd stage infective larvae would be found in the non-steroid-treated group. Although this was the case and a dose response could be established for both mucus and contents, the effect was found in all groups, including the uninfected control group, and did not differ between them. This finding suggested that the effect was probably not immune-mediated, but rather caused by some unspecific and physical properties of the mucus and contents. The fact that LC_{50} values were lower in intestinal contents than in mucus, and the effect therefore more potent or concentrated in the former, further supports this, as one would have expected any immune-mediated effect to be more concentrated in the mucus. As a large proportion of unhatched eggs were generally present (some embryonated) in the LDA wells with low development, the presence of varying concentrations of mucus and contents may have caused a complete to partial exclusion of available oxygen for the developing eggs and larval stages. This would impair or reduce the ability of the free-living stages to develop, as oxygen availability is known to be one of the limiting factors for the development from egg to larvae (Rose, 1961; Young *et al.*, 1980b).

Somewhat more mucus could be recovered from animals in the non-steroid-treated group, particularly at week 15 after the start of trickle infection. It has been shown that numbers of mucus producing goblet cells and hence mucus production, is increased in response to infection with nematodes in rats and mice (reviewed by Miller, 1987).

However, no correlation between numbers of goblet cells and mucus production has been demonstrated in sheep infected with *T. colubriformis* (Douch *et al.*, 1986). More recently, $CD4^+$ lymphocytes have been suggested to be responsible for the control of the amount of intestinal mucus present in response to infection with *Nippostrongylus brasiliensis* in mice and that reduced amounts of mucus interfere with the spontaneous cure against the same parasite (Khan *et al.*, 1995).

If, in spite of the findings presented here, there is an adverse effect on larval development mediated by mucus, it seems likely that the extraction method used in this study was not sensitive enough, or did not purify the mucus sufficiently. To which factor or factors a possible effect of intestinal mucus on larval development may be ascribed, is uncertain. It is possible that immunoglobulins or one or more immune factors or messenger mediators (cytokines) secreted by Type 2 T-helper cells are of importance. Certainly, in lambs that are immune to *T. colubriformis*, the expulsion of challenge is associated with increased concentrations of IgG_1, IgG_2 and IgA in intestinal mucus (McClure *et al.*, 1992). Furthermore, antibody (IgA, IgM and IgG) extracted from faeces of rabbits infected with *Obeliscoides cuniculi* has tentatively been linked to a reduction in developmental success of eggs in vitro (Wedrychowicz and Kowalczyk, 1991).

Overall, the findings in this experiment did not help to locate the effect of host immunity on larval development, but instead highlighted some unexpected problems with the use of cortico-steroids as an immunosuppressant in artificial infections with *T. colubriformis*. It is suggested that the effects of intestinal mucus and contents on larval development shown here, were not immune-mediated but more likely due to some physical and unspecific properties of the mucus and contents.

CHAPTER EIGHT

GENERAL DISCUSSION

The work presented in this thesis was prompted by recent experimental findings that there was a variation between host animals in the proportion of trichostrongylid eggs developing to 3rd stage larvae, and that this variation may be attributed to differences in host immunity (Jorgensen *et al.*, 1998). Variations in the development of the free-living stages of parasites have in the past been attributed only to external factors such as temperature, humidity and oxygen availability. The aim of this project was to confirm and further quantify the earlier findings that developmental success may be influenced by host immunity, firstly in sheep in the field carrying mixed nematode infections and subsequently in housed sheep carrying a single species infection. In addition, an attempt was made to locate the origin of the effect in the host animal.

Support for the hypothesis that increased host immunity has an adverse effect on the developmental success of the free-living stages was found in animals in the field as their age and exposure to parasites on pasture increased. In adult ewes, this was especially the case at the time of the PPR. In addition, the effect was found in eggs from 14-month-old housed lambs that were relatively immune to *O. circumcincta*. Unfortunately, group sizes were small in this study and variation between animals large, which meant that a difference in developmental success was only demonstrated in a larval development assay and not in larval cultures.

Dexamethasone, a long-acting gluco-corticoid was used, in two indoor trials, to create groups of lambs that differed in immunity to *T. colubriformis*. Although indications were that the groups differed markedly in their immunity to *T. colubriformis*, it was not possible to demonstrate the expected differences in the effect on the developmental success of the free-living stages. As developmental success decreased in both the immune and immunosuppressed groups after 8-10 weeks of trickle infection in both groups and dexamethasone treatment in one group, this would, according to the hypothesis, mean that both groups increased in their immunity to *T. colubriformis*. This might indicate that there are some hitherto unknown effects of one or more of the dexamethasone metabolites excreted in faeces, and that although many aspects of immunity are suppressed, that which causes the effects on the free-living stages may not be. Evidence that long-term treatment of lambs with cortico-steroids increase the sensitivity of *T. colubriformis* eggs to ivermectin, both after single and trickle infections was presented by Hoza (1998). The use of dexamethasone to suppress immunity may in fact be inappropriate for studies involving the measurement of parameters related to the development and fitness of free-living stages. Using animals reared worm-free or housing field-reared animals well in advance of starting an experiment to allow their immunity to wane, instead of using immunosuppressed field reared animals, may prove to be better options.

The epidemiological consequences of the developmental success of eggs being significantly lower in more immune animals are potentially great. Not only will genetically resistant or more immune sheep shed fewer eggs onto pasture, but these eggs are also less likely to develop into infective larvae. Under normal drenching practices, ewes are considered, generally, to be greater contributors to pasture larval contamination than lambs, primarily because of the larger volume of faeces they produce per day (West, 1982; Familton, 1991). However, as these studies have not considered the seasonal variation in developmental success and the removal of larvae from pasture by the ewes (due to their larger intake of grass, compared to lambs), and are essentially summing FEC over time to assess pasture larval contamination levels, the contribution of the adult ewe is likely to have been overestimated.

Grazing genetically nematode resistant or susceptible lines of lambs separately has, in the present study and in other studies, been shown to result in vast differences in larval infestations on pature within a few months (Leathwick, pers.comm.; Bisset *et al.*, 1997). It would appear that grazing genetically parasite resistant animals is not only an effective way to reduce larval challenge on pasture but may also reduce the number of anthelmintic treatments needed. In the Perendale experiment, the Low FEC Line lambs could possibly have been drenched less frequently, but one also has to bear in mind the higher immune responsiveness in these resistant animals and that pathological changes in their gastrointestinal system are elicited at lower infection levels than is the case for random-bred or susceptible animals (Bisset *et al.*, 1997). Selection for both high resistance to parasites and high production is presently being carried out in New Zealand, with some success (McEwan *et al.*, 1997). In any event, it would have been interesting to perform worm counts on lambs from both lines to relate these to FEC, pathological changes in the gastrointestinal system and resistance level, but unfortunately this was not possible at the time.

In an attempt to locate the origin of the effect of host immunity on the developing stages, eggs were cultured to 3rd stage larvae in an LDA in the presence of various concentrations of intestinal mucus or contents. Although an adverse effect was present, this was unexpectedly found to be more concentrated or potent in the intestinal contents than in the intestinal mucus and did not differ between highly immune and immunosuppressed groups of lambs. As the effect was uniform for both groups and for an uninfected control group, it seemed likely that some physical or chemical property of the mucus and contents, digesta, or metabolites thereof, adversely affected the developing stages, effectively 'overshadowing' any effects of intestinal mucus and contents due to differences in immunity. Another possibility is that in spite of large differences in host immunity, there is no direct effect on larval development transmitted via the mucus/contents. Also, an LDA may not be the preferred medium for investigating effects of mucus or contents on developmental success of the free-living stages, due to variations in immunity.

Technical problems were encountered with some of the parasitological methods applied in this thesis. The indication that in egg counts lower than 500 epg, the FEC is generally underestimated by up to 50%, is of particular concern and is likely to have caused developmental success measured in these samples, to have been overestimated by the same percentage. As it was of interest to examine larval development in faeces in most of the work for this thesis, it was necessary to use a modified McMaster method for estimating FEC. In spite of the shortcomings of this method, it was probably the only option available. Although in some instances, eggs were cultured in an LDA, in addition to in larval cultures, this was generally not possible for samples taken from animals in the field as they usually had quite low FEC. A certain minimum concentration of eggs in faeces is required in order to recover a sufficient number for the *in vitro* test. The applicability of the LDA was therefore very limited.

Developmental success in indoor experiments was generally much higher than that found in samples from animals grazing pasture. Development from egg to 3rd stage larvae has been shown to be suppressed in water logged faeces (Young *et al.*, 1980b; Gruner and Suryahadi, 1993). This may be caused by a reduction in available oxygen, which is crucial for the developing stages. Faecal moisture content of 60-70% is thought to be suitable for the development from egg to 3rd stage larva (Silverman and Campbell, 1959). In samples from field animals (Perendale ewes), the faecal moisture content was 70% or more, whereas in housed animals it was between 50-70%. Although faeces could not be characterised as 'water logged' in samples from Perendale ewes, they nevertheless, on average, had a higher moisture content than 70%, suggesting that in some faeces there may have been an excess moisture content that could adversely affect the development from egg to 3rd stage larva. To explain why outdoor grazing animals have higher moisture content in their faeces, one would have to consider their diet, which in New Zealand consists of predominantly ryegrass/clover. Breakdown products from fresh green feed may themselves have some adverse effects on the developing larval stages in the faeces. Housed animals, on the other hand, are often fed hay and lucerne pellets, both of which are feed items with a lower water content than green grass (~80% water) and clover. Faecal pellets from housed sheep appear more fibrous and less dense, and this may more easily allow oxygen to reach the developing stages in the pellets.

An interesting observation made in all experiments for this thesis was the large and often significant variation between animals within a line, age and/or treatment group. This variation appears to be a natural phenomenon and was found in both housed animals and animals in the field. Treatment with dexamethasone generally decreased the variation between animals within a group with respect to the measured parameters, a finding which supports the idea that the variation is a natural phenomenon. The variation between non-immunosuppressed animals meant that rendering a housed group of animals homogeneously immune to a particular parasite was very difficult. Part of this problem may also be ascribed to the way we attempt to measure immunity. One may question the

value of FEC, a frequently used indicator of immunity, for this purpose. Although FEC may reflect the size of worm burdens relatively accurately at certain stages of the development of immunity to *T. colubriformis* (Chiejina and Sewell, 1974b; Steel *et al.*, 1980), it has been reported to be a poor indicator of worm burdens and immunity level, particularly in infections with *O. circumcincta* (Coop *et al.*, 1977 and 1985; Jackson and Christie, 1979).

The natural variation between animals in response to infection with gastrointestinal nematodes has major implications for the design of experiments. As 'host animal' has often been shown to be a significant factor when measuring and analysing various responses to parasitic infection (this thesis; Leathwick pers.comm.; Thamsborg, pers.comm.), one cannot disregard this when designing experiments. As a consequence, experiments must contain sufficient replication at the animal level to ensure an adequate statistical power.

The question remains, how an effect of host immunity may be translated into an effect on the development of the free-living stages. A direct effect of one or more immune products, secreted into and present in the mucus, contents and later faeces, could be responsible. In view of findings that proteins extracted from faeces of rabbits infected with *Obeliscoides cuniculi* inhibited egg development *in vitro* (Wedrychowicz and Kowalczyk, 1991), one could consider extracting and purifying antibody proteins from mucus, gut contents and faeces and testing these on larval development in an LDA. However, there is also the possibility of an indirect effect. For instance, changes in the chemical and physiological environment in the gastrointestinal system, brought about by an immune response to parasitic infection, could potentially have an effect on the fertility of male and female adult worms and/or the sex ratio. This could in turn result in the production of less viable and infertile eggs. In that case, an adverse effect on developmental success, due to increased host immunity, would not be detected in mucus, contents or faeces. In order to separate an effect of reduced fertility from an effect present in mucus, contents or faeces, one would have to recover eggs directly from adult females in the gastrointestinal system, thus avoiding any contact with mucus and digesta, and culturing them in an LDA.

Overall, these studies support and describe further the phenomenon of an adverse effect of host immunity on the developmental success of the free-living stages of some common trichostrongylid parasites in sheep. This finding adds to the list of known effects of the host immune response on parasitic stages, and may, in future studies, aid our understanding of the complex and fascinating interaction between host and parasite. Although the work presented in this thesis raises a number of questions regarding some of the methods and procedures commonly used in experimental parasitology, it would nevertheless seem that the adverse effect of host immunity on the free-living stages of nematode parasites is a real phenomenon.

APPENDICES

Appendix 1a Development of immunity to *Ostertagia circumcincta*

Age of sheep	Breed	Dose given (*O. circumcincta* L3s)	Period of infection Challenge/Drench?	Major findings	Reference
ewes, >5 years some reared parasite-free	Blackface	50000	Challenge with or without preceding treatment with BZ, at different times of the year	ewes were more resistant to challenge than sheep reared worm-free ewes remained immune on challenge also after BZ treatment	Reid and Armour, 1975
2 ½ - 3 months 4 months reared worm-free	Blackface -Border Leicester x Suffolk	1000/3000/5000/day 4000/day	8 weeks 14 weeks	>1000 L3/day → decrease in performance FEC = poor indicator of worm burden	Coop *et al.*, 1977
4 months reared worm-free	Blackface -Border Leicester x Suffolk	a) 100 5x/week b) 320 5x/week c) 4000/day	a) 20 weeks b) 20 weeks c) 20 weeks	no significant difference in FEC between any of the groups ⇔ FEC is independent of larval intake	Jackson and Christie, 1979
7 months, worm-free until 6 months, then 1 month on pasture	cross-bred	drenched with BZ, Lev. and Mor. before infections started 1000 x2/week 10000 x2/week	infected for up to 140 days	Build-up and maintenance of nematode populations were regulated and related to infection level No differences in L4 numbers, worm length, FEC or *in utero* egg counts	Callinan and Arundel, 1982
ewes, 5-7 years old field-reared	Blackface	a) 7000/day b) unchallenged c) unchallenged + treated with BZ	a) infected for 7 + 9 days All animals killed 21 days after challenge in group a)	Larval challenge associated with marked pathophysiological changes, i.e. increase in plasma pepsinogen + loss of plasma protein into lumen. Impaired production possible.	Yakoob *et al.*, 1983
3 ½ months old reared indoors; weaned onto new ley	Blackface -Border Leicester x Suffolk	500/1500/3000/5000 daily 5x/week All grazing clean pasture	12 weeks all animals killed 14 weeks after start of infection	L4 proportion increased with increasing larval intake. FEC: little relation to worm burden. >1500 L3/day: depressed growth rate ≥3000 L3/ day:increased plasma pepsinogen	Coop *et al.*, 1985
10-18 months old, reared worm-free	Welsh Mountain Greyface x Suffolk	half given 2000 5x/week	10 weeks drenched with BZ. All challenged with 50000 and killed 9 days later.	lymphocytes transferred i.v. to naive sheep just challenged, transferred partial immunity, i.e. smaller worms, loss of worms and increases in local IgA and MMC	Smith *et al.*, 1986
3 ½ months old reared worm-free	Dorset Horn rams + ewes	3000/10000/33000 in single doses	lambs killed from day 10 to day 77 p.i.	worm burdens: plateau phase followed by loss phase. The higher the dose the shorter the plateau phase, the shorter the worms and the smaller the worm burden	Hong *et al.*, 1986
4 months old reared worm-free	Dorset Horn rams + ewes	250/500/1000 /day	up to 140 days Lambs killed at different intervals from day 30-140	number of worms in animals appeared to be related to the rate of intake of larvae. Population of worms turned over rapidly	Hong *et al.*, 1987

4 year old pregnant ewes grazed pasture 9 months old reared worm-free	Dorset Suffolk x	most drenched with BZ,	Challenged with 10000 killed 2, 4 and 6 weeks after challenge	ewes were as susceptible to infection as naïve animals with respect to the size and stage of development of their worm populations	Jackson *et al.*, 1988
5 months old reared worm-free	Greyface x Suffolk	1000/day	4, 8 and 12 weeks challenged with 3 doses of 1000 radio-labelled L3s on consecutive days	first sign of immunity = retardation of worms; happens after 4 weeks >4-8 weeks: resistance to establishment of incoming larvae develops + turn-over at 12 weeks: animals almost completely immune to incoming worms; increases in serum-IgG and globule leukocytes	Seaton *et al.*, 1989
5 moths old reared worm-free	Finn x Dorset	vaccination with either L3 surface or somatic extract and Freund's or BeOH adjuvants	Vaccinated at 4 weeks intervals x 3 then challenged with 50000	71% protection by vaccination with L3 surface extracts and BeOH as adjuvant IgG increased in all sheep vaccinated with L3 surface antigen serum-IgA: react with whole surface of exsheathed larvae and excretory pores IgG: strong reaction with anterior and posterior pores of L3s	Wedry-chowicz *et al.*, 1992
as above	as above	as above	as above	Vaccination: induced high levels of serum-IgG. IgA levels low but increase after infection IgG response was stage specific in animals vaccinated with adult surface extracts	Wedry-chowicz *et al.*, 1994
4 ½ to 5 months old reared worm-free	Greyface x Suffolk	a) 2000/day +casein-infusion b) 2000/day c) control	a) + b) : 8 weeks All drenched with LEV Challenged w/ 50000 killed 10 days later	casein-infusion encouraged development of immunity. MMC increased in numbers. worm length was better indicator of immunity than worm burden. Age-dependent immunity may be due to relative protein deficiency in young, growing lambs	Coop *et al.*, 1995
5-6 months at housing reared on pasture	Blackface High FEC and Low FEC animals	natural infection on pasture, before housing	Drenched w/BZ Challenged w/ 50000 Monitored for 38 weeks Challenged w/ 50000 Monitored for 8 weeks	FEC output different between the groups Naturally resistant lambs were better at delaying worm development than naturally susceptible lambs	Stear *et al.*, 1995a

9 months old housed when 6 months old field-reared	Blackface ewes		Challenged after housing w/ 50000. Wait 8 weeks. Drenched w/ LEV + BZ Wait 4 weeks Challenged with 50000 killed after 8 weeks	Variation between animals in worm burdens, worm size, L4 numbers, histology, serology. Positive correlations between increases in MMC, GL, EOS, IgA-prod. plasma cells, and specific IgA in mucosa. Female worm length ~ eggs *in utero* Negative corr. between GL and worm burden. FEC ~ worm burden	Stear *et al.*, 1995b
9 months field-reared until 6 months old, then housed	Blackface ewes		Challenged after housing w/ 50000. Wait 8 weeks. Drenched w/ LEV + BZ Wait 4 weeks Challenged with 50000 killed after 8 weeks	Variation between animals in IgA and IgG. Local IgA response to somatic extract of L4 – very similar to response to L4 E/S products. Antibody responses in plasma cannot predict antibody responses in mucus	Sinski *et al.*, 1995
12 months old field-reared	Romney	5 groups; 2 infected as described later, 2 uninfected but drenched, 1 uninfected + not drenched all grazing contaminated pasture during trial	40000 T.c. +20000 O.c. drench w/ BZ or IVO, wait 2 weeks, then inf. w/ 60000 T.c. + 30000 O.c.; drench with BZ or IVO	significant protection after BZ abbreviated infections as measured by FEC and worm burdens No significant protection after ivermectin-abbreviated infections	Stankiewicz *et al.*, 1996a
9 months old housed when 6 months old field-reared	Blackface ewes		Challenged after housing w/ 50000. Wait 8 weeks. Drenched w/ LEV + BZ Wait 4 weeks Challenged with 50000 killed after 8 weeks	Variation between animals in recognition of parasite molecules from L3 and adults, by serum-antibody. May explain some of the variation between animals in resistance to Ostertagia circumcincta	McCririe *et al.*, 1997
6 months old < 1 week old	Blackface	*in vitro* experiments on abomasal tissue		E/S products from adult worms => release of pepsinogen => contraction of smooth muscle No responses in muscle from parasite naive animals	Scott and McKellar, 1998
6 months old parasite-free	Suffolk x	a) 4000/day BZ-res b) 4000/day IVO+Bzres c) 4000/day susc.	8 weeks all drenched w/ LEV, wait 1 week challenge w/ 10000	plasma pepsinogen increased by day 14 p.i. in infected groups Susceptible strain became patent before resistant No difference after challenge with respect to worm burden or MMC Pathogenicity appears to be the same for resistant as susceptible isolates	Barrett *et al.*, 1998

14 months old field-reared drenched at housing	Romney	a) 5000 O.c./week b) 5000 O.o./week c) uninfected	wait 4 weeks challenge with 50000 O.c.	No effect of previous challenge on FEC or parasite establishment Egg viability reduced in both trickle infected groups. Differences in female worm length, a < b < c *In utero* egg counts lower in a) Increase in IgG and eosinophils in trickle infected groups	Sutherland *et al.*, 1999b
mixed age ewes single bearing housed at parturition	Romney	drenched w/ BZ at housing	challenged with oxfen-resistant L3: 12000 T.c. +12000 O.c. either 2, 4 or 6 weeks after parturition	Establishment of resistant parasites was low at all times. Lactating ewes exhibited a substantial ability to prevent establishment of ingested larvae	Leathwick *et al.*, 1999
10 months old housed at 6 months of age	Blackface		challenged after housing w/ 50000. Wait 8 weeks. Drenched w/ LEV + BZ Wait 4 weeks challenged with 50000 killed after 8 weeks	Heterogeneous pattern in the recognition of antigens from L4 by plasma IgA. Two antigens associated w/ reduction in adult worm length Immunological mechanism controlling worm length is likely to be the parasite specific IgA response or something closely associated	Strain and Stear, 1999

Appendix 1b Development of immunity to *Trichostrongylus colubriformis*

Age of sheep	Breed	Dose given (*T. colubriformis* L3s)	Period of infection Challenge/Drench?	Major findings	Reference
8-10 weeks old reared worm-free	?	2000 5x/week	24 weeks TBZ given weekly / every 4 weeks every 12 weeks/at 24 weeks Killed at end of trial	Weekly anthelmintic treatment prevented development of resistance to infection Resistance developed in other groups	Gibson *et al.*, 1970
8-36 weeks old reared worm-free	?	2000 5x/week	1 to 28 weeks Killed at end of trial	Ability to develop resistance to infection is well developed when lambs are 36 weeks old Good resistance from when they are 24-28 weeks old	Gibson and Parfitt, 1972
11-16 weeks old	Dorset-Horn	2000 5x/week	5-45 weeks Killed at end of trial	5-20 weeks of trickle infection: increasing worm burdens >25 weeks trickle infection: decreasing worm burdens Resistance to establishment of infection develops during the first 30 weeks of life In smaller worm burdens, *in utero* egg counts were lower	Gibson and Parfitt, 1973
24 months old field-reared 30 mated, 13 not mated	Merino ewes	drenched with TBZ + tetramisole: 200 *H. contortus* + 1000 T. colubriformis x2/week	2 months	More worms established in lactating and pregnant ewes Reduction in worm burden correlated with increase in GL Higher MMC counts in ewes showing host response to infection	O'Sullivan and Donald, 1973
3-19 weeks worm-free	?	intitially 10 x6/week, increasing to 5000 x6/week until week 10. Week 10-16: 5000 x6/week	up to 16 weeks	FEC+worm burden: exponential trend until week 12 of infection (=cumulative worm burdens) Resistance to re-infections: very strong >16 weeks p.i. No rapid turn-over of adults Threshold suggested for immunological control: 100000 or age-related	Chiejina and Sewell, 1974a
3, 5 and 5 months old reared worm-free	Dorset-Finn crossbred	5000/day	15-20 weeks	worm burdens cumulative during first 4-8 weeks p.i.; >8 weeks: strong resistance to re-infection Considerable loss of adult worms by week 8-15 p.i.; stunting of adult worms, especially in females decrease in *in utero* egg counts	Chiejina and Sewell, 1974b

6-8 months old reared worm-free	Merino ewes	Day 0: drenched w/ TBZ Day 7: vaccinated with 20000 irradiated L3 Day 28: vaccinated with 20000 irradiated L3 Day 56+58: TBZ drench	Day 67: challenged w/ 20000 normal L3 Day 105: killed	High level of protection against single-species challenge with same species. No significant protection against genetically unrelated species Good protection against several species if challenged w/ all at once Specific antigenic trigger required but terminal effector mechanism ~ not immunologically specific	Dineen *et al.*, 1977
3 months 10 months reared worm-free	Menno	Vaccinated on days 0 and 14 with 20000 irradiated L3. Compared with unvaccinated animals	Challenged from day 35 for 4 weeks Killed at different times	Poor protection in 3 months old lambs. Good protection in 10 months old. IgG levels not related to unresponsiveness MMC present also in unvaccinated and unchallenged lambs Many GL's in adult sheep resistant to challenge infection. Few GL's in lambs that responded poorly to vacc.	Gregg *et al.*, 1978
9-10 months old reared worm-free	Menno ewes and wethers	Vaccinated w/ 3x 20000 irradiated L3, given 2 weeks apart Half of animals drenched w/ TBZ	Day 56 after first vaccination: challenged w/ 40000 normal L3 or 2000/week for 4 weeks	97-99% protection against single challenge and sequential challenge	Gregg and Dineen, 1978
3-6 years old, some non- lactating 7-8 months old tracer lambs	Frisian ewes and ram lambs	larval challenge on pasture. Mixed infections	3, 5 or 9 months	In non-lactating ewes, the majority of *Trichostrongylus* spp was inhibited as L3s. In lambs hardly any L3s were found	Eysker, 1978
4 months old reared worm-free	Blackface -Border Leicester x Suffolk	*T. vitrinus*!! 2500/day	90 days killed 2 weeks after last dose	Histological findings were quite similar to lesions caused by *T. colubriformis* Most severe changes in first 1.5 m of small intestine. Many GLs in mucosa	Coop *et al.*, 1979
3-5 months old reared worm-free	Merino Merino x	0/300/950/3000/9500/ 30000 /week	24 weeks	Week 1-12: FEC increased, reflecting larval intake. >week 12, FEC decreased. At week 24 most animals were resistant to establishment >3000/week: food intake depressed max effect week 8-12, normal by week 20-24. Protein synthesis in liver+muscle, depressed if 30000/week	Steel *et al.*, 1980
3 months old reared worm-free	Suffolk x	*T. vitrinus*!! 2500 x5/week	4, 6, 8 and 13 weeks killed one week later	Severe villus atrophy, epithelial erosion and cellular infiltration in first 2-3 m of intestine in lambs killed at week 5 and 7. No change in linear distribution of worms. Lambs killed at week 14: resistant!	Jackson *et al.*, 1983

17 and 21 weeks reared worm-free	Merino wethers	vaccinated w/ 80000 irradiated L3 at 17 or 21 weeks of age	Drenched w/ LEV at 25 weeks of age Challenged at 26 weeks w/ 30000 normal L3 Killed 4 weeks later	Lambs fed a high plane nutrition of lucerne had higher AB-titres after vaccination + lower FEC and worm burdens, lower *in utero* egg counts than lambs fed low nutrition plane. Developing immune response competes with weight gain for limited physiological resources	Wagland *et al.*, 1984
3 months old ram lambs 15 months old ewes and wethers all worm-free	Merino	lambs vaccinated with different size doses of irradiated L3 at 12 and 16 or 8 and 12 weeks of age. Ewes and wethers grazed pasture for 6 weeks	drenched at 10 or 16 weeks of age challenged with 10000-60000 or 5000 and 80000 Killed 4 weeks after challenge	Threshold for response to both vaccination and challenge is exceeded by 5000 L3. Response independent of challenge dose size Ram lambs: less responsive immunologically than ewe lambs at higher challenge levels Filed study: response to vaccination only apparent if sheep are transferred to heavily contaminated pasture	Windon *et al.*, 1984
18 months old field-reared 5-6 months old reared worm-free	Romney	Pen trial: High FEC and Low FEC line sheep: given 5000 5x/week Field trial w/ field-reared sheep and 5-6 months old sheep Pen trial w/ mixed species infection given once	Pen trial: some steroid-treated. Trickle for 4 weeks Animals killed at different intervals	Nematode cholinesterase activity: higher in sheep w/ High FEC and in female nematodes Decline in cholinesterase activity in female worms: associated w/ increasing age of animals, decline in worm length, *in utero* egg counts, and worm burdens Steroid-treatment alleviated these effects. LowFEC sheep had more GL's and higher mucus LMI activity than HighFEC sheep	Douch *et al.*, 1988
8-16 weeks reared worm-free	Romney ewes	vaccinated at age: 8 weeks w/ 2x28000 12 weeks w/ 2x35000 16 weeks w/ 2x42000	Two vaccination doses given 16 days apart; drench w/ TBZ after 12 days Challenged with same size dose 14 days after drench. Killed 42 days later	Lambs; 'separated' into responders and non-responders. Decrease in worm burden w/ increasing age. Number of GL's increased in older lambs and reflected individual responsiveness to immunisation	Douch, 1988
5 months old reared worm-free	Suffolk x Finn-Dorset	some given 2500/day some uninfected	34 weeks	Inappetence: weeks 6 – 13 + marked eosinophilia at this time Plasma-N leakage into the intestine was higher in infected lambs from week 8 to 14	Kimambo *et al.*, 1988a
21 months old some worm-free some had been given 2500L3/day for 34 weeks	Suffolk x Finn-Dorset	trickle infected animals were then given no larvae for 24 weeks	All sheep challenged with 2500/day for 10 weeks	No positive FEC during challenge period, but rapid development of eosinophilia	Kimambo *et al.*, 1988b

as above	as above	as above	as above	Mucus from both small intestine and abomasum paralysed and inhibited larval migration significantly more than mucus from worm-free animals LMI activity also in digesta and in faeces at some times Mucus and other substances secreted into the lumen of the gut remain potent during passage through the small intestine	Kimambo and MacRae, 1988
5 months old reared worm-free	Romney	immunized w/ 2 x 200000 15 days apart TBZ drench 12 days after 2nd immunisation dose	challenged w/ 20000 two weeks later monitored 3 – 7 weeks after challenge Some grazed pasture for 4 weeks instead	Significant protection against mixed natural challenge. Immunized sheep: significantly more GL, MMC and EOS and higher LMI activity in mucus Haematological parameters reflected parasite challenge and were unrelated to acquired worm burden	Douch, 1989
9 months old reared worm-free	Saanen goats!! wethers	10000/week	10 weeks, then drenched w/ IVO challenged twice w/ 50000 (drenched in-between)	Significantly lower worm burdens in trickle- + challenge-infected group than in challenge only groups Decrease in fecundity and M/F-ratio trickle infected group FEC and worm burden: highly correlated	Pomroy and Charleston, 1989
	guinea pigs	detergent soluble fraction from 3rd stage larvae	antigens used to 'immunise' guinea pigs	One antigen w/ molecular weight 41000, induced 43 – 51 % protection	O'Donnell *et al.*, 1989a
	guinea pigs and sheep	E/S-products from exsheathed L3's	used to 'immunise' guinea pigs and sheep	Some degree of immunity in guinea pigs. IgG to antigen in sheep and guinea pigs. IgA also found in intestinal lymph of sheep. Activity associated with L3's only	O'Donnell *et al.*, 1989b
16 months old reared worm-free on pasture	Merino wethers	day 0: 20000 irradiated L3 either *T. colubriformis* or *Haemonchus contortus* day 28: 20000 irradiated L3 day 56: oxfendazole drench	day 63: challenged with 20000 normal *T. colubriformis* or 10000 normal *H. contortus*	Protection only against challenge infection with the species used for vaccination	Adams *et al.*, 1989
5 months old reared worm-free	Merino wethers	2000/632/200 5x/week susceptible larvae replaced with resistant at different times after start	1/4/7/10 weeks	65% establishment in previously uninfected sheep low establishment levels after 7/10/14 weeks at the 3 infection levels threshold worm burden required before resistance developed Threshold worm burden~3000-3500	Dobson *et al.*, 1990a

12/20/28/36 weeks of age reared worm-free + field-reared lambs	Merino ewe lambs	2000 x5/week susceptible larvae replaced with resistant at different times after start	up to 9 weeks	Rate of development of resistance to new infection was faster in older than in younger animals. 12 weeks old: 9 weeks trickle needed 20 weeks old: 6 weeks or less needed Immunity after natural infection not effectively expressed until lambs are 20-35 weeks old. 1) A threshold worm burden must be exceeded before any substantial resistance develops 2) When threshold is reached: rate of development of resistance is age-dependent only	Dobson *et al.*, 1990b
5 – 6 months old reared worm-free	Merino ewes	2000/1124/632/200 5x/week susceptible larvae replaced with resistant at different times after start	up to 20 weeks	Rejection of adult worms began at week 7, took about 9 weeks to complete, at dose levels >200 x5/week.	Dobson *et al.*, 1990c
Model				prediction of establishment from infection rate and host age was used to estimate worm burden, worm rejection and arrested development	Dobson *et al.*, 1990d Barnes and Dobson, 1990
9 – 12 months old reared worm-free	Merino Merino x Border-Leicester ewes and wethers	Immunised w/ 3 x 60000/80000 irradiated L3 given 4 weeks apart. Drenched with LEV 12 weeks after first immunisation	challenged one week after drench w/ 40000 normal L3	Sheep were solidly immune when having been immunised and challenged Rejection of incoming larvae by immune sheep is associated w/ an intestinal inflammatory response involving secretion of biogenic amines and plasma loss	Steel *et al.*, 1990
2 years old reared worm-free	Merino wethers	Immunised w/ 3 x 30000 normal L3 given 4 – 6 weeks apart. Drenched with LEV 13 weeks after first dose	challenged one week after drench w/ 30000 exsheathed L3, by surgical transfer Sheep killed at intervals after challenge	Immune sheep rejected most of their larvae within 1 day Assoc. w/ local appearance of GL and increases in IgG_1 and IgG_2 in mucus. Rejection of remaining worms happened day 3-14 p.i and was assoc. w/ increases in IgA and IgG_2 in mucus, T-cell infiltration activation and differentiation and epithelial necrosis.	McClure *et al.*, 1992

6-18 months old reared worm-free	Merino ewes and wethers	a) 6 months old: 30000 L3; drenched 13 weeks p.i. b) 8 months old: truncated infections c) 8 months old: 10000 x5 at 10-day intervals. Drench 7 days after last infection	a) challenged week 14/19/26/39 w/ 30000; then adult worms were surgically transferred b) challenge w/ 30000 1 week after drench c) 30000 exsheathed L3 transferred surgically	a) suppressed fecundity, establishment and survival of adoptively transferred worms b) 4x7 or 4x10 day truncated infections protected animals significantly c) challenge larval infection given intra-duodenally was expelled within 3 days after challenge. Stage-specific antigens produced by early L3 – L4, effectively immunised sheep against larval challenge but may less effective against adults	Emery *et al.*, 1992a
a) 8 months b) 12 months c) 12 months all reared worm-free	Merino wethers	a) 20000 normal or irradiated L3 and adult worms given twice, 4 weeks apart b) 9000 adult worms c) 9000 adult worms, drenched	a) drenched, then challenged with 20000 L3 b) no drench; inoculated intra-dudenally w/ 30000 L3 c) challenged w/ 30000 exsheathed intraduodenally	a) adoptive transfer of adults gave significant protection against challenge worm rejection did not occur until 7-10 days after challenge antigens that elicited a response were stage-specific and only present/produced in sufficient amounts when parasites had developed for a week	Emery *et al.*, 1992b
6-9 months old reared worm-free	Merino wethers	a) 6 months old: truncated infections or 5x 100000 L3, 2 weeks apart, drenched 1 week after each infection b) 8 months old: infected w/ 10000 adult worms; drenched after 16 weeks	a) challenged with *T. colubriformis* or *N. spathiger* or with both and *O. circumcincta* at two dose levels b) challenged 1 week later w/ *T. colubriformis*, *H. contortus* and *N. spathiger*	>90% protection against *T. colubriformis* in sheep immunised with that species, but no protection against unrelated species If immunised w/ intestine residing species, then no protection against abomasal species Non-specific rejection of unrelated parasites living in the same downstream niches in the gut, when the nematode used to induce immunity is included in the challenge infection	Emery *et al.*, 1993
14/20/26 weeks old reared worm-free	Merino	6000/week	18/12/6 weeks At 32 weeks sheep were killed or drenched Challenged w/ 2 abomasal nematodes + *T. colubriformis* or challenged w/ 10000 at week 6/12/18 after the drench Killed 17 days after last challenge	Low establishment of challenge in groups given 18 or 12 weeks primary infection, at all challenge times Animals w/ 6 weeks primary infection: establishment low only at the first two challenge times Immunity to *T. colubriformis* gave little protection against other species	Barnes and Dobson, 1993

a) 18 months, worm-free b) 2 years, worm-free c) 9 months, field-reared d) 7-8 months, worm-free	Romney	a) infected every 3 weeks w/ increasing doses b) half given 4x100000 + OXFEN 10 days after each dose, next dose 4 days later c) natural challenge on pasture d) 10000 L3 on days 0, 5 and 10. 10000 *O. circumcincta* L3 given same days	a) challenged w/ 20000/40000/ 60000/80000; killed 2 weeks later b) challenged w/ 50000 4 days after last drench c) d) drenched w/ IVO on day 13; challenged on ay 20 w/ 10000 of each *T. colubriformis* and *O. circumcincta*	High numbers of GL in intestinal lumen; assoc. w/ parasite infection and protective immune response Lumen GL ~ GL in epithelium Positive correlation between lumen GL and LMI activity and between lumen GL and eosinophils and IgG Negative correlation between worm burden and lumen GL and *in utero* egg counts	Stankiewicz *et al.*, 1993
8 months old reared worm-free	Romney	5000 twice a week dexamethasone treatment in one group from 1 week before infection started, until day 77, in another dexamethasone treatment started at day 77	up to 210 days	Resistance measured by FEC, was expressed 35 days after infection began, but not in dexamethasone treated sheep. Increases in IgG_1 to larval and adult antigen. Dexamethasone treatment prevented the antibody responses	Douch *et al.*, 1994
3 months old field-reared	Merino wethers	drenched w/ IVO at housing Fed different levels of fish meal (protein) 1000 x3/week of L3	up to day 140 animals killed a days 35, 70, 105 and 140	Infection lowered liveweight gain at lower level protein diets FEC was significantly lower in animals fed high protein diets Worm expulsion rate was higher in protein supplemented animals Rate of expulsion correlated w/ circulating EOS and mast cell proteinase concentrations	Houtert *et al.*, 1995
4 months old field-reared	Merino Merino x Border-Leicester	lambs grazing pasture contaminated w/ *T. colubriformis* and *H. contortus*	IgG_1 levels were measured	Lambs could be separated into sire groups by their response to *T. colubriformis* and partly to *H. contortus* High and Low responder groups could be differentiated in lamb populations with respect to antibodies to both the parasites and to *Lucilia cuprina*	Hohenhaus *et al.*, 1995
10-12 months old reared worm-free	?	some lambs rendered resistant between 5-10 months of age by truncated infections: 30000 every 5 weeks; drench 4 weeks after each dose	all lambs tested with various compounds and drugs given from 5 days prior to and 4 days after challenge w/ 20000 L3	Corticosteroids inhibited rejection of the challenge infection by ~ 70%	Emery and McClure, 1995

8-26 weeks old 31-51 weeks old all worm-free	Dorset Down x Coop-worth rams	animals fed different protein levels in diet	Challenged w/ 30000	Young lambs on a low protein diet have lower protective immunity Increased levels of $CD4^+$, $CD5^+$ and $CD8^+$ with increasing age More $CD4^+$ in immune than non-immune animals	Kambara and McFarlane, 1996
15 months old reared worm-free	Romney	10000 given on days 0, 21 and 42. Abbreviated w/ OXFEN or LEV	challenged on day 70 w/ 60000	>90% protection in sheep immunised w/ 3, 15 or 7 day OXFEN-abbreviated infections Higher worm burdens and FEC in sheep immunised by 7-day LEV-abbreviated infections than in 7-day OXFEN-abbreviated infections	Stankiewicz *et al.*, 1996a
6-9 months old reared worm-free	Merino wethers	some lambs immunised w/ 4x 20000 L3 given w/ 1 month interval, from when lambs were 5-8 months old	challenged w/ 10000/ 20000/40000/80000 or 30000 + drench and a 2nd dose of 30000	In susceptible animals, 90% of worms were located in the first 3 m of the small intestine In immune sheep, worms were relocated posteriorly from the first 3 m to the next 6 m of the intestine	Wagland *et al.*, 1996
?	Suffolk x Blackface	2500/day animals fed high or low protein diet	drenched w/ OXFEN + LEV Challenged w/ 30000	Some blood parameters were affected by parasitism, e.g. albumin Increases in circulating EOS and mast cell proteinase in parasitised animals. Development of acquired immunity appeared to be unaffected by previous nutritional treatment	Kyriazakis *et al.*, 1996
3-4 months old field-reared	Romney	lambs given 3 OXFEN-abbreviated infections w/ *T. colubriformis* and *O. circumcincta*		FEC lower in all immunised animals Not as good a response to immunisation as in older animals	Hadas and Stankiewicz, 1998
7 months old field-reared	Romney rams	2 groups given CRC, 2 not given CRC Day 0: all drench w/ combination drench 3000 x2/week, either BZ resistant or susceptible L3	10 weeks trickle infection Week 11: drench Week 12: challenge with 20000 susceptible Week 17: kill	larval challenge alone resulted in incomplete though substantial protection against subsequent challenge. CRC-treatment ~ reduced level of immunity by removing some or all larval and adult antigen	Sutherland *et al.*, 1999a
5-12 months old reared worm-free	Romney	immunised with 3 truncated (14 day) infections w/ 30000, each terminated w/ OXFEN and next dose given 1 week later	Canulae fitted in anterior duodenum Challenged w/ 20000 or 40000 exsheathed L3; intestinal fluid collected for up to 24 hours	Immune sheep had fewer larvae in the intestine, in some, challenge infections were expelled within 2 hours. Mucus: increases in IgG, histamine and LMI activity Intestinal fluids: increases in IgG and histamine. Histology: increases in MMC and GL in immune sheep Evidence for an intermediate hypersensitivity reaction in the intestine of immune sheep and direct anti-larval properties of mucus	Harrison *et al.*, 1999

In vitro studies		E/S product of *T. colubriformis* tested in assay on different cell lines		increase in cell numbers found with the three types of epithelial intestinal cells	Huby *et al.*, 1999
1 day to 7 weeks old 3 months old reared worm-free	Merino Wethers	2000 x3/week or inoculated w/ recombinant *T. colubriformis* antigen	up to 7 or 22 weeks	85-91% reductions in worm burden in trickle immunised neonates compared to 50% protection in those vaccinated with a recombinant antigen	Emery *et al.*, 1999
9-11 weeks old field-reared, housed when 9 weeks old	Texel x Greyface	2500 x5/week Animals fed different protein level diets during trickle infection period	up to 20 weeks Animals killed at different times	requirements of immune functions appeared to have priority over those of growth FEC, worm burdens and fecundity were unaffected by changes to metabolisable protein supply	Kahn *et al.*, 2000

Appendix 1c Paper describing results of 1995-1996 study

Appendix 2a Modified McMaster Method for counting strongyle eggs

METHOD:

1. Weigh out 2 g (±0.1 g) of faeces into a 100 ml container[1]
2. Add 28 ml of saturated NaCl solution (specific gravity 1.2) and mix well to a fine suspension using an electric stirrer[2]
3. Pour mixture through a small coarse sieve (aperture approximately 0.85 mm) into a high-edged petri dish[3]
4. Mix well while taking out a subsample, using a pasteur pipette, to fill one chamber of a McMaster counting slide[4]
5. Each egg counted in one chamber represents 50 eggs per g faeces

[1] Labserv plastic specimen container

[2] Heidolph RZR 2040, John Morris scientific Ltd.

[3] Labserv petri dish; diameter = 85 mm; height = 24 mm

[4] 3 chamber x 0.3 ml; J.A. Whitlock and Co., PO Box 51, Eastwood NSW 2122, Australia

Appendix 2b Culturing eggs to 3rd stage larvae in faeces

Larval cultures were set up as soon as possible after obtaining the faecal samples, either on the same day of sampling or the next morning. Samples were stored at 4°C until processed. The method used has been modified from a method developed at AgResearch, Grasslands (Leathwick, pers.comm.).

METHOD:

1. Weigh out 10 g (±1.0 g) of faeces. By setting up three cultures per animal sample and using the mean of the three in the statistical analysis, the variation due to culture size was reduced
2. Place faeces in the base of a 55 mm diameter petri dish[1] placed in the base of a 85-mm-diameter petri dish containing 5 ml of distilled water
3. Place the lid on the larger dish to maintain the humidity as high as possible during the incubation time. The lid of the petri dish is not fitted tightly and thus allows diffusion of oxygen into the dish and evaporation out of the dish to take place.
4. Incubate cultures at 25°C for 10 days in an incubator[2]
5. Check the cultures after 5-6 days of incubation and add more distilled water if necessary, to maintain humidity levels.
6. Extract larvae as described in Appendix 2c. If cultures can not be put on Baermann funnels immediately after the end of the incubation time, they should be stored at 4°C until processing, to avoid further development.

[1] Labserv

[2] Contherm Precision Environmental Chamber

Appendix 2c Baermann procedure for extracting 3rd stage larvae from faecal cultures

METHOD:

1. Remove larval cultures from 25°C incubator after 10 days of incubation.
2. Place a sieve on top of each glass funnel[1] in the Baermann set-up. Put a single sheet (one layer) of tissue[2] in each sieve.
3. Using forceps, break down pelleted faeces into halves (two to three smaller pieces) and empty out into the sieve. Rinse all parts of the petri dish at least 3 times with a washbottle, containing tap water, and wash into the sieve. Make sure that faeces are covered by water.
4. Leave Baermanns to sediment for 24 hours.
5. Run off approximately 50 ml of the sediment through the bottom of the funnel and into a 50-ml Falcon tube.
6. Allow samples to settle for 24 hours at 4°C.
7. Carefully siphon off the supernatant of the sample until a volume of 20 ml is left in the Falcon tube, taking care to avoid stirring up the sample whilst doing this

The samples are now ready for larval identification and counting.

[1] Pyrex® glass funnels with 60° bowl angle,; top external diameter = 125 mm; stem diameter = 12 mm; stem length = 125 mm; overall height = 220 mm; fitted with soft red rubber tubing and clamp

[2] Hygenex Royale white 2-ply tissues, Carter Holt Harvey

Appendix 2d Lugol's Iodine

- 2 g Potassium Iodide (Analar, BHD Laboratory Supply, England; KI = 166.0)
- 1 g Iodine (Analar, BHD Laboratory Supply, England; I = 126.90)
- 100 ml of distilled water

Dissolve potassium iodide in water and add iodine. Mix well and keep in cool dark place when not in use.

Appendix 2e Counting third stage larvae

Larvae are recovered from faecal cultures as described in Appendix 2c.

METHOD:

1. Mix the sample well and take out a sub-sample of 2 ml by means of an automatic pipette[1]
2. Place the sample in a glass counting slide to which a drop of Lugol's iodine has already been added to kill the larvae. Free-living nematodes present in the sample will take up the iodine immediately and more rapidly than the 3rd stage larvae of strongylid nematodes and thus are readily distinguishable.
3. Place the slide in the compound microscope and leave to settle for about 30 seconds
4. Count 3rd stage larvae in the whole area of the slide. If fewer than 10 larvae are found, then a larger volume is counted. If more than 300-400 larvae are present in 2 ml, then a smaller volume is counted. Adjust for aliquot factor to get the total number of larvae in the sample.
5. **'% developmental success'** is calculated as follows:
   ((total number of 3rd stage larvae in sample/(mean FEC * culture weight)) * 100

[1] Jencons automatic pipette, 1000 – 5000 µl

Appendix 2f Identifying third stage larvae

METHOD:

1. Leave samples to settle at 4°C for at least 3 - 4 hours to concentrate the larvae.
2. Using a pasteur pipette, transfer a small volume of the sample from the tip of the Falcon tube on to a glass slide.
3. Add one drop of Lugol's iodine and place a cover-slip on top.
4. Identify larvae to genera and species level using the x10 and the x40 objective, with x10 eyepieces of which one has a scale for measuring larval length.
5. Where possible identify 100 third stage larvae to genus level.

Key for the identification of the 3rd stage larvae of common gastro-intestinal nematodes of sheep (adapted from 'Manual of veterinary parasitological laboratory techniques, Ministry of Agriculture, Fisheries and Food, p 37)

1.	Oesophagus rhabditiform	Free-living nematode
	Oesophagus not rhabditiform	2
2.	Without sheath; oesophagus ~ half the length of body	*Strongyloides*
	With sheath; oesophagus less than ¼ the length of body	3
3.	Tail of sheath short, non-filamentous	4
	Tail of sheath medium length, non-filamentous	5
	Tail of sheath filamentous	6
4.	Head of larva tapered, tail indistinctly rounded or bearing one or two tuberosities, < 720 µm	*Trichostrongylus sp.*
	Head of larva squared, tail indistinctly rounded, 'shoulders' just below head of larva, >720 µm	*Ostertagia sp.*
5.	Head of larva squared, bearing refractile bodies or band	
	Tail of sheath tapering almost to a filament or abruptly becoming a fine point	*Cooperia sp.*
	Head of larva narrow rounded, tail of sheath off-set	*Haemonchus contortus*
6.	Head broad rounded, 32 gut cells	*Oesophagostomum/Chabertia spp.*

Appendix 2g Analysis for Faecal Dry Matter Content (% D.M.)

METHOD:

1. Weigh out 10 – 20 g of faeces in 100 ml container[1]
2. Oven dry at approximately 60°C[2] for a minimum of 7 days
3. Weigh again to record dry weight.
4. Calculate the faecal dry matter content (%D.M) as follows:

%D.M. = ((dry weight - weight of container) / (wet weight - weight of container))*100 %

[1] Labserv plastic specimen containers. Weights: Small = 11.5 g ± 0.1 g (n = 30); Large = 18.2 g ± 0.1 g (n = 30)

[2] Oven: Qualtex REG, Sydney, 250 V, 740 W; supplier: Andrew Thomas Limited

Appendix 2h Collection of Blood Samples

Blood was collected from the jugular vein into a vacutainer tube[1] containing sodium heparin. A soon as possible after sampling, the samples were placed at 4 °C until further processing (eosinophil counts and/or centrifugation).

METHOD:

1. To collect plasma for antibody assays centrifuge[2] samples at 1065 G[3] for 7 minutes
2. Collect plasma and store at –20°C until assayed for specific antibody levels.

$$\underline{\text{Centrifugal force}} = G = \left(\frac{2\pi}{60}\right)^2 \times \left(\frac{1}{9.81 m/s^2}\right) \times rpm^2 \times r \ ,$$

where *rpm* = revolutions per minute and *r* = radius (m).

(Bronstein and Semendjajew, 1987).

[1] Becton Dickinson Vacutainer Systems; PrecisionGlide needles; Vacutainer Tubes with Sodium Heparin as Additive; Needle Holder

[2] Eppendorf Centrifuge 5810

[3] 2300 rpm; max. radius = 18 cm

Appendix 2i ELISA Method

Serum samples were analysed for specific antibody levels (IgG_1) using an enzyme-linked immunosorbent assay (ELISA) similar to that described by Adams *et al.* 1989. This method had been further modified at AgResearch, Wallaceville, Upper Hutt (R. Green, pers. comm.) where all ELISAs for this project were carried out.

PREPARATION OF PLATES:

ELISA plates were coated with ES antigen (200ng protein - determined on the basis of absorbance at 230/260nm) in 100ml of coating buffer. Plates were then incubated at 37°C for 2 hours and afterwards washed 3 times in de-ionised distilled water containing Tween 20 detergent (0.1%) - washing solution.

Plates were blotted with "Blotto" (10mM phosphate buffer at pH 7.2 to which is added Tween 20[1] (to 0.5%) and bovine skim milk powder (to 5%, purchased at a local retail outlet). Plates were washed 6 times in the above washing solution and could then be stored at -20°C until required. Blocking (blotting) was carried out to reduce non-specific binding at sites not used by the antigen. Multichannel pipettes were used for these and the following steps of the assay[2].

METHOD:

1. Store the plates in a humidity chamber in the walk in freezer until ready to use (up to 3 months).
2. Thaw sheep sera to be tested in the assay
3. Dilute the sheep sera 1 in 500 in ELISA buffer (PBS, (10mM phosphate buffered (0.15M) saline), pH 7.2 and Tween 20 (to 0.5%).
4. Add 75 µl of ELISA buffer to each well in all plates to be used for assay
5. Add 25 µl of serum dilution to each of 3 wells, i.e. the sera are now diluted 1:2000 in ELISA buffer
6. Add standard sheep serum[3] to wells H2-H4. Incubate at 37°C for 1 hour. Then wash 6 times in washing solution (with 0.1% Tween-20).
7. Add 100µl of an enzyme labelled anti-sheep rabbit immunoglobulins conjugated with Horse Radish Peroxidase (DAKO®; diluted 1/1000 in ELISA buffer) to each well. This is incubated at 37°C for 2 hours. Then wash 6 times in washing solution (with 0.1% Tween 20).
8. Warm the 3,3',5,5'-Tetramethylbenzidine (TMB) Buffer (100mM Sodium acetate/ Citric acid pH5.2) in cell culture bottle in warm water, until luke warm. Add the 1µl/ml H_2O_2 (30%) and TMB stock (1mg TMB in 100µl Dimethyl sulphoxide /10ml buffer) to

[1] Tween 20 : Polyethylene Sorbitan Monolaureate (Sigma® P-1379 500 ml Lot 88H0469)

[2] Biohit Proline Multichannel Pipettes, Models '250' and '1200'

[3] Standard sheep serum

the buffer. Shake to mix and add 100 µl of this enzyme substrate per plate well. Develop for 10 minutes. Stop by adding 50 µl 1M (2N) Sulphuric acid to each well.

9. Wipe the plates with tissues to remove drops of water from the bottom.
10. The resulting colour which is related to the amount of antibody present was measured in an ELISA plate reader (Dynatech MR5000) using 450 nm filter as sample filter and 630nm filter for reference. Results, expressed as units of optical density, were means of triplicate assays (an algorithm was used to identify and discard outlier readings).

Appendix 2j Assessment of the faecal egg counting technique

THE EFFECT OF STORAGE TEMPERATURE ON FEC

Introduction

As a developmental success rate of more than 100% was recorded in some samples from several of the experiments described in this thesis, it was necessary to try and find out why this could happen. One possibility was that if samples had been kept at temperatures higher than 10°C for a sufficient amount of time, for instance when spending a day sampling animals in the field, eggs would start to hatch. This could result in an underestimation of the number of eggs originally present in the samples and the hatched larvae would continue to develop in the cultures and be included in the final larval count. Any developmental success rate obtained under these circumstances would then tend to be overestimated.

Aim

The aim of the experiment was to test the effect on FEC of exposure of eggs to different temperatures.

Methodology

Mean FEC (see Appendix 2a) of four counts were estimated in freshly obtained faeces (<1 hour 'old') from four housed sheep infected with *T. colubriformis*. The faeces were stored at different temperatures and FEC repeated after different time intervals (duplicate counts for each time interval). The temperatures were chosen to simulate those likely to be encountered while collecting samples in the field where cooling facilities are not always readily available. To standardize the results, the FEC at x hours were divided by the FEC at 0 hours and expressed as a percentage. This percentage was then plotted against storage time (see Figure A).

Results

The data are shown in Table A below and represented graphically in Figure A. Generally, vermiform embryos appeared after 8 – 10 hours of storage at 25°C and 43°C and later at the lower storage temperatures (Table A). As the error bars shown in the figure are in some instances derived from as few as four counts, they can only be regarded as indicative. Bearing that in mind, there appeared to be no significant effect of the various storage temperatures on FEC within the time intervals included in this experiment.

Discussion

The current experiment considered only the effect of temperature on FEC in samples containing *T. colubriformis* eggs. This was relevant for much of the work in this thesis. However, in situations where eggs of other species were present, as was the case in some of the outdoor experiments, it is possible that eggs of some species could hatch

earlier or later than those of *T. colubriformis* according to their differing temperature requirements (Crofton, 1963).

It was concluded that storing samples at temperatures of 10°C or above, for up to 10 hours probably did not affect the outcome of the FEC carried out in this thesis. Therefore the explanation for developmental success rates of more than 100% was likely to be found elsewhere.

The effect of storage at various temperatures on FEC

Time = number of hours stored for

Storage temp. = 10°C

Time	Tag	FEC 1	FEC 2	FEC 3	FEC 4	FECmean	
0	2	2700	3600	3500	3650	3362.5	
0	9	2300	2200	1950	1950	2100	
4	2	3050	2500			2775	morula
4	9	1700	1650			1675	morula
8	2	2700	2750			2725	morula
8	9	2200	1550			1875	morula

Storage temp. = 18°C

Time	Tag	FEC 1	FEC 2	FEC 3	FEC 4	FECmean	
0	2	2500	1900	1850	1800	2012.5	
0	9	1650	1850	1900	1500	1725	
0	15	50	0	0	0	12.5	
0	20	250	650	550	450	475	
24	2	1250	1550			1400	50% vermiform
24	9	1850	2150			2000	50% vermiform
24	15	0	0			0	
24	20	600	550			575	vermiform

Storage temp. = 25°C

Time	Tag	FEC 1	FEC 2	FEC 3	FEC 4	FECmean	
0	15	200	0	100	0	75	
0	20	500	300	500	750	512.5	
3	15	0	0			0	morula
3	20	850	700			775	morula
6	15	100	50			75	morula
6	20	200	550			375	morula
26	15	50	0			25	morula
26	20	350	350			350	many vermiform
28	15	50	50			50	morula
28	20	500	500			500	vermiform
30	15	50	200			125	morula
30	20	650	800			725	vermiform

Storage temp. = 25°C

Time	Tag	FEC 1	FEC 2	FEC 3	FEC 4	FECmean	
0	2	2050	2250	2850	2400	2387.5	
0	9	2350	2450	3100	2600	2625	
0	20	200	300	400	200	275	
4	2	2450	2650			2550	morula
4	9	2700	1900			2300	morula
4	20	250	150			200	morula
6	2	1900	2050			1975	morula
6	9	2750	2750			2750	morula
6	20	300	250			275	morula
9	2	2950	2600			2775	morula
9	9	2300	2300			2300	morula
9	20	300	150			225	morula

Storage temp. = 25°C

Time	Tag	FEC 1	FEC 2	FEC 3	FEC 4	FECmean	
0	2	2850	2500	3100	3850	3075	
0	9	3650	4550	2400	3000	3400	
4	2	3000	2800			2900	morula
4	9	2400	2500			2450	morula
10	2	2100	2550			2325	late morula/vermiform
10	9	3350	3700			3525	late morula/vermiform

Storage temp. = 43°C

Time	Tag	FEC 1	FEC 2	FEC 3	FEC 4	FECmean	
0	2	1500	2500	1300	2350	1912.5	
0	9	2000	2550	2500	2250	2325	
0	15	50	0	50	0	25	
0	20	400	550	300	300	387.5	
2	2	1500	1450			1475	morula
2	9	2350	1950			2150	morula
2	15	150	0			75	morula
2	20	550	300			425	morula
4	2	1200	1650			1425	morula
4	9	1850	1850			1850	morula
4	15	0	0			0	morula
4	20	700	650			675	morula
6	2	1500	1600			1550	morula
6	9	1750	1600			1675	morula
6	15	0	0			0	morula
6	20	500	750			625	morula
8	2	2550	1600			2075	morula
8	9	1750	1650			1700	morula
8	15	50	0			25	morula
8	20	450	550			500	morula
20	2	1500	1550			1525	some vermiform
20	9	1200	1750			1475	some vermiform
20	15	0	0			0	
20	20	500	400			450	some vermiform

Storage temp. = 43°C

Time	Tag	FEC 1	FEC 2	FEC 3	FEC 4	FECmean	
0	2	2850	2500	3100	3850	3075	
0	9	3650	4550	2400	3000	3400	
4	2	2150	3000			2575	morula
4	9	3500	3550			3525	morula
10	2	2550	2700			2625	few vermiform
10	9	2650	3450			3050	few vermiform

Table A The effect of storage temperature on FEC

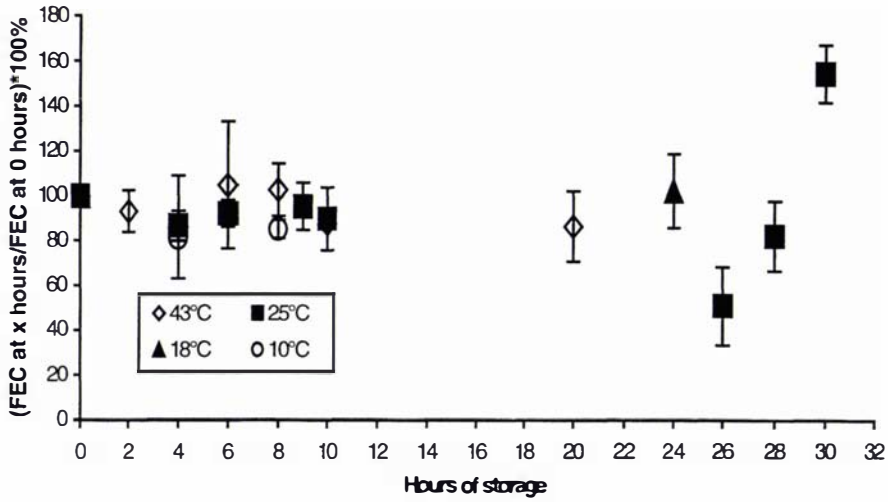

Figure A The relative change in FEC after storage at various temperatures (Mean ± S.E.)

SENSITIVITY OF THE MODIFIED MCMASTER METHOD IN FAECES FROM HOUSED ANIMALS

Introduction

Another possible explanation for recording a developmental success of more than 100% in some samples, is that the modified McMaster method was underestimating the actual number of eggs per gram present in faecal samples.

Aim

To test the sensitivity of the egg counting technique.

Methodology

Eggs used for this experiment were recovered as described in Appendix 5a from faeces of two housed lambs artificially infected with *T. colubriformis*. Three concentrations of eggs were prepared containing approximately: 4000 eggs/ml, 2000 eggs/ml and 500 eggs/ml. Parasite-free faeces were obtained from three housed animals. The faeces from each of these three animals were weighed out into eighteen 2-g samples. To each set of 18 samples, 0.5 ml of the first egg concentration was added to six samples, 0.5 ml of the second egg concentration to the next six samples and 0.5 ml of the third egg concentrations to the last six samples. This gave a total of 3x6 = 18 samples for counting for each egg concentration. **The theoretical FEC** in the samples were approximately 1000, 500 and 125 epg, respectively. The pellets of the parasite-free faeces were halved before the addition of eggs, to facilitate the absorption of the egg suspensions into the faeces. This was allowed to take place for approximately eight hours at 4°C. To obtain **the actual FEC**, the number of eggs in each sample was estimated using the modified McMaster method (Appendix 2a). The actual FEC (obtained with modified McMaster method) was then compared with the theoretical FEC (according to the number of eggs added to the sample) and the efficiency of the McMaster method indicated by = Actual FEC/Theoretical FEC expressed as a percentage. To compare the possible variation in efficiency at different levels of FEC, the results were plotted against the theoretical FEC in Figure B below, in which each point represents six counts from one animal faecal sample.

Results

The results from the experiment are summarised in Figure B. It was found that an underestimation of FEC was mainly a problem at lower egg counts (<500 epg) where approximately 50 % of the added eggs were detected. At higher egg counts a greater proportion of eggs (although not all) were detected. The regression relationship between the theoretical and actual egg counts was also examined. A log relationship was found to provide a better fit ($R^2 = 0.69$) than a linear one ($R^2 = 0.51$).

Discussion

The findings suggested that developmental success could be overestimated particularly in samples where FEC is lower than 500 epg. In the Perendale experiment

(Chapter 2), this could mean that developmental success in the Low FEC Line would often have been lower than that actually recorded. In other experiments, when FEC was higher than 500 epg in most animals, it is likely that there would be a smaller error in the FEC recorded, and the results for developmental success would be more precise. Obviously, where FEC did not differ between treatment groups, any error associated with the level of egg count would be comparable. A large variation between replicated faecal egg counts, especially for those at 500 epg or below has been reported previously (Brambell, 1963), reflecting sampling errors inherent in the method and this was also seen in the present experiment (see Figure B). In order to allow for this, wherever possible and particularly in the main experiments described in this thesis, estimates of FEC were based on multiple counts, usually six per animal sample.

A log relationship provided a relatively good fit ($R^2 = 0.69$) of the data. This might reflect that these were distributed according to a Poisson distribution as is often the case with data recorded as counts.

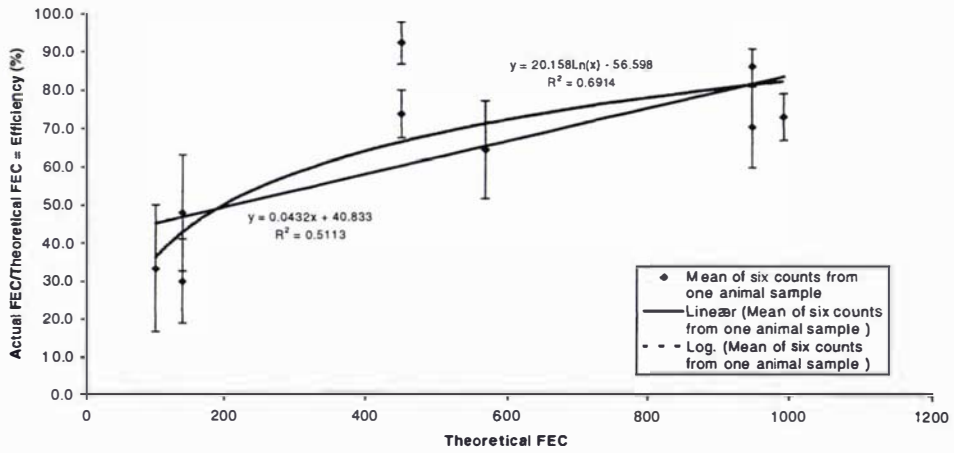

Figure B Sensitivity of the McMaster method in housed animals (Means ± S.E.)

EXTRACTION EFFICIENCY OF EGGS FROM FAECES FROM HOUSED VERSUS FROM OUTDOOR ANIMALS

Introduction

The consistency of faeces differs between outdoor and housed sheep with that of the latter being more fibrous and less dense. As this could have some effect on how well eggs can be detected in faeces from outdoor animals versus in faeces from housed animals, an experiment was carried out to investigate this.

Aim

To compare egg extraction efficiencies in samples from outdoor animals with samples from housed animals and to relate the resulting egg concentrations to those obtained with a modified McMaster method.

Methodology

FEC was first estimated using the modified McMaster method (see Appendix 2a) in 5 x 2g faecal samples from each of ten housed animals infected with *O. circumcincta* and in 5 x 2 g faecal samples from each of eight outdoor animals carrying mixed nematode infections. Then the contents of slides, faecal matter in sieve and faecal suspension left in the mixing bowl after taking the subsample for counting were used to recover the total number of eggs present in the faeces, using the method described in Appendix 5a. This gave an indication of the efficiency of the McMaster method in detecting the number of eggs actually present in the samples. In addition, faecal dry matters (%D.M.) were also measured as described in Appendix 2g.

Results

Results are presented graphically in Figure C below. Each data point in the figure represents the result from one animal sample, i.e. the mean FECx10/eggs recovered from one animal. The estimates of efficiency were found to be considerably more variable at lower FEC and on two occasions the McMaster method indicated a larger number of eggs than were subsequently recovered. There was a significant difference in %D.M. ($p<0.05$), with samples from outdoor animals showing a mean ($\pm$ S.E.) of 15.5 ± 1.5 and samples from housed animals a mean of 31.3 ± 0.9 but no significant correlation between extraction efficiency and %D.M.

Discussion

That the estimates of efficiency were considerably more variable at lower FEC probably reflects a variation inherent in the egg counting technique (Brambell, 1963). Given the relatively small number of samples examined and the above mentioned variability of the results at low FEC, the data obtained in this experiment must be interpreted with caution. However, within the limits of the experiment, there did not appear to be any difference in the efficiency of the McMaster method being used in the detection of eggs in faeces from housed and pastured animals.

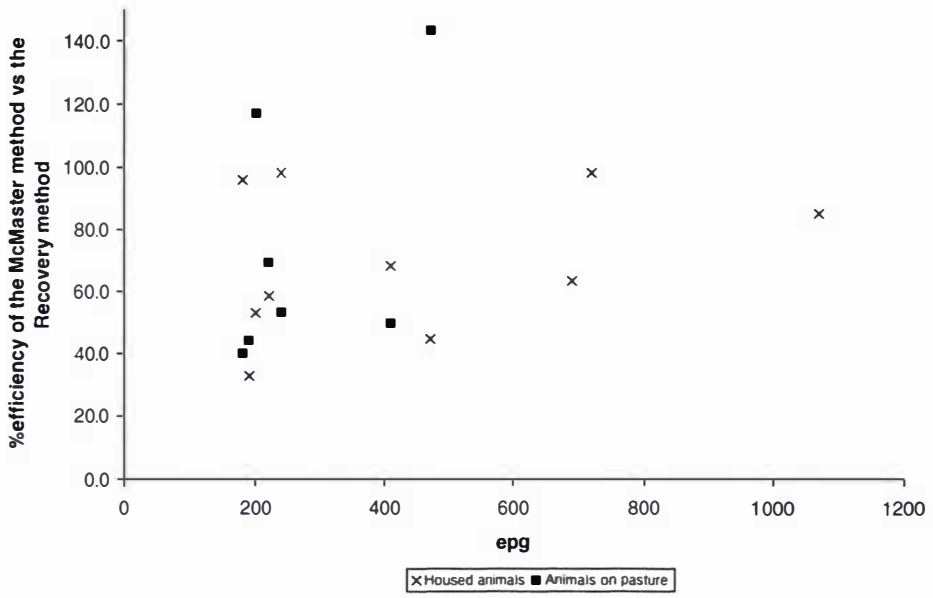

Figure C The Modified McMaster method compared with a total recovery method

Appendix 2k Modifications to the Culturing and Extraction Technique

Details of some preliminary experiments used for the development and assessment of the culturing method and the extraction of third stage larvae are given below

VARIATION IN DEVELOPMENTAL SUCCESS IN FAECAL CULTURES

Introduction

It was anticipated that there would be some variation in developmental success between replicates from individual animal samples. It was important to get an idea of the magnitude of this variation. A very large variation between within-animal (replicate) samples would mean than differences between animal samples and between treatment groups could be obscured.

Aim

The aim was to assess the level of variation in developmental success of eggs to infective larvae in faecal samples from outdoor animals.

Methodology

Faeces were collected from six Perendale ewes, all carrying mixed natural nematode infections. For each animal sample, six FEC were carried out (see Appendix 2a) and ten 5-g cultures were incubated at 25°C for 10 days (see Appendix 2b). Larvae were recovered in Baermann funnels and the developmental success calculated.

Results

The results for the mean developmental success in individual animal samples are presented in both Table B and in Figure D below, whereas FEC are presented only in Table B. Substantial variation was found between within-animal (replicate) samples, as reflected by the size of the standard errors. A large variation was also found between animal samples. However, within the limits of the experiment, there was no detectable relationship between the mean level of developmental success and either the variation between replicate cultures or differences in FEC.

Discussion

A major difficulty in this experiment was the need to use adult sheep with very low faecal egg counts. Ideally, it would have been carried out over a wider range of FEC and preferably with young, non-immune animals but circumstances precluded this. As FEC were below 500 epg in all samples, much of the variation recorded for developmental success, including that for within animal (replicate) samples, may be attributable to sampling errors inherent in the egg counting method (see Appendix 2j). The large variation between samples from different animals may, at least in part, be the consequence of sampling ewes with varying levels of immunity to parasites since there is

evidence that this can affect larval developmental success (Jorgensen *et al.*, 1998; see also Appendix 1c). Another contributing factor could be differences between animals in the proportions of eggs of various parasite genera if their eggs differed in ability to develop under the culture conditions used. Any such differences would potentially have a more marked influence on overall developmental success at low egg counts.

Although the experiment did not provide data that could be used to quantify the level of variation between replicate cultures that might be expected or its relationship to FEC, it did indicate that, particularly at low egg counts, the potential for variation was very substantial. It underlined the importance of replicating cultures as far as practicable in an attempt to moderate the effects of this. This was done throughout this study although the level of replication possible was limited by the practicality of processing large numbers of cultures. The results also indicated that the potential for variation was such that it could be difficult to detect subtle differences in developmental success between different treatments, particularly with small treatment groups.

	Tag 1095	Tag 523	Tag 1085	Tag 469	Tag 2971	Tag 3018
FECmean	75	116.5	25	8.3	8.3	75
%Dev.Succ.	1.9	3.7	12.2	15.1	20.5	24.8
Std.dev.	1.6	2.6	5.5	9.0	14.6	22.5
S.E.	0.5	0.8	1.7	2.9	4.6	7.1
Variance	2.5	6.6	30.5	81.5	213.6	505.8
CV (%)	**83**	**70**	**45**	**60**	**71**	**91**

Table B Variation in developmental success in samples from Perendale ewes.
C.V. = coefficient of variation = standard deviation/%dev.succ.; S.E. = standard errors

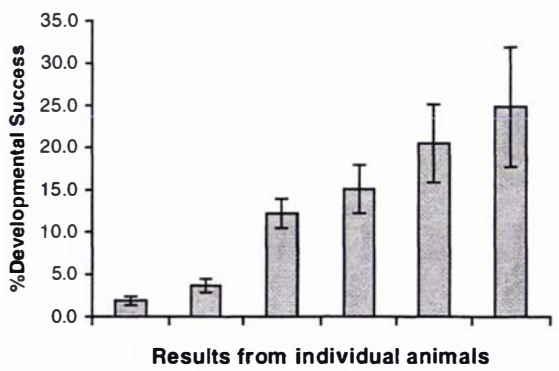

Figure D Variation in developmental success in samples from Perendale ewes (Means ± S.E.).

THE EFFECT OF COLD STORAGE ON SUBSEQUENT DEVELOPMENTAL SUCCESS

Introduction

In the Perendale experiment (Chapter 2), the large number of samples on each sampling occasion meant that processing all these on the same day as sampling was at times a problem. Logistical problems at the beginning of this trial meant that on one occasion, samples had been stored at 4°C for four days. This was unfortunate as this prolonged cold storage was expected to have some effect on the subsequent developmental success of eggs. Some faecal cultures were found to contain *H. contortus*, although usually in small proportions. Eggs of *H. contortus* are known to be particularly sensitive to storage at lower temperatures (McKenna, 1998) and would the first genus to be affected by a prolonged storage at 4°C.

Aim

The aim of this experiment was to assess the effect of prolonged storage of eggs in faecal samples at 4°C, on the subsequent developmental success in either mixed nematode infections or in infections with *H. contortus*.

Methodology

Faeces collected from three Perendale ewes carrying mixed nematode infections were stored at 4°C for up to six days. Daily, from the day of collecting the samples and for up to 6 days, FEC were performed in duplicate (Appendix 2a) and 5-g faecal cultures set up in duplicate (Appendix 2b) from each of the animal samples. Results are presented in Figure E below, where each point represents the mean of two counts.

Faecal samples were also taken from a housed lamb infected with *H. contortus*. Samples were treated as described above, but in addition to storing samples at 4°C, each individual sample was split into halves, with one half being stored at 4°C and the other half at 10°C. Results are presented in Figure F below.

Results

In mixed infection samples, there was a decline in developmental success already after one or two days of storage at 4°C, after which the developmental success remained approximately the same for the days to follow.

In samples from the *H. contortus* infected animal, there was a dramatic decrease in developmental success already after one day of storage in those stored at 4°C, whereas for those stored at 10°C, developmental success decreased substantially only after two days of storage.

Discussion

These two experiments underlined the importance of processing faecal samples as soon as possible after sampling in order to obtain reliable results for developmental success. In practice, this meant setting up cultures on the same day as taking the faecal samples. In the Perendale experiment (Chapter 2), cultures were set up on the day of sampling, starting from January 1997. In all other experiments, cultures were always set up on the day of sampling.

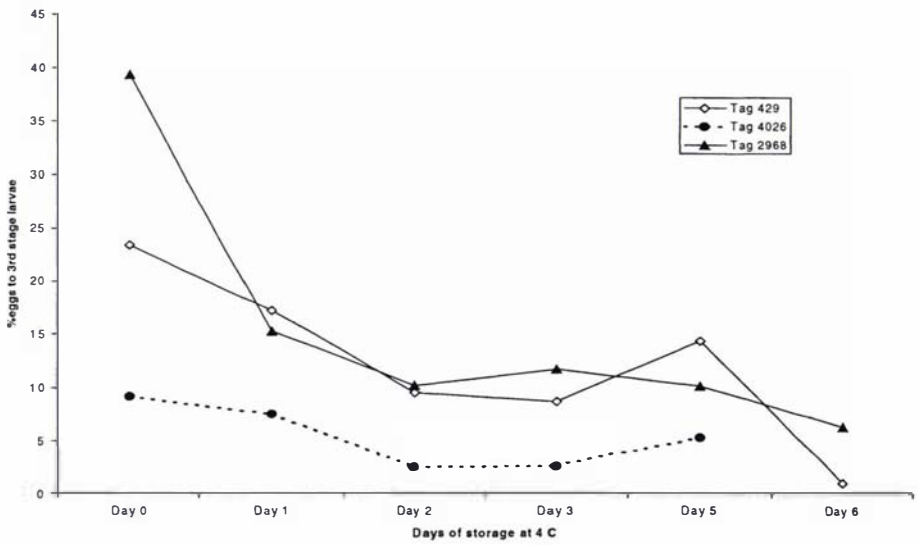

Figure E Developmental success in samples from Perendale ewes

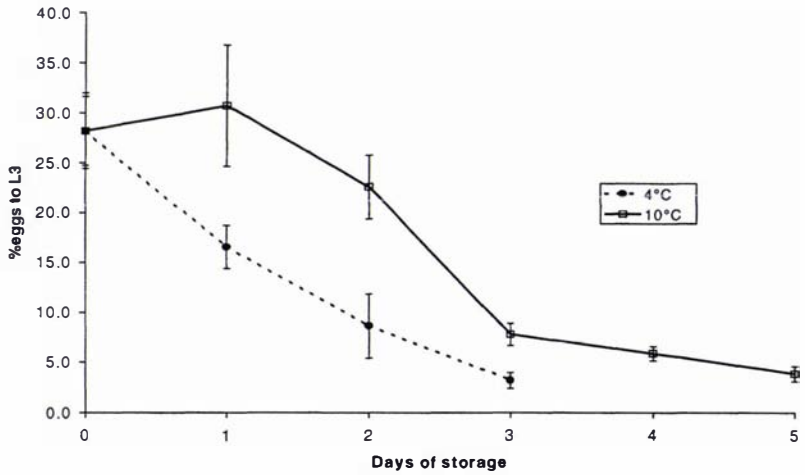

Figure F The Effect of Cold Storage on the Developmental Success of *Haemonchus contortus* eggs

THE EFFECT OF CULTURE SIZE ON SUBSEQUENT DEVELOPMENTAL SUCCESS

Introduction

Five-gram faecal cultures had been used in a previous experiment (Jorgensen *et al.*, 1998). However, as some animals in the Low-FEC Line had very low faecal egg counts and the resulting number of larvae would therefore be too low to perform larval identification, it was decided to change to larger culture sizes.

Aim

The aim of this experiment was to assess the possible effect of changing culture sizes from 5 to 10 g of faeces.

Methodology

Faecal samples were taken from a housed lamb infected with *T. colubriformis*. FEC were estimated in three counts. Ten 5-g (±1.0 g) and ten 10-g (±1.0 g) larval cultures were set up as described in Appendix 2b. Larvae were extracted and counted to obtain results for developmental success.

Results

Results are presented in Table C. The size of the faecal culture did not significantly ($p<0.14$) affect the percentage development of eggs to 3rd stage larvae.

Discussion

The culture size was changed from 5 to 10 g, starting from the November 1996 sampling in Chapter 2, and throughout the rest of the study.

Culture	Weight	FECmean	%egg to L3		Culture	Weight	FECmean	%egg to L3
1	10.4	200	4.6		1	5.2	200	109.6
2	10.5	200	62.4		2	5.2	200	123.6
3	9.9	200	100.0		3	5.7	200	34.2
4	9.9	200	80.1		4	5	200	55.0
5	10.1	200	37.4		5	5.5	200	55.0
6	10.9	200	15.8		6	5.8	200	71.1
7	9.8	200	99.7		7	5.7	200	84.6
8	10.9	200	91.7		8	5.3	200	108.0
9	10	200	78.3		9	5.8	200	101.3
10	10.1	200	60.9		10	5.8	200	107.3
		mean=	63.1				mean=	85.0
		S.D.	33.9				S.D.	29.7
		S.E.	10.7				S.E.	9.4

Table C The effect of culture size on developmental success

THE EFFECT OF ADDING VERMICULITE ON SUBSEQUENT DEVELOPMENTAL SUCCESS

Introduction

Vermiculite is routinely added when culturing large amounts of faeces, and is thought to ensure that enough oxygen is available for the further development of eggs.

Aim

The aim was to test whether adding Vermiculite to the faeces before culturing would increase developmental success.

Methodology

Faecal samples were taken from a housed lamb infected with *T. colubriformis*. FEC were estimated in three counts. Ten 10-g (±1.0 g) larval cultures with vermiculite added and ten without vermiculite added were set up as described in Appendix 2b, except that faeces were mashed before adding vermiculite. Larvae were extracted and counted to obtain results for developmental success.

Results

Results are presented in Table D. Adding Vermiculite to 10-g faecal cultures containing *T. colubriformis* eggs did not significantly increase the number of 3rd stage larvae recovered ($p<0.12$).

Discussion

It was decided to culture faeces without adding Vermiculite. Not having to add Vermiculite to cultures meant that it was possible to set up all cultures on the same day as sampling, as the processing time had been shortened dramatically.

Culture	FECmean	+ Vermiculite	No Vermiculite
1	200	106.7	43.5
2	200	50.2	34
3	200	39.1	41.5
4	200	83.3	20.9
5	200	31.4	40.8
6	200	79	32
7	200	39.6	37.2
8	200	24	57
9	200	52.5	57.7
10	200	39.7	32.3
	Mean=	54.6	39.7
	S.E.	8.4	3.6

Table D The effect of adding Vermiculite to cultures

EXTRACTION EFFICIENCY IN THE BAERMANN FUNNELS

Introduction

It was of interest to find out how faeces should be prepared after incubation, if one wanted to extract a maximum number of larvae. A time effective method was desirable, as large numbers of samples had to be dealt with in the experiments described in most chapters.

Aim

The aim of this experiment was to investigate how sheep faeces should be processed after incubation, in order to most efficiently recover larvae in the Baermann funnels.

Methodology

Pelleted faeces from a housed lamb infected with *T. colubriformis* were cultured in 10-g cultures (Appendix 2b). A total of 30 cultures were set up. FEC were also estimated. After incubation, the faecal pellets were treated in three different ways before extracting larvae in the Baermann funnels. In ten cultures the pellets were left whole, in ten cultures they were halved (=divided into two to three smaller parts) and in the remaining ten cultures the pellets were mashed. Larvae were extracted and counted to obtain results for developmental success.

Results

The results are presented in the Table E. Leaving faecal pellets whole, resulted in a significantly lower recovery rate ($p<0.05$) than in the other two treatments, as judged by a lower developmental success, whereas there was no significant difference ($p=0.47$) between halving or mashing the faecal pellets.

Discussion

The results suggested that pellets should be either halved or mashed before extracting larvae in Baermann funnels. As large numbers of samples were dealt with in most chapters, pellets were halved rather than mashed, as the former procedure was less time consuming and not inferior to the latter.

Culture	FEC	Whole pellets	Halved pellets	Mashed pellets
1	833	38.7	70.7	109.6
2	833	34.8	87.2	97.2
3	833	27.2	71.6	34.8
4	833	45.8	85.3	75.3
5	833	33.7	77.3	41.7
6	833	29.9	61.3	53.4
7	833	31.3	57.4	36.7
8	833	29.8	22.6	70.9
9	833	29.2	64.8	19.1
10	833	34.7	57.4	41.2
	Mean	33.5	65.6	58.0
	St.dev	5.5	18.4	29.3
	S.E.	1.7	5.8	9.3

Table E The effect of various degrees of breaking up pellets when extracting L3s in Baermann funnels

Appendix 21 Data from Chapter 2

PERENDALE EWES

PERENDALE EWES, sampled 21/8 - 96																						
High FEC Line										Total	Low FEC Line											Total
Tag	FEC	FEC	Mean	Dev.Succ.	Haem	Ost	Trich	Coop	L.T.	id.'ed	Tag	FEC	FEC	Mean	Dev.Succ.	Haem	Ost	Trich	Coop	L.T.	id.'ed
409	0	0	25	10.6						0	437	0	100	50	4.8						0
409	100	0	25	8.3						0	437	150	0	50	3.3						0
409	0	50	25	9.3						0	437	0	50	50	2.7						0
429	50	200	108	17.6						0	489	100	100	133	10.4						0
429	150	100	108	14.4						0	489	150	100	133	13.9						0
429	50	100	108	5.8						0	489	100	250	133	11.0						0
469	300	250	208	18.0	3	65	2	30	0	100	523	50	100	75	10.5						0
469	150	200	208	11.5							1008	0	0	8	3.9						0
469	200	150	208	16.6	11	48	0	41	0	100	1008	0	0	8	6.6						0
479	50	50	142	1.8						0	1008	50	0	8	0.0						0
479	200	100	142	1.7						0	1018	0	0	25	142.3	3	66	0	31	0	100
479	400	50	142	3.6						0	1018	0	100	25	117.8	3	79	2	16	0	100
498	200	100	200	12.0	6	26	2	54	12	50	1018	50	0								
498	50	200	200		4	14	0	72	10	50	1039	900	400	650	1.1						0
498	350	300	200	11.9							1043	0	0	16	8.9						0
504	450	250	290	0.7						0	1043	50	0	16	7.4						0
504	200	150	290	6.4						0	1043	0	50	16	12.1						0
504	400		290	6.1						0	1057	50	100	45	4.6						0
1015	750	900	733	3.8						0	1057	150	100	45	0.4						0
1015	850	250	733	1.5						0	1057	0	250	45	1.6						0
1015	700	950	733	18.3	3	33	1	46	17	100	1091	850	250	450	2.0						0
2207	0	0	8	1.8						0	1091	650	50	450	2.4						0
2207	0	0	8	31.7						0	1095	50	0	16	10.8						0
2207	0	50	8	23.6						0	1095	0	0	16	30.6						0
2251	400	400	263	9.6	4	28	0	60	8	50	1095	0	50	16	20.1						0
2251	150	100	263	21.7	7	22	14	48	9	100	1107	250	150	200	7.3	2	32	0	66	0	50
2263	600	250	458	30.3	1	52	2	45	0	100	2915	0	100	58	6.1						0
2263	500	600	458	11.8	4	62	0	32	2	50	2915	0	100	58	4.3						0
2263	300	500	458	2.1						0	2915	50	100	58	0.0						0
2266	200	100	133	0.5						0	2971	800	100	392	3.7						0
2266	50	100	133	3.4						0	2971	300	250	392	2.6						0
2942	200	150	83	9.3						0	2971	450	450	392	3.2						0
2942	150	100	83	6.9						0	2980	50	50	75	0.3						0
2942	50	50	83	5.1						0	2980	200	50	75	1.6						0
2965	0	150	175	4.6						0	2980	50	50	75	0.5						0
2965	150	250	175	4.2						0	3008	0	50	33	0.0						0
2965	100	300	175	7.6						0	3008	50	50	33	11.9						0
2968	150	100	400	13.4						0	3008	50	0	33	0.6						0
3018	1200	950	1260	2.2						0	3190	200	100	133	1.8						0
3018	1050	1300	1260	11.8	7	19	0	74	0	100	3190	100		133	2.3						0
3018	1800		1260		0	16	0	84	0	50	4054	100	150	92	1.2						0
3056	1250	1050	1608	1.4						0	4054	50	100	92	1.3						0
3056	1400	1500	1608	1.6	8	8	0	82	2	50	4054	150	0	92	0.0						0
3056	1550	1650	1608	3.0						0	4110	100	50	158	0.1						0
3085	1250	200	1058	0.2						0	4110	100	250	158	0.0						0
3085	900	1400	1058	1.3							4110	350	100	158	0.0						0
3085	1400	1200	1058								4172	200	200	200	2.5						0
3140	900	800	708	5.7	2	20	0	60	18	50	7035	250	300	250	2.6						0
3140	450	250	708	1.6						0	7035	350	200	250	44.0	16	78	0	4	2	50
3140	600	1300	708	2.7	8	10	0	80	2	50	7035	200	200								
3165	0	50	100	0.2						0	7047	100	100	75	8.6						0
3165	150	250	100	2.9						0	7047	0	100	75	2.6						0
3165	100	50	100	14.9	8	14	0	78	0	50	7047	100	50	75	2.3						0
4026	2700	1850	2550	5.3						0	9203	50	50	83	6.2						0
4026	3250	2300	2550	0.5						0	9203	100	100	83	3.3						0
4026	2750	2450	2550	6.5	0	19	1	80	0	100	9203	50	150	83	8.7						0
4065	0	0	8	13.6						0	9238	0	50	25	13.0						0
4065	50	0	8	11.1						0	9272	500	700	775	1.1						0
4065	0	0	8	1.9						0	9272	1200	700	775	0.6						0
4097	0	0	42	2.9						0	9278	0	0	8	4.9						0
4097	50	100	42								9278	50	0	8	2.5						0
4097	0	100	42								9278	0	0	8	5.1						0
4165	0	100	50	5.1						0											
4165	100	0	50	2.6						0				135.7	9.8						
4165			50	7.1						0						6	63	0.5	29.25	0.5	
4205	550	650	450	0.8						0											
4205	500	200	450	0.9						0											
4205	200	600	450	1.7						0											
7112	100	200	192	7.3						0											
7112	250	150	192	5.3						0											
7112	150	300	192	6.8						0											
7135	150	150	200	27.2	0	3	3	94	0	100											
7136	300	100	200	22.6	0	2	2	96	0	100											
7135	400	100	200	17.9	0	0	0	100	0	100											
9309	50	0	25	22.0						0											
9309	50	0	25	20.8						0											
9309	0	50	25	8.6						0											
			431.6	8.5																	

PERENDALE EWES , sample date: 17/9 - 1996

High FEC Line

Tag	FEC	FEC	Mean	dev.succ.	Haem	Ost	Trich	Coop	L.T.	Total Id.'ed
409	0	0	17	9.1				1		1
409	50	50	17	17.6						0
409	0	0	17	12.7						0
429	0	0	8	4.6						0
429	0	0	8	4.7						0
429	50	0	8	25.9		3		3		6
469	50	0	8	22.1		3	1	20		24
469	0	0	8	16.2				2		2
479	150	100	100	3.2						0
479	50	150	100	6.9				2		2
479	0	150	100	6.4						0
498	50	0	50	87.5	1	6		40	2	50
498	150	100	50	35.8		8		12	1	21
498	0	0	50	42.8	1	11		16	1	29
504	300	200	208	6.8		23		1		24
504	150	250	208	9.7	2	68		10	6	86
504	300	50	208	9.6		41		7	2	50
1015	300	0	150	8.0		12	1	37		50
1015	150	200	150	17.0		1		1		2
2207	0	100	33	9.4		6		5		11
2207	0	100	33	11.3		2		1		3
2207	0	0	33	3.0						0
2251	300	600	425	5.4		27	19	52	2	91
2251	650	350	425	9.9		19		30	1	50
2251	200	450	425	8.8						
2263	250	0	67	186.6	2	53	2	43	0	100
2263	50	0	67	107.0	4	14	1	26	5	50
2263					1	40	0	52	7	100
2266	0	0	25	11.4						0
2266	0	50	25	0.0						0
2266	0	100	25	16.6		7		5		12
2942	0	100	58	0.0				4		4
2942	50	50	58	4.3						0
2942	100	50	58	4.2						
3018	0	100	75	62.9	1	2	0	97	0	100
3018	100	100	75	34.5	12	5		61		78
3018	0	150	75	64.9	1	4	0	95	0	100
3045	50	50	67	20.3	3	11		36		50
3045	50	50	67	15.7	12	13	1	7		33
3045	50	150	67	64.6	14	13	1	21	1	50
3140	250	300	167	9.4		12	1	33		46
3140	100	0	167	7.9	1	8		41		50
3140	200	150	167	15.0		6	1	38	5	50
3165	50	0	42	6.5						0
3165	50	100	42	5.0				5		5
3165	50	0	42	0.4						0
4026	500	150	433	7.3	1	4	1	94	0	100
4026	500	650	433	18.0		1		11		12
4026	500	300	433	2.0		4		4		8
4097	0	50	58	42.6	3	17		29	1	50
4097	100	0	58	44.2	1	11		10		22
4097	200	0	58	26.8						0
4205	0	100	50	36.5	2	4		2		8
4205	0		50	59.2			1	49		50
4205	100		50	60.4	1	5	1	43		50
7112	350	750	467	0.5				1		1
7112	300	350	467	0.3						0
7112	300	750	467	0.4		1		2		3
		Mean	128	23.3						

Low FEC Line

Tag	FEC	FEC	Mean	dev.succ.	Haem	Ost	Trich	Coop	L.T.	Total Id.'ed
489	500	150	367	3.7	17	17	3	13		50
489	400	350	367	9.2	9	11	2	28		50
489	400	400	367	13.2	8	34	2	56	0	100
523	200	0	200	2.9		10	1	16		27
523	50	50	200	3.8			10	29	1	40
523	200	200	200	6.1		14	11	54	1	80
1018	450	50	167	7.6		5		14		19
1018	200	100	167	8.0		5		18		23
1018	100	100	167	6.3				7		7
1039	100	50	92	0.0						0
1039	150	100	92	1.0						0
1039	150	0	92	1.3		5	1			6
1057	150	250	225	3.6	10	9		2	3	24
1057	250	300	225	1.8						0
1057	200	200	225	2.3	1	11		5		17
1085	50	50	25	12.5						0
1085	50	0	25	13.6		1	7	8		16
1085	0	0	25	10.2		5	2	3		10
1095	100	0	75	3.7		3		2		5
1095	100	50	75	5.5		1		2	2	5
1095	100	100	75	1.9	1	1		1	1	4
1107	200	100	167	2.8		2		5	1	8
1107	150	200	167	0.5				1		1
1107	150	200	167	0.8						0
2303	0	0	8	0.0						0
2303	0	50	8	24.0		5	1	4		10
2303	0	0	8	2.5						0
2971	0	0	8	18.9		3		2		5
2971	50	0	8	57.5		3	3	9		15
2971	0	0	8	0.0						0
2980	0	0	17	9.0						0
2980	50	0	17	9.3				5		5
2980	50	0	17	8.2				3		3
3008	0	50	8	2.4						0
3008	0	0	8	0.0						0
3008	0	0	8	4.5						0
3131	50	50	33	0.0						0
3131	0	50	33	5.3		1		4		5
3131	0	50	33	7.2	2	4		2		8
3190	100	200	125	2.4		1		3		4
3190	100	250	125	4.4	3	4		20		27
3190	50	50	125	0.0						0
4110	100	0	17	39.2		1		5		6
4110	0	0	17	59.9		5		18		23
4110	0	0	17	1.1						0
4172	150	0	33	7.6		1		3		4
4172	50	0	33	6.1	1			1		2
4172	0	0	33	5.5		1		4		5
7047	50	50	50	16.2	6	6	1	25		38
7047	0	150	50	11.5						0
7047	50	0	50	7.5		3		8		11
9203	50	0	25	0.8						0
9203	50	0	25	4.5		2		1		3
9203	50	0	25	8.0		3		1		4
9238	0	0	8	2.6				1		1
9238	0	0	8	5.0				2		2
9238	0	50	8	18.5				2		2
9272	400	350	367	0.5		1		3		4
9272	350	450	367	0.4						0
9272	450	200	367	0.7		1		5		6
9278	0	0	8	2.3						0
9278	50	0	8	9.6				4		4
9278	0	0	8	22.7						0
		Mean	96	8.1						

PERENDALE EWES, sample date: 14/10 - 1996																					
High FEC Line										total	Low FEC Line										total
Tag	FEC	FEC	Mean	dev.succ.	Haem.	Ost.	Trich.	Coop.	LT.	id.'ed	Tag	FEC	FEC	Mean	dev.succ.	Haem.	Ost.	Trich.	Coop.	LT.	id.'ed
409	400	250	333	5.7	0	42	1	7	0	50	437	50	0	75	4.2		1		7		8
	250	300	333	6.1	0	38	1	11	0	50		50	50	75	1.3		1				1
	450	350	333	5.8	6	39		28	2	75		250	50	75	5.1		1		12		13
429	250	100	125	20.0		62			3	65	489	50	100	67	4.2		2	1	3		6
	150	50	125	16.8		24		1	2	27		0	50	67	6.9		7	1	10		18
	50	150	125	3.7		21		2		23		50	150	67	7.6		5		11		16
469	0	50	33	21.1	1	3		17	1	27	523	50	50	67	2.5				2		2
	0	100	33	25.6		5		3	7	15		50	100	67	5.6	1	3		8		12
	0	50	33	21.8		6	3	4	3	16		50	100	67	3.7		4		9		13
479	150	300	192	1.4		6				6	1018	50	150	67	3.8						0
	100	300	192	1.0		1		2		3		0	200	67	8.1		1		1		2
	150	150	192	0.3						0		0	0	67	36.1		36	1	47	4	88
498	0	150	125	7.2		7		19	4	30	1039	50	50	42	3.2				2		2
	200	100	125	5.9	1	11	1	20	9	42		0	0	42	2.4				2		2
	300	0	125	40.6	1	7	1	53	38	100		0	150	42	3.0		5		2		7
504	300	250	258	30.2	0	38	1	6	5	50	1085	50	0	17	4.6		3		1		4
	200	400	258	23.9	5	68	3	8	16	100		0	50	17	17.2		4		6		10
	100	300	258	24.9	1	89	0	1	9	100		0	0	17	33.2		9		5		14
1015	350	350	383	19.8	0	24	0	23	3	50	1095	0	50	8	38.4		11	1			11
	350	250	383	14.7	2	56	0	24	18	100		0	0	8	28.1		3		2		5
	600	400	383	8.7		4		4	4	12		0	0	8	28.8		9		1		10
2207	100	150	150	14.0		28	1	2	6	37	1107	100	0	67	6.7		1	1	12	5	19
	50	100	150	16.2		75	2	13	9	100		50	0	67	3.4		3	1		3	7
	300	150	150	4.8		13		6	6	25		100	150	67	54.9	0	90	0	10	0	100
2251	350	200	292	7.4						0	2303	50	0	25	3.8		1	1	3		5
	150	250	292	7.0	0	45	3	2	0	50		100	0	25	11.8		6		4		10
	300	500	292	3.0		20				20		0	0	25	1.3						0
2263	100	300	300	9.3	0	38	2	9	1	50	2915	50	0	25	5.9		2				2
	650	300	300	9.0	1	66		15	1	83		0	100	25	22.5		4	1	10		15
	100	300	300	39.9	2	71	1	25	1	100		0	0	25	27.7		3		20		23
2266	50	50	25	2.4		3				3	2971	0	150	58	7.1		5		6		11
	0	50	25	1.4		1			1	2		50	50	58	15.2		17	1	5		23
	0	0	25	0.7		1				1		100	0	58	9.4		13		3		16
2965	100	100	83	3.2		2			5	7	2980	0	0	33	0.0		1		1		2
	100	0	83	3.7		3		4	1	8		100	50	33	1.7		2				2
	150	50	83	0.8						0		50	0	33	4.5			1	2		3
3018	350	700	558	7.4	0	32	0	66	2	100	3008	0	0	17	1.1				1		1
	450	650	558	9.1	1	13	0	36	0	50		0	50	17	1.1				1		1
	800	400	558	7.8	1	22	0	75	2	100		0	50	17	2.1						0
3045	50	50	25	14.4		6		5		11	3124	0	100	50	1.1				1		1
	0	50	25	25.4	2	8	1	9		20		50	50	50	0.4						0
	0	0	25	36.9	5	16		25	2	48		50	50	50	0.0						0
3056	250	250	267	2.7		3		14		17	3131	50	50	17	0.0						0
	250	400	267	6.2	0	12	1	37	0	50		0	0	17	0.0						0
	200	250	267	7.8	1	22	2	68		93		0	0	17	1.1		1				1
3140	1650	1200	1433	2.8				5		5	3190	0	100	58	5.3		2		15		17
	1350	1050	1433	0.8	3	11		24	1	39		0	100	58	3.4				2		2
	1800	1550	1433	1.8	0	14	1	35	0	50		50	100	58	5.1		2		6		8
3165	0	50	50	11.0		3		5		8	4110	0	100	42	8.6		2		1	1	4
	0	150	50	4.0		1		3		4		50	0	42	9.7		1	1	4		6
	50	50	50	3.5								50	50	42	18.0	1	19	1	11		32
4026	350	ns	483	16.2	1	4	0	95	0	100	4172	150	150	117	3.0		1		16		17
	600	ns	483	13.4	1	32	1	65	1	100		50	150	117	2.0		1		9		10
	500	ns	483	8.6		5		30		35		100	100	117	6.5		2	1	20		23
4097	250	300	300	5.4		1		1		2	7047	0	150	92	0.2				1		1
	450	150	300	3.8		20		6		26		50	100	92	0.6		1		2		3
	250	400	300	4.3	3	28		4		35		150	100	92	8.7		4		37		41
4205	250	600	492	0.7		2		6		8	9203	50	0	25	26.0		12	1	13	1	27
	400	650	492	1.3		2		19		21		50	0	25	1.5						0
	350	700	492	0.8		3		6		9		0	50	25	21.1		11		9	1	21
7112	350	200	150	1.1		3		2		5	9238	50	0	8	11.5	1	3		1		5
	350	300	150	0.2		1		1		2		0	0	8	6.1		1				1
	550	150	150	1.1				6		6		0	0	8	5.0				1		1
9286	300	100	92	1.7		6		3		9	9272	450	250	392	2.1		10	1	1		12
	50	0	92	3.9		1		1		2		250	500	392	4.1	1	29	0	20	0	50
	50	50	92	5.1				15		15		400	500	392	2.4		5		7		12
											9278	0	0	8	2.4						0
		Mean	280	9.7								0	50	8	2.5				1		1
																	50		2	1	53
													Mean	61	8.6						

PERENDALE EWES, sampled 18/11 1996																					
High FEC Line										total	Low FEC Line										total
Tag	FEC	FEC	Mean	Dev.Succ.	Haem.	Ost.	Trich.	Coop.	L.T.	Id.'ed	Tag	FEC	FEC	Mean	Dev.Succ.	Haem.	Ost.	Trich.	Coop.	L.T.	Id.'ed
409	100	50	67	12.4		10		15	11	36	489	100	200	150	12.9	3	82	1	13	1	100
	50	50	67	15.1		17		10	2	29		150	200	150	12.4	2	69	5	6	18	100
	100	50	67	13.8	0	19	0	17	14	50		0	250	150	15.8	0	42	0	6	2	50
429	0	50	33	2.1				1		1	523	0	50	42	4.6	1	7		5		13
	0	100	33	0.3				1		1		0	150	42	7.9				1		1
	50	0	33	3.9		9				9		0	50	42	4.2		3		4	2	9
469	50	0	33	23.9		5		7	4	16	1018	0	200	117	9.9	0	5	0	45	0	50
	50	0	33	36.5		3		12	7	22		100	100	117	8.0		9		12		21
	50	50	33	56.1	0	6	0	15	29	50		50	250	117	4.3		3		4		7
479	0	50	50	15.2		25		12		37	1039	150	100	75	11.0	0	26	1	23	0	50
	0	100	50	11.5		11		10		21		150		75	11.2	0	11	2	37	0	50
	100	50	50	2.0		4		2		6		50		75	3.7		15		7	1	23
498	50	0	42	6.1		2		2		4	1043	0	50	8	22.8		2		10	1	13
	50	50	42	4.0		6		2		8		0	0	8	34.4		2		3	20	25
	50	50	42	3.2				3		3		0	0	8	132.5	0	9	0	41	0	50
504	250	150	150	5.8	0	25	0	21	4	50	1057	350	100	275	6.2	3	28		1		32
	0	200	150	20.1	0	22	2	34	42	100		300	250	275	1.3		14		6		20
	100	200	150	8.7		10		8	5	23		250	400	275	3.1	0	47	0	3	0	50
1015	600	550	483	4.8	0	19	0	28	3	50	1085	50	0	17	1.1						0
	400	600	483	2.5	0	24	0	26	0	50		0	0	17	3.4		4		1		5
	150	150	483	5.8	0	23	0	72	5	66		0	50	17	2.3		1		1		2
2207	100	50	83	8.2		7		11		18	1091	50	0	17	31.2		5		1	18	24
	50	100	83	8.0		29		10		39		0	0	17	30.3		34				34
	150	50	83	1.2		5		1		6		50	50	17	26.9		18		2	13	33
2251	800	700	492	8.0		12		16	3	31	1095	850	550	725	4.0	1	92	7	0	0	100
	450	350	492	8.2	0	17	2	20	11	50		1050	800	725	0.6		20	1	4		25
	200	450	492	3.0	1	23	0	13	13	50		550	550	725	1.9	3	44	0	3	0	50
2263	750	450	470	5.6	0	22	0	27	1	50	1107	150	250	200	3.6	0	21	0	29	0	50
	250	500	470	3.8	0	14	0	35	1	50		350	150	200	2.7				4	1	5
	400		470	3.1	0	12	2	32	4	50		200	100	200	4.5		15		10		25
2266	0	0	17	4.3		3		2	1	6	2303	50	0	8	22.5		1		1	6	8
	0	100	17	2.9		1		3		4		0	0	8	35.0		2		11	7	20
	0	0	17	25.1	1	10		10	9	30		0	0	8	18.4		2			3	5
2942	50	200	92	13.4	0	22	0	23	5	50	2915	0	100	33	1.7		1		1	1	3
	50	100	92	20.2	0	9	0	33	8	50		100	0	33	3.5		3				3
	50	100	92	22.5	0	8	0	42	0	50		0	0	33	1.7		1		2	1	4
2965	200	100	183	14.7	1	33	0	66	0	100	2971	150	250	250	2.6		28	1	6		35
	200	300	183	2.7		8		16		24		250	350	250	4.1	0	53	2	43	2	62
	150	150	183	2.7		8		9		17		250	250	250	18.0	0	91	4	4	1	100
2968	650	300	533	19.5	0	85	5	9	1	100	2980	50	0	17	1.2				1		1
	450	700	533	22.0	1	88	8	3	0	100		50	0	17	5.6		1		3	3	7
	500	600	533	24.9	1	90	7	2	0	100		0	0	17	4.2		1		1	2	4
3018	650	550	788	11.9	0	18	0	80	2	100	3008	0	0	8	6.2		1		2	1	4
	900	1050	788	11.7	0	17	0	77	6	100		0	0	8	5.2				1		1
	1050		788	10.7	3	9	0	88	0	100		50	0	8	13.5				5		5
3045	100	150	117	9.8	0	27	0	23	0	50	3124	50	50	33	1.5		1		4		5
	200	0	117	2.4		6				6		50	0	33	3.4		5	1	4		10
	250	0	117	34.0	0	57	3	38	2	100		0	50	33	2.4		3		2		5
3056	450	550	467	0.9		2		15		17	3131	50	0	17	1.1				1		1
	450	400	467	13.7	1	15	0	84	0	100		0	0	17	16.9		14		11		25
	600	350	467	1.3		3		19		22		0	50	17	7.6		5		9		14
3085	300	900	833	12.1	4	11	0	85	0	100	3190	100	0	17	24.4				3		3
	900	800	833	28.4	2	6	1	91	0	100		0	0	17	11.4		4		1		5
	1300	800	833	30.3	1	7	0	91	1	100		0	0	17	11.2				9		9
3140	700	1050	900	2.4	0	25	0	7	18	50	4075	50	0	17	5.4		4		2		6
	550	1250	900	1.2	1	20	0	24	5	50		50	0	17	24.2		11				11
	800	1050	900	2.2	0	17	1	80	2	100		0	0	17	18.1		4		9		13
3165	150	50	150	2.0		5		10		15	4110	50	0	42	5.3		9		1		10
	150	150	150	4.0		5	3	21		29		0	50	42	2.9						0
	200	200	150	2.4		1		6		7		50	100	42	2.3		1		1		2
4026	300	500	517	14.5	0	13	0	87	0	100	4172	150	50	100	5.3		1		20		21
	500	650	517	12.6	0	7	0	93	0	100		100	50	100	6.4		9		14		23
	650	500	517	2.4		2		1		3		100	150	100	11.5	1	17	1	17		36
4065	50	50	83	19.2	0	2	0	69	29	100	7035	50	0	17	21.9		7		10	15	32
	150	100	83	15.2	1	11	0	19	19	50		0	0	17	7.4		1		4	2	7
	100	50	83	19.0	1	2	0	48	49	100		0	50	17	22.1		7	1	7		15
4097	100	150	175	29.7	0	16	0	24	10	50	7047	100	50	150	2.1		4		3		7
	100	50	175	11.5		17		9	8	34		100	150	150	0.5		1				1
	300	350	175	18.1	0	16	0	28	6	50		200	300	150	9.5		22		6	3	31
4165	350	350	508	7.6	0	75	1	9	15	100	9203	0	0	8	3.6		1				1
	500	600	508	20.6	1	55	0	25	29	100		0	50	8	3.7				2		2
	700	550	508	10.7	0	52	1	9	38	100		0	0	8	4.9		2		1		3
4205	150	250	350	17.2	0	36	0	45	19	100	9238	0	0	8	3.8		3				3
	550	300	350	4.1		5		11	4	20		0	0	8	25.3		4		5		9
	500	350	350	16.2	1	8	0	91	0	100		50	0	8	6.9		1		1		2
7112	750	1100	633	7.9	0	83	3	13	1	100	9272	200	250	300	0.6		1		9		10
	650	450	633	2.3	0	31	0	15	4	50		350	300	300	0.4		5		3		8
	450	400	633	9.4	1	36	3	57	3	100		350	350	300	0.2		3	3	1		4
9286	0	0	8	115.3		26		2		28											
	0	0	8	84.6	1	11		5		17			Mean	102	10.7						
	50	0	8	45.7		16		2		18											
9309	0	0	17	24.0		23		5		28											
	100	0	17	7.8		5		2		7											
	0	0	17	6.0		4				4											
		Mean	296	14.1																	

PERENDALE EWES, sampled 6/1 1997

High FEC Line

Tag	FEC	FEC	Mean	dev.succ.	Haem.	Ost.	Trich.	Coop.	LT.	nr. id'ed
469	50	0	8	312.0				2	98	100
	0	0	8	168.3		1	2	1	46	50
	0	0	8	81.3				2	20	22
498	0	0	8	52.9		11		4		15
	0	50	8	154.2		28	1	8		37
	0	0	8	10.6	1	2		1		4
504	0	50	25	10.3		3		1		4
	50	0	25	15.5				1	15	16
	50	0	25	29.4		4		2	27	33
1015	50	100	42	184.1		11	14	58	17	100
	0	0	42	155.3		13	1	35	51	100
	50	50	42	119.3		8	4	74	14	100
1027	50	50	25	8.0		2		8		10
	50	0	25	0.0						0
	0	0	25	2.4					1	1
2207	50	0	8	53.2	1	2	1	6	23	33
	0	0	8	315.5		4		74	22	100
	0	0	8	79.4		2		5	11	18
2251	300	250	250	44.5		6			94	100
	200	250	250	41.8		29	6	14	51	100
	250	250	250	26.8		12	5	16	67	100
2263	50	0	8	29.2		1			4	5
	0	0	8	7.1		1				1
	0	0	8	10.0		1		1	1	3
2266	50	0	8	70.1		1		1	33	35
	0	0	8	23.5		4		5	5	14
	0	0	8	0.0						0
2942	0	0	16	15.8				1	10	11
	0	50	16	6.7		6				6
	50	0	16	1.8					3	3
2968	1000	700	775	25.6		94	2		4	100
	650	750	775	11.2		87	3	8	2	100
	750	800	775	19.0	1	83	9	6	1	100
3045	0	50	42	0.5		1			1	2
	100	50	42	1.9		3				3
	0	50	42	3.4						0
3056	50	0	8	25.5				13	8	21
	0	0	8	117.8		2	1	11	9	23
	0	0	8	67.7		4		11	39	54
3085	50	50	50	74.8					100	100
	0	50	50	69.0				2	98	100
	50	100	50	114.1					89	89
3140	0	0	8	25.0		1		2	3	6
	0	0	8	26.3		1				1
	50	0	8	9.4		1		5	2	8
3165	0	0	8	23.1		3		1		4
	0	50	8	27.4		1		1	1	3
	0	0	8	15.0		7			1	8
4026	50	0	25	39.6		35			5	40
	0	0	25	32.8		58				58
	100	0	25	3.5		3				3
4065	50	150	67	110.1				10	90	100
	100	50	67	86.4				4	96	100
	0	50	67	58.9		1		2	97	100
4097	250	150	133	20.9		5	1		94	100
	150	50	133	23.4		2			98	100
	150	50	133	20.6				2	98	100
4165	50	200	117	166.1					100	100
	150	150	117	160.7					100	100
	50	100	117	195.4					100	100
7112	100	100	75	110.2		15		5	80	100
	100	150	75	115.3		14		1	85	100
	0	0	75	106.7		14	2	4	80	100
5056	0	150	50	27.7		1			49	50
	50	0	50	26.9		2			74	76
	50	50	50	3.1					2	2
5089	0	50	8	16.8		4		1	4	9
	0	0	8	28.2		2			11	13
	0	0	8	36.9		2			19	21
5102	0	0	8	9.8					3	3
	50	0	8	45.3		2			25	27
	0	0	8	42.1		3			27	30
5104	100	100	70	74.8			3		97	100
	150	0	70	39.4		4	2		94	100
	0		70	81.9		5			95	100
5105	0	0	8	38.8		2		2	28	32
	50	0	8	10.5		4				4
	0	0	8	12.1						0
5127	150	100	133	56.4		5	4	3	88	100
	250	50	133	66.0		3			47	50
	200	50	133	57.3		22		3	65	90
5164	150	150	67	22.4		8	2	5	85	100
	0	50	67	12.6		22	2	9	49	82
	0	50	67	24.4		3	1	1	25	30
5034	0	0	0							
	0	0	0							
	0	0	0							
		Mean	71	55.4						

Low FEC Line

Tag	FEC	FEC	Mean	dev.succ.	Haem.	Ost.	Trich.	Coop.	LT.	nr. id'ed
489	0	50	8	85.5		8		2	35	45
	0	0	8	221.2		7		9	34	50
523	0	0	8	6.0				4	1	5
	0	0	8	7.5					1	1
	50	0	8	68.5					12	12
1043	0	0	8	95.0					21	21
	0	0	8	79.9		1		1	17	19
	50	0	8	51.0					12	12
4172	0	100	33	25.5				45		45
	50	0	33	10.3				10	1	11
	0	50	33	7.4		1		6	4	11
7035	0	0	8	54.9		3		13	16	32
	50	0	8	38.1		8		2	7	17
	0	0	8	7.4					1	1
9272	0	50	33	5.7		3		2	4	9
	50	100	33	0.6				2		2
	0	0	33	1.7		1				1
5027	100	0	42	2.7				1	1	2
	100	50	42	7.9					1	1
	0	0	42	2.7		1		1		2
5156	50	0	8	46.3		1		14	2	17
	0	0	8	15.9		3		10		13
	0	0	8	12.0				5		5
5184	0	50	16	8.0		1		5	2	8
	0	0	16	11.1		6		3		9
	50	0	16	6.9		3	1		2	6
437	0	0	0							
	0	0	0							
1008	0	0	0							
	0	0	0							
	0	0	0							
1018	0	0	0							
	0	0	0							
1039	0	0	0							
	0	0	0							
	0	0	0							
1057	0	0	0							
	0	0	0							
1085	0	0	0							
	0	0	0							
1091	0	0	0							
	0	0	0							
	0	0	0							
1095	0	0	0							
	0	0	0							
1125	0	0	0							
	0	0	0							
	0	0	0							
2303	0	0	0							
	0	0	0							
	0	0	0							
2915	0	0	0							
	0	0	0							
	0	0	0							
2971	0	0	0							
	0	0	0							
2980	0	0	0							
	0	0	0							
	0	0	0							
3008	0	0	0							
	0	0	0							
3124	0	0	0							
	0	0	0							
3131	0	0	0							
	0	0	0							
3150	0	0	0							
	0	0	0							
	0	0	0							
3190	0	0	0							
	0	0	0							
4054	0	0	0							
	0	0	0							
	0	0	0							
9203	0	0	0							
9238	0	0	0							
	0	0	0							
9278	0	0	0							
	0	0	0							
	0	0	0							
5161	0	0	0							
	0	0	0							
	0	0	0							
		Mean	5	33.8						

PERENDALE EWES, sampled 17/2 1997

High FEC Line

Tag	FEC	FEC	Mean	Dev.Succ.	Haem.	Ost.	Trich.	Coop.	L.T.	nr. id.'ed
469	0	0	8	293.5				13	87	100
	0	50	8	470.2						
	0	0	8	453.0						
479	50	0	25	42.4		41		22		63
	0	50	25	36.9	1	29	2	43		75
	0	50	25	25.9	3	12		31		46
504	0	0	25	65.4		36		19	45	100
	50	50	20	137.3						
1015	50	50	33	85.8		74	4	20	2	100
	0	0	33	227.3						
	0	100	33	145.5						
2251	100	150	125	19.0	1	11	12	45	31	100
			125	12.5						
2942	50	0	33	87.7	3	85	4	7	1	100
	50	50	33	68.2						
	0	50	33	39.0						
2968	950	850	1008	9.3		56	38	2	4	100
	950	1050	1008	11.7						
	1200	1050	1008	21.3						
3045	50	0	16	0.6					1	1
	50	0	16	0.0						0
	0	0	16	0.6					1	1
3056	0	0	8	110.4			1	50	15	66
	50	0	8	119.0		3		66	31	100
	0	0	8	85.8				40	5	45
3085	50	50	58	4.4		1	5	4	16	26
	50	50	58	32.5						
	100	50	58	22.7		3		10	87	100
3140	0	0	8	2.4		1			1	2
	0	0	8	5.0		1		2	1	4
	50	0	8	25.2		1		18	2	21
4026	50	0	20	104.8		28	6	32	34	100
	0	50	20	12.3						
	0		20	83.2	4	13	10	9	64	100
4065	0	50	30	13.3						
	50	50	30	10.9			1	21	1	23
	0		30	17.3		15	1	5	1	22
4097	250	850	417	7.2		9	2	1	88	100
	550	300	417	15.2						
	350	200	417	14.6						
4165	50	400	117	3.6					36	36
	100	100	117	9.3		1			83	84
	0	50	117	6.5		4			54	58
4205	100	100	113	32.7		2	6	61	31	100
	150	100	113	49.8						
			113	33.4		7	4	72	17	100
5034	100	200	133	11.3	1	9	1	69	20	100
	200	100	133	13.4	3	16		57	24	100
	50	150	133	36.9						
5056	0	50	33	2.1		1			6	7
	0	50	33	2.0		2			5	7
	0	100	33	4.5				3	7	10
5089	150	150	158	11.2	2	1		58	16	77
	150	200	158	4.5	4			15		19
	250	50	158	2.8		6	1	18	1	26
5102	150	50	170	10.8		1	1		98	100
	300	100	170	7.3						
	250		170	18.6					100	100
5104	0	0	13	41.5		12	2	4	6	22
	50	0	13	12.5		4	2	1	5	12
			13	11.7	1	6	1	1	7	16
5105	100	300	200	23.3		40		7	53	100
	100	150	200	38.5						
	150	400	200	34.0						
5127	400	250	283	4.5		4	6	55	17	82
	400	200	283	4.5		8		80	12	100
	150	300	283	1.3	1	1	1	15	7	25
5164	900	700	1100	35.5	4	14	1	78	3	101
	1200	1100	1100	41.2						
	1700	1000	1100	51.2						
9286	0	0	0							
	0	0	0							
	0	0	0							
409	0	0	0							
	0	0	0							
	0	0	0							
429	0	0	0							
	0	0	0							
498	0	0	0							
	0	0	0							
	0	0	0							
1027	0	0	0							
2267										
	0	0	0							
2965	0	0	0							
	0	0	0							
3018	0	0	0							
	0	0	0							
	0	0	0							
3165	0	0	0							
	0	0	0							
	0	0	0							
	Mean	130	50.0							

Low FEC Line

Tag	FEC	FEC	Mean	Dev.Succ.	Haem.	Ost.	Trich.	Coop.	L.T.	nr. id.'ed
7035	50	0	8	57.7				2	31	33
	0	0	8	36.3		4			15	19
	0	0	8	28.3		3		2	19	24
9203	0	0	8	8.6					7	7
	0	50	8	12.5		1			4	5
	0	0	8	0.0						
9272	100	50	87.5	7.5	1	56		3	1	61
	100	100	88	8.2		24			8	32
489	0	50	25	37.8	3	15	5	65	1	88
	0	50	25	37.2	1	10	4	20		35
			25	22.7	1	5		16		22
523	0	0	8	23.8		2		3	4	9
	50	0	8	6.1		1		3	1	5
	0	0	8	5.0		1		1	2	4
1043	0	0	8	78.0		14		9		23
	0	50	8	13.0			1	1	4	6
	0	0	8	10.8			1	2	6	9
1107	0	0	25	198.4		6		94		100
	50	50	25	234.3						
2915	0	0	8	14.2			1			1
	0	0	8	7.3						0
	50	0	8	0.0						0
3190	0	0	8	34.3		10		7	1	18
	0	50	8	11.8		3	1		1	5
	0	0	8	15.0		1		1		2
4172	0	0	13	50.0	1	3		13		17
	50	0	13	35.2		3		20		23
5018	100	50	50	8.4				6	39	45
	0	100	50	27.2		4		96		100
	50	0	50	18.8						
5027	0	0	33.33	39.2		17	2	26	26	71
	50	50	33	29.2		14	2	22	14	52
	0	100	33	31.4		16	8	51	25	100
5161	50	50	33	147.2		3	1	17	79	100
	50	0	33	111.0						
	50	0	33	147.1						
5194	50	0	50	35.0		32	19	9	40	100
	50	100	50	31.0						
	50	50	50	32.4						
9238	0	0	0							
	0	0	0							
	0	0	0							
475	0	0	0							
	0	0	0							
1008	0	0	0							
	0	0	0							
1018	0	0	0							
	0	0	0							
1039	0	0	0							
	0	0	0							
	0	0	0							
1057	0	0	0							
	0	0	0							
	0	0	0							
1085	0	0	0							
	0	0	0							
1091	0	0	0							
	0	0	0							
	0	0	0							
1095	0	0	0							
	0	0	0							
	0	0	0							
1125	0	0	0							
	0	0	0							
	0	0	0							
2303	0	0	0							
	0	0	0							
	0	0	0							
2971	0	0	0							
	0	0	0							
2980	0	0	0							
	0	0	0							
	0	0	0							
3008	0	0	0							
	0	0	0							
3131	0	0	0							
	0	0	0							
	0	0	0							
3150	0	0	0							
	0	0	0							
	0	0	0							
4110	0	0	0							
	0	0	0							
	0	0	0							
5032	0	0	0							
	0	0	0							
	0	0	0							
5060	0	0	0							
	0	0	0							
	0	0	0							
5156	0	0	0							
			Mean	10	42.4					

PERENDALE EWES, sampled 14/4 1997																						
High FEC Line											Low FEC Line											
Tag	FEC	FEC	Mean	Dev.Succ.	Haem.	Ost.	Trich.	Coop.	LT	nr. kl'ed	Tag	FEC	FEC	Mean	Dev.Succ.	Haem.	Ost.	Trich.	Coop.	LT	nr. kl'ed
469	50	50	17	30.0				6	12	18	489	0		33	50.9	1	4	3	27	3	38
	0	0	17	38.8		5		13	13	31		0		33	37.4		5		32		37
	0	0	17	30.8								100		33	135.5						
479	100	50	33	5.8		1		4		5	523	200	50	42	3.4		1		14		15
	0	0	33	27.4		6		44		50		0	0	42	7.2		14		17		31
504	50	0	75	17.8	1			4	20	25		0	0	42	44.3		61		39		100
	250	50	75	23.0		1		7	42	50	1018	0	100	25	10.7		1		1		2
	50	50	75	5.7								0	50	25	25.0		27		21	2	50
1015	250	350	233	38.1		23	1	46	30	100		0	0	25	7.9						
	150	250	233	48.1							1057	0	50	25	8.7		2		8	11	21
	300	100	233	63.8										25	49.5		6		14	80	100
2251	500		275	38.7		8	5	93	18	124				25	29.2						
	50		275	30.3							1107	200	100	117	23.5		2		65	8	75
			275	8.0								100	200	117	23.0						
2942	0	50	17	12.4				7		7		50	50	117	15.5						
	0	0	17	28.5		9		18	2	29	3008	0	0	17	12.4		13	1	5	2	21
	0	50	17	27.2		3		41		44		50	0	17	5.2		1		3		4
2968	4000	3250	3550	9.2	1	9	67	14	9	100		0	50	17	8.3		7		8		15
	3250	3700	3550	17.5							4172	150	50	67	7.0		1		12		13
			3550	22.7								50	50	67	4.5				7		7
3045	0	0	10	87.0		12	3	7	5	27		0	100	67	6.1		5		6		11
	0	50	10	49.5		13		12	6	31	5027	50	50	50	21.8		5		41	4	50
	0		10	905.9								150	50	50	9.9						
3056	0		17	382.4		1	34		65	100		0	0	50	10.5						
	50		17	161.8							5161	100	50	42	15.3				35		35
	0		17	84.4								50	0	42	10.8		8		10	1	19
3085	100	100	100	10.6		5		42	7	54		0	50	42	4.1		6		7		13
	50	100	100	4.5							5194	0		17	2.8						0
	100	150	100	6.3								0		17	0.6		1				1
3140	100	50	25	68.6	2	5		30	13	50		50		17	4.4		1		2		3
	0	0	25	27.2																	
	0	0	25	7.5							1008	0	0	0							
4026	0	150	108	118.6		18	12	70		100		0	0	0	19.8						
	150	100	108	78.5								0	0	0							
	100	150	108	73.5							1043	0	0	0							
4065	0	50	42	27.7			3	16	38	57		0	0	0							
	50	50	42	5.6							1085	0	0	0							
	50	50	42	6.9							1091	0	0	0							
4097	550	200	492	27.5		5			95	100		0	0	0							
	850	600	492	10.8								0	0	0							
	400	350	492	13.5							1095	0		0							
4165	0	50	100	48.9		3			97	100	1125	0		0							
	150	250	100	64.2							2303	0	0	0							
	50	100	100	28.2									0	0							
4205	150	200	175	83.2		4	2	87	7	100	2915	0	0	0							
	300	200	175	73.1							2971	0	0	0							
	150	50	175	76.0								0	0	0							
5034	850	450	642	5.8				29	21	50		0	0	0							
	600	650	642	9.5							2980	0	0	0							
	550	750	642	11.2								0	0	0							
5056	50	0	83	5.4				25	6	31		0	0	0							
	250	50	83	9.7		15		21	7	43	3131	0	0	0							
	50	100	83	11.9								0	0	0							
5089	800	650	683	16.4	2		1	97		100		0	0	0							
	600	850	683	12.9							3150	0	0	0							
	600	600	683	15.9								0	0	0							
5102	50	50	25	58.8		1		3	96	100		0	0	0							
	0	0	25	76.5							3190	0	0	0							
			25	79.1								0	0	0							
5105	600	550	683	0.4		3		12		15		0	0	0							
	900	600	683	1.0		20		30		50	4054	0	0	0							
	750	700	683	0.6								0	0	0							
5127	350	450	390	19.2		6	9	35	50	100	4110	0	0	0							
	400	200	390	8.1								0	0	0							
	550		390	3.5							5018	0	0	0							
5164	450	500	667	68.8	3	1	2	79	15	100		0	0	0							
	850	750	667	124.7								0	0	0							
	750	700	667	62.5							5032	0	0	0							
												0	0	0							
5104	0	0	0								5060	0	0	0							
	0	0	0									0	0	0							
												0	0	0							
		Mean	361.3	53.8									Mean	18	19.8						

PERENDALE EWES, sampled 19/5 1997

High FEC Line

Tag	FEC	FEC	Mean	Dev.Succ.	Haem.	Ost.	Trich.	Coop.	LT.	nr. kl'ed
479	50	0	25	5.2		2	6			8
479	0	50	25	1.2				3		3
479	0	50	25	130.4				100		100
504	100	100	108	8.1						
504	150	100	108	4.9		44		4	2	50
504	100	100	108	1.8						
1015	150	400	342	32.2		21	4	74	1	100
1015	350	400	342	24.4						
1015	250	500	342	16.4						
2251	150	250	150	30.6		21	6	70	4	100
2251	100	150	150	13.3						
2251	50	200	150	40.8						
2942	50	0	25	20.0		3	1	12		16
2942	50	50	25	37.8		6	3	41		50
2942	0	0	25	14.8						
2965	100	0	58	23.4		8		42		50
2965	200	0	58	22.8						
2965	50	0	58	10.3						
3045	50	0	8	36.8		13		10	2	25
3045	0	0	8	11.7		1		9		10
3045	0	0	8	15.5		2		1		3
3056	300	350	338	3.9				50		50
3056	250	450	338	4.7						
3056			338	5.0						
3085	550	400	500	18.7		6	1	93		100
3085	500	350	500	10.1						
3085	550	650	500	14.1						
3140	200	300	183	4.4		3		26		29
	200	150	183	7.7		3		87		100
	200	50	183	8.3						
4026	0	200	108	8.8		14	5	31		50
	200	150	108	13.8						
	100	0	108	12.7						
4065	50	50	58	10.2	2	11		37		50
	50	50	58	4.9						
	50	100	58	15.4						
4097	300	250	183	4.0		42	5	2	1	50
	250	100	183	2.2						
	50	150	183	2.7						
4165	450	500	433	15.7		36	5	59		100
	550	350	433	9.2						
	250	500	433	7.7						
4205	50	50	108	25.0		3		72		75
	100	200	108	24.3						
	50	200	108	35.1						
5034	300	350	342	1.7		3		10	4	17
	300	250	342	0.8		2		19	1	22
	550	300	342	0.9		2		20	1	23
5056	50	50	67	8.1		6	2	31		39
	50	100	67	2.9				11		11
	50	100	67	3.5						
5089	150	600	358	1.8				50		50
	350	500	358	1.3						
	350	200	358	4.5						
5102	50	50	16	0.0						0
	0	0	16	0.6				1		1
	0	0	16	3.4				1		1
5104	100	50	83	8.8		1	2	53	1	57
	100	100	83	4.8						
	0	150	83	14.8						
5105	100	100	258	2.8	2	5		43		50
	350	400	258	7.7						
	400	200	258	11.1						
5127	100	200	150	3.1				7		7
			150	2.7		2	2	29	2	35
			150	2.0						0
5164	1000	1250	1033	0.8		8	7	34	1	50
	1000	800	1033	0.3						
	1300	850	1033	1.3						
469	0		0							
	0		0							
	0		0							
		Mean	205.6	12.5						

Low FEC Line

Tag	FEC	FEC	Mean	Dev.Succ.	Haem.	Ost.	Trich.	Coop.	LT.	nr.kl'ed
489	150	200	233	7.0		9	2	39		50
	450	300	233	15.6						
	150	150	233	6.2						
1018	0		33	14.6		7	1	36	1	45
	100		33	22.1		3		30		33
	0		33	38.2						
1057	0	0	8	36.4		3	3	2	12	20
	0	0	8	15.0		3		5	4	12
	50	0	8	29.1			1	3		4
1091	0	0	8	51.9				2	27	29
	0	50	8	13.6				6		6
	0	0	8	37.7		2			20	22
1095	0	0	16	12.0						
	50	0	16	26.4	1	2	3	24	14	44
	0	50	16	11.3		4	5	2	8	19
1107	150	50	100	13.0		1		51	2	54
	200	50	100	73.3						
	150	0	100	33.5						
1125	0	0	8	10.7			1		8	9
	0	50	8	9.7				6	2	8
	0	0	8	20.4					2	2
4172	0	100	58	4.8		3		10		13
	50	50	58	4.5		1		15		16
	100	50	58	15.6				12		12
5027	500	250	325	1.1		3	1	23	1	28
	400	300	325	1.2		1		22		23
	350	150	325	6.2						
5161	100	0	58	10.3						
	100	0	58	5.3		1		20		21
	50	100	58	8.3		2		18		20
5194	0	0	8	11.0		7	2			9
	50	0	8	1.3				1		1
	0	0	8	12.3		1		4		5
1008	0	0	0							
	0	0	0	17.6						
	0	0	0							
1039	0	0	0							
	0	0	0							
	0	0	0							
1043	0	0	0							
	0	0	0							
	0	0	0							
1085	0	0	0							
	0	0	0							
	0	0	0							
2303	0	0	0							
	0	0	0							
	0	0	0							
2915	0	0	0							
	0	0	0							
	0	0	0							
2971	0	0	0							
	0	0	0							
	0	0	0							
2980	0	0	0							
	0	0	0							
	0	0	0							
3008	0	0	0							
	0	0	0							
	0	0	0							
3131	0	0	0							
	0	0	0							
	0	0	0							
3150	0	0	0							
	0	0	0							
	0	0	0							
3190	0	0	0							
	0	0	0							
	0	0	0							
4054	0	0	0							
	0		0							
4110	0	0	0							
	0	0	0							
	0	0	0							
5018	0	0	0							
	0	0	0							
	0	0	0							
5032	0	0	0							
	0	0	0							
	0	0	0							
5060	0	0	0							
	0	0	0							
	0	0	0							
5156	0	0	0							
	0	0	0							
	0	0	0							
		Mean	30	17.6						

PERENDALE EWES, sampled 30/6 1997

High FEC Line

Tag	FEC	FEC	Mean	Dev.Succ.	Haem.	Ost.	Trich.	Coop.	LT.	nr. kl'ed
409	0	0	17	5.7		5	1	4		10
	0	100	17	18.3	2	9	6	15	2	34
	0	0	17	6.9		1	2	3	1	7
469	0	50	33	4.2						
	0	0	33	8.5		1	2	21		24
	100	50	33	9.6		2		7		9
479	0	100	50	0.0						0
	50	0	50	0.4				2		2
	100	50	50	0.0						0
498	200	50	75	6.9	1		3	10		14
	50	100	75	2.1				7		7
	50	0	75	0.6						0
504	1500	1400	92	10.5	36	38	1	7		82
	1350	1350	92	80.5						
	1500	1650	92	29.7						
1015	1000	750	117	101.2		33	2	64	1	100
	900	1050	117	192.3						
	900	1200	117	184.5						
1027	0	50	17	3.5		2		4		6
	0	0	17	0.6				1		1
	0	50	17	6.6	1	1	1	4		7
2263	150	200	167	4.1		5	4	35		44
	200	50	167	3.8		6				6
	350	50	167	3.5						
2266	0	50	8	6.9				1		1
	0	0	8	3.8		1		2		3
	0	0	8	14.7		1		1		2
2942	50	50	33	2.0				2		2
	0	50	33	8.0		2		26		28
	0	50	33	5.1				18		18
2965	50	0	33	3.3				1		1
	0	100	33	19.3	2	2		56		60
3018	50	100	83	8.2		1	2	47		50
	100	100	83	2.8						
	100	50	83	6.9						
3045	50	0	25	0.8				1	1	2
	0	0	25	1.1				3		3
	100	0	25	2.0				5		5
3056	600	400	542	1.0	2	4		27	1	34
	400	550	542	2.5		6		93	1	100
	700	600	542	1.5						
3140	150	250	192	0.8	1		2	8		11
	150	150	192	0.8		1		4		5
3165	0	50	50	10.2			2	16		18
	100	50	50	5.1		2		21		23
	50	50	50	7.0		4		18		22
4026	100	300	142	2.2		6	4	8		18
	150	150	142	5.4		17	6	27		50
	100	50	142	5.9						
4065	50	100	83	11.1			4	46		50
	0	250	83	15.1						
	50	50	83	15.4						
4097	150	200	175	6.5		32	2	15	1	50
	250	200	175	6.3						
	150	100	175	11.5						
4165	150	150	190	6.1		14	1	70		85
	150	200	190	52.1						
	300		190	12.9						
4205	200	100	167	1.7		1		29		30
	50	300	167	4.7		6		31		37
	200	150	167	2.4						
5034	500	600	550	0.3	1		2	8		11
	400	500	550	0.1		2		6		8
	700	600	550	0.3		1		14		15
5056	0	200	83	1.0				3		3
	150	0	83	0.9		2		6		8
	100	50	83	0.9		2		1		3
5089	300	400	258	1.8				40		40
	150	200	258	5.9		1	4	29		34
	250	250	258	1.9						
5164	250	300	283	2.2		5	1	28		34
	300	300	283	2.0		2		35		37
	350	200								
429	0	0	0							
	0	0	0							
	0	0	0							
2207	0	0	0							
	0	0	0							
	0	0	0							
5102	0	0	0							
	0	0	0							
	0	0	0							
		Mean	122.1	13.5						

Low FEC Line

Tag	FEC	FEC	Mean	Dev.Succ.	Haem.	Ost.	Trich.	Coop.	LT.	nr. kl'ed
489	100	50	67	5.9		14		13		27
	50	100	67	13.8	2	19	4	22		47
	50	50	67	12.1		7	2	20		29
1039	0	0	8	2.5				2		2
	0	50	8	6.1						0
	0	0	8	0.0						0
1057	0	0	8	34.3		14	4		5	23
	0	50	8	12.4		3		2		5
	0	0	8	0.0						0
1085	0	50	17	1.7			2	1		3
	0	50	17	5.5			3	2		5
	0	0	17	6.1				1		1
1095	0	0	25	5.6		3	3	9		15
	0	50	25	0.8				1	1	2
	0	100	25	1.1		2		1		3
1107	150	50	125	4.2				32		32
	200	200	125	25.6		2		98		100
	150	0	125	8.8						
1125	0	0	17	0.6		1				1
	50	0	17	0.6		1				1
	50	0	17	0.0						0
2980	0	0	33	0.9				3		3
	50	100	33	1.1		1		3		4
	0	50	33	0.6				2		2
3008	0	0	8	7.5		1	1	4		6
	0	50	8	0.0						0
	0	0	8	0.0						0
3150	0	0	8	0.0						0
	0	50	8	1.2						1
	0	0	8	1.2						1
3190	50	50	67	2.2		1	1	3		5
	100	100	67	0.7						0
	50	50	67	0.8				6		6
4110	0	0	8	0.0						0
	0	50	8	0.0						0
	0	0	8	7.5				1		1
4172	250	300	225	0.6				4		4
	150	200	225	0.8		1		18		19
	150	300	225	0.5			3	3		6
5027	200	450	342	3.0		5		45		50
	500	300	342	1.3						
	200	400	342	0.7						
5156	0	0	33	2.1		6		1		7
	100	0	33	1.5		5				5
	100	0	33	1.2		2	1	1		4
5161	150	100	142	5.6		5		45		50
	200	100	142	8.5						
	100	200	142	2.4						
5184	150	50	158	0.8		3		5		8
	200	100	158	10.2	24	8	3	65		100
	200	250	158	2.6	5	2	2	8	2	19
1008	0	0	0							
	0	0	0							
	0	0	0							
1018	0	0	0							
	0	0	0							
	0	0	0							
1043	0	0	0							
	0	0	0							
	0	0	0							
1091	0	0	0							
	0	0	0							
	0	0	0							
2303	0	0	0							
	0	0	0							
	0	0	0							
2915	0	0	0							
	0	0	0							
	0	0	0							
2971	0	0	0							
	0	0	0							
	0	0	0							
3131	0	0	0							
	0	0	0							
	0	0	0							
4054	0	0	0							
	0	0	0							
	0	0	0							
			Mean	50	4.2					

Perendale ewes , sampled September 11 1997

High FEC Line

Tag	FEC	FEC	Mean	Dev.Succ.	Haem	Ost	Trich	Coop	LT	Nr. id'ed
409	550		833	47.8		28	4	32	36	100
	1050		833	35.7						
	900		833	20.5						
429	100	100	42	1.1		3	2			5
	50	0	42	8.1		14	13	1	7	35
	0	0	42	3.0		5	5	1	2	13
469	250	50	125	35.8	1	1	6	31	61	100
	150	150	125	59.0						
	0	150	125	44.6						
479	50	150	108	1.6	3	6		8		17
	100	100	108	9.7	12	24		38		75
	100	150	108	0.0						0
498	300	200	175	2.7	2	8	2	11	7	30
	50	150	175	6.0	4	2	2	31	22	61
	50	300	175	3.2						
504	250		333	2.4	20	25	3	15	5	68
	350		333	7.3						
	400		333	1.1						
1015	0	150	233	242.2		34	2	43	21	100
	900	100	233	115.8	2	58	7	19	14	100
	50	200	233	11.8						
2266	50		25	9.4	12	2				14
	0		25	0.8	2					2
			25	7.3	4			3		7
2942	50		50	2.6			2	18		20
	50		50	6.1		6		13	3	22
	50		50	18.8		3		21		24
2965	50	0	42	21.5		1	2	52	9	64
	0	0	42	25.7		4		87	7	98
	150	50	42	39.3						
3018	150	50	92	6.5	11	1		40		52
	0	100	92	7.0	10			16	1	27
	50	200	92	13.0						
3056	350	450	433	1.9		4		22		26
	500		433	1.2	7	11	1	23	2	44
3140	350		317	8.1						
	350		317	21.6		26	7	67		100
	250		317	11.8						
3165	100	0	92	3.5						
	100	100	92	9.7		1	1	13		15
	100	150	92	21.3		41	7	27		75
4026	350	350	300	18.1						
	300	350	300	5.0	1	19	3	77		100
	250	200	300	8.4						
4065	0	0	42	27.8						
	100	0	42	19.0		5		81		86
	50	100	42	27.5		4		36		40
4165	700		800	2.3						
	900		800	5.8	5	18	2	8	1	34
4205	152	250	167	1.2	2	45	10	41	2	100
	200	300	167	7.7						
	100	0	167	6.0			2	18		20
5034	400	400	550	1.0						0
	500	800	550	2.2	6	5		39		50
	600	600	550	2.5	1	10	1	15		27
5056	0	100	50	4.2	1	25	5	58		89
	0	0	50	6.9						
	50	150	50	5.9		1		6		7
5089	200	300	217	1.8		8	2	16		26
	300	150	217	1.5		3		12		15
	250	100	217	5.3			2	8		10
5164	100		83	24.0				5		5
	100		83	1.5	1	1		42		44
	50		83	22.7		1		48		49
					2	1	1	10		14
2207	0	0	0							
	0	0	0							
	0	0	0							
3045	0		0							
	0		0							
	0		0							
5102	0	0	0							
	0	0	0							
	0	0	0							
		Mean	193.1	17.3						

Low FEC Line

Tag	FEC	FEC	Mean	Dev.Succ.	Haem	Ost	Trich	Coop	LT	Nr. id'ed
489	450	400	375	1.6	8	8	1	6		23
	350	450	375	2.7	18	23	13	26	3	83
	300	300	375	0.9		5	1	18	2	26
1018	50	50	67	6.8		2		17		19
	200	0	67	5.2			1	14	1	16
	50	50	67	7.5		3		38		41
1039	0	0	8	37.0		1		11		12
	50	0	8	33.4		2		25		27
	0	0	8	36.7				12		12
1057	250	0	117	1.3	10		1	2	2	16
	250	50	117	1.5	10	1	1	2	5	19
	0	150	117	1.3	6					6
1085	100	50	42	1.0		1	2	1		4
	0	50	42	0.7	1	1		1		3
	0	50	42	2.5		1				1
1091	50	50	17	36.1	12	5		5		22
	0	0	17	40.0	39	18	7	6		70
	0	0	17	7.8	3	1				4
1095	50	100	58	9.9		11		3	6	20
	200	0	58	1.5	2	5	1	1		9
	0	0	58	7.4		3	1	3		7
1107	50	100	67	11.9				34		34
	100	0	67	13.2				23		23
	50	100	67	8.5				9		9
2915	100	50	33	0.3	1					1
	0	0	33	3.3		2		9		11
	50	0	33	0.3	1					1
3008	0	0	33	13.5		6		10		16
	100	50	33	31.2	1	7	2	29		39
	0	50	33	21.2		1		19		20
3150	0	0	8	12.6				1		1
	0	50	8	2.4		1		1		2
	0	0	8	0.0						0
3190	0	50	50	7.9		13	5	4		22
	150	100	50	8.3		1		2		3
	0	0	50	8.0		3	3	15		23
4110	50	0	8	134.9		5		2	2	9
	0	0	8	21.0		3		3	1	7
	0	0	8	20.4		1		6		7
4172	0	100	25	0.4				1		1
	0	0	25	9.3				4		4
	0	50	25	15.8	1	1		10		12
5018	0	50	17	11.4		9		1		10
	50	0	17	5.8		3		7		10
	0	0	17	11.9		3	8			11
5027	100		150	0.9		2		2		4
	200		150	4.3				1		1
5032	50	50	17	4.1		5		2		7
	0	0	17	10.0		16	2			18
	0	0	17	18.1		20	2	1		23
5156	0	0	25	17.4	1	3	2			6
	0	100	25			100				100
	50	0	25	14.3			14	2		16
5161	100	200	192	5.3	2	5	3	14		24
	200	150	192	4.9	1	12		5		18
	300	200	192	12.9		13	8	79		100
1008	0	0	0							
	0	0	0							
	0	0	0							
1043	0	0	0							
	0	0	0							
	0	0	0							
	0	0	0							
2303	0	0	0							
	0	0	0							
	0	0	0							
2971	0	0	0							
	0	0	0							
	0	0	0							
3131	0	0	0							
	0	0	0							
	0	0	0							
4054	0	0	0							
	0	0	0							
	0	0	0							
5060	0	0	0							
	0	0	0							
	0	0	0							
		Mean	47	12.9						

Perendale ewes , sampled 6 October 1997

High FEC Line

Tag	FEC	FEC	Mean	Dev.Succ.	Haem	Ost	Trich	Coop	LT	Nr. id'ed
429	100	100	58	41.0						
	50	0	58	12.2		14	5	2		21
	50	50	58	37.0		64	20	9		93
469	250		183	18.5		2	18	48	32	100
	150		183	31.0						
	150		183	23.1						
498	500	350	467	13.0		5	8	86	1	100
	400	450	467	3.6						
	400	700	467	8.4						
504	500	350	542	36.2	5	15	10	18	52	100
	850	550	542	20.1						
	300	700	542	39.1						
1015	850	1400	1200	28.9		2	9	58	31	100
	1350		1200	28.8						
2207	0	50	8	31.0						
	0	0	8	43.2	1	2		3		6
	0	0	8	3.5	2	9	4	2		17
2266	50	150	50	20.0		3				3
	50	0	50	58.5	4	57	4	28		93
	50	0	50	32.7						
2942	200	100	83	58.7						
	100	50	83	73.6		28	7	65		100
	50	0	83	117.0						
2965	300		217	5.7						
	50		217	17.5		5	4	48		57
	300		217	31.8						
3018	150		100	106.9						
	50		100	32.7	2	4		94		100
	100		100	87.9						
3056	1150		1150	19.8						
3140	500	300	517	79.7	1	2	3	85	8	100
	700	800	517	85.2						
	300	500	517	90.1						
3165	200	50	117	34.4		16	12	73	5	100
	100	150	117	44.0						
	100	100	117	58.9						
4026	1050		883	24.6	3	3	23	71		100
	950		883	16.8						
	650		883	41.4						
4065	150	200	108	20.7		12	9	79		100
	100	100	108	19.4						
	50	50	108	23.1						
4165	450		375	38.8		2	1	41		44
	300		375	49.0		13		49		62
			375	42.9						
4205	100	150	117	114.9		3	47	50		100
	50	200	117	98.3						
	50	150	117	123.1						
5034	850		825	13.0			3	97		100
	800		825	13.0						
5056	50	100	142	8.7						
	150	100	142	12.3	3	6	14	76		100
	300	150	142	20.8						
5089	650	950	817	49.2						
	600	750	817	52.3	2	4	5	8		19
	750	1200	817	67.5		28	1	53	8	91
3045	0		0		3			97		100
	0		0							
5102	0	0	0							
	0		0							
5164	0	0	0							
	0		0							
		Mean	315.4	41.7						

Low FEC Line

Tag	FEC	FEC	Mean	Dev.Succ.	Haem	Ost	Trich	Coop	LT	Nr. id'ed
489	50	100	67	11.9	1	2	2	4		9
	100	50	67	12.6		5	1	2		8
	0	100	67	4.7		6		8		14
1018	50	0	25	40.0	3	1	2	35	11	52
	0	0	25	123.1						
	100	0	25	47.5						
1043	0	0	17	14.0		4				4
	0	50	17	8.7				15		15
	50	0	17	12.5				3		3
1057	50	50	83	15.5		29	1	22		52
	150	150	83	9.8						
	50	50	83	5.8						
1085	50		17	8.1		3		1		4
	0		17	11.6		14		6		20
	0		17	6.3				1		1
1095	50		25	28.0		31	3	7	1	42
	100		25	14.2		15	2			17
	0		25	6.0		12	2	1		15
1107	250	50	100	47.6		2		142		144
	50	150	100	55.1						
	50	50	100	47.6						
2915	0		50	7.8		13		37		50
	50		50	10.0						
	100		50	20.0						
2971	0	0	17	2.9		3		2		5
	0	50	17	8.8		1	1	3		5
	0	50	17	62.2		6	1	4		11
3190	50	0	42	7.1		2	1	8		11
	0	50	42	5.4				4		4
	50	100	42	7.7		2		1		3
4172	100		50	23.8		1	2	65		68
	0		50	33.6						
	50		50	14.0						
5032	50	100	42	2.4		9	1			10
	0	50	42	6.3		13	5			18
	0	50	42	0.7		1		2		3
5156	50	50	50	6.8		4	21	9	1	35
	50		50	24.8		16	2	6		24
5161	100	150	175	2.2						
	150	150	175	4.8		11	4	10		25
	150	350	175	8.5		34	11	27		72
5184	150	150	133	1.4		1	1	8		10
	250	50	133	1.4						0
	100	100	133	2.6		1		16		17
1008	0	0	0							
	0	0	0	18.2						
	0	0	0							
1039	0	0	0							
1091	0	0	0							
1125	0	0	0							
	0	0	0							
	0	0	0							
2303	0	0	0							
	0	0	0							
	0	0	0							
3008	0	0	0							
3131	0	0	0							
	0	0	0							
3150	0	0	0							
	0	0	0							
	0	0	0							
4054	0	0	0							
	0	0	0							
	0	0	0							
4110	0	0	0							
	0	0	0							
	0	0	0							
5018	0	0	0							
	0	0	0							
	0	0	0							
5027	0	0	0							
	0	0	0							
5060	0	0	0							
	0	0	0							
	0	0	0							
		Mean	35	18.1						

Perendale ewes , sampled 5 November 1997

High FEC Line

Tag	FEC	FEC	Mean	Dev.Succ.	Haem	Ost	Trich	Coop	LT	Nr. id'ed
429	0	50	42	3.5		6		10		16
	50	50	42	15.3		29	3	3		35
	100	0	42	4.5						
469	100	350	242	7.2		12	2	83		97
	250	50	242	2.0						
	550	150	242	6.8						
479	300	100	117	2.3		3	1	2		6
	100	0	117	6.1		4		9		13
498	250	200	250	7.4		34		66		100
	350	300	250	15.1						
	200	200	250	8.5						
504	100	400	250	1.5		21		14	2	37
	300	250	250	4.2		76		22	2	100
	300	150	250	3.0						
1015	1150	1750	1350	6.4	1	39	2	58		100
	1050	1250	1350	8.1						
	1350	1550	1350	3.3						
2207	0	100	92	1.4		1	1	1		3
	150	100	92	3.1						0
2942	100	0	50	14.8		7		49		56
	50	100	50	26.4						
	0		50	26.9						
2965	150	200	92	13.5		19		66		85
	0	100	92	35.9						
	0	100	92	33.9						
3018	100	0	42	9.8		7		16		23
	50	100	42	8.7				24	3	27
	0	0	42	6.7						
3056	450	400	492	7.4		3		97		100
	450	550	492	5.8						
	750	350	492	10.4						
3140	550	850	667	2.7		13	1	50	3	67
	450	550	667	3.2						
	1150	450	667	4.6						
3165	100	0	17	0.6		1				1
	0	0	17	3.5		6				6
	0	0	17	2.2		1		3		4
4026	650	550	600	8.5		17	1	82		100
	550	850	600	9.4						
4065	500	900	642	1.4		9	1	50		60
	550	750	642	3.4						
	650	500	642	0.9						
4165	1250	1050	1000	4.1		16		84		100
	800	1050	1000	10.0						
	950	900	1000	13.0						
4205	150	100	150	4.7				12		12
	250	100	150	2.2				32	1	33
	200	100	150	0.1				2		2
5034	150	500	333	1.9		2		14		16
	350	300	333	0.4		2		3		5
	250	450	333	1.2				11		11
5056	50	100	108	1.3		10		4		14
	300	100	108	2.6		3		6		9
	50	50	108	0.4		2		3		5
5089	500	400	383	10.2		17	1	83		101
	450	300	383	9.8						
	200	450	383	12.9						
2266	0	0	0							
	0	0	0							
	0	0	0							
3045	0	0	0							
	0	0	0							
	0	0	0							
5102	0	0	0							
	0	0	0							
	0	0	0							
5164	0	0	0							
	0	0	0							
	0	0	0							
		Mean	289.1	7.5						

Low FEC Line

Tag	FEC	FEC	Mean	Dev.Succ.	Haem	Ost	Trich	Coop	LT	Nr. id'ed
489	50		50	14.5		18		36		54
	50		50	6.0						
	50		50	3.7						
1008	0	0	8	3.5				3		3
	50	0	8	0.0						0
	0	0	8	0.0						0
1018	50	0	17	5.9		7			4	11
	0	50	17	9.4				3	4	7
	0	0	17	0.0						0
1039	0	100	17	15.8				8		8
	0	0	17	0.6				1		1
	0	0	17	2.8				5		5
1057	250	50	67	2.3		3		3		6
	0	100	67	0.4		2		1		3
	0	0	67	0.0						0
1085	50	50	25	1.5		3		1		4
	0	0	25	0.8				2		2
	50	0	25	0.0						0
1095	0	50	33	0.8		3				3
	50	50	33	1.8		1		5		6
	50	0	33	0.6				2		2
1107	350	50	167	7.0		13		58		71
	200	100	167	8.7		2		33		35
	50	250	167	12.1						
2915	0	0	17	7.3				3		3
	0	50	17	0.6				1		1
	0	50	17	2.3				4		4
2971	0	50	17	0.6				1		1
	0	0	17	1.7				3		3
	50	0	17	2.7				5		5
3190	50	50	25	2.4		1		5		6
	0	0	25	4.7		1		1		2
	0	50	25	8.5		2				2
4110	0	0	8	0.0						0
	50	0	8	1.2		1				1
	0	0	8	0.0						0
4172	0	50	25	7.4		1		8		9
	0	0	25	1.1		1		2		3
	50	50	25	0.4				1		1
5018	0	0	8	15.9		3				3
	0	50	8	2.3		2				2
	0	0	8	2.4		1		1		2
5027	300	250	283	3.2		21	1	56		78
	350	50	283	6.5						
	400	350	283	2.1						
5156	50	0	33	9.7		7	2	3		12
	50	100	33	3.6		1		1		2
	0	0	33	7.0		2		1		3
5161	50	200	133	9.8		17	2	57		76
	150	150	133	0.7						
	150	100	133	0.7						
5184	100	150	100	2.4		3		2		5
	0	50	100	1.0						0
	200	100	100	0.5		4		1		5
1043	0	0	0							
	0	0	0							
	0	0	0							
1091	0	0	0							
	0	0	0							
	0	0	0							
1125	0	0	0							
	0	0	0							
	0	0	0							
2303	0	0	0							
	0	0	0							
	0	0	0							
3008	0	0	0							
	0	0	0							
	0	0	0							
3131	0	0	0							
	0	0	0							
	0	0	0							
3150	0	0	0							
	0	0	0							
	0	0	0							
4054	0	0	0							
	0	0	0							
	0	0	0							
5032	0	0	0							
	0	0	0							
	0	0	0							
5060	0	0	0							
	0	0	0							
	0	0	0							
		Mean	37	3.8						

PERENDALE EWES - Faecal Dry Matter Pecentage (%)

Oct 1996		Oct 1996		Nov 1996		Nov 1996		Jan 1997		Jan 1997		Feb 1997		Feb 1997		Apr 1997		Apr 1997	
High FEC Line		Low FEC Line		High FEC Line		Low FEC Line		High FEC Line		Low FEC Line		High FEC Line		Low FEC Line		High FEC Line		Low FEC Line	
Tag	%D.M	Tag	%D.M	Tag	%D.M	Tag	%D.M	Tag	%D.M	Tag	%D.M	Tag	%D.M	Tag	%D.M	Tag	%D.M	Tag	%D.M
409	18.8	437	39.8	429	37.7	489	36.9	469	25.0	437	34.0	409	14.3	489	17.6	469	19.2	489	26.7
429	27.4	489	20.1	469	22.6	523	19.1	498	18.1	489	45.0	469	22.6	523	14.9	479	0.0	523	11.1
469	23.6	523	17.0	479	22.8	1018	22.5	504	38.7	523	26.7	479	18.3	1008	0.0	504	17.0	1008	16.5
479	14.8	1018	66.0	498	22.6	1099	23.2	1015	27.7	1008	24.3	504	24.7	1043	40.4	1015	18.3	1018	24.1
498	26.4	1099	22.6	504	21.5	1043	49.6	1027	22.0	1018	25.2	1015	16.1	1107	34.6	2251	18.2	1043	21.6
504	26.1	1095	20.8	1015	25.9	1057	53.7	2207	28.1	1099	19.0	2942	17.5	1125	18.8	2942	15.6	1057	25.0
1015	30.3	1091	28.1	2207	21.3	1095	39.4	2251	24.8	1043	26.2	2968	22.5	3190	19.4	2968	26.2	1095	40.0
2207	30.4	1125	17.8	2251	27.6	1091	38.0	2263	16.0	1057	22.3	3045	18.2	5018	22.0	3045	36.4	1091	45.0
2251	23.6	2915	27.1	2266	19.2	1095	25.0	2266	17.2	1095	15.1	3056	20.0	5027	18.5	3056	20.0	1107	16.0
2263	24.5	2971	24.3	2942	19.2	1107	17.8	2942	21.2	1099	23.2	3095	17.0	5032	6.3	3095	14.5	1125	13.3
2266	20.0	2980	19.2	2965	16.3	2303	67.8	2968	19.0	1107	18.7	3140	13.3	5060	11.9	3140	18.0	2303	16.7
2965	17.8	3008	48.9	2968	33.3	2915	77.1	3045	21.1	1125	14.7	3165	13.1	5161	23.1	4026	20.7	2915	12.5
2968	16.2	3124	48.6	3045	18.2	2971	17.8	3056	25.1	2303	26.3	4026	22.2	5194	17.2	4065	14.1	2971	53.8
3018	17.3	3131	28.7	3056	35.5	2980	37.4	3095	19.8	2915	23.2	4065	15.2	7095	26.9	4097	18.9	2980	15.0
3045	56.2	3150	24.2	3095	37.8	3008	16.4	3140	18.3	2971	22.8	4097	20.3			4165	20.0	3008	14.8
3056	16.9	3190	40.5	3140	19.6	3124	25.1	3165	16.2	2980	18.1	4205	36.0			4205	27.9	3131	11.1
3095	21.3	4054	19.8	3165	37.4	3131	26.4	4026	28.9	3008	22.8	5034	13.8			5034	16.5	3150	16.8
3140	19.0	4075	30.0	4026	29.7	3190	19.1	4065	28.0	3124	17.5	5056	18.0			5056	14.1	3190	19.5
3165	30.1	4110	31.5	4065	26.4	4075	26.1	4097	19.3	3131	14.4	5089	4.2			5089	18.4	4054	17.4
4026	23.2	4172	17.3	4165	45.3	4110	15.8	4165	22.8	3150	17.8	5102	28.8			5102	28.6	4110	28.6
4065	26.4	7095	30.6	4205	20.5	4172	23.5	5034	20.8	3190	21.4	5104	17.4			5104	16.7	4172	15.3
4097	23.5	7047	9.8	7112	32.6	7095	40.3	5056	18.3	4054	16.0	5105	23.8			5105	14.4	5018	11.7
4165	34.0	9203	68.7	9286	61.2	7047	53.5	5089	21.8	4172	19.6	5127	18.4			5127	22.2	5027	8.3
4205	11.2	9238	18.6			9203	38.8	5102	19.4	5027	12.4	5164	22.5			5164	24.1	5032	28.1
7112	20.2	9272	21.0			9238	22.6	7112	24.4	5156	18.2							5060	14.3
9286	35.4	9278	32.7			9272	32.9	5105	18.1	5161	22.4							5156	24.0
								5164	18.8	5184	18.1							5161	12.7
										7035	21.5							5194	25.6
										9203	31.0								
										9238	28.6								
										9272	12.0								
										9278	21.7								
Mean	24.4		29.8		28.4		33.3		22.2		21.9		19.1		19.4		19.2		20.9

May 1997		May 1997		Jun 1997		Jun 1997		Sept 1997		Sept 1997		Oct 1997		Oct 1997		Nov 1997		Nov 1997	
High FEC Line		Low FEC Line		High FEC Line		Low FEC Line		High FEC Line		Low FEC Line		High FEC Line		Low FEC Line		High FEC Line		Low FEC Line	
Tag	%D.M	Tag	%D.M	Tag	%D.M	Tag	%D.M	Tag	%D.M	Tag	%D.M	Tag	%D.M	Tag	%D.M	Tag	%D.M	Tag	%D.M
469	11.5	489	16.3	409	15.5	489	18.9	409	35.3	489	26.9	429	30.6	489	26.0	429	23.5	489	15.5
479	21.0	1008	18.9	429	16.9	1008	18.3	429	27.0	1018	19.4	469	45.2	1008	37.9	469	23.2	1008	22.2
504	21.0	1018	30.4	469	22.1	1018	21.9	469	30.7	1099	24.3	498	20.2	1018	28.1	479	21.7	1018	18.0
1015	20.0	1099	24.4	479	16.0	1099	15.2	479	26.7	1057	25.9	504	26.8	1099	29.3	498	17.1	1099	16.8
2251	26.9	1043	33.3	498	16.7	1043	21.2	498	18.5	1095	15.4	1015	27.8	1043	35.5	504	15.8	1043	19.3
2942	16.5	1057	27.1	504	22.4	1057	15.5	504	23.4	1091	17.1	2207	31.8	1057	21.1	1015	17.0	1057	18.2
2965	22.7	1095	17.8	1015	18.3	1095	16.0	1015	39.5	1095	27.3	2266	17.9	1095	30.3	2207	23.9	1095	23.0
3045	14.8	1091	29.2	1027	19.5	1091	24.8	2207	29.9	1107	16.8	2942	32.3	1091	30.1	2266	11.9	1091	21.5
3056	14.3	1095	17.9	2207	23.1	1095	12.6	2266	28.6	1125	11.8	2965	18.8	1095	25.2	2942	20.8	1095	15.9
3095	19.2	1107	5.9	2263	19.5	1107	15.2	2942	24.1	2303	35.7	3018	28.2	1107	34.5	2965	24.2	1107	18.0
3140	14.6	1125	18.8	2266	15.1	1125	14.3	2965	19.2	2915	27.8	3045	33.3	1125	25.0	3018	15.8	1125	17.7
4026	23.2	2303	21.8	2942	19.8	2303	12.8	3018	21.8	2971	29.6	3140	33.9	2303	28.6	3045	20.2	2303	19.3
4065	20.0	2915	28.6	2965	20.3	2915	18.0	3045	30.2	3008	29.9	3165	20.2	2915	34.4	3056	18.3	2915	16.3
4097	17.2	2971	17.5	3018	17.2	2971	21.1	3056	21.3	3131	16.0	4026	25.0	2971	20.8	3140	16.0	2971	15.7
4165	17.3	2980	19.6	3045	22.5	2980	15.3	3140	18.3	3150	27.8	4065	29.2	3008	22.1	3165	9.6	3131	12.8
4205	25.6	3008	23.5	3056	16.7	3008	14.0	3165	15.5	3190	18.4	4165	37.1	3131	10.4	4026	26.0	3150	14.8
5034	15.9	3131	11.3	3140	17.0	3131	13.6	4026	22.8	4054	18.2	4205	32.7	3150	23.3	4065	18.6	3190	13.5
5056	23.3	3150	13.6	3165	13.9	3150	14.0	4065	20.2	4110	40.3	5034	20.0	3190	22.3	4165	23.9	4054	20.8
5089	12.9	3190	22.2	4026	17.3	3190	17.5	4165	26.8	4172	18.3	5056	24.1	4054	18.4	4205	13.8	4110	11.5
5102	13.9	4054	7.1	4065	19.2	4054	15.1	4205	31.8	5018	17.7	5089	24.1	4110	25.9	5034	12.5	4172	15.6
5104	9.2	4110	17.2	4097	19.7	4110	15.0	5034	13.1	5027	22.0	5102	37.5	4172	26.7	5056	12.8	5018	12.0
5105	25.6	4172	10.0	4165	16.5	4172	15.2	5056	11.9	5032	20.6	5164	32.7	5018	24.8	5089	20.4	5027	14.5
5127	12.7	5018	15.6	4205	16.7	5027	17.7	5089	21.6	5060	23.1			5027	33.3	5102	9.2	5032	17.9
5164	14.5	5027	12.2	5034	11.7	5156	15.4	5102	26.1	5156	20.4			5032	25.7	5164	21.2	5060	19.5
		5032	21.4	5056	15.5	5161	19.6	5164	21.6	5161	16.7			5060	19.6			5156	18.6
		5060	18.3	5089	15.3	5184	20.8							5156	41.9			5161	15.6
		5156	17.8	5102	10.9									5161	18.2			5184	11.7
		5161	15.3	5164	24.3									5184	14.0				
		5194	16.7																
Mean	18.1		19.0		17.8		16.9		24.2		22.7		28.6		26.2		18.2		16.9

Perendale Ewes - IgG1 levels

Time	Line	Tag	Oc L3	Oc Ad	Cc L3	Cc Ad	Time	Line	Tag	Oc L3	Oc Ad	Cc L3	Cc Ad	Time	Line	Tag	Oc L3	Oc Ad	Cc L3	Cc Ad
Oct-96	Hi	469	0.52	0.82	0.59	0.80	Jun-97	Hi	409	0.75	0.86	0.65	0.78	Nov-97	Hi	429	0.93	0.82	0.92	0.86
Oct-96	Hi	479	0.56	0.94	0.63	0.78	Jun-97	Hi	469	0.64	0.72	0.71	0.75	Nov-97	Hi	469	0.59	0.74	0.56	0.74
Oct-96	Hi	2207	0.47	0.59	0.51	0.59	Jun-97	Hi	479	0.62	0.61	0.90	0.64	Nov-97	Hi	479	0.60	0.83	0.71	0.67
Oct-96	Hi	3165	0.53	0.93	0.53	0.81	Jun-97	Hi	498	0.74	0.57	0.60	0.59	Nov-97	Hi	498	0.70	0.63	0.60	0.64
Oct-96	Hi	9286	0.61	0.72	0.65	0.86	Jun-97	Hi	504	0.85	0.88	0.79	0.74	Nov-97	Hi	504	0.67	0.87	0.81	0.69
Oct-96	Lo	1039	0.57	0.87	0.49	0.83	Jun-97	Hi	1015	0.98	0.95	0.67	0.92	Nov-97	Hi	1015	0.79	1.10	0.69	0.91
Oct-96	Lo	2980	0.60	0.45	0.37	0.54	Jun-97	Hi	1027	0.62	0.65	0.60	0.71	Nov-97	Hi	2207	0.50	0.60	0.59	0.58
Oct-96	Lo	3131	0.32	0.68	0.39	0.60	Jun-97	Hi	2263	0.42	0.66	0.38	0.70	Nov-97	Hi	2942	0.82	0.80	0.51	0.77
Nov-96	Hi	409	0.81	0.92	0.69	0.81	Jun-97	Hi	2266	0.67	0.86	0.63	0.69	Nov-97	Hi	2965	0.71	0.65	0.51	0.74
Nov-96	Hi	469	0.57	0.69	0.52	0.76	Jun-97	Hi	2942	0.86	0.73	0.52	0.71	Nov-97	Hi	3018	0.71	0.93	0.56	0.77
Nov-96	Hi	479	0.49	0.73	0.50	0.73	Jun-97	Hi	2965	0.76	0.51	0.42	0.58	Nov-97	Hi	3056	0.81	0.98	0.62	0.91
Nov-96	Hi	2207	0.39	0.49	0.41	0.47	Jun-97	Hi	3018	0.59	0.77	0.43	0.51	Nov-97	Hi	3140	0.53	0.56	0.34	0.49
Nov-96	Hi	2942	0.44	0.66	0.40	0.66	Jun-97	Hi	3045	0.54	0.72	0.67	0.68	Nov-97	Hi	3165	0.67	0.82	0.54	0.81
Nov-96	Hi	3045	0.38	0.61	0.46	0.72	Jun-97	Hi	3056	0.45	0.54	0.41	0.37	Nov-97	Hi	4026	0.72	1.02	0.46	0.96
Nov-96	Hi	3165	0.48	0.66	0.43	0.70	Jun-97	Hi	3140	0.44	0.43	0.36	0.36	Nov-97	Hi	4065	0.69	0.93	0.65	0.89
Nov-96	Hi	9286	0.54	0.55	0.57	0.76	Jun-97	Hi	3165	0.60	0.72	0.51	0.65	Nov-97	Hi	4165	0.66	0.97	0.48	0.86
Nov-96	Lo	1043	0.45	0.83	0.35	0.83	Jun-97	Hi	4026	0.47	0.72	0.33	0.81	Nov-97	Hi	4205	0.69	0.89	0.40	0.87
Nov-96	Lo	1091	0.38	0.76	0.39	0.91	Jun-97	Hi	4065	0.47	0.57	0.53	0.52	Nov-97	Hi	5034	0.66	0.80	0.74	0.75
Nov-96	Lo	1107	0.37	0.53	0.27	0.31	Jun-97	Hi	4097	0.52	0.67	0.45	0.61	Nov-97	Hi	5056	0.82	0.71	0.82	0.87
Nov-96	Lo	2980	0.47	0.45	0.36	0.43	Jun-97	Hi	4165	0.65	0.78	0.57	0.74	Nov-97	Hi	5089	0.99	0.68	0.56	0.79
Nov-96	Lo	3131	0.24	0.48	0.29	0.43	Jun-97	Hi	4205	0.59	0.64	0.32	0.76	Nov-97	Lo	489	0.65	0.84	0.56	0.72
Nov-96	Lo	3190	0.26	0.41	0.24	0.33	Jun-97	Hi	5034	0.64	0.82	0.68	0.65	Nov-97	Lo	1008	0.45	0.73	0.48	0.72
Feb-97	Hi	469	0.91	0.92	0.86	0.92	Jun-97	Hi	5056	1.11	0.85	0.96	0.97	Nov-97	Lo	1018	0.70	0.98	0.73	0.81
Feb-97	Hi	479	0.68	0.83	0.92	0.87	Jun-97	Hi	5089	1.07	0.66	0.59	0.70	Nov-97	Lo	1039	0.58	0.90	0.56	0.80
Feb-97	Hi	504	0.93	0.99	0.89	0.76	Jun-97	Hi	5164	0.49	0.48	0.47	0.47	Nov-97	Lo	1057	0.62	0.71	0.48	0.76
Feb-97	Hi	2251	1.16	0.85	0.92	0.88	Jun-97	Lo	489	0.57	0.68	0.53	0.69	Nov-97	Lo	1085	0.68	0.60	0.53	0.70
Feb-97	Hi	2942	0.68	0.86	0.65	0.82	Jun-97	Lo	1039	0.73	0.99	0.74	0.76	Nov-97	Lo	1095	0.59	0.72	0.58	0.69
Feb-97	Hi	2968	0.80	0.90	0.61	0.78	Jun-97	Lo	1057	0.51	0.56	0.44	0.53	Nov-97	Lo	1107	0.50	0.56	0.35	0.48
Feb-97	Hi	3045	0.65	0.88	0.92	0.98	Jun-97	Lo	1095	0.57	0.54	0.56	0.51	Nov-97	Lo	2915	0.57	0.58	0.44	0.60
Feb-97	Hi	3056	0.99	1.16	0.94	0.94	Jun-97	Lo	1107	0.41	0.45	0.29	0.41	Nov-97	Lo	2971	0.81	0.76	0.56	0.79
Feb-97	Hi	3085	0.76	1.07	0.88	0.86	Jun-97	Lo	1125	0.70	1.13	0.73	0.97	Nov-97	Lo	3190	0.36	0.74	0.33	0.60
Feb-97	Hi	3140	0.63	0.66	0.65	0.73	Jun-97	Lo	2980	0.68	0.52	0.36	0.51	Nov-97	Lo	4110	0.93	0.73	0.57	0.60
Feb-97	Hi	4026	0.69	0.95	0.55	1.03	Jun-97	Lo	3008	0.42	0.63	0.41	0.58	Nov-97	Lo	4172	0.53	0.60	0.38	0.50
Feb-97	Hi	4065	1.00	0.95	0.97	0.97	Jun-97	Lo	3150	0.84	0.81	0.74	0.88	Nov-97	Lo	5018	0.75	0.69	0.91	1.02
Feb-97	Hi	4097	0.96	0.91	0.83	0.78	Jun-97	Lo	3190	0.48	0.75	0.29	0.62	Nov-97	Lo	5027	0.67	0.56	0.62	0.71
Feb-97	Hi	4165	0.85	1.10	0.79	1.02	Jun-97	Lo	4110	0.69	0.49	0.48	0.33	Nov-97	Lo	5156	0.63	0.93	0.70	0.99
Feb-97	Hi	4205	0.54	0.90	0.44	0.87	Jun-97	Lo	5027	0.71	0.60	0.81	0.68	Nov-97	Lo	5161	0.36	0.62	0.34	0.58
Feb-97	Hi	5034	0.76	1.13	0.80	0.89	Jun-97	Lo	5156	0.61	0.98	0.93	0.97	Nov-97	Lo	5184	0.52	0.60	0.49	0.60
Feb-97	Hi	5056	1.17	1.02	0.99	0.97	Jun-97	Lo	5161	0.39	0.64	0.70	0.40							
Feb-97	Hi	5089	1.04	0.85	0.78	0.80	Jun-97	Lo	5184	0.66	0.70	0.60	0.84							
Feb-97	Hi	5102	0.88	1.02	0.84	0.93	Sep-97	Hi	409	0.72	0.93	0.58	0.95							
Feb-97	Hi	5104	0.65	0.59	0.41	0.67	Sep-97	Hi	469	0.72	0.77	0.72	0.93							
Feb-97	Hi	5105	0.84	0.97	0.81	0.97	Sep-97	Hi	479	0.62	1.07	0.87	0.89							
Feb-97	Hi	5127	0.78	0.88	0.52	0.91	Sep-97	Hi	498	0.93	0.74	0.74	0.87							
Feb-97	Hi	5164	0.55	0.66	0.49	0.79	Sep-97	Hi	1015	1.01	0.96	0.72	1.01							
Feb-97	Lo	523	1.06	0.63	0.77	0.71	Sep-97	Hi	3140	0.46	0.65	0.48	0.77							
Feb-97	Lo	1043	0.59	0.94	0.85	0.94	Sep-97	Hi	3165	0.58	0.84	0.58	0.87							
Feb-97	Lo	1107	0.66	0.98	0.43	0.51	Sep-97	Hi	4026	0.65	1.05	0.56	1.01							
Feb-97	Lo	2915	0.73	0.69	0.67	0.65	Sep-97	Hi	5034	0.52	1.03	0.78	0.86							
Feb-97	Lo	3190	0.41	0.74	0.36	0.70	Sep-97	Hi	5056	1.09	1.03	1.04	1.02							
Feb-97	Lo	4172	0.35	0.49	0.36	0.41	Sep-97	Hi	5164	0.72	0.80	0.64	0.78							
Feb-97	Lo	5018	0.75	0.83	0.83	0.71	Sep-97	Lo	1018	0.79	0.87	0.69	0.91							
Feb-97	Lo	5027	0.98	0.78	0.81	0.71	Sep-97	Lo	1057	0.69	0.61	0.47	0.70							
Feb-97	Lo	5161	0.42	0.74	0.64	0.51	Sep-97	Lo	1085	0.55	0.57	0.47	0.75							
Feb-97	Lo	5194	0.74	0.65	0.77	0.61	Sep-97	Lo	1091	0.48	0.73	0.59	0.95							
Feb-97	Lo	7035	0.89	0.96	0.85	0.82	Sep-97	Lo	1095	0.63	0.50	0.65	0.69							
Feb-97	Lo	9203	0.65	0.71	0.94	0.82	Sep-97	Lo	1107	0.70	0.57	0.45	0.63							
May-97	Hi	479	0.69	0.71	0.99	0.76	Sep-97	Lo	3150	0.55	0.95	0.67	0.92							
May-97	Hi	504	0.82	0.83	0.92	0.69	Sep-97	Lo	3190	0.39	0.81	0.30	0.74							
May-97	Hi	1015	1.09	0.91	0.80	0.91	Sep-97	Lo	4172	0.49	0.52	0.46	0.55							
May-97	Hi	2251	1.07	0.85	0.90	0.72	Sep-97	Lo	5018	0.74	0.81	0.69	0.77							
May-97	Hi	2942	0.84	0.69	0.59	0.73	Sep-97	Lo	5027	0.73	0.82	0.85	0.84							
May-97	Hi	2965	0.79	0.60	0.70	0.75	Sep-97	Lo	5032	0.51	0.76	0.85	0.72							
May-97	Hi	3045	0.65	0.73	0.80	0.83	Sep-97	Lo	5156	0.56	1.14	0.88	1.02							
May-97	Hi	3056	0.78	0.97	0.67	0.72	Sep-97	Lo	5161	0.31	0.65	0.57	0.73							
May-97	Hi	3085	0.72	1.00	0.89	0.74	Oct-97	Hi	429	1.05	1.01	1.01	1.00							
May-97	Hi	3140	0.58	0.63	0.57	0.64	Oct-97	Hi	469	0.64	0.90	0.61	0.94							
May-97	Hi	4026	0.68	1.00	0.55	0.97	Oct-97	Hi	498	1.06	0.81	0.72	0.80							
May-97	Hi	4065	0.81	0.84	0.80	0.83	Oct-97	Hi	504	0.79	0.80	0.69	0.74							
May-97	Hi	4097	0.76	0.88	0.68	0.60	Oct-97	Hi	2207	0.52	0.71	0.67	0.72							
May-97	Hi	4165	0.83	1.12	0.73	0.91	Oct-97	Hi	2266	0.85	1.00	0.76	0.84							
May-97	Hi	4205	0.70	0.85	0.44	0.82	Oct-97	Hi	2942	0.88	1.13	0.60	0.95							
May-97	Hi	5034	0.81	1.17	0.84	0.89	Oct-97	Hi	2965	0.82	0.76	0.62	0.79							
May-97	Hi	5056	1.14	0.93	1.05	0.97	Oct-97	Hi	3018	0.77	1.07	0.59	0.82							
May-97	Hi	5089	1.24	0.78	0.90	0.80	Oct-97	Hi	3056	0.73	0.92	0.66	0.83							
May-97	Hi	5102	0.98	0.98	0.94	1.08	Oct-97	Hi	3140	0.45	0.69	0.50	0.65							
May-97	Hi	5104	0.68	0.74	0.41	0.67	Oct-97	Hi	3165	0.61	0.87	0.58	0.88							
May-97	Hi	5105	1.11	1.08	0.90	0.51	Oct-97	Hi	4026	0.67	1.05	0.54	1.01							
May-97	Hi	5127	0.60	0.96	0.54	0.63	Oct-97	Hi	4065	0.58	0.89	0.70	0.85							
May-97	Hi	5164	0.81	0.68	0.71	0.69	Oct-97	Hi	5034	0.70	1.06	0.90	0.88							
May-97	Lo	489	0.88	0.82	0.72	0.83	Oct-97	Hi	5056	1.02	0.95	0.95	0.93							
May-97	Lo	1018	0.79	1.01	0.82	0.85	Oct-97	Hi	5089	1.18	0.86	0.67	0.85							
May-97	Lo	1057	0.69	0.67	0.68	0.67	Oct-97	Lo	489	0.72	0.95	0.66	0.89							
May-97	Lo	1091	0.49	0.95	0.80	0.95	Oct-97	Lo	1018	0.70	0.98	0.50	0.97							
May-97	Lo	1095	0.63	0.65	0.76	0.59	Oct-97	Lo	1043	0.57	1.05	0.71	1.03							
May-97	Lo	1107	0.54	0.68	0.46	0.47	Oct-97	Lo	1057	0.74	0.86	0.52	0.84							
May-97	Lo	1125	0.63	1.03	0.56	0.79	Oct-97	Lo	1085	0.65	0.67	0.62	0.72							
May-97	Lo	4172	0.41	0.39	0.36	0.43	Oct-97	Lo	1095	0.69	0.98	0.67	0.84							
May-97	Lo	5027	0.88	0.71	0.91	0.73	Oct-97	Lo	1107	0.59	0.76	0.47	0.69							
May-97	Lo	5161	0.53	0.65	0.98	0.82	Oct-97	Lo	2915	0.68	0.84	0.55	0.80							
							Oct-97	Lo	2971	0.76	0.94	0.62	0.84							
							Oct-97	Lo	3190	0.34	0.97	0.37	0.81							
							Oct-97	Lo	4172	0.45	0.72	0.43	0.66							
							Oct-97	Lo	5032	0.61	0.77	0.86	0.71							
							Oct-97	Lo	5156	0.52	1.16	0.90	0.98							
							Oct-97	Lo	5161	0.34	0.86	0.52	0.77							
							Oct-97	Lo	5184	0.61	0.86	0.66	0.84							

PERENDALE LAMBS

PERENDALE LAMBS, sampled 25/11/96

High FEC Line											Low FEC Line										
Tag	FEC	FEC	Mean	Dev.Succ.	Haem.	Ost.	Trich.	Coop.	L.T.	Total Id.'ed	Tag	FEC	FEC	Mean	Dev.Succ.	Haem.	Ost.	Trich.	Coop.	L.T.	Total Id.'ed
302	100	350	308	2.7		15	1		4	20	318	0	0	8	12.0		4		1		5
	250	300	308	1.9		14				14		0	0	8	12.0		4		1		5
	300	550	308	1.6		32		1	1	34		50	0	8	6.9		1				1
332	300	200	208	6.9		10				10	425	50	50	25	0.0						0
	350	50	208	20.4		15				15		50	0	25	2.0						0
	150	200	208	4.4		26				26		0	0	25	0.0						0
342	900	500	475	2.0		22	1			23	454	150	100	150	1.0		10				10
	450	400	475	1.3		40		3		43		200	50	108	3.8		38		1	1	40
	200	400	475	0.5		10				10		100	50	108	0.9						0
363	250	350	325	1.3	3	20				23	461	0	100	25	9.9		10				10
	250	300	325	2.8	2	67		3		72		50	0	25	21.8		38	1		2	29
	350	450	325	4.0	5	87		8		100		0	0	25	3.9						0
372	450	800	508	1.6		59		1		60	475	150	250	158	0.3		10				10
	500	450	508	2.3		92				92		50	300	158	0.0		26	1		2	29
	350	500	508	1.5		11				11		100	100	158	0.5						0
374	750	550	625	1.8		89	1	10		100	497	0	0	16	7.7		8				8
	600	650	625	0.7		8		2	1	11		0	0	16	3.0						0
	700	500	625	0.5	1	15				16		0	100	16	3.4		1				1
377	300	400	233	1.2	3	15	1	1		20	6336	50	50	42	6.6		24				24
	100	350	233	0.7		11		1		12		0	100	42	11.1		4				4
	150	100	233	0.9		15		2		17		0	50	42	0.0						0
433	300	200	225	1.4		27	1		1	29	327	50	0	50	4.8		13			1	14
	200	100	225	1.5		26			2	28		0	50	50	11.1		29				29
	200	350	225	1.2		24		1		25		100	100	50	9.4		31			1	32
463	300	200	200	3.8		48	2			50	369	450	450	330	1.3		10				10
	250	100	200	3.0		5				5		300	250	330	0.0		1				1
	150	200	200	1.3		18		5		23		200		330	0.3		3				3
317	550	400	483	1.4		11		1		12	386	150	150	125	4.8		3				3
	550	500	483	1.6		10		1		11		100	150	125	3.5						0
	500	400	483	12.7	4	82		4	8	100		100	100	125	3.5		1				1
360	200	450	317	1.6		6				6	415	200	350	250	0.3		6		2		8
	400	400	317	2.4		7				7		300	250	250	1.1		22		2		24
	150	300	317	1.1		5				5		200	200	250	0.0						0
407	0	0	8	9.8		3				3	420	0	100	100	13.4		71				71
	0	50	8	7.3		1				1		100	150	100	15.9		75	1			76
	0	0	8	12.5		4		1		5		200	50	100	6.8		46		2		48
416	100	100	100	5.6		8	1	1	7	17	424	150	50	125	1.7		11				11
	150	50	100	7.2		2		1	6	9		250	100	125	4.1		31		2		33
	100		100	2.9		6		1		7		150	50	125	3.6	1	40		2		43
430	550	500	567	1.1		10		3	4	17	447	50	0	33	4.2	1	7		7		15
455	100	50	100	7.9		4				4		50	0	33	8.4	3	19		2		24
	50	200	100	10.1		29	1	4		35		50	50	33	15.0	4	56		40		50
470	250	200	158	6.5		13		5	9	27	449	350	250	266.7	0.5		4				4
	100	200	158	0.5		9				9		300	400	267	1.2		4				4
498	1050	950	900	5.9		89	2		9	100		150	150	267	0.8		2				2
	1150	750	900	3.0		87	5	5	3	100	472	50	50	92	4.5		1				1
	1000	500	900	4.2		63	5	1	4	73		50	150	92	10.6	1	31				32
320	150	100	125	10.6		46		8	13	67		200	50	92	23.3		39				39
	150	150	125	25.3		88		6	6	100	477	100	150	133	16.7	2	48				50
	100	100	125	25.9	1	82		5	12	100		200	150	133	7.9		50				50
331	0	0	66.67	3.3		12				12		150	50	133	12.3		98		2		100
	100	150	67	1.1		2				2	339	0	100	25	3.4		2				2
	100	50	67	2.4		6				6		50	0	25	13.3	1	21		5		27
352	100	50	117	6.0		24		2		26		0	0	25	9.6		4				4
	50	150	117	8.9		17		3	8	28	376	100	0	33.33	18.2	1	32		1		34
	100	250	117	4.5		1				1		0	50	33	12.4		4	1	1		6
365	250	250	192	5.2	1	42	2	5		50		0	50	33	0.0						0
	100	150	192	2.7	1	3	1	4		9	432	0	0	8	15.8		10	2	1		13
	100	300	192	5.2		83	1	10		94		0	0	8	6.0		4	1			5
368	250	200	242	93.4		98	1	1		100		0	50	8	73.1	1	47		4		52
	150	250	242	115.6		99	1			100	435	200	150	133	60.2	2	97		1		100
	400	200	242	133.8		99	1			100		100	200	133	18.2	4	93		3		100
413	300	300	267	3.2		20		1	4	25		50	100	133	43.3		100				100
	400	250	267	2.1		11		3		14	452	100	200	142	1.5		18				18
	200	150	267	2.3		4			1	5		0	100	142	1.6	1	12	2			15
491	100	100	142	2.7		17		2	10	29		250	200	142	1.8	1	11				12
	150	100	142	14.3		18	1		2	22											
	200	200	142	18.1		24		8	2	34	408	0	0	0							
												0	0	0							
		Mean	283.6	9.8								0	0	0							
											370	0	0	0							
												0	0	0							
												0	0	0							
											439	0	0	0							
												0	0	0							
												0	0	0							
											492	0	0	0							
												0	0	0							
												0	0	0							
													Mean	86	8.7						

PERENDALE LAMBS, sampled 19/12 1996																						
High FEC Line										Total	Low FEC Line											Total
Tag	FEC	FEC	Mean	Dev.Succ.	Haem.	Ost.	Trich.	Coop.	L.T.	Id.'ed	Tag	FEC	FEC	Mean	Dev.Succ.	Haem.	Ost.	Trich.	Coop.	L.T.	Id.'ed
302	300	350	217	31.7		81		19		100	318	0	0	8	35.0		11		4		15
	50	50	217	76.5		41		39		80		50	0	8	27.9		14		4		18
	250	300	217	114.2		24		8		32		0	0	8	15.0		1		6		7
332	100	200	175	6.5		2				2	425	0	0	8	24.5		1		5		6
	300	50	175	3.7		1		3		4		0	50	8	46.6		1		2		3
	250	150	175	7.2		19		10		29		0	0	8	27.5		5		2		7
342	200	250	267	100.2		88	1	11		100	454	0	0	8	85.3		12		4		16
	400	200	267	7.4	1	34	1	3		38		50	0	8	80.4		36		4		40
	300	250	267	37.5		79		21		100		0	0	8	78.1				5		5
363	300	250	183	48.3		24		79		100	475	0	0	25	33.3		45		15		60
	50	50	183	93.7		37		76		100		0	50	25	28.7		30		1		31
	200	250	183	77.8		57	1	63		100		0	100	25	22.8		15		2		17
372	450	100	275	124.1		57	1	42		100	6336	50	0	42	112.5		1		3		4
	400	150	275	78.3	1	78		21		100		150	0	42	64.4		6				6
	450	100	275	108.7		88		12		100		0	50	42	88.3		6				6
374	200	300	240	103.5		31		69		100	327	50	0	25	27.5		7		1		8
	50	600	240	65.0		31		69		100		50	0	25	9.5		3		1		4
	50		240	120.6		58		42		100		50	0	25	51.5		48		20		68
377	350	600	358	54.6		43		57		100	386	0	0	33	13.7		12		1		13
	300	150	358	77.9		46		54		100		50	100	33	4.9		2				2
	250	500	358	67.5		10		10		20		50	0	33	11.1		3		1		4
433	300	150	167	42.7		11		68		79	420	0	50	16	22.7		9		1		10
	150	100	167	43.8		26		74		100		0	0	16	98.6		15		3		18
	150	150	167	49.8		2		98		100		50	0	16	12.7		1				1
463	100	50	67	49.0		14		12		26	424	0	100	50	2.3		3				3
	150	50	67	33.9		1				1		0	50	50	0.0						0
	50	0	67	34.8		2				2		100	50	50	1.3		2				2
317	400	50	208	14.3		8		1		9	449	100	50	67	31.8		45		22		67
	300	200	208	14.4		70		5		75		100	100	67	5.5		18				18
	150	150	208	14.1		99		1		100		50	0	67	24.6		23				23
360	150	50	67	52.8		96		4		100	432	0	0	8	32.3		3		1		4
	100	50	67	42.2		96		4		100		0	0	8	56.1		18		10		28
	50	0	67	80.8		99		1		100		50	0	8	50.5		21		11		32
416	0	50	33	27.4		9		41		50	435	50	0	8	149.8		80		7		87
	0	50	33	17.9		4		6		10		0	0	8	217.5		29		12		41
	50	50	33	15.2		5		13		18		0	0	8	57.9		6		9		15
430	500	300	325	31.5		49		51		100	452	0	50	16	11.6		10		4		14
	450	150	325	34.6		81		19		100		0	50	16	12.2		1				1
	250	300	325	39.2		45		28		73		0	0	16	43.1		25		4		29
470	100	0	25	16.8		2				2	492	0	0	8	86.6		6		14		20
	0	0	25	8.3	1	2		7		10		0	0	8	91.0		35		15		50
	0	50	25	7.0		4		5		9		50	0	8	87.2		3		8		11
498	250	150	183	59.7		66		4		70											
	150	200	183	31.0		5		4		9	409	0	0	0							
	200	150	183	101.9		68		32		100		0	0	0							
320	100	250	208	21.2		77	3	20		100		0	0	0							
	200	250	208	5.1						0	461	0	0	0							
	200	250	208	12.5		60		6		66		0	0	0							
365	0	0	25	7.2		3				3		0	0	0							
	50	50	25	20.9		5		1		6	497	0	0	0							
	0	50	25	8.5		1		1		2		0	0	0							
368	150	100	167	150.9		94		6		100		0	0	0							
	150	250	167	107.6		81		19		100	415	0	0	0							
	300	50	167	107.8		91		9		100		0	0	0							
413	450	700	458	29.9		5		2		7		0	0	0							
	400	500	458	26.4		44		56		100	447	0	0	0							
	350	350	458	14.7		38		37		76		0	0	0							
491	150	200	225	27.9		100				100		0	0	0							
	250	200	225	28.2		99		1		100	472	0	0	0							
	300	250	225	40.8		92		8		100		0	0	0							
												0	0	0							
407	0	0	0								477	0	0	0							
	0	0	0									0	0	0							
	0	0	0									0	0	0							
331	0	0	0								339	0	0	0							
	0	0	0									0	0	0							
	0	0	0									0	0	0							
											370	0	0	0							
		Mean	176	48.0								0	0	0							
												0	0	0							
													Mean	14	47.2						

Perendale lambs sampled 13/1/97																					
High FEC Line										Total		Low FEC Line									Total
Tag	FEC	FEC	Mean	Dev.Succ.	Haem.	Ost.	Trich.	Coop.	LT.	Id'ed		Tag	FEC	FEC	Mean	Dev.Succ.	Haem.	Ost.	Trich.	Coop.	LT.	Id'ed
320	650	900	933	46.0	1	78	4	16	1	100		318	0	50	16	2.9						0
	950	1200	933	7.5		96		4		100			0	50	16	43.5				3		3
	1000	900	933	40.8		92		8		100			0	0	16	1.8		3				3
331	0	0	16	10.9		8				8		336	150	0	100	4.9		10				10
	50	50	16	12.6		5	1	5		11			150	50	100	9.1		25		12		37
	0	0	16	3.0		3		1	1	5			200	50	100	9.5	1	31		12		44
352	250	250	175	18.8		35	5	60		100		409	0	0	16	35.0	1	6	1	23		31
	50	200	175	28.4		39	3	24		66			50	0	16	15.8				16		16
	50	250	175	29.8	1	26	6	67		100			50	0	16	42.5	1	6		31		38
365	150	150	133	2.9		15		25		40		454	50	0	42	7.2		8				8
	100	150	133	1.1		4		7		11			50	50	42	12.6		36		14		50
	150	100	133	6.6	1	10	6	32		49			0	100	42	4.4		11	1	2	1	15
368	550	350	775	13.4		71		26	3	100		461	0	0	8	13.6		3		8		11
	1200	1050	775	19.5		68		32		100			0	0	8	8.3		2				2
	500	1000	775	18.8		65		35		100			0	50	8	1.2		1				1
491	350	100	183	0.4		6		1		7		475	50	50	58	7.8		14				14
	100	200	183	1.7		3	1	4		8			50	100	58	18.4		40		30		70
	300	50	183	2.1		10				10			50	50	58	8.8		19		7		26
302	900	1100	1000	3.2	1	36		63		100		497	50	50	42	14.8		16		28		44
	1100	1000	1000	1.1	2	9		18	1	30			50	0	42	2.4		4	1	1		6
	1100	800	1000	1.6	5	41		39		85			0	100	42	2.4				1		1
332	100	950	592	1.5	1	37		26		64		327	0	0	8	2.4				2		2
	550	450	592	2.4		26		14		40			50	0	8	28.9	2	3		15		20
	700	800	592	0.5		10		10		20			0	0	8	24.0		8	1	6		15
342	1850	1100	1525	2.4		58	1	41		100		369	0	50	25	3.6				9		9
	1450	1650	1525	1.7	4	32		64		100			0	0	25	2.6		2				2
	1100	2000	1525	0.8		20		21		41			50	50	25	4.5		1		1		2
363	800	950	975	22.3		49		51		100		386	0	50	16	8.8		1		4		5
	900	1350	975	17.0	1	39		60		100			0	50	16	7.8		3		5		8
	1100	750	975	17.8	3	50		57		110			0	0	16	8.4	1	4		9		14
372	1100	1500	1217	0.7		13		14		27		415	0	150	58	4.1						0
	1050	1500	1217	0.1		11		3		14			0	50	58	0.3				2		2
	1150	1000	1217	0.5		27		21		48			50	100	58	0.3		2				2
374	2050	2250	1583	2.1		41		59		100		420	50	0	33	6.1		2		4		6
	1300	1150	1583	3.4		50		11		61			100	50	33	5.1		1		2		3
	1450	1300	1583	2.2		46	2	52		100			0	0	33	4.1		2	2	5		9
377	1100	950	917	9.7		25		75		100		424	100	100	58	11.3		4		8		12
	750	900	917	15.8		27	3	70		100			50	0	58	5.2		8	3	6		17
	1050	750	917	14.6	3	35	15	45	2	100			50	50	58	4.9		12		13		25
433	150	450	242	29.4		42		58		100		447	0	0	16	38.0		5	1	12		18
	250	250	242	14.6	1	40	1	58		100			0	50	16	14.0		1		1		2
	100	250	242	8.0		42		58		100			50	0	16	24.0		13		12		25
463	1250	600	983	0.7	2	20		24		46		472	0	0	16	7.4		1		6		7
	1200	800	983	1.8		54		46		100			0	0	16	13.2	3	1		8		12
	1250	800	983	0.1				1		1			0	100	16	4.8	2	1		5		8
317	350	1000	592	14.7		84	5	11		100		477	0	50	8	16.4				13	1	14
	500	750	592	5.8	1	77	6	21	1	106			0	0	8	12.7		2		8	1	11
	500	450	592	11.4	1	74	1	24		100			0	0	8	8.1		1		1		2
360	1050	1000	883	25.2		98		2		100		339	0	0	8	32.1		6		1		7
	1150	950	883	74.1		98	1	1		100			0	50	8	7.4		1				1
	550	600	883	54.0		99		1		100			0	0	8	25.7		7		4		11
416	150	300	200	5.1		24	3	48	2	77		370	0	0	8	21.6	1	3		4		8
	250	350	200	3.9		14		28	1	43			50	0	8	9.5		5		3		8
	50	100	200	5.1		4		18		22			0	0	8	35.4	1	9		10		20
430	3750	2100	2992	1.7		60	1	38	1	100		432	100	150	75	83.6		57	1	41	1	100
	3000	2800	2992	2.5		22		9		31			50	100	75	71.2		83	3	13		100
	3300	3000	2992	2.6	1	56		43		100			0	50	75	98.5		47		53		100
470	50	100	58	8.5		9		5		14		439	0	0	8	154.8						0
	100	0	58	12.0		11		10		21			50	0	8	18.8		13		2		15
	0	100	58	14.4		40		43		83			0	0	8	67.7		26	1	2		29
498	300	700	517	17.2		73		27		100		452	0	50	33	22.4		42		7		49
	650	500	517	9.9		85		15		100			0	50	33	40.9		40		10		50
	350	600	517	18.3		94	1	5		100			50	50	33	7.8		22		4		26
												492	50	200	92	10.9		50	3	21	1	75
		Mean	785	11.5									0	50	92	13.5		10		2		12
													100	150	92	11.4	1	42		24		67
												435	0	0	0							
													0	0	0							
													0	0	0							
														Mean	32	19.3						

PERENDALE LAMBS sampled 5/3 1997																						
High FEC Line										Total	Low FEC Line											Total
Tag	FEC	FEC	Mean	Dev.Succ.	Haem.	Ost.	Trich.	Coop.	LT	id.'ed	Tag	FEC	FEC	Mean	Dev.Succ.	Haem.	Ost.	Trich.	Coop.	LT		id.'ed
320	1050	700	808	4.9		53	20	27		100	318	0	0	92	6.0	1	44	5	1			51
	950	1000	808	0.8		29	9	26		63		250	0	92	8.7							
	450	700	808	11.6	1	77	5	14	3	100		50	250	92	1.0							
331	300	250	317	1.6	3	33	9		1	45	336	50	200	100	19.4	3	78	15	4			100
	300	450	317	1.7		17	1	1		19		200	50	100	13.8							
	300	300	317	2.0		33	7	1		41		0	100	100	44.1							
352	1250	1150	1183	1.4		38	7	24	1	70	409	0	50	8	25.7		9		2	1		12
	1300	1500	1183	0.5								0	0	8	14.0			1	1			2
	950	950	1183	1.6								0	0	8	62.5		47	1	1	4		53
365	300	400	292	10.7	2	43	2	53		100	454	0	50	25	102.6		91	3	5	1		100
	300	300	292	1.6								50	50	25	27.2							
	150	300	292									0	0	25	93.1							
368	700	1350	1117	16.6	1	40	23	35	1	100	461	50	100	83	3.4		29	1				30
	1500	1250	1117	10.6								0	100	83	0.4	1	1	1				3
	900	1000	1117	15.5								200	50	83	13.8	4	27	5	26	4		66
491	300	300	433	1.2							475	0	0	33	12.3		2	1	5			8
	400	700	433	0.1		10		3		13		50	50	33	28.9		28	38				66
	450	450	433									100	0	33	10.4	2	9	11	3			25
302	600	1300	1133	32.9	3	34	14	40	9	100	369	0	0	42	12.2		16		11			27
	600	1500	1133	35.4								100	0	42	29.0	1	15	2	47	3		68
	1300	1500	1133									100	50	42	24.0	2	31		52	1		86
332	1550	1450	1650	7.1	2	23	2	70	3	100	386	0	0	16	4.2		1		1			2
	2050	1700	1650	10.9								50	0	16	9.5	1	1	2	2			6
	1550	1600	1650	9.1								0	50	16	7.6		3					3
342	1750	2000	1767	1.9	1	25	6	67		100	420	0	0	25	11.2		9	1	7	1		18
	1500	2050	1767	1.3								0	50	25	1.9	2	2	1				5
	1800	1500	1767	1.5								50	50	25	3.1				2	1		3
363	2450	2050	2183	26.8		20	1	79		100	424	100	0	33	4.6		1					1
	2400	1950	2183	41.4								0	50	33	5.3		4		8	7		19
	1900	2350	2183	38.1								50	0	33	5.7		1		4			5
372	2150	2000	1967	4.3	1	37	3	59		100	447	50	50	50	4.5		6	1	7			14
	2450	1800	1967	3.8								50	100	50	4.4				2			2
	1900	1500	1967	7.2								50	0	50	3.8	1	2	2	1			6
374	2100	2350	2200	65.3		12	4	84		100	472	50	150	67	23.3		40	4	50	6		100
	2250	2500	2200	57.0								50	50	67	10.2							
	1950	2050	2200	18.9								50	50	67	7.1							
377	850	1550	1083	5.7		11	19	68	2	100	477	50	0	33	22.2		6		11			17
	1050	1200	1083	8.6								50	100	33	12.9		12	4	8			24
	600	1250	1083	4.5	9	9	9	72	1	100		0	0	33	19.2		8		13			21
433	850	750	1000	13.6		39		60	1	100	339	0	50	25	4.3		1	2	2			5
	1000	800	1000	16.9								50	50	25	0.7		1		1			2
	1500	1100	1000	21.2								0	0	25	4.9		9	1	3			13
463	2400	3250	3167	0.7		67	6	27		100	370	0	50	8	3.8				2	1		3
	3000	3500	3167	0.6								0	0	8	1.2	1						1
	3250	3600	3167	0.5								0	0	8	13.5				1			1
317	450	200	508	7.6		36	1	55	8	100	432	50	0	75	22.2	3	42		55			100
	850	800	508	9.1								150	50	75	26.7							
	400	350	508	0.4								100	100	75	26.3							
360	600	450	558	10.5		4				4	452	0	0	8	38.0		19		1	1		21
	650	450	558	3.7	1	50	5	2	2	60		0	50	8	15.9		3					3
	500	700	558	3.9	5	78	9	1	7	100		0	0	8	31.3		8	2		1		11
430	600	1100	858	3.9	1	27		66	1	100	492	50	50	33	9.4	5	1		2			8
	900	900	858	0.9								50	0	33	28.0	29	5	1	14	1		50
	800	850	858	1.8								0	50	33	7.5	20	3	2				25
470	150	300	233	2.4	1	29		10	10	50												
	250	200	233	3.4							497	0	0	0								
	150	350	233	4.7								0	0	0								
498	600	700	767	11.5	2	78	6	11	3	100		0	0	0								
	850	750	767	24.6	5	54	10	28	3	100	327	0	0	0								
	850	850	767	27.6	2	46	12	34	6	100		0	0	0								
												0	0	0								
		Mean	1161	11.1							415	0	0	0								
												0	0	0								
												0	0	0								
											435	0	0	0								
												0	0	0								
												0	0	0								
											439	0	0	0								
												0	0	0								
												0	0	0								
														Mean	33	17.5						

PERENDALE LAMBS sampled 9/4 1997																					
High FEC Line										Total	Low FEC Line										Total
Tag	FEC	FEC	Mean	Dev.Succ.	Haem.	Ost.	Trich.	Coop.	LT.	Id'ed	Tag	FEC	FEC	Mean	Dev.Succ	Haem.	Ost.	Trich.	Coop.	LT.	Id.'ed
320	300	900	492	36.4		50	1	49		100	318	150	50	33	25.2		31	9	10		50
	400	400	492	38.0								0	0	33	0.0						
	500	450	492									0	0	33	27.9						
352	750	1450	1183	9.2	2	22		76		100	409	50	0	25	26.8		35		2		37
	900	1000	1183	6.2								0	50	25	7.2		9	2	2		13
	1500	1500	1183	10.7								50	0	25							
365	800	250	492	55.8		29		71		100	454	50	0	25	17.7		19	3	5		27
	400	850	492	0.6								0	100	25	17.4		26	3	7		36
	250	400	492	2.0								0	0	25	44.0						
368	5000		5520	3.4		57	3	40		100	461	50	0	16	92.3		45	5	50		100
	5850		5520									0	0	16	57.1						
	5700		5520									50	0	16	46.7						
491	2850	2500	2267	1.2		84	10	6		100	475	0	0	16	97.0		43	56	1		100
	1750	2300	2267	1.2								100	0	16	9.4						
	1950	2250	2267	0.6								0	0	16							
302	3550	4550	5150	36.0	1	54	5	37	3	100	497	100	50	175	42.7		52	17	31		100
	7350	4550	5150	33.4								150	400	175							
	6050	4850	5150											175							
332	2750	3500	2858	4.7		47	1	52		100	327	0	100	92	14.5		53	6	41		100
	3100	3200	2858	2.2								50	100	92	23.3						
	2100	2500	2858	1.1								250	50	92	40.3						
342	4800	4200	4133	0.4		24	1	25		50	369	200	50	100	2.5		3		3		6
	4750	3350	4133	4.8								100	0	100	4.6		26	1	9		36
	5000	2700	4133									50	200	100	3.7		15	1	14		30
363	1350	2100	1858	6.6		19		81		100	386	250	100	133	21.1		9		41		50
	1900	2150	1858	16.2								200	100	133	5.2						
	2550	1100	1858	9.8								100	50	133	10.2						
372	5450	4600	3908	0.4		23	6	21		50	420	50	100	150	2.8		21	5	12		38
	4100	3550	3908	1.2								100	250	150							
	3150	2600	3908	1.0								100	300	150							
374	1850	2050	1925	0.1		3		2		5	424	300	300	225	0.5		4		4		8
	2250	1450	1925	0.2		9	1	3		13		100	200	225	2.6		30	3	10		43
	1500	2450	1925											225							
377	1500	1750	1417	3.7		20	7	73		100	435	50	50	42	17.3		43	5	2		50
	1250	800	1417	26.1								0	100	42	25.2						
	1550	1650	1417	18.6								50	0	42	28.0						
433	4050	4350	4042	20.8		36	4	60		100	449	50	50	58	25.6	1	73	6	20		100
	3800	4150	4042	11.8								50	50	58	49.4						
	3900	4000	4042	10.2								100	50	58	23.0						
463	1150	700	883	0.8		17	4	29		50	472	50	0	58	0.7		2		2		4
	800	750	883	2.6								50	50	58	0.5		3				3
	900	1000	883	1.9								150	50	58							
317	800	850	775	4.4	1	42	6	51		100	477	0	100	25	41.1		29		63		92
	1100	500	775	3.3								0	0	25	29.9						
	850	550	775	8.3								0	50	25							
360	1300	700	917	6.0		90	6	4		100	432	100	150	125	95.7		33	5	61	1	100
	900	850	917	7.1								150	100	125	21.2						
	700	1050	917											125	106.7						
416	200	400	275	122.7		34	8	58		100	439	0	0	16	23.9		6		12	1	19
	200	200	275	67.3								0	50	16	65.6		22	2	26		50
	300	350	275	83.2								0	50	16	3.1						
430	2500	1900	2350	1.4		58	10	32		100	452	50	50	33	20.8		38	5	7		50
	3050	2400	2350	2.5								50	0	33	13.7						
	2100	2150	2350	3.5								50	0	33	0.0						
470	650	700	767	2.5		81	1	16	2	100	492	300	250	275	13.8		21	3	76		100
	850	950	767	3.8								350	200	275							
	650	800	767	3.1										275							
		Mean	2169	14.0							339	0	0	0							
												0	0	0							
												0	0	0							
											370	0	0	0							
												0	0	0							
												0	0	0							
													Mean	77	27.1						

PERENDALE LAMBS sampled 12/5 1997

High FEC Line

Tag	FEC	FEC	Mean	Dev.Succ.	Haem.	Ost.	Trich.	Coop.	LT	Total Id.'ed
331	100	150	133	0.0						0
331	0	300	133	0.1			1			1
331	200	50	133	0.7						0
352	150	550	517	13.8		6	71	23		100
352	400	600	517	41.2						
352	600	800	517	22.4						
365	50	250	108	0.8		1	2	1		4
365	50	100	108	0.9		1				1
365	200	0	108	0.4						0
368	950	1350	1025	1.5		3	5	1	2	11
368	1250	950	1025	13.1						
368	800	850	1025	3.5						
491	1250	2250	1675	1.3		19	30	2		51
491	1800	1650	1675	0.3						
491	1600	1500	1675	1.0						
302	1450	1000	1158	1.7		8	42	50		100
302	1550	1150	1158	5.5						
302	950	850	1158	10.9						
332	2950	1700	2300	1.9		14	24	62		100
332	2650	2100	2300	4.3						
332	2450	1950	2300	6.4						
342	2550	1700	1875	0.5		16	56	28		100
342	1950	1800	1875	3.9						
342	2100	1150	1875	1.9						
363	2600		3550	18.5		3	20	77		100
363	4500		3550	15.8						
372	2150	200	1917	0.1		4	1	6		11
372	2050	2400	1917	0.1		2	1	3		6
372	2050	2650	1917	0.1			5	3		8
374	1950	1500	1675	1.3		6	56	38		100
374	2250	1250	1675	0.2						
374	1750	1350	1675	0.6						
377	1700	1250	1200	4.5		17	29	54		100
377	850	900	1200	14.8						
377	1300	1200	1200	9.0						
463	650	700	750	2.8		6	8	39		53
463	550	1000	750	1.6						
463	800	800	750	1.9						
317	400	200	283	0.5			9	5		14
317	150	350	283	1.0			5	5		10
317	350	250	283	0.5		6	10	21		37
360	350	550	317	8.9		30	60	10		100
360	350	150	317	22.4						
360	150	350	317	10.4						
416	350	450	425	4.8		20	40	39		99
416	550	350	425	13.0						
416	550	300	425	21.8						
430	750	1150	958	1.2		50	40	10		100
430	800	1500	958	0.6						
430	900	650	958	0.6						
470	150	300	125	0.8		9		2		11
470	100	50	125	0.1			1			1
470	50	100	125	0.9			1	1		2
		Mean	1065	5.6						

Low FEC Line

Tag	FEC	FEC	Mean	Dev.Succ.	Haem.	Ost.	Trich.	Coop.	LT	Total Id.'ed
318	150	150	100	3.9		8	33			41
318	100	50	100	12.4		9	56			65
318	0	150	100	11.3		5	43			48
6336	100	0	100	3.6		8	15	6		29
6336	150	150	100	11.3		1	62			63
6336	100	100	100	7.0						
409	0	50	25	1.9						0
409	50	0	25	1.9						0
409	50	0	25	0.0						0
454	200	150	142	3.6		15	37			52
454	150	0	142	3.9						
454	100	250	142	4.8						
475	0		50	2.0			4	1		5
475	100		50	2.2			1			1
327	250	50	133	30.1		72	2	24		100
327	100	200	133	15.1			92	8		100
327	150	50	133	33.9						
369	50		25	21.7		1	5	1		7
369	0		25	24.9		8	26	15		49
369			25	10.6		1	3	3		7
386	50		50	3.8		4	15			19
386	50		50	10.8		2	2	2		6
386			50	16.7		6	10	21		37
415	0	0	25	0.0						0
415	100	0	25	2.6			2			2
415	0	50	25	0.8			2			2
420	0	100	75	3.2		1	8	1		10
420	50	100	75	7.1		5	8			13
420	150	50	75	6.4		8	8			16
435	100	100	75	6.5		3	37	1		41
435	50	100	75	8.5		8	18	2		28
435	50	50	75	4.0			2			2
447	200	50	83	19.4		3	56			59
447	0	100	83	15.9						
447	50	100	83	4.7						
472	150	50	125	4.7		10	27	2		39
472	200	100	125	12.5		2	91	7		100
472			125	16.9						
432	450	450	563	7.7		6	92	2		100
432	600	750	563	10.9						
432			563	6.6						
439	150	200	83	3.3		1	7	1		9
439	0	150	83	4.0		6	10			16
439	0	0	83	3.3			2	2		4
492	300	100	192	8.0		8	34			42
492	200	250	192	2.7		1	21	2		24
492	200	100	192	7.3		9	36			45
477	0	0	0							
477	0	0	0							
477	0	0	0							
370	0	0	0							
370	0	0	0							
370	0	0	0							
452	0	0	0							
452	0	0	0							
		Mean	100	8.6						

PERENDALE LAMBS sampled 3/3 1998

High FEC Line

Tag	FEC	FEC	Mean	Dev.Succ.	Haem.	Ost.	Trich.	Coop.	LT	Total Id'ed	%D.M.
512	250	150	200	8.8		83	18	5	15	121	24.8
	200	250		33.3							
	100	250		29.2							
520	300	350	283	85.7		76	10	11	3	100	28.6
	100	500		102.3							
	300	150		42.2							
533	700	700	667	9.2		73	8	18	1	100	21.6
	600	1100		14.4							
	400	500		16.8							
548	200	100	217	8.0		25	7	15	3	50	25.3
	500	100		40.3							
	200	200		44.4							
549	700	800	767	19.6		65	7	25	3	100	21.9
	1100	950		8.8							
	450	600		11.9							
559	350	200	256	6.7	3	41	8	2	1	55	19.1
	150	300		8.6							
	350	200		10.5							
582	950	700	642	2.2		49	16	22	1	88	17.0
	300	600		4.2							
	300	1000		5.7							
583	450	200	233	3.2		5	2	2	1	10	11.0
	250	50		2.0		21	2	5		29	
	350	100		1.9		24	3	5	2	34	
592	550	400	375	20.0	2	70	11	4	13	100	22.2
	450	350		19.3							
	350	150		19.5							
623	300	500	308	4.0		39	5	3	3	50	15.5
	150	300		4.9							
	450	150		2.2							
627	200	350	242	1.4		8	5	2		15	11.1
	150	150		0.5		11				11	
	350	250		1.9		8				8	
651	650	350	508	3.0		40	8	5		53	12.5
	300	700		2.9							
	250	800		3.6							
654	50	150	117	21.5		38	4	10	4	56	23.4
	50	150		20.6							
	150	150		23.8							
670	150	300	275	9.0		97	21	3	13	134	22.2
	150	350		6.9							
	200	500		17.3							
676	200	150	167	10.5		56	22	2		80	19.2
	50	250		10.7							
	50	300		8.3							
		Mean	351	16.3							19.7

Low FEC Line

Tag	FEC	FEC	Mean	Dev.Succ.	Haem.	Ost.	Trich.	Coop.	LT	Total Id'ed	%D.M.
517	150	50	92	1.4		12	2			14	15.0
	100	150	92	3.0		26	2			28	
	50	50	92	5.2		35	2	4		41	
526	150	50	33	3.2		9	1	1		11	15.0
	0	0	33	4.5		5				5	
	0	0	33	1.1		3	1			4	
541	50	250	92	0.2		2				2	16.8
	100	0	92	7.4		39	2			41	
	100	50	92	4.6		12				12	
557	0	50	17	0.0						0	12.3
	50	0	17	0.0						0	
	0	0	17	0.0						0	
562	100	0	33	0.8		2	1			3	
	50	0	33	4.5		5		1		6	
	0	50	33	0.0						0	
574	100	0	17	0.6		1				1	9.9
	0	0	17	0.0						0	
	0	0	17	0.0						0	
585	50	150	100	1.8		14	2			18	16.0
	50	0	100	2.7		15	1	1		17	
	250	100	100	7.0		34	5	2		41	
590	100	0	50	3.8		11	9			20	14.9
	100	50	50	1.2		6				6	
	50	0	50	8.1		33	7		1	41	
628	0	50	17	16.0		5	3		1	9	16.4
	0	0	17	0.0						0	
	50	0	17	10.0		6	2			8	
646	0	50	25	10.6		8	1			9	19.8
	50	0	25	11.9		9	2			11	
	50	0	25	8.8		11	2			13	
648	50	50	25	1.1		3				3	13.8
	0	50	25	5.9		6				6	
	0	0	25	17.8		4		1		5	
658	100	100	42	2.5		10	1			11	12.6
	0	50	42	0.5		2				2	
	0	0	42	2.3						0	
675	50	100	58	52.4		73	5	13		91	
	0	150	58	26.9		52	4	34		90	
	50	0	58	41.8							
526	0	0									19.1
	0	0		6.9							
	0	0									
570	0	0									14.2
	0	0									
	0	0									
665	0	0									15.8
	0	0									
	0	0									
		Mean	46	6.9							15.2

Perendale ewe lambs, sampled 9/4/97								
High FEC Line					Low FEC Line			
Tag	FEC	Dev.Succ.	%D.M.		Tag	FEC	Dev.Succ.	%D.M.
355	250	8.2	24.0		406	150	189.9	17.6
350	350	4.5	17.1		427	100	2.3	19.2
422	1000	6.1	18.9		434	0		18.5
423	150	4.9	23.1		489	100	9.9	24.4
446	100	45.8	26.6		308	150	7.7	23.1
310	600	1.1	19.3		359	400	2.1	18.2
312	100	14.8	16.1		465	0		19.4
315	350	2.3			321	250	15.6	14.3
357	550	8.9	19.8		366	0		22.5
431	200	6.0	76.5		462	0		19.9
Mean	365	10.3	26.8			115	37.9	19.7

Perendale lambs, Faecal Dry Matter Percentage																								
	Sampled 25/11/96				Sampled 19/12/96				Sampled 13/1/97				Sampled 5/3/97				Sampled 9/4/97				Sampled 12/5/97			
	High FEC Line		Low FEC Line		High FEC Line		Low FEC Line		High FEC Line		Low FEC Line		High FEC Line		Low FEC Line		High FEC Line		Low FEC Line		High FEC Line		Low FEC Line	
	Tag	%D.M.	Tag	%D.M.	Tag	%D.M.	Tag	%D.M.	Tag	%D.M.	Tag	%D.M.	Tag	%D.M.	Tag	%D.M.	Tag	%D.M.	Tag	%D.M.	Tag	%D.M.	Tag	%D.M.
	302	41.4	318	69.6	302	38.9	318	40.6	320	20.6	318	13.8	320	15.7	318	27.2	320	41.2	497	30.8	331	17.5	318	20.1
	317	68.5	327	55.6	332	39.7	425	25.4	331	12.1	336	18.6	331	13.1	336	22.8	352	22.4	327	23.9	352	22.3	6336	20.2
	320	45.4	369	42.0	342	43.4	454	36.4	352	19.1	409	24.4	352	15.6	409	18.6	365	20.9	369	16.2	365	15.6	409	12.4
	331	50.8	376	74.5	363	46.7	475	34.0	365	15.3	454	15.8	365	18.4	454	18.0	368	40.0	386	21.9	368	20.5	454	17.0
	332	46.6	386	54.2	372	26.8	6336	27.7	368	20.6	461	13.7	368	15.5	461	21.7	491	12.7	420	20.0	491	15.3	475	23.1
	342	43.9	409	68.0	374	25.4	327	19.9	491	8.6	475	21.0	491	11.4	475	19.6	302	40.7	424	20.7	302	26.6	327	23.1
	352	78.7	415	47.5	377	41.8	386	48.1	302	9.3	497	13.5	302	28.6	327	19.6	332	15.4	435	24.8	332	21.0	369	20.0
	360	86.7	420	51.7	433	29.1	420	16.7	332	11.1	327	17.6	332	17.5	369	14.8	342	16.4	449	16.5	342	22.6	386	25.0
	363	56.5	424	46.7	463	36.6	424	49.1	342	18.4	369	16.8	342	14.3	386	13.2	363	26.1	447	18.7	372	12.5	415	15.3
	365	49.7	425	54.4	317	22.5	449	31.4	363	23.9	386	11.0	372	13.8	415	11.2	372	11.6	472	17.7	374	17.1	420	22.6
	368	67.2	432	55.6	360	27.8	432	25.3	372	11.2	415	12.6	374	24.5	420	17.1	374	13.9	477	35.3	377	21.5	435	19.1
	372	58.0	435	53.7	407	48.4	435	41.9	374	17.8	420	12.3	377	20.4	424	14.4	377	22.0	339	19.0	463	20.6	447	23.3
	374	54.2	439	50.9	430	46.2	452	35.8	377	26.7	424	16.1	433	18.0	435	12.9	433	18.2	370	21.3	317	15.5	472	26.7
	377	44.2	447	40.6	470	35.4	492	42.2	433	20.9	435	14.5	463	18.8	427	14.5	463	13.9	432	50.0	360	23.5	477	14.8
	407	44.1	449	38.6	498	36.0			463	14.5	447	13.4	317	13.3	472	15.8	317	15.5	439	28.8	416	24.5	370	23.5
	413	50.0	452	42.9	320	24.3			317	22.8	472	11.6	360	15.6	477	19.6	360	21.2	452	22.2	430	16.6	432	31.8
	416	59.0	454	47.5	331	24.5			360	33.1	477	16.0	430	13.8	339	16.0			492	30.8	470	22.0	439	16.1
	430	91.6	461	44.1	352	34.2			416	19.9	339	14.4	470	13.8	370	13.7							452	26.7
	433	44.4	472	75.0	365	18.3			430	19.1	370	13.5	498	17.1	432	21.8							492	18.1
	463	48.2	475	44.0	368	28.5			470	21.1	432	26.1			439	11.3								
	470	87.4	477	63.6	413	21.4			498	20.8	439	17.8			452	13.9								
	491	59.1	492	48.5	491	28.8					452	15.2			492	14.2								
	498	60.3	497	43.9							492	17.2												
			6336	46.5																				
Mean		58.1		52.5		32.9		33.9		18.4		16.0		16.8		16.9		22.0		24.6		19.7		21.0

Perendale lambs, IgG levels

Tag	Date	Line	ccl3	ocl3	ocad	tcl3	tcad	Tag	Date	Line	ccl3	ocl3	ocad	tcl3	tcad
302	Nov-96	High	0.30	0.13	0.18	0.23	0.29	309	Nov-96	Low	0.3661	0.1247	0.22	0.3343	0.3509
305	Nov-96	High	0.36	0.16	0.27	0.26	0.38	321	Nov-96	Low	0.3892	0.1431	0.2053	0.3038	0.2899
313	Nov-96	High	0.38	0.15	0.29	0.28	0.34	322	Nov-96	Low	0.3329	0.1521	0.2029	0.2594	0.3006
319	Nov-96	High	0.40	0.14	0.29	0.29	0.40	324	Nov-96	Low	0.2731	0.1296	0.1496	0.2463	0.2159
332	Nov-96	High	0.27	0.18	0.20	0.25	0.29	337	Nov-96	Low	0.272	0.1469	0.1593	0.2525	0.2074
334	Nov-96	High	0.41	0.20	0.29	0.29	0.41	339	Nov-96	Low	0.2856	0.1466	0.1716	0.3062	0.2368
342	Nov-96	High	0.44	0.17	0.34	0.29	0.40	340	Nov-96	Low	0.2759	0.1358	0.1678	0.2272	0.237
350	Nov-96	High	0.37	0.17	0.28	0.31	0.36	354	Nov-96	Low	0.4142	0.1836	0.2648	0.2871	0.3428
351	Nov-96	High	0.46	0.17	0.33	0.29	0.47	366	Nov-96	Low	0.2555	0.1233	0.172	0.239	0.222
353	Nov-96	High	0.27	0.12	0.23	0.23	0.29	370	Nov-96	Low	0.3878	0.2787	0.2668	0.3794	0.3257
363	Nov-96	High	0.34	0.16	0.21	0.31	0.30	405	Nov-96	Low	0.4136	0.1883	0.2286	0.4741	0.2572
372	Nov-96	High	0.24	0.19	0.18	0.28	0.23	408	Nov-96	Low	0.3029	0.215	0.229	0.3157	0.2655
375	Nov-96	High	0.50	0.22	0.42	0.43	0.50	411	Nov-96	Low	0.306	0.1753	0.2188	0.3221	0.3455
377	Nov-96	High	0.26	0.17	0.17	0.26	0.20	414	Nov-96	Low	0.2931	0.1807	0.1929	0.2881	0.2643
385	Nov-96	High	0.27	0.17	0.22	0.26	0.26	419	Nov-96	Low	0.336	0.198	0.2122	0.3025	0.256
422	Nov-96	High	0.30	0.19	0.29	0.28	0.29	421	Nov-96	Low	0.3147	0.2037	0.2048	0.2979	0.2354
423	Nov-96	High	0.41	0.24	0.26	0.46	0.42	426	Nov-96	Low	0.2684	0.1737	0.1999	0.2542	0.2393
433	Nov-96	High	0.39	0.21	0.32	0.33	0.36	432	Nov-96	Low	0.3015	0.2067	0.2118	0.2934	0.2215
451	Nov-96	High	0.52	0.26	0.38	0.43	0.47	436	Nov-96	Low	0.4024	0.313	0.2651	0.4725	0.3431
460	Nov-96	High	0.40	0.21	0.41	0.37	0.45	439	Nov-96	Low	0.4714	0.3173	0.3242	0.3971	0.3851
463	Nov-96	High	0.30	0.19	0.21	0.30	0.25	448	Nov-96	Low	0.3631	0.185	0.2848	0.3232	0.3645
302	Jan-97	High	0.57	0.37	0.46	0.67	0.55	462	Nov-96	Low	0.4572	0.3298	0.3307	0.5338	0.4056
317	Jan-97	High	0.87	0.60	0.88	0.90	1.08	476	Nov-96	Low	0.3302	0.1854	0.353	0.3357	0.4683
320	Jan-97	High	0.76	0.51	0.90	0.75	0.92	492	Nov-96	Low	0.4509	0.2557	0.3173	0.3486	0.3544
331	Jan-97	High	0.63	0.51	0.75	0.73	0.81	318	Jan-97	Low	0.9587	0.5602	0.4095	1.1646	0.6724
332	Jan-97	High	0.37	0.32	0.29	0.41	0.40	327	Jan-97	Low	0.4051	0.2938	0.2607	0.4479	0.3476
342	Jan-97	High	0.75	0.32	0.89	0.53	1.05	336	Jan-97	Low	0.6443	0.2341	0.6454	0.4468	0.8422
352	Jan-97	High	0.78	0.36	0.63	0.82	0.71	339	Jan-97	Low	0.5933	0.569	0.5215	0.6196	0.5847
360	Jan-97	High	0.77	0.35	0.89	0.61	0.88	369	Jan-97	Low	0.4752	0.1955	0.4547	0.3933	0.5838
363	Jan-97	High	0.32	0.26	0.20	0.43	0.33	370	Jan-97	Low	0.4489	0.3328	0.752	0.4135	1.05
365	Jan-97	High	0.77	0.44	0.72	0.56	0.82	386	Jan-97	Low	0.6944	0.5262	0.8223	0.7092	0.9638
368	Jan-97	High	0.71	0.64	0.73	0.57	0.80	409	Jan-97	Low	1.108	0.7008	1.072	1.1342	1.2492
372	Jan-97	High	0.49	0.43	0.66	0.47	0.60	415	Jan-97	Low	0.6771	0.481	0.803	0.6013	0.8858
374	Jan-97	High	0.74	0.59	0.86	0.62	1.09	420	Jan-97	Low	0.6948	0.7539	0.7867	0.5938	0.8403
377	Jan-97	High	0.53	0.56	0.67	0.53	0.73	424	Jan-97	Low	0.9458	0.9662	0.893	0.8105	1.0389
416	Jan-97	High	0.81	0.88	0.92	0.70	1.22	425	Jan-97	Low	1.2643	1.0197	1.0187	1.1399	1.1513
430	Jan-97	High	0.69	0.62	0.78	0.57	0.85	432	Jan-97	Low	0.6379	0.6664	0.8483	0.5453	0.8588
433	Jan-97	High	0.65	0.44	0.70	0.66	0.77	435	Jan-97	Low	0.8291	0.4626	0.789	0.8825	0.8559
463	Jan-97	High	0.65	0.30	0.60	0.56	0.72	439	Jan-97	Low	0.8107	1.0442	0.599	0.8092	0.6799
470	Jan-97	High	0.83	0.57	1.09	0.90	1.28	447	Jan-97	Low	0.91	0.8485	1.099	0.703	1.216
491	Jan-97	High	0.88	0.54	1.02	0.94	1.18	449	Jan-97	Low	0.464	0.2263	0.4291	0.301	0.448
498	Jan-97	High	0.64	0.35	0.55	0.60	0.66	452	Jan-97	Low	0.8688	0.3534	0.7039	0.764	0.8277
302	Mar-97	High	0.57	0.48	0.50	0.66	0.55	454	Jan-97	Low	0.3959	0.3186	0.8409	0.3869	1.061
317	Mar-97	High	1.09	0.89	0.99	0.99	1.14	461	Jan-97	Low	1.015	0.2412	0.7965	1.3233	0.9037
320	Mar-97	High	0.74	0.87	0.64	0.77	0.66	472	Jan-97	Low	0.8285	0.7644	0.8827	0.5901	0.9163
331	Mar-97	High	0.90	0.68	0.59	0.90	0.86	475	Jan-97	Low	0.8499	0.4884	0.9868	0.8546	1.2721
332	Mar-97	High	0.61	0.50	0.35	0.60	0.29	477	Jan-97	Low	0.7516	0.3085	0.9971	0.8422	1.2506
342	Mar-97	High	0.99	0.76	0.97	0.79	1.19	492	Jan-97	Low	1.0009	0.6514	0.9153	1.0191	1.0182
352	Mar-97	High	0.82	0.66	0.47	0.89	0.53	497	Jan-97	Low	0.7327	0.3549	0.8903	0.5803	0.8387
360	Mar-97	High	0.93	0.52	0.80	0.84	1.01	318	Mar-97	Low	1.3066	0.5967	0.5813	1.4914	0.878
363	Mar-97	High	0.37	0.45	0.23	0.44	0.35	336	Mar-97	Low	0.603	0.5097	0.6509	0.5276	0.6929
365	Mar-97	High	0.78	0.48	0.62	0.80	0.82	339	Mar-97	Low	0.8499	0.7292	0.6504	1.0012	0.6676
368	Mar-97	High	0.86	1.06	0.71	0.80	0.80	369	Mar-97	Low	0.8044	0.4056	0.6218	0.9131	0.8737
372	Mar-97	High	0.70	0.72	0.53	0.68	0.64	370	Mar-97	Low	0.983	0.5462	1.2051	0.8918	1.3474
374	Mar-97	High	0.88	1.03	0.57	0.77	0.82	386	Mar-97	Low	0.9458	1.1515	0.7344	0.9086	0.9072
377	Mar-97	High	0.74	0.67	0.73	0.77	0.81	409	Mar-97	Low	1.1231	0.9407	1.204	1.0876	1.3873
430	Mar-97	High	0.95	1.04	0.93	0.89	1.01	420	Mar-97	Low	0.9462	1.0577	0.8452	0.8112	0.8998
433	Mar-97	High	0.83	0.70	0.78	0.83	0.85	424	Ma -97	Low	1.1718	1.3253	0.9884	0.9536	1.0334
463	Mar-97	High	0.55	0.49	0.42	0.62	0.44	432	Mar-97	Low	1.0859	1.0871	1.0791	1.0717	0.9872
470	Mar-97	High	1.11	0.83	1.19	0.96	1.36	447	Mar-97	Low	1.0265	0.8168	1.0722	0.8907	1.2781
491	Mar-97	High	1.06	1.07	1.00	0.89	1.19	452	Mar-97	Low	1.1127	0.6636	0.547	1.2426	0.7397
498	Mar-97	High	0.69	0.67	0.73	0.67	0.91	454	Mar-97	Low	0.7639	0.4944	0.8135	0.6991	1.1256
302	Apr-97	High	0.71	0.57	0.66	0.72	0.85	461	Mar-97	Low	1.3257	0.5379	0.8755	1.219	1.0764
317	Apr-97	High	1.29	1.14	1.10	1.07	1.23	472	Mar-97	Low	0.8992	0.9613	0.9009	0.7955	1.0232
320	Apr-97	High	0.73	1.02	0.61	0.79	0.53	475	Mar-97	Low	1.5059	1.0748	1.2135	1.2897	1.458
331	Apr-97	High	0.93	0.75	0.77	0.91	0.88	477	Mar-97	Low	1.1444	0.961	1.3904	1.0657	1.5246
332	Apr-97	High	0.64	0.72	0.46	0.65	0.48	492	Mar-97	Low	1.2988	0.995	1.0486	1.2634	1.2851
342	Apr-97	High	1.15	0.96	1.03	0.90	1.11	318	Apr-97	Low	1.452	0.8391	0.8769	1.3079	0.9531
352	Apr-97	High	0.90	0.81	0.72	1.02	0.84	327	Apr-97	Low	1.0183	0.4811	0.5238	1.0252	0.6431
360	Apr-97	High	0.91	0.69	0.96	0.94	1.09	336	Apr-97	Low	0.5349	0.53	0.6651	0.5647	0.7144
363	Apr-97	High	0.44	0.53	0.33	0.72	0.41	339	Apr-97	Low	1.3895	1.0201	0.8306	1.4056	0.8858
365	Apr-97	High	0.88	0.59	0.84	0.97	0.80	369	Apr-97	Low	1.1459	0.49	0.8883	1.2152	1.2094
368	Apr-97	High	0.81	1.02	0.67	0.92	0.65	386	Apr-97	Low	1.1003	1.1811	0.8215	1.1571	0.9203
372	Apr-97	High	0.81	0.79	0.76	0.85	0.81	409	Apr-97	Low	1.0721	0.9113	1.155	1.1026	1.2215
374	Apr-97	High	1.03	1.04	0.81	0.93	0.96	415	Apr-97	Low	0.8446	0.6218	0.8375	0.9504	0.5508
377	Apr-97	High	0.89	0.77	0.91	1.00	1.00	420	Apr-97	Low	0.9929	1.0358	0.8493	1.0085	0.8624
416	Apr-97	High	0.96	1.07	0.85	1.02	0.93	318	May-97	Low	1.368	0.8031	1.0224	1.4027	1.1364
430	Apr-97	High	0.94	1.02	0.93	0.94	0.97	327	May-97	Low	1.0573	0.6091	0.5508	1.144	0.7037
433	Apr-97	High	0.95	0.81	0.86	0.97	0.95	336	May-97	Low	0.7259	0.6481	0.7865	0.7765	0.8788
463	Apr-97	High	0.58	0.54	0.43	0.65	0.44	369	May-97	Low	1.2934	0.5257	1.0597	1.3137	1.2825
470	Apr-97	High	1.00	0.82	1.21	0.96	1.41	386	May-97	Low	1.1291	0.8928	0.8716	1.1606	0.9204
491	Apr-97	High	0.96	0.97	0.96	0.93	1.04	409	May-97	Low	1.3738	0.9441	1.3128	1.2945	1.3821
498	Apr-97	High	0.63	0.56	0.55	0.68	0.67	415	May-97	Low	1.3826	0.84	0.9358	1.3793	0.9058
302	May-97	High	0.73	0.52	0.59	0.82	0.61	420	May-97	Low	1.143	0.9441	0.9371	1.1119	0.905
317	May-97	High	1.11	0. 2	1.03	1.05	1.05	432	May-97	Low	1.4304	1.1902	1.2261	1.3547	1.1641
331	May-97	High	1.07	0.81	0.90	1.03	1.06	435	May-97	Low	1.182	0.7437	1.0784	1.1704	1.0039
342	May-97	High	1.17	0.90	1.11	1.02	1.24	439	May-97	Low	1.2556	0.916	0.9417	1.3022	1.0878
352	May-97	High	1.03	0.69	0.96	1.06	0.96	447	May-97	Low	1.2255	0.8851	1.0422	1.1284	1.1583
360	May-97	High	0.96	0.71	0.90	0.90	1.02	454	May-97	Low	1.1207	0.736	0.8288	1.1176	1.0837
363	May-97	High	0.68	0.54	0.37	0.78	0.51	472	May-97	Low	1.0193	0.9994	0.9322	1.0789	0.933
365	May-97	High	1.02	0.54	0.74	1.07	0.91	475	May-97	Low	1.5495	0.9099	1.1481	1.5841	1.3083
368	May-97	High	0.81	0.71	0.86	0.90	0.81	492	May-97	Low	1.5705	0.9787	1.0283	1.5169	1.1103
372	May-97	High	0.84	0.73	0.76	0.89	0.76	424	May-97	Low	1.0268	1.2417	0.8999	0.9655	0.888
374	May-97	High	0.99	1.04	0.89	0.92	0.97	432	May-97	Low	1.1442	1.1164	0.9668	1.0868	1.0006
377	May-97	High	0.91	0.65	0.97	0.95	0.95	435	May-97	Low	1.036	0.8492	0.8671	0.9877	0.921
416	May-97	High	0.92	0.79	0.82	0.97	0.99	439	May-97	Low	1.214	1.0259	0.8645	1.23	0.8989
430	May-97	High	1.11	0. 2	0.92	1.08	0.96	447	May-97	Low	0.902	0.6391	0.9705	0.8095	1.2098
463	May-97	High	0.73	0.90	0.52	0.83	0.58	449	May-97	Low	0.5507	0.4887	0.475	0.5183	0.5208
470	May-97	High	1.02	0.80	1.22	1.01	1.31	452	May-97	Low	1.0323	0.7128	0.5471	1.01	0.6492
491	May-97	High	0.98	0.85	0.90	0.96	1.03	454	May-97	Low	0.8553	0.5344	0.7532	0.782	1.0876
								472	May-97	Low	0.7463	0.708	0.6587	0.7933	0.7029
								475	May-97	Low	1.3903	1.0144	1.2614	1.3947	1.4849
								477	May-97	Low	1.193	0.8969	1.3499	1.1763	1.5014
								492	May-97	Low	1.2167	0.9072	0.8876	1.2737	1.0068
								497	May-97	Low	0.8587	0.6429	0.7857	0.8963	0.8009

Appendix 2m Statistical Analysis – Chapter 2

PERENDALE EWES

Dependent Variable: ln(FEC+1)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	98	622.1664414	6.3486372	8.85	0.0001
Error	384	275.3134881	0.7169622		
Corrected Total	482	897.4799295			

R-Square	C.V.	Root MSE	LOGFEC Mean
0.693237	19.75539	0.846736	4.286103

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TIME	11	77.8546077	7.0776916	9.87	0.0001
LINE	1	154.6168080	154.6168080	215.66	0.0001
ANIMAL(LINE)	75	372.1485483	4.9619806	6.92	0.0001
TIME*LINE	11	17.5464773	1.5951343	2.22	0.0127

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TIME	11	131.8912769	11.9901161	16.72	0.0001
LINE	1	153.5708497	153.5708497	214.20	0.0001
ANIMAL(LINE)	75	373.9749278	4.9863324	6.95	0.0001
TIME*LINE	11	17.5464773	1.5951343	2.22	0.0127

Tests of Hypotheses using the Type III MS for ANIMAL(LINE) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LINE	1	153.5708497	153.5708497	30.80	0.0001

Dependent Variable: ln(developmental success + 1)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	98	304.9479805	3.1117141	4.16	0.0001
Error	384	287.4349132	0.7485284		
Corrected Total	482	592.3828937			

R-Square	C.V.	Root MSE	LOGPERC Mean
0.514782	36.33192	0.865175	2.381309

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TIME	11	168.6179842	15.3289077	20.48	0.0001
LINE	1	13.2938927	13.2938927	17.76	0.0001
ANIMAL(LINE)	75	109.0312706	1.4537503	1.94	0.0001
TIME*LINE	11	14.0048331	1.2731666	1.70	0.0711

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TIME	11	128.6586390	11.6962399	15.63	0.0001
LINE	1	11.9659137	11.9659137	15.99	0.0001
ANIMAL(LINE)	75	107.5758406	1.4343445	1.92	0.0001
TIME*LINE	11	14.0048331	1.2731666	1.70	0.0711

Tests of Hypotheses using the Type III MS for ANIMAL(LINE) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LINE	1	11.96591367	11.96591367	8.34	0.0051

TIME=2
Dependent Variable: LOGPERC

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LINE	1	6.92253065	6.92253065	7.70	0.0084

TIME=11
Dependent Variable: LOGPERC

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LINE	1	7.25988067	7.25988067	12.73	0.0011

TIME=12
Dependent Variable: LOGPERC

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LINE	1	2.01027884	2.01027884	4.33	0.0447

Dependent Variable: ln(%D.M. + 1)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	91	26.34068891	0.28945812	2.80	0.0001
Error	285	29.43119055	0.10326734		
Corrected Total	376	55.77187946			

R-Square	C.V.	Root MSE	LOGDM Mean
0.472293	10.37572	0.321352	3.097159

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TIME	9	13.24624311	1.47180479	14.25	0.0001
LINE	1	0.00453654	0.00453654	0.04	0.8341
ANIMAL(LINE)	72	12.00513689	0.16673801	1.61	0.0033
TIME*LINE	9	1.08477237	0.12053026	1.17	0.3159

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TIME	9	8.19913461	0.91101496	8.82	0.0001
LINE	1	0.10359452	0.10359452	1.00	0.3174
ANIMAL(LINE)	72	12.10977277	0.16819129	1.63	0.0028
TIME*LINE	9	1.08477237	0.12053026	1.17	0.3159

Tests of Hypotheses using the Type III MS for ANIMAL(LINE) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LINE	1	0.10359452	0.10359452	0.62	0.4351

Dependent Variable: OcL3

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	78	7.38139842	0.09463331	11.75	0.0001
Error	146	1.17604143	0.00805508		
Corrected Total	224	8.55743986			

R-Square	C.V.	Root MSE	OCL3 Mean
0.862571	13.23245	0.089750	0.678257

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TIME	7	1.75357213	0.25051030	31.10	0.0001
LINE	1	0.94536103	0.94536103	117.36	0.0001
ANIMAL(LINE)	63	4.62695637	0.07344375	9.12	0.0001
TIME*LINE	7	0.05550890	0.00792984	0.98	0.4449

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TIME	7	0.55481167	0.07925881	9.84	0.0001
LINE	1	0.29552466	0.29552466	36.69	0.0001
ANIMAL(LINE)	63	4.56809190	0.07250940	9.00	0.0001
TIME*LINE	7	0.05550890	0.00792984	0.98	0.4449

Tests of Hypotheses using the Type III MS for ANIMAL(LINE) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LINE	1	0.29552466	0.29552466	4.08	0.0478

Dependent Variable: OcAd

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	78	5.68185613	0.07284431	9.03	0.0001
Error	146	1.17829360	0.00807050		
Corrected Total	224	6.86014973			

R-Square	C.V.	Root MSE	OCAD Mean
0.828241	11.31308	0.089836	0.794089

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TIME	7	1.41801493	0.20257356	25.10	0.0001
LINE	1	0.37687481	0.37687481	46.70	0.0001
ANIMAL(LINE)	63	3.76148549	0.05970612	7.40	0.0001
TIME*LINE	7	0.12548090	0.01792584	2.22	0.0357

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TIME	7	1.14825441	0.16403634	20.33	0.0001
LINE	1	0.28335482	0.28335482	35.11	0.0001
ANIMAL(LINE)	63	3.69801530	0.05869866	7.27	0.0001
TIME*LINE	7	0.12548090	0.01792584	2.22	0.0357

Tests of Hypotheses using the Type III MS for ANIMAL(LINE) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LINE	1	0.28335482	0.28335482	4.83	0.0317

Dependent Variable: CcL3

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	78	6.74076241	0.08642003	12.81	0.0001
Error	146	0.98532051	0.00674877		
Corrected Total	224	7.72608292			

R-Square	C.V.	Root MSE	CCL3 Mean
0.872468	13.04592	0.082151	0.629706

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TIME	7	1.80949415	0.25849916	38.30	0.0001
LINE	1	0.25080863	0.25080863	37.16	0.0001
ANIMAL(LINE)	63	4.60965962	0.07316920	10.84	0.0001
TIME*LINE	7	0.07080001	0.01011429	1.50	0.1721

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TIME	7	0.99190737	0.14170105	21.00	0.0001
LINE	1	0.02704958	0.02704958	4.01	0.0471
ANIMAL(LINE)	63	4.55908887	0.07236649	10.72	0.0001
TIME*LINE	7	0.07080001	0.01011429	1.50	0.1721

Tests of Hypotheses using the Type III MS for ANIMAL(LINE) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LINE	1	0.02704958	0.02704958	0.37	0.5431

Dependent Variable: CcAd

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	78	4.81525702	0.06173406	10.15	0.0001
Error	146	0.88786402	0.00608126		
Corrected Total	224	5.70312104			

R-Square	C.V.	Root MSE	CCAD Mean
0.844320	10.31167	0.077982	0.756254

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TIME	7	1.10424116	0.15774874	25.94	0.0001
LINE	1	0.40826198	0.40826198	67.13	0.0001
ANIMAL(LINE)	63	3.13343383	0.04973704	8.18	0.0001
TIME*LINE	7	0.16932005	0.02418858	3.98	0.0005

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TIME	7	0.90495967	0.12927995	21.26	0.0001
LINE	1	0.12942330	0.12942330	21.28	0.0001
ANIMAL(LINE)	63	3.09237205	0.04908527	8.07	0.0001
TIME*LINE	7	0.16932005	0.02418858	3.98	0.0005

Tests of Hypotheses using the Type III MS for ANIMAL(LINE) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LINE	1	0.12942330	0.12942330	2.64	0.1094

By time

TIME=3
Dependent Variable: CCL3

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LINE	1	0.05124507	0.05124507	13.77	0.0100

TIME=4
Dependent Variable: OCL3

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LINE	1	0.08093799	0.08093799	5.54	0.0365

Dependent Variable: CCL3

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LINE	1	0.10954311	0.10954311	16.17	0.0017

TIME=6
Dependent Variable: OCL3

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LINE	1	0.17441814	0.17441814	4.83	0.0352

Dependent Variable: OCAD

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LINE	1	0.18904000	0.18904000	8.93	0.0053

Dependent Variable: CCAD

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LINE	1	0.31930785	0.31930785	23.47	0.0001

TIME=8
Dependent Variable: OCL3

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LINE	1	0.24193708	0.24193708	7.64	0.0095

TIME=10
Dependent Variable: OCL3

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LINE	1	0.13644842	0.13644842	4.81	0.0386

Dependent Variable: OCAD

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LINE	1	0.15969268	0.15969268	5.78	0.0247

Dependent Variable: CCAD

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LINE	1	0.09558216	0.09558216	7.24	0.0130

TIME=11
Dependent Variable: OCL3

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LINE	1	0.27485051	0.27485051	9.04	0.0053

TIME=12
Dependent Variable: OCL3

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LINE	1	0.11070009	0.11070009	6.25	0.0171

Dependent Variable: OCAD

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LINE	1	0.09800165	0.09800165	5.07	0.0305

Dependent Variable: *Haemonchus*

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	83	3573.364902	43.052589	1.78	0.0006
Error	202	4889.054679	24.203241		
Corrected Total	285	8462.419580			

R-Square	C.V.	Root MSE	HAEM Mean
0.422263	296.8414	4.919679	1.657343

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TIME	11	1066.161488	96.923772	4.00	0.0001
LINE	1	5.941754	5.941754	0.25	0.6208
ANIMAL(LINE)	60	2245.891370	37.431523	1.55	0.0136
TIME*LINE	11	255.370290	23.215481	0.96	0.4850

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TIME	11	1128.314039	102.574004	4.24	0.0001
LINE	1	0.000196	0.000196	0.00	0.9977
ANIMAL(LINE)	60	2365.327957	39.422133	1.63	0.0066
TIME*LINE	11	255.370290	23.215481	0.96	0.4850

Tests of Hypotheses using the Type III MS for ANIMAL(LINE) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LINE	1	0.00019575	0.00019575	0.00	0.9982

Dependent Variable: *Ostertagia*

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	83	109713.3842	1321.8480	3.39	0.0001
Error	202	78719.6123	389.7011		
Corrected Total	285	188432.9965			

R-Square	C.V.	Root MSE	OST Mean
0.582241	77.54268	19.74085	25.45804

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TIME	11	37524.31375	3411.30125	8.75	0.0001
LINE	1	1633.60603	1633.60603	4.19	0.0419
ANIMAL(LINE)	60	65986.80664	1099.78011	2.82	0.0001
TIME*LINE	11	4568.65782	415.33253	1.07	0.3907

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TIME	11	20062.10628	1823.82784	4.68	0.0001
LINE	1	1991.33292	1991.33292	5.11	0.0249
ANIMAL(LINE)	60	65117.12976	1085.28550	2.78	0.0001
TIME*LINE	11	4568.65782	415.33253	1.07	0.3907

Tests of Hypotheses using the Type III MS for ANIMAL(LINE) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LINE	1	1991.332922	1991.332922	1.83	0.1806

Dependent Variable: *Trichostrongylus*

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	83	8094.569787	97.524937	2.51	0.0001
Error	202	7843.475667	38.829087		
Corrected Total	285	15938.045455			

R-Square	C.V.	Root MSE	TRICH Mean
0.507877	169.2452	6.231299	3.681818

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TIME	11	1990.005014	180.909547	4.66	0.0001
LINE	1	28.172134	28.172134	0.73	0.3953
ANIMAL(LINE)	60	5796.415735	96.606929	2.49	0.0001
TIME*LINE	11	279.976904	25.452446	0.66	0.7792

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TIME	11	990.604473	90.054952	2.32	0.0106
LINE	1	0.945491	0.945491	0.02	0.8762
ANIMAL(LINE)	60	5814.263200	96.904387	2.50	0.0001
TIME*LINE	11	279.976904	25.452446	0.66	0.7792

Tests of Hypotheses using the Type III MS for ANIMAL(LINE) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LINE	1	0.94549106	0.94549106	0.01	0.9216

Dependent Variable: *Cooperia*

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	83	222518.3527	2680.9440	7.39	0.0001
Error	202	73329.8291	363.0190		
Corrected Total	285	295848.1818			

R-Square	C.V.	Root MSE	COOP Mean
0.752137	35.70419	19.05306	53.36364

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TIME	11	87360.5049	7941.8641	21.88	0.0001
LINE	1	9.5761	9.5761	0.03	0.8711
ANIMAL(LINE)	60	131226.0475	2187.1008	6.02	0.0001
TIME*LINE	11	3922.2243	356.5658	0.98	0.4638

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TIME	11	31522.7491	2865.7045	7.89	0.0001
LINE	1	930.2530	930.2530	2.56	0.1110
ANIMAL(LINE)	60	128459.1935	2140.9866	5.90	0.0001
TIME*LINE	11	3922.2243	356.5658	0.98	0.4638

Tests of Hypotheses using the Type III MS for ANIMAL(LINE) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LINE	1	930.2529980	930.2529980	0.43	0.5123

Dependent Variable: *Chabertia/Oesophagostomum*

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	83	158455.2700	1909.0996	5.63	0.0001
Error	202	68464.0062	338.9307		
Corrected Total	285	226919.2762			

R-Square	C.V.	Root MSE	LT Mean
0.698289	116.2570	18.41007	15.83566

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TIME	11	107370.9618	9760.9965	28.80	0.0001
LINE	1	1194.4260	1194.4260	3.52	0.0619
ANIMAL(LINE)	60	44374.1251	739.5688	2.18	0.0001
TIME*LINE	11	5515.7571	501.4325	1.48	0.1413

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TIME	11	39550.71307	3595.51937	10.61	0.0001
LINE	1	231.48952	231.48952	0.68	0.4095
ANIMAL(LINE)	60	45533.45029	758.89084	2.24	0.0001
TIME*LINE	11	5515.75706	501.43246	1.48	0.1413

Tests of Hypotheses using the Type III MS for ANIMAL(LINE) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LINE	1	231.4895181	231.4895181	0.31	0.5828

PERENDALE LAMBS

Dependent Variable: ln(FEC+1)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	59	558.0879877	9.4591184	18.70	0.0001
Error	173	87.5074576	0.5058235		
Corrected Total	232	645.5954453			

R-Square	C.V.	Root MSE	LOGFEC Mean
0.864455	14.17903	0.711213	5.015946

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TIME	5	64.0240474	12.8048095	25.31	0.0001
LINE	1	355.2295149	355.2295149	702.28	0.0001
ANIMAL(LINE)	48	112.2965323	2.3395111	4.63	0.0001
TIME*LINE	5	26.5378931	5.3075786	10.49	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TIME	5	72.5833781	14.5166756	28.70	0.0001
LINE	1	258.5926427	258.5926427	511.23	0.0001
ANIMAL(LINE)	48	101.5755860	2.1161580	4.18	0.0001
TIME*LINE	5	26.5378931	5.3075786	10.49	0.0001

Tests of Hypotheses using the Type III MS for ANIMAL(LINE) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LINE	1	258.5926427	258.5926427	122.20	0.0001

Dependent Variable: ln(developmental success + 1)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	59	174.7178101	2.9613188	3.83	0.0001
Error	173	133.6121147	0.7723244		
Corrected Total	232	308.3299248			

R-Square	C.V.	Root MSE	LOGPERC Mean
0.566659	37.95854	0.878820	2.315210

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TIME	5	82.22473411	16.44494682	21.29	0.0001
LINE	1	11.96719506	11.96719506	15.50	0.0001
ANIMAL(LINE)	48	76.09332333	1.58527757	2.05	0.0004
TIME*LINE	5	4.43255756	0.88651151	1.15	0.3371

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TIME	5	73.49417288	14.69883458	19.03	0.0001
LINE	1	6.32139096	6.32139096	8.18	0.0047
ANIMAL(LINE)	48	74.68277698	1.55589119	2.01	0.0006
TIME*LINE	5	4.43255756	0.88651151	1.15	0.3371

Tests of Hypotheses using the Type III MS for ANIMAL(LINE) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LINE	1	6.32139096	6.32139096	4.06	0.0495

TIME=3
Dependent Variable: LOGPERC

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LINE	1	3.84412793	3.84412793	4.24	0.0458

TIME=5
Dependent Variable: LOGPERC

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LINE	1	7.08578234	7.08578234	5.57	0.0238

Dependent Variable: ln(%D.M. + 1)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	58	44.77497026	0.77198225	12.72	0.0001
Error	160	9.71316896	0.06070731		
Corrected Total	218	54.48813921			

R-Square	C.V.	Root MSE	LOGDM Mean
0.821738	7.569784	0.246389	3.254895

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TIME	5	40.02073121	8.00414624	131.85	0.0001
LINE	1	0.00155336	0.00155336	0.03	0.8731
ANIMAL(LINE)	47	4.28116748	0.09108867	1.50	0.0337
TIME*LINE	5	0.47151821	0.09430364	1.55	0.1763

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TIME	5	35.72873770	7.14574754	117.71	0.0001
LINE	1	0.02226847	0.02226847	0.37	0.5456
ANIMAL(LINE)	47	4.22978585	0.08999544	1.48	0.0381
TIME*LINE	5	0.47151821	0.09430364	1.55	0.1763

Tests of Hypotheses using the Type III MS for ANIMAL(LINE) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LINE	1	0.02226847	0.02226847	0.25	0.6212

Dependent Variable: CcL3

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	52	11.97633956	0.23031422	20.38	0.0001
Error	108	1.22046083	0.01130056		
Corrected Total	160	13.19680039			

R-Square	C.V.	Root MSE	CCL3 Mean
0.907518	12.17132	0.106304	0.873398

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TIME	4	6.09686908	1.52421727	134.88	0.0001
LINE	1	1.25575548	1.25575548	111.12	0.0001
ANIMAL(LINE)	43	4.21353790	0.09798925	8.67	0.0001
TIME*LINE	4	0.41017711	0.10254428	9.07	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TIME	4	3.82976939	0.95744235	84.73	0.0001
LINE	1	0.27568376	0.27568376	24.40	0.0001
ANIMAL(LINE)	43	4.26424551	0.09916850	8.78	0.0001
TIME*LINE	4	0.41017711	0.10254428	9.07	0.0001

Tests of Hypotheses using the Type III MS for ANIMAL(LINE) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LINE	1	0.27568376	0.27568376	2.78	0.1027

Dependent Variable: OcL3

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	52	10.84910681	0.20863667	18.26	0.0001
Error	108	1.23411162	0.01142696		
Corrected Total	160	12.08321843			

R-Square	C.V.	Root MSE	OCL3 Mean
0.897866	15.62848	0.106897	0.683988

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TIME	4	5.59803996	1.39950999	122.47	0.0001
LINE	1	0.11157357	0.11157357	9.76	0.0023
ANIMAL(LINE)	43	4.98998941	0.11604627	10.16	0.0001
TIME*LINE	4	0.14950386	0.03737597	3.27	0.0142

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TIME	4	4.01173154	1.00293289	87.77	0.0001
LINE	1	0.01520128	0.01520128	1.33	0.2513
ANIMAL(LINE)	43	5.07163466	0.11794499	10.32	0.0001
TIME*LINE	4	0.14950386	0.03737597	3.27	0.0142

Tests of Hypotheses using the Type III MS for ANIMAL(LINE) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LINE	1	0.01520128	0.01520128	0.13	0.7213

Dependent Variable: OcAd

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	52	10.41368999	0.20026327	15.20	0.0001
Error	108	1.42255035	0.01317176		
Corrected Total	160	11.83624034			

R-Square	C.V.	Root MSE	OCAD Mean
0.879814	14.77506	0.114768	0.776770

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TIME	4	3.79599909	0.94899977	72.05	0.0001
LINE	1	0.47862552	0.47862552	36.34	0.0001
ANIMAL(LINE)	43	5.91953735	0.13766366	10.45	0.0001
TIME*LINE	4	0.21952803	0.05488201	4.17	0.0035

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TIME	4	1.72653729	0.43163432	32.77	0.0001
LINE	1	0.06497180	0.06497180	4.93	0.0284
ANIMAL(LINE)	43	5.90931003	0.13742581	10.43	0.0001
TIME*LINE	4	0.21952803	0.05488201	4.17	0.0035

Tests of Hypotheses using the Type III MS for ANIMAL(LINE) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LINE	1	0.06497180	0.06497180	0.47	0.4954

Dependent Variable: TcL3

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	52	12.33312434	0.23717547	24.03	0.0001
Error	108	1.06599977	0.00987037		
Corrected Total	160	13.39912411			

R-Square	C.V.	Root MSE	TCL3 Mean
0.920443	11.60787	0.099350	0.855883

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TIME	4	6.95877990	1.73969498	176.25	0.0001
LINE	1	1.16504750	1.16504750	118.03	0.0001
ANIMAL(LINE)	43	3.86995866	0.08999904	9.12	0.0001
TIME*LINE	4	0.33933828	0.08483457	8.59	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TIME	4	4.73429773	1.18357443	119.91	0.0001
LINE	1	0.26921031	0.26921031	27.27	0.0001
ANIMAL(LINE)	43	3.92856167	0.09136190	9.26	0.0001
TIME*LINE	4	0.33933828	0.08483457	8.59	0.0001

Tests of Hypotheses using the Type III MS for ANIMAL(LINE) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LINE	1	0.26921031	0.26921031	2.95	0.0933

Dependent Variable: TcAd

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	52	12.47169032	0.23984020	15.39	0.0001
Error	108	1.68310942	0.01558435		
Corrected Total	160	14.15479974			

R-Square	C.V.	Root MSE	TCAD Mean
0.881093	14.14825	0.124837	0.882352

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TIME	4	4.18464579	1.04616145	67.13	0.0001
LINE	1	0.63933316	0.63933316	41.02	0.0001
ANIMAL(LINE)	43	7.48969010	0.17417884	11.18	0.0001
TIME*LINE	4	0.15802127	0.03950532	2.53	0.0443

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TIME	4	1.43574898	0.35893725	23.03	0.0001
LINE	1	0.11729627	0.11729627	7.53	0.0071
ANIMAL(LINE)	43	7.38717542	0.17179478	11.02	0.0001
TIME*LINE	4	0.15802127	0.03950532	2.53	0.0443

Tests of Hypotheses using the Type III MS for ANIMAL(LINE) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LINE	1	0.11729627	0.11729627	0.68	0.4132

TIME=4
Dependent Variable: CCL3

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LINE	1	0.55079930	0.55079930	12.58	0.0011

Dependent Variable: OCAD

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LINE	1	0.47642739	0.47642739	7.69	0.0087

Dependent Variable: TCL3

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LINE	1	0.48899180	0.48899180	13.54	0.0008

Dependent Variable: TCAD

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LINE	1	0.61578208	0.61578208	7.92	0.0079

TIME=5
Dependent Variable: CCL3

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LINE	1	0.31946262	0.31946262	7.44	0.0099

Dependent Variable: TCL3

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LINE	1	0.23285665	0.23285665	7.54	0.0095

TIME=6
Dependent Variable: CCL3

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LINE	1	0.70451743	0.70451743	21.45	0.0001

Dependent Variable: OCAD

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LINE	1	0.16928594	0.16928594	4.24	0.0480

Dependent Variable: TCL3

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LINE	1	0.66829617	0.66829617	29.13	0.0001

Dependent Variable: *Haemonchus*

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	52	1119.239469	21.523836	1.05	0.4102
Error	117	2404.084060	20.547727		
Corrected Total	169	3523.323529			

R-Square	C.V.	Root MSE	HAEM Mean
0.317666	531.4505	4.532960	0.852941

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TIME	5	240.2044818	48.0408964	2.34	0.0460
LINE	1	41.9856093	41.9856093	2.04	0.1555
ANIMAL(LINE)	41	617.7560878	15.0672217	0.73	0.8712
TIME*LINE	5	219.2932902	43.8586580	2.13	0.0661

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TIME	5	382.7957383	76.5591477	3.73	0.0036
LINE	1	12.1658217	12.1658217	0.59	0.4432
ANIMAL(LINE)	41	658.4333272	16.0593494	0.78	0.8149
TIME*LINE	5	219.2932902	43.8586580	2.13	0.0661

Tests of Hypotheses using the Type III MS for ANIMAL(LINE) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LINE	1	12.16582175	12.16582175	0.76	0.3892

Dependent Variable: *Ostertagia*

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	52	104437.6082	2008.4155	6.95	0.0001
Error	117	33818.9801	289.0511		
Corrected Total	169	138256.5882			

R-Square	C.V.	Root MSE	OST Mean
0.755390	31.04464	17.00150	54.76471

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TIME	5	70681.38122	14136.27624	48.91	0.0001
LINE	1	1867.43751	1867.43751	6.46	0.0123
ANIMAL(LINE)	41	30686.48712	748.45091	2.59	0.0001
TIME*LINE	5	1202.30231	240.46046	0.83	0.5295

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TIME	5	55637.37431	11127.47486	38.50	0.0001
LINE	1	668.60674	668.60674	2.31	0.1310
ANIMAL(LINE)	41	31106.98495	758.70695	2.62	0.0001
TIME*LINE	5	1202.30231	240.46046	0.83	0.5295

Tests of Hypotheses using the Type III MS for ANIMAL(LINE) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LINE	1	668.6067383	668.6067383	0.88	0.3534

Dependent Variable: *Trichostrongylus*

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	52	70056.43152	1347.23907	13.77	0.0001
Error	117	11445.36259	97.82361		
Corrected Total	169	81501.79412			

R-Square	C.V.	Root MSE	TRICH Mean
0.859569	88.26241	9.890582	11.20588

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TIME	5	57833.18340	11566.63668	118.24	0.0001
LINE	1	1357.34682	1357.34682	13.88	0.0003
ANIMAL(LINE)	41	6184.20253	150.83421	1.54	0.0378
TIME*LINE	5	4681.69877	936.33975	9.57	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TIME	5	54351.99294	10870.39859	111.12	0.0001
LINE	1	931.02851	931.02851	9.52	0.0025
ANIMAL(LINE)	41	6661.69438	162.48035	1.66	0.0185
TIME*LINE	5	4681.69877	936.33975	9.57	0.0001

Tests of Hypotheses using the Type III MS for ANIMAL(LINE) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LINE	1	931.0285073	931.0285073	5.73	0.0213

Dependent Variable: *Cooperia*

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	52	78304.21247	1505.85024	5.50	0.0001
Error	117	32055.19930	273.97606		
Corrected Total	169	110359.41176			

R-Square	C.V.	Root MSE	COOP Mean
0.709538	51.63079	16.55222	32.05882

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TIME	5	28366.34761	5673.26952	20.71	0.0001
LINE	1	6513.42439	6513.42439	23.77	0.0001
ANIMAL(LINE)	41	38705.08368	944.02643	3.45	0.0001
TIME*LINE	5	4719.35679	943.87136	3.45	0.0061

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TIME	5	24432.40335	4886.48067	17.84	0.0001
LINE	1	2876.65208	2876.65208	10.50	0.0016
ANIMAL(LINE)	41	39768.35695	969.95993	3.54	0.0001
TIME*LINE	5	4719.35679	943.87136	3.45	0.0061

Tests of Hypotheses using the Type III MS for ANIMAL(LINE) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LINE	1	2876.652081	2876.652081	2.97	0.0926

Dependent Variable: *Chabertia/Oesophagostomum*

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	52	784.7990355	15.0922891	2.21	0.0002
Error	117	799.2068469	6.8308278		
Corrected Total	169	1584.0058824			

R-Square	C.V.	Root MSE	LT Mean
0.495452	242.7921	2.613585	1.076471

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TIME	5	302.2159353	60.4431871	8.85	0.0001
LINE	1	36.1283082	36.1283082	5.29	0.0232
ANIMAL(LINE)	41	362.2521011	8.8354171	1.29	0.1445
TIME*LINE	5	84.2026909	16.8405382	2.47	0.0366

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TIME	5	205.1633490	41.0326698	6.01	0.0001
LINE	1	39.9605963	39.9605963	5.85	0.0171
ANIMAL(LINE)	41	353.8780892	8.6311729	1.26	0.1670
TIME*LINE	5	84.2026909	16.8405382	2.47	0.0366

Tests of Hypotheses using the Type III MS for ANIMAL(LINE) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LINE	1	39.96059632	39.96059632	4.63	0.0374

Ram lambs versus ewe lambs

Dependent Variable: ln(FEC+1)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	159.783891	53.261297	29.00	0.0001
Error	54	99.164157	1.836373		
Corrected Total	57	258.948048			

R-Square	C.V.	Root MSE	LOGFEC Mean
0.617050	25.81886	1.35513	5.24860

Source	DF	Type I SS	Mean Square	F Value	Pr > F
LINE	1	133.952855	133.952855	72.94	0.0001
SEX	1	24.100764	24.100764	13.12	0.0006
LINE*SEX	1	1.730272	1.730272	0.94	0.3360

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LINE	1	112.235082	112.235082	61.12	0.0001
SEX	1	24.100764	24.100764	13.12	0.0006
LINE*SEX	1	1.730272	1.730272	0.94	0.3360

Dependent Variable: ln(developmental_success + 1)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	8.42274685	2.80758228	2.21	0.0990
Error	50	63.65737993	1.27314760		
Corrected Total	53	72.08012677			

R-Square	C.V.	Root MSE	LOGPERC Mean
0.116853	47.56085	1.12834	2.37241

Source	DF	Type I SS	Mean Square	F Value	Pr > F
LINE	1	7.74777516	7.74777516	6.09	0.0171
SEX	1	0.20564905	0.20564905	0.16	0.6895
LINE*SEX	1	0.46932263	0.46932263	0.37	0.5465

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LINE	1	4.61037678	4.61037678	3.62	0.0628
SEX	1	0.32368906	0.32368906	0.25	0.6163
LINE*SEX	1	0.46932263	0.46932263	0.37	0.5465

Perendale lambs sampled in March 1998

Dependent Variable: ln(FEC + 1)

Source	DF	Sum of Squares	F Value	Pr > F
Model	1	88.09759153	268.73	0.0001
Error	80	26.22629073		
Corrected Total	81	114.32388226		

R-Square	C.V.	LOGFEC Mean
0.770597	12.03647	4.75690284

Source	DF	Type I SS	F Value	Pr > F
LINE	1	88.09759153	268.73	0.0001

Source	DF	Type III SS	F Value	Pr > F
LINE	1	88.09759153	268.73	0.0001

Dependent Variable: ln(developmental success + 1)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	27	87.66367017	3.24680260	9.61	0.0001
Error	56	18.92546009	0.33795464		
Corrected Total	83	106.58913026			

R-Square	C.V.	Root MSE	LOGPERC Mean
0.822445	30.06130	0.581339	1.933844

Source	DF	Type I SS	Mean Square	F Value	Pr >
LINE	1	18.59007621	18.59007621	55.01	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LINE	1	18.59007621	18.59007621	55.01	0.0001

Appendix 3a Data from Chapter 3

P.T. ram lambs sampled 20/1/99

									Developmental success			Mean	Generic Composition (%)					%D.M.	Antibody-levels			
Tag	Line	FEC 1	FEC 2	FEC 3	FEC 4	FEC 5	FEC 6	FECmean	Culture 1	Culture 2	Culture 3	dev. success	Haem	Ost	Trich	Coop	LT	%DM	Tc L3	Tc Ad	Oc L3	Oc Ad
1	Control	600	650	650	700	150	500	542	23.3	19.5	16.8	19.9	0	60	28	8	4	16.8	1.20	0.52	0.57	0.20
2	Control	150	0	0	0	50	100	50	156.4	89.1	93.1	112.9	0	42	9	2	47	38.5	0.90	0.32	0.14	0.13
12	Control	300	300	200	450	250	100	267	7.0	12.4	4.6	8.0	0	54	32	3	10	19.1	0.93	0.29	0.27	0.13
17	Control	50	100	0	150	0	150	75	250.4	219.6	106.7	192.2	0	81	12	7	0	24.7	0.88	0.26	0.12	0.12
40	Control	850	550	700	350	950	600	667	14.1	25.9	4.8	15.0	0	68	16	8	8	14.6	0.92	0.47	0.28	0.15
42	Control	100	0	0	0	0	0	17	187.9	207.7	110.7	168.8	0	15	16	32	37	33.0	0.99	0.27	0.26	0.13
49	Control	50	100	100	100			88	324.7	286.3	315.5	302.1	0	57	13	19	11		0.49	0.20	0.11	0.12
57	Control	300	400	350	500	250	600	400	41.9	26.7	61.3	43.3	0	59	26	13	1	22.0	1.15	0.55	0.20	0.18
61	Control	650	950	450	750	1400	750	825	3.8	4.1	2.7	3.5	0	67	31	1	1	14.4	0.86	0.34	0.24	0.15
63	Control	100	100	0	50	50	100	67	251.0	257.4	340.5	282.9	0	89	7	4	0	31.0	0.35	0.20	0.10	0.10
65	Control	250	150	350	100	150	100	183	89.0	88.6	70.6	82.7	0	61	23	0	16	24.5	0.39	0.19	0.11	0.10
68	Control	300	550	400	400	450	300	400	99.5	98.1	78.5	92.0	5	70	8	8	9	27.5	0.29	0.16	0.13	0.09
79	Control	0	50	100	100	50	100	67	52.9	10.2	25.2	29.4	0	20	21	19	30	20.3	0.66	0.15	0.11	0.09
113	Control	250	250	150	200	150	100	183	136.4	150.5	92.3	126.4	0	74	8	4	14	27.0	0.52	0.17	0.12	0.10
122	Control	350	200	50	100	0	150	142	97.9	152.4	177.2	142.5	0	46	19	5	29	27.5	0.42	0.20	0.11	0.16
131	Control	150	200	200	250	50	300	192	169.5	153.9	114.8	146.1	0	27	11	24	38	35.0	0.21	0.11	0.06	0.10
154	Control	400	400	450	950			550	25.2	10.6	22.4	19.4	0	67	19	14	0	17.9	0.38	0.19	0.14	0.11
157	Control	500	850	750	550	550	1200	733	156.9	123.5	132.9	137.8	0	72	12	9	6	21.7	0.25	0.11	0.08	0.10
170	Control	350	100	300	200	300	400	275	86.8	126.5	81.3	98.2	0	80	12	4	4	25.0	0.27	0.20	0.07	0.11
184	Control	200	200	100	50	50	300	150	187.6	284.7	263.5	245.3	0	89	11	0	0	26.8	0.18	0.09	0.06	0.10
							Mean=	294			Mean=	113.4					Mean	24.6	0.61	0.25	0.16	0.12
22	HFW	250	50	150	200	150	200	167	121.9	98.2	81.5	100.6	0	64	24	2	9	25.3	0.49	0.23	0.11	0.15
23	HFW	450	250	400	150	250	450	325	35.4	50.8	44.4	43.5	1	60	29	10	0	17.9	0.59	0.29	0.11	0.13
46	HFW	0	0	100	100	0	100	50	40.0	47.7	30.5	39.4	0	61	31	8	0	18.5	0.52	0.19	0.10	0.16
72	HFW	50	50	150	250	100	250	142	22.3	4.8	19.8	15.6	0	73	25	2	0	35.9	0.26	0.15	0.07	0.09
83	HFW	550	350	750	600	450	600	550	3.1	12.2	11.3	8.9	0	80	16	0	4	17.3	0.57	0.30	0.12	0.12
98	HFW	450	400	350	250	550	200	367	22.8	4.1	33.8	20.2	0	28	24	9	39	19.0	0.42	0.23	0.13	0.11
99	HFW	350	250	250	600	500	400	392	39.1	68.7	47.1	51.6	0	50	38	3	9	20.8	0.74	0.20	0.11	0.16
101	HFW	150	550	450	400	300	300	358	91.0	45.0	119.7	85.2	0	73	18	6	3	26.6	0.38	0.22	0.10	0.12
103	HFW	100	300	150	150	300	200	200	173.6	132.5	104.2	136.8	0	50	24	0	26	24.7	0.45	0.24	0.11	0.12
111	HFW	500	350	200	400	200	250	317	2.0	3.4		1.8	0	39	17	9	35		1.01	0.55	0.18	0.17
117	HFW	450	600	500	600	400	250	467	13.8	14.6	32.5	20.3	0	75	22	0	3	20.2	0.36	0.18	0.18	0.12
120	HFW	450	450	550	1150	1150	400	692	28.9	27.5	31.6	29.4	0	74	5	5	16	17.5	0.25	0.33	0.08	0.10
140	HFW	500	550	600	750	750		630	74.1	77.8	110.2	87.4	0	51	42	7	0		0.74	0.25	0.14	0.13
142	HFW	900	700	900	1100	900	850	892	31.0	36.4	13.8	27.1	0	46	30	5	19	14.7	0.50	0.22	0.11	0.11
145	HFW	200	200	400	450	250	400	317	51.5	80.4	107.1	79.7	0	86	4	9	1	24.5	0.76	0.36	0.22	0.16
152	HFW	350	650	650	150	300	300	400	80.5	64.3	96.1	80.3	0	84	14	2	0	23.9	0.78	0.36	0.13	0.13
153	HFW	150	150	50	100	0	50	83	89.3	128.2	153.5	123.7	0	48	20	3	29	19.1	0.62	0.15	0.08	0.13
158	HFW	750	300	650	900	350	300	542	129.6	7.4	58.0	65.0	0	58	18	1	23	23.4	0.36	0.30	0.07	0.10
168	HFW	700	800	550	450	400	350	542	0.4	2.0	2.3	1.6	0	89	18	1	23	21.0	0.52	0.24	0.09	0.11
177	HFW	50	100	150	200	250	200	158	36.8	95.0	128.7	86.9	0	60	35	4	1	21.8	0.61	0.21	0.16	0.11
							Mean=	379			Mean=	55.2					Mean=	21.8	0.54	0.26	0.12	0.13

P.T. ram lambs sampled 23/2/99

									Developmental success			Mean	Generic Composition (%)					%D.M.	Antibody-levels			
Tag	Line	FEC 1	FEC 2	FEC 3	FEC 4	FEC 5	FEC 6	FECmean	Culture 1	Culture 2	Culture 3	dev. success	Haem	Ost	Trich	Coop	LT	%DM	Tc L3	Tc Ad	Oc L3	Oc Ad
1	Control	350	550	700	250	450	500	467	133.2	150.8	201.3	161.8	1	41	42	9	7	31.6	1.01	0.32	0.41	0.15
2	Control	50	250	50	50	300	150	142	42.6	25.9	37.3	35.3	1	59	11		29	35.8	0.70	0.31	0.09	0.10
12	Control	400	200	250	350	450	200	308	146.7	101.1	156.0	134.6	5	51	15	1	28	31.3	0.78	0.25	0.16	0.10
17	Control	100	100	100	100	200	150	125	114.5	109.2	93.5	105.7	1	90	7		2	27.8	0.89	0.28	0.10	0.11
40	Control	400	300	200	250	200	100	242	95.0	132.5	102.4	110.0	6	35	25	8	26	29.7	0.98	0.37	0.21	0.13
42	Control	50	50	100	150	100	100	92	84.8	73.1	42.1	66.7	7	65	8	4	16	28.9	0.77	0.18	0.10	0.10
49	Control	350	550	300	150	200		310	83.6	13.1	14.1	36.9	1	72	11		16		0.56	0.17	0.10	0.11
57	Control	300	350	650	350	450	450	425	215.4	155.0	157.9	176.1	3	70	16	2	9	25.6	1.01	0.36	0.15	0.16
61	Control	600	400	250	350	600	600	467	68.6	47.1	39.7	51.8	4	57	10	3	26	20.7	0.82	0.33	0.19	0.14
63	Control	100	150	200	300	100	100	158	68.3	60.0	53.6	60.7	3	69	20		8	37.2	0.40	0.28	0.13	0.09
65	Control	100	200	100	50	100	200	125	184.1	183.5	145.9	171.2	3	62	19	12	4	30.7	0.39	0.12	0.07	0.08
68	Control	300	150	100	200	150	200	183	46.6	92.2	79.4	72.7	1	82	15		2	33.7	0.26	0.11	0.07	0.08
79	Control	250	250	150	100	100	250	183	194.0	149.9	232.3	192.1	2	78	12		8	23.9	0.69	0.16	0.11	0.08
113	Control	0	0	0	520	0	0	87	42.0	42.7	45.9	43.5		84	14		2	29.6	0.30	0.09	0.08	0.08
122	Control	200	150	100	0	150	50	108	34.6	64.3	46.2	48.3	4	73	16		7	33.7	0.67	0.30	0.10	0.12
131	Control	250	200	200	150	150	150	183	142.7	206.4	157.9	169.0	8	69	13	4	6	32.6	0.29	0.14	0.06	0.09
154	Control	500	350	500	600	800	500	542	23.0	10.5	22.8	18.8	2	67	14	3	14	22.9	0.52	0.15	0.12	0.09
157	Control	200	50	50	50	300	350	167	197.8	211.4	200.2	203.1		78	20		2	26.7	0.34	0.19	0.07	0.10
170	Control	100	300	100	200	100	150	158	90.7	147.0	145.3	127.7		71	16		13	24.0	0.80	0.45	0.17	0.16
184	Control	100	200	150	250	100	250	175	47.2	134.9	132.0	104.7	2	85	3		10	28.2	0.31	0.10	0.06	0.08
							Mean=	232			Mean	104.5					Mean=	29.2	0.63	0.23	0.13	0.11
22	HFW	350	400	450	200	300	350	342	49.4	60.3	52.0	53.9	1	74	21		4	18.9	1.03	0.25	0.11	0.17
23	HFW	100	100	100	200	200	50	125	63.9	51.8	54.1	56.6		88	10		2	24.2	0.66	0.15	0.08	0.11
46	HFW	100	350	250	300	200	100	217	104.6	92.3	100.1	99.0	1	72	19	5	3	26.9	0.89	0.24	0.10	0.18
72	HFW	0	0	50	100	50	0	33	297.0	210.3	265.7	257.7	3	82	11	3	1	33.3	0.34	0.14	0.08	0.09
83	HFW	200	550	200	200	400	200	292	57.7	58.0	84.9	66.9		54	21	2	23	24.5	0.76	0.61	0.11	0.11
98	HFW	50	50	200	50	150	200	117	168.8	173.1	153.6	165.2	3	56	19	2	20	28.2	0.62	0.21	0.10	0.11
99	HFW	200	150	50	200	250	100	158	103.6	172.8	133.1	136.5	1	80	9	1	9	25.3	0.81	0.19	0.10	0.14
101	HFW	150	150	150	450	300	200	233	95.0	59.3	137.5	97.3	1	73	15	3	8	27.6	0.29	0.11	0.07	0.09
103	HFW	100	200	100	100	50	0	92	94.1	112.2	107.0	104.4	2	77	11	4	6	23.6	0.58	0.24	0.09	0.12
111	HFW	1050	900	950	550	550	850	808	66.6	33.1	39.3	46.3	1	73	15	3	8	22.7	0.81	0.36	0.13	0.14
117	HFW	100	250	500	250	150	250	250	116.9	30.0	52.4	66.4	1	75	17		7	22.2	0.40	0.16	0.12	0.10
120	HFW	350	200	550	500	600	250	408	92.4	184.9	190.4	155.9	1	71	20	3	5	30.4	0.35	0.23	0.07	0.10
140	HFW	100	50	100	300	100	200	142	141.0	133.4	158.7	144.4	1	70	20	4	5	26.0	0.64	0.17	0.09	0.10
142	HFW	650	550	1000	550	400	300	575	124.5	84.6	69.6	92.9		75	10		15	24.7	0.86	0.22	0.14	0.13
145	HFW	200	250	400	200	100	100	208	24.0	46.6		35.3		87	11	1	1	26.9	0.83	0.32	0.18	0.15
152	HFW	550	200	150	100	100	100	200	53.0	150.5	81.9	95.1	2	68	20	3	7	23.6	0.69	0.35	0.12	0.13
153	HFW	250	150	150	0	0	100	108	76.2	139.8	69.0	95.0	6	43	20	2	29	26.3	0.39	0.16	0.08	0.12
158	HFW	450	450	200	400	350	300	358	48.1	113.5	144.7	102.1	1	31	33		35	30.8	0.31	0.20	0.07	0.11
168	HFW	200	200	200	350	100	400	242	161.4	177.5	190.4	176.4	2	57	19	1	21	29.5	0.41	0.18	0.09	0.11
177	HFW	200	250	300	550	200	550	342	61.2	95.5	87.2	81.3	4	66	12	1	17	26.5	0.88	0.17	0.12	0.10
							Mean=	263			Mean	106.4					Mean	26.1	0.63	0.23	0.10	0.12

P.T. ram lambs sampled 12/5/99																						
									Developmental success			Mean	Generic Composition (%)					%D.M.	Antibody-levels			
Tag	Line	FEC 1	FEC 2	FEC 3	FEC 4	FEC 5	FEC 6	FECmean	Culture 1	Culture 2	Culture 3	dev. success	Haem	Ost	Trich	Coop	LT	%DM	Tc L3	Tc Ad	Oc L3	Oc Ad
1	Control	no sample																	1.16	0.26	0.40	0.12
2	Control	1100	950	750	1300	650	1050	967	15.4	50.8	23.6	29.9	0	44	34	4	18	40.9	1.48	0.72	0.27	0.18
12	Control	1850	2150	1150	1600	3000	2800	2092	36.6	19.7	36.9	31.1	0	20	13	10	57	30.9	1.38	0.57	0.40	0.19
17	Control	2500	2150	1800	1500	1850	2100	1983	13.9	5.9	4.2	8.0	0	52	26	3	19	22.8	1.31	0.66	0.25	0.20
40	Control	2850	2550	2800	3900	2700	3800	3100	11.5	15.7	20.2	15.8	0	66	19	5	9	25.5	1.70	0.71	0.53	0.27
42	Control	850	1300	1050	1900	1300	1800	1367	8.8	44.2	48.0	33.7	0	59	28	12	1	29.9	1.23	0.46	0.23	0.16
49	Control	5950	7900	6100	4500	5400	6900	6125	2.4	5.0	4.3	3.9	0	44	12	9	35		0.99	0.25	0.19	0.12
57	Control	1800	2700	1600	1750	1950	2350	2025	5.3	11.5	12.5	9.8	0	11	5	7	77	25.0	1.54	0.76	0.46	0.20
61	Control	3550	3750	3600	3250	3800	3300	3542	2.0	1.5	0.9	1.5	0	63	18	12	7	16.3	1.17	0.49	0.40	0.19
63	Control	2150	1750	800	2000	2050	1600	1725	4.1	4.0	4.0	4.0	0	28	2	2	68	22.5	1.22	0.52	0.29	0.13
65	Control	1700	1400	2300	1550	2650	1550	1858	3.1	3.3	3.2	3.2	0	73	18	4	5	22.4	1.11	0.34	0.19	0.13
68	Control	1200	1500	1050	1250	1950	1800	1458	45.7	46.7	38.1	43.5	0	28	28	9	35	29.6	0.69	0.21	0.12	0.11
79	Control	1250	1550	1250	2200	1500	1500	1542	6.0	8.0	8.6	7.5	0	27	26	9	38	23.7	1.19	0.29	0.26	0.11
113	Control	750	600	950	350	400	700	625	32.9	17.1	21.8	23.9	0	47	20	7	26	36.5	0.58	0.17	0.09	0.10
122	Control	2650	1700	2400	2250	2550	4850	2733	20.8	15.7	13.8	16.8	0	34	23	29	34	29.9	1.36	0.43	0.24	0.17
131	Control	2000	2550	2350	1950	3000	2450	2383	1.3	1.2	1.1	1.2	0	66	14	14	6	15.1	0.79	0.33	0.09	0.11
154	Control	2350	2400	3800	4450	3950	2900	3308	1.6	9.6	4.6	5.3	0	34	10	4	2	23.3	0.99	0.19	0.26	0.10
157	Control	1900	1950	1700	1150	1850	1300	1642	39.8	24.7	29.9	31.4	0	15	18	1	66	27.5	0.58	0.19	0.10	0.09
170	Control	1200	900	1450	1600	1800	1950	1483	28.6	31.1	41.8	33.8	0	28	48	4	20	30.2	0.65	0.34	0.09	0.10
184	Control	1700	2150	2650	2400	1550	1800	2042	38.1	30.5	47.6	38.7	0	53	28	11	7	29.5	1.02	0.27	0.44	0.12
							Mean=	2211			Mean	18.0500434					Mean=	26.7	1.11	0.41	0.27	0.14
22	HFW	1500	1750	2500	2200	1900	1700	1925	4.5	10.8	5.7	7.0	0	53	12	6	29	20.3	1.37	0.47	0.29	0.21
23	HFW	1150	1500	1750	2000	1500	1400	1550	3.1	2.0	1.7	2.3	0	39	20	4	36		1.24	0.47	0.30	0.19
46	HFW	950	1200	2400	2050	950	2900	1742	17.5	15.8	32.3	21.9	0	69	17	11	3	28.7	1.22	0.33	0.19	0.32
72	HFW	1750	1800	1550	1800	1800	2500	1867	9.9	13.1	6.4	9.8	0	62	29	9	0	21.1	1.08	0.33	0.14	0.13
83	HFW	2700	3500	3700	2950	2550	3900	3217	0.9	1.3	1.6	1.2	2	34	11	13	40	15.6	1.35	0.60	0.28	0.15
98	HFW	2100	3150	2750	3000	2550	3350	2817	8.3	22.8	17.9	16.3	0	56	34	9	1	27.6	1.33	0.39	0.30	0.19
99	HFW	3100	3000	2250	4100	2400	3500	3058	10.0	5.3	2.8	6.0	0	53	13	13	21	23.0	1.14	0.42	0.19	0.18
101	HFW	4100	3750	3600	2550	2850	4650	3583	2.3	2.2	4.5	3.0	0	60	14	8	18	17.1	0.50	0.37	0.19	0.15
103	HFW	1700	2200	2350	2050	1900	2550	2125	9.4	9.0	9.4	9.3	0	62	22	3	10	23.1	1.24	0.70	0.34	0.28
111	HFW	3200	3000	4050	2600	2600	2950	3067	14.0	18.9		16.5	0	24	12	11	53		1.05	0.55	0.25	0.17
117	HFW	1900	1450	1750	1750	2050	2250	1858	46.1	36.5	33.5	38.7	0	43	25	20	12	30.4	0.94	0.36	0.32	0.18
120	HFW	2600	4550	3650	4350	5750	5300	4367	3.3	2.5	6.2	4.0	0	57	19	0	24	21.9	0.81	0.41	0.12	0.10
140	HFW	4000	5100	5850	4950	3700	5550	4858	2.8	3.9	4.8	3.9	0	56	10	6	28	18.6	1.16	0.46	0.30	0.18
142	HFW	4400	3050	3550	3450	3900	3650	3667	25.0	12.0	18.7	18.6	1	44	32	12	11	23.9	1.33	0.36	0.36	0.16
145	HFW	4000	3650	3150	3650	4150	3600	3700	42.0	30.6	32.1	36.3	0	36	16	4	44	30.6	1.30	0.63	0.32	0.23
152	HFW	3850	3700	3150	4550	5850	3850	4158	6.9	11.9		9.4	0	20	22	25	33		1.00	0.46	0.21	0.14
153	HFW	1950	1950	1700	1400	1950	1800	1792	4.6	3.7	6.0	4.7	0	13	10	4	73	22.0	0.98	0.30	0.14	0.12
158	HFW	2550	1850	3250	2750	3350	3700	2908	7.1	7.8	9.3	8.1	0	22	6	11	61	26.4	1.03	0.64	0.23	0.19
168	HFW	3650	5050	6200	3950	5250	5800	4983	22.8	21.1	16.3	20.1	2	30	27	25	15	29.6	0.96	0.48	0.20	0.14
177	HFW	no sample																	1.39	0.52	0.32	0.16
							Mean=	3013			Mean	12.5					Mean=	23.7	1.12	0.46	0.25	0.18

Appendix 3b Statistical analysis – Chapter 3

Dependent Variable: ln(FEC+1)

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TIME	2	142.4906066	71.2453033	157.64	0.0001
LINE	1	2.5740082	2.5740082	5.70	0.0187
TAG(LINE)	2	2.7959147	1.3979573	3.09	0.0493
TIME*LINE	2	0.9311555	0.4655778	1.03	0.3604

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TIME	2	143.0844745	71.5422372	158.29	0.0001
LINE	1	0.1496278	0.1496278	0.33	0.5662
TAG(LINE)	2	2.8254201	1.4127100	3.13	0.0478
TIME*LINE	2	0.9311555	0.4655778	1.03	0.3604

Tests of Hypotheses using the Type III MS for TAG(LINE) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LINE	1	0.14962778	0.14962778	0.11	0.7757

Dependent Variable: ln(developmental success + 1)

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TIME	2	89.45662891	44.72831446	52.60	0.0001
LINE	1	2.16921756	2.16921756	2.55	0.1131
TAG(LINE)	2	1.55365756	0.77682878	0.91	0.4041
TIME*LINE	2	2.71306190	1.35653095	1.60	0.2075

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TIME	2	89.50060035	44.75030018	52.62	0.0001
LINE	1	0.48315678	0.48315678	0.57	0.4526
TAG(LINE)	2	1.55818187	0.77909093	0.92	0.4031
TIME*LINE	2	2.71306190	1.35653095	1.60	0.2075

Tests of Hypotheses using the Type III MS for TAG(LINE) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LINE	1	0.48315678	0.48315678	0.62	0.5135

Dependent Variable: DM

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TIME	2	364.8203240	182.4101620	6.73	0.0018
LINE	1	241.9408309	241.9408309	8.93	0.0035
TAG(LINE)	2	35.4962165	17.7481082	0.66	0.5215
TIME*LINE	2	0.4617698	0.2308849	0.01	0.9915

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TIME	2	376.3623436	188.1811718	6.95	0.0015
LINE	1	141.0475656	141.0475656	5.21	0.0246
TAG(LINE)	2	35.5867483	17.7933741	0.66	0.5206
TIME*LINE	2	0.4617698	0.2308849	0.01	0.9915

Tests of Hypotheses using the Type III MS for TAG(LINE) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LINE	1	141.0475656	141.0475656	7.93	0.1064

Dependent Variable: TCL3

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TIME	2	7.02235183	3.51117591	69.84	0.0001
LINE	1	0.01034349	0.01034349	0.21	0.6510
TAG(LINE)	2	2.40100171	1.20050085	23.88	0.0001
TIME*LINE	2	0.04185442	0.02092721	0.42	0.6605

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TIME	2	7.02235183	3.51117591	69.84	0.0001
LINE	1	0.32113165	0.32113165	6.39	0.0129
TAG(LINE)	2	2.40100171	1.20050085	23.88	0.0001
TIME*LINE	2	0.04185442	0.02092721	0.42	0.6605

Tests of Hypotheses using the Type III MS for TAG(LINE) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LINE	1	0.32113165	0.32113165	0.27	0.6565

Dependent Variable: TCAD

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TIME	2	0.98261363	0.49130682	34.62	0.0001
LINE	1	0.01310848	0.01310848	0.92	0.3386
TAG(LINE)	2	0.32200643	0.16100322	11.34	0.0001
TIME*LINE	2	0.01684362	0.00842181	0.59	0.5542

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TIME	2	0.98261363	0.49130682	34.62	0.0001
LINE	1	0.05943399	0.05943399	4.19	0.0431
TAG(LINE)	2	0.32200643	0.16100322	11.34	0.0001
TIME*LINE	2	0.01684362	0.00842181	0.59	0.5542

Tests of Hypotheses using the Type III MS for TAG(LINE) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LINE	1	0.05943399	0.05943399	0.37	0.6053

Dependent Variable: OCL3

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TIME	2	0.45840686	0.22920343	35.25	0.0001
LINE	1	0.02421384	0.02421384	3.72	0.0562
TAG(LINE)	2	0.15285093	0.07642547	11.75	0.0001
TIME*LINE	2	0.00422730	0.00211365	0.33	0.7232

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TIME	2	0.45840686	0.22920343	35.25	0.0001
LINE	1	0.07145677	0.07145677	10.99	0.0012
TAG(LINE)	2	0.15285093	0.07642547	11.75	0.0001
TIME*LINE	2	0.00422730	0.00211365	0.33	0.7232

Tests of Hypotheses using the Type III MS for TAG(LINE) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LINE	1	0.07145677	0.07145677	0.93	0.4356

Dependent Variable: OCAD

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TIME	2	0.04815187	0.02407593	21.10	0.0001
LINE	1	0.00843028	0.00843028	7.39	0.0076
TAG(LINE)	2	0.02060181	0.01030090	9.03	0.0002
TIME*LINE	2	0.00423552	0.00211776	1.86	0.1610

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TIME	2	0.04815187	0.02407593	21.10	0.0001
LINE	1	0.00369163	0.00369163	3.24	0.0747
TAG(LINE)	2	0.02060181	0.01030090	9.03	0.0002
TIME*LINE	2	0.00423552	0.00211776	1.86	0.1610

Tests of Hypotheses using the Type III MS for TAG(LINE) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LINE	1	0.00369163	0.00369163	0.36	0.6102

Antibodies by Line

LINE=1 control

Dependent Variable: TCL3

Source	DF	Type I SS	F Value	Pr > F
TIME	2	3.17231002	29.50	0.0001
TAG(LINE)	1	2.36142827	43.93	0.0001
Source	DF	Type III SS	F Value	Pr > F
TIME	2	3.17231002	29.50	0.0001
TAG(LINE)	1	2.36142827	43.93	0.0001

Dependent Variable: TCAD

Source	DF	Type I SS	F Value	Pr > F
TIME	2	0.37154565	10.93	0.0001
TAG(LINE)	1	0.31870649	18.75	0.0001
Source	DF	Type III SS	F Value	Pr > F
TIME	2	0.37154565	10.93	0.0001
TAG(LINE)	1	0.31870649	18.75	0.0001

Dependent Variable: OCL3

Source	DF	Type I SS	F Value	Pr > F
TIME	2	0.20335876	9.78	0.0002
TAG(LINE)	1	0.15163507	14.58	0.0003
Source	DF	Type III SS	F Value	Pr > F
TIME	2	0.20335876	9.78	0.0002
TAG(LINE)	1	0.15163507	14.58	0.0003

Dependent Variable: OCAD

Source	DF	Type I SS	F Value	Pr > F
TIME	2	0.01375841	6.13	0.0039
TAG(LINE)	1	0.01260563	11.23	0.0014

Source	DF	Type III SS	F Value	Pr > F
TIME	2	0.01375841	6.13	0.0039
TAG(LINE)	1	0.01260563	11.23	0.0014

LINE=3 HFW

Dependent Variable: TCL3

Source	DF	Type I SS	F Value	Pr > F
TIME	2	3.89189622	41.59	0.0001
TAG(LINE)	1	0.03957343	0.85	0.3617

Source	DF	Type III SS	F Value	Pr > F
TIME	2	3.89189622	41.59	0.0001
TAG(LINE)	1	0.03957343	0.85	0.3617

Dependent Variable: TCAD

Source	DF	Type I SS	F Value	Pr > F
TIME	2	0.62791160	27.57	0.0001
TAG(LINE)	1	0.00329994	0.29	0.5925

Source	DF	Type III SS	F Value	Pr > F
TIME	2	0.62791160	27.57	0.0001
TAG(LINE)	1	0.00329994	0.29	0.5925

Dependent Variable: OCL3

Source	DF	Type I SS	F Value	Pr > F
TIME	2	0.25927540	49.72	0.0001
TAG(LINE)	1	0.00121586	0.47	0.4975

Source	DF	Type III SS	F Value	Pr > F
TIME	2	0.25927540	49.72	0.0001
TAG(LINE)	1	0.00121586	0.47	0.4975

Dependent Variable: OCAD

Source	DF	Type I SS	F Value	Pr > F
TIME	2	0.03862898	16.66	0.0001
TAG(LINE)	1	0.00799618	6.90	0.0111

Source	DF	Type III SS	F Value	Pr > F
TIME	2	0.03862898	16.66	0.0001
TAG(LINE)	1	0.00799618	6.90	0.0111

Dependent Variable: *Haemonchus*

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TIME	2	104.0811998	52.0405999	32.41	0.0001
LINE	1	4.1016949	4.1016949	2.55	0.1128
TAG(LINE)	2	2.8813605	1.4406802	0.90	0.4106
TIME*LINE	2	10.2202582	5.1101291	3.18	0.0453

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TIME	2	103.4291833	51.7145916	32.21	0.0001
LINE	1	5.6771512	5.6771512	3.54	0.0627
TAG(LINE)	2	2.9204188	1.4602094	0.91	0.4057
TIME*LINE	2	10.2202582	5.1101291	3.18	0.0453

Tests of Hypotheses using the Type III MS for TAG(LINE) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LINE	1	5.67715116	5.67715116	3.89	0.1874

Dependent Variable: *Ostertagia*

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TIME	2	13278.28031	6639.14015	24.44	0.0001
LINE	1	62.67797	62.67797	0.23	0.6319
TAG(LINE)	2	1932.78978	966.39489	3.56	0.0318
TIME*LINE	2	8.96539	4.48269	0.02	0.9836

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TIME	2	13608.75505	6804.37753	25.05	0.0001
LINE	1	1836.14591	1836.14591	6.76	0.0106
TAG(LINE)	2	1931.27129	965.63564	3.56	0.0319
TIME*LINE	2	8.96539	4.48269	0.02	0.9836

Tests of Hypotheses using the Type III MS for TAG(LINE) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LINE	1	1836.145907	1836.145907	1.90	0.3019

Dependent Variable: *Trichostrongylus*

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TIME	2	343.9389831	171.9694915	2.36	0.0994
LINE	1	97.0254237	97.0254237	1.33	0.2512
TAG(LINE)	2	58.2374301	29.1187151	0.40	0.6718
TIME*LINE	2	326.4970573	163.2485286	2.24	0.1115

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TIME	2	346.4446220	173.2223110	2.38	0.0977
LINE	1	3.2686569	3.2686569	0.04	0.8327
TAG(LINE)	2	64.8335954	32.4167977	0.44	0.6423
TIME*LINE	2	326.4970573	163.2485286	2.24	0.1115

Tests of Hypotheses using the Type III MS for TAG(LINE) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LINE	1	3.26865691	3.26865691	0.10	0.7809

Dependent Variable: *Cooperia*

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TIME	2	1022.448617	511.224309	16.19	0.0001
LINE	1	39.186441	39.186441	1.24	0.2677
TAG(LINE)	2	11.207914	5.603957	0.18	0.8376
TIME*LINE	2	241.520415	120.760207	3.82	0.0248

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TIME	2	1027.409179	513.704589	16.27	0.0001
LINE	1	32.793042	32.793042	1.04	0.3104
TAG(LINE)	2	12.214770	6.107385	0.19	0.8244
TIME*LINE	2	241.520415	120.760207	3.82	0.0248

Tests of Hypotheses using the Type III MS for TAG(LINE) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LINE	1	32.79304216	32.79304216	5.37	0.1464

Dependent Variable: *Chabertia/Oesophagostomum*

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TIME	2	6091.352944	3045.676472	12.38	0.0001
LINE	1	22.915254	22.915254	0.09	0.7608
TAG(LINE)	2	1706.023273	853.011637	3.47	0.0346
TIME*LINE	2	3.216440	1.608220	0.01	0.9935

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TIME	2	6311.716578	3155.858289	12.83	0.0001
LINE	1	1539.551744	1539.551744	6.26	0.0138
TAG(LINE)	2	1705.978652	852.989326	3.47	0.0346
TIME*LINE	2	3.216440	1.608220	0.01	0.9935

Tests of Hypotheses using the Type III MS for TAG(LINE) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LINE	1	1539.551744	1539.551744	1.80	0.3113

Appendix 4a Data from Chapter 4

Henson's ewe lambs sampled 3/3/99																		
								Mean	Developmental success			Mean	Generic Composition (%)					
Tag	Drench	FEC 1	FEC 2	FEC 3	FEC 4	FEC 5	FEC 6	FEC	Culture 1	Culture 2	Culture 3	Dev.Succ.	Haem	Ost	Trich	Coop	LT	%DM
507	Albendazole	50	50	100	50	0	100	58	0.0	12.8	9.7	7.5	16	22	0	18	44	24.1
510	Albendazole	0	0	0	50	0	0	8	0.0	6.0	5.8	3.9	0	0	0	0	0	
511	Albendazole	0	0	0	50	0	0	8	96.0	156.0	154.5	135.5	32	3	12	47	6	23.4
512	Albendazole	0	0	0	150	0	0	25	0.0	0.8	5.6	2.1						16.0
514	Albendazole	0	50	0	0	0		10	0.0	30.0	34.7	21.6	0	35	18	41	6	
515	Albendazole	50	0	50	50	0	0	25	0.0	10.2	24.8	11.7	10	22	8	18	42	16.0
517	Albendazole	0	0	50	50	0	50	25	0.0	13.7	5.8	6.5						
518	Albendazole	100	50	50	50	0	200	75	10.5	38.3	21.0	23.2	16	51	9	20	4	19.3
519	Albendazole	50	50	0				33	0.0	86.1	212.5	99.5	0	9	4	69	18	
520	Albendazole	50	0	50	0			25	0.0	2.0	8.0	3.3						21.4
521	Albendazole	50	0	0	100			38	0.0	37.0	24.6	20.5	4	18	1	72	5	
523	Albendazole	100	0	0	0	0	0	17	17.2	41.2	64.7	41.0						21.6
526	Albendazole	0	0	0	0			0										
527	Albendazole	0	100	50	0	0		30	0.0	13.3	18.5	10.6	3	60	5	28	5	
528	Albendazole	50	50	0	0	0		20	0.0	90.0	85.0	58.3	4	17	1	70	8	
530	Albendazole	0	0	50	0	0	0	8	62.3	161.5	23.1	82.3	47	29	3	15	6	12.8
531	Albendazole	50	50	0	0	50	100	42	0.0	42.8	25.4	22.7	2	51	12	35	0	20.9
532	Albendazole	0	0	0	100	0	0	17	180.6	205.9	270.0	218.8	40	29	0	23	8	28.7
533	Albendazole	50	0	50	50	0		30	79.2	60.0	34.0	57.7	49	25	9	15	2	
534	Albendazole	100	50	50	100	0	0	50	0.0	413.6	290.0	234.5	6	34	1	53	6	27.6
							Mean=	27			Mean	55.9	15	27	5	35	11	21.1
269	Ivermectin	50	50	0	0	50	50	33	1.2	1.4	4.2	2.3						14.7
270	Ivermectin	0	0	0	0	0	0	0										15.7
271	Ivermectin	100	0	0	0	50	0	25	0.0	28.0	36.9	21.6	0	93	3	4	0	28.6
272	Ivermectin	0	0	0	0	50	0	8	0.0	11.4	2.4	4.6						14.6
274	Ivermectin	0	50	0	0	100	0	25	0.0	47.1	100.9	49.3	23	56	1	18	2	
275	Ivermectin	0	0	0	0	0		0										
306	Ivermectin	0	0	0	0	0	0	0										20.0
307	Ivermectin	0	0	0	0	0	0	0										19.0
310	Ivermectin	0	0	0	0	0		0										26.7
312	Ivermectin	50	0	0	0	0	0	8	0.0	53.5	52.9	35.5	0	92	8	0	0	27.9
314	Ivermectin	0	50	50	50	0	50	33	0.0	23.1	24.8	15.9	0	98	0	2	0	24.6
315	Ivermectin	50	0	0	0	0	0	8	0.0	44.4	17.1	20.5	0	94	4	2	0	25.4
318	Ivermectin	0	0	0	0	0	0	0										29.8
320	Ivermectin	0	0	50	0	0	0	8	0.0	29.4	9.5	13.0	20	68	2	6	4	7.1
321	Ivermectin	0	0	0	0	0	0	0										19.2
322	Ivermectin	0	0	0	0	0		0										10.0
323	Ivermectin	0	0	0	0	0	0	0										27.3
324	Ivermectin	0	0	0	0	0	0	0										25.3
351	Ivermectin	0	0	0	0	0		0										
352	Ivermectin	0	0	0	0	0	0	0										26.7
							Mean=	8			Mean	20.3	7	84	3	5	1	21.3

Henson's ewe lambs sampled 1/4/99																		
								Mean	Developmental success			Mean	Generic Composition (%)					
Tag	Drench	FEC 1	FEC 2	FEC 3	FEC 4	FEC 5	FEC 6	FEC	Culture 1	Culture 2	Culture 3	Dev.Succ.	Haem	Ost	Trich	Coop	LT	%DM
507	Albendazole	100	150	50	0	300	0	100	38.2	51.9	45.3	45.1	0	20	0	66	14	25.2
510	Albendazole	150	0	0	0	50	0	33	8.6	27.9	17.6	18.1	0	76	8	16	0	25.9
511	Albendazole	300	150	50	50	100	150	133	8.3	9.8	8.1	8.7	0	30	42	20	0	17.9
512	Albendazole	0	0	0	0	0	0	0										24.1
514	Albendazole	50	0	0	0	50	50	25	15.8	42.7	31.1	29.9	0	54	15	31	0	26.6
515	Albendazole	100	50	0	100	200	100	92	0.0	2.2	1.6	1.3	0	38	15	31	15	22.9
517	Albendazole	0	0	50	50	0	50	25	28.0	42.3	55.6	42.0	0	34	6	52	8	22.9
518	Albendazole	50	0	0	100	0	0	25	72.0	56.0	71.0	66.3	2	30	5	60	3	29.2
519	Albendazole	50	0	0	0	0	0	8	104.9	228.0	176.5	169.8	0	56	18	26	0	32.6
520	Albendazole	0	0	50	150	0	0	33	8.8	17.6	22.6	16.4	6	54	6	34	0	25.6
521	Albendazole	0	0	0	50	0	50	17	0.0	3.0	5.7	2.9						27.4
523	Albendazole	50	0	0	0	0	100	25	7.6	66.0	46.2	39.9	0	42	2	56	0	
526	Albendazole	50	250	0	50	50	50	75	11.1	23.3	10.8	15.1	0	50	0	38	13	19.1
527	Albendazole	150	0	100	100	50	100	83	14.4	27.6	14.3	18.8	0	73	9	18	0	24.7
528	Albendazole	50	0	0	50	0	0	17	115.4	198.1	165.7	159.7	0	53	19	28	0	34.9
530	Albendazole	200	300	100	100	100	200	167	44.0	39.2	21.2	34.8	0	70	12	18	0	17.4
531	Albendazole	0	150	0	200	100	100	92	81.8	71.7	99.5	84.3	0	80	9	10	1	27.1
532	Albendazole	150	250	50	300	150	150	175	264.3	340.1	240.6	281.7	6	35	3	43	13	33.0
533	Albendazole	0	200	0	0	0	50	42	104.6	120.0	153.6	126.1	0	13	8	79	0	31.8
534	Albendazole	150	100	100	50	50	50	83	2.4	1.7	0.6	1.6	0	69	12	19	0	23.8
							Mean=	63			Mean=	61.2	1	49	11	36	4	25.9
269	Ivermectin	50	50	0	0	0	0	17	12.0	8.9	3.0	8.0	0	0	0	100	0	20.0
270	Ivermectin	150	50	0	0	0	100	50	5.8	6.6	5.0	5.8	0	40	0	60	0	20.9
271	Ivermectin	50	50	250	50	150	150	117	18.1	36.7	35.8	30.2	0	41	3	56	0	29.9
272	Ivermectin	0	0	0	0	0	0	0										
274	Ivermectin	150	100	50	0	0	0	50	115.4	49.0	52.4	72.3	6	15	8	71	0	26.5
275	Ivermectin	0	0	0	0	0	0	0										19.1
306	Ivermectin	0	50	0	0	0	0	8	24.0	8.4	5.6	12.7	0	64	7	29	0	26.8
307	Ivermectin	0	0	0	0	0	0	0										33.3
310	Ivermectin	0	0	0	0	0	0	0										30.8
312	Ivermectin	0	0	0	0	0	0	0										
314	Ivermectin	50	50	0	0	0	0	17	56.8	123.7	96.6	92.4	12	50	0	38	0	31.3
315	Ivermectin	50	50	0	50	0	0	25	18.9	49.1	47.5	38.5	2	24	2	70	2	25.2
318	Ivermectin	100	200	0	50	100	50	83	46.8	76.5	120.0	81.1	2	39	10	48	1	34.8
320	Ivermectin	50	0	50	150	150	100	83	19.1	29.2	85.4	44.5	2	49	4	44	2	30.2
321	Ivermectin	100	0	100	0	50	50	50	59.6	61.4	8.8	43.3	2	48	2	48	0	30.4
322	Ivermectin	50	0	0	0	0	0	8	14.6	10.9		12.8	0	60	0	40	0	
323	Ivermectin	150	0	50	100	50	0	58	9.8	5.1	9.3	8.1	0	14	0	80	6	19.1
324	Ivermectin	0	0	0	0	0	0	0										33.3
351	Ivermectin	0	0	0	50	50	0	17	76.5	80.8	112.9	90.0	0	94	4	2	0	25.0
352	Ivermectin	0	0	0	0	0	0	0										32.6
							Mean=	29			Mean=	41.5	2	41	3	53	1	27.6

Appendix 4b Statistical analysis – Chapter 4

Dependent Variable: ln(FEC+1)

Source	DF	Type I SS	F Value	Pr > F
TRT	1	55.62589650	29.11	0.0001
TAG(TRT)	2	8.77265368	2.30	0.1079
TIME	1	16.52018145	8.64	0.0044
TIME*TRT	1	1.40141331	0.73	0.3946

Source	DF	Type III SS	F Value	Pr > F
TRT	1	2.35994745	1.23	0.2701
TAG(TRT)	2	8.77265368	2.30	0.1079
TIME	1	16.52018145	8.64	0.0044
TIME*TRT	1	1.40141331	0.73	0.3946

Tests of Hypotheses using the Type III MS for TAG(TRT) as an error term

Source	DF	Type III SS	F Value	Pr > F
TRT	1	2.35994745	0.54	0.5396

Dependent Variable: ln(developmental success + 1)

Source	DF	Type I SS	F Value	Pr > F
TRT	1	0.53754596	0.41	0.5240
TAG(TRT)	2	14.37222902	5.50	0.0067
TIME	1	0.51835836	0.40	0.5314
TIME*TRT	1	0.47503365	0.36	0.5491

Source	DF	Type III SS	F Value	Pr > F
TRT	1	7.64284046	5.85	0.0190
TAG(TRT)	2	13.30078418	5.09	0.0095
TIME	1	0.83124027	0.64	0.4286
TIME*TRT	1	0.47503365	0.36	0.5491

Tests of Hypotheses using the Type III MS for TAG(TRT) as an error term

Source	DF	Type III SS	F Value	Pr > F
TRT	1	7.64284046	1.15	0.3959

Dependent Variable: DM

Source	DF	Type I SS	F Value	Pr > F
TRT	1	1.78544669	0.06	0.8067
TAG(TRT)	2	173.78718164	2.94	0.0608
TIME	1	489.95053884	16.58	0.0001
TIME*TRT	1	8.50129054	0.29	0.5938

Source	DF	Type III SS	F Value	Pr > F
TRT	1	15.84257618	0.54	0.4670
TAG(TRT)	2	175.67547626	2.97	0.0591
TIME	1	472.48495582	15.99	0.0002
TIME*TRT	1	8.50129054	0.29	0.5938

Tests of Hypotheses using the Type III MS for TAG(TRT) as an error term

Source	DF	Type III SS	F Value	Pr > F
TRT	1	15.84257618	0.18	0.7124

Dependent Variable: *Haemonchus*

Source	DF	Type I SS	F Value	Pr > F
TRT	1	167.94258373	1.54	0.2212
TAG(TRT)	2	247.12007050	1.13	0.3313
TIME	1	1577.49711541	14.45	0.0004
TIME*TRT	1	240.48495981	2.20	0.1446

Source	DF	Type III SS	F Value	Pr > F
TRT	1	224.42264164	2.06	0.1584
TAG(TRT)	2	237.92250723	1.09	0.3449
TIME	1	1037.63275276	9.50	0.0035
TIME*TRT	1	240.48495981	2.20	0.1446

Tests of Hypotheses using the Type III MS for TAG(TRT) as an error term

Source	DF	Type III SS	F Value	Pr > F
TRT	1	224.42264164	1.89	0.3033

Dependent Variable: *Ostertagia*

Source	DF	Type I SS	F Value	Pr > F
TRT	1	3023.7100049	8.87	0.0046
TAG(TRT)	2	3184.0831952	4.67	0.0143
TIME	1	0.6911919	0.00	0.9643
TIME*TRT	1	11919.3792016	34.95	0.0001

Source	DF	Type III SS	F Value	Pr > F
TRT	1	541.9378278	1.59	0.2138
TAG(TRT)	2	3961.9939355	5.81	0.0056
TIME	1	1399.7974812	4.10	0.0486
TIME*TRT	1	11919.3792016	34.95	0.0001

Tests of Hypotheses using the Type III MS for TAG(TRT) as an error term

Source	DF	Type III SS	F Value	Pr > F
TRT	1	541.93782778	0.27	0.6531

Dependent Variable: *Trichostrongylus*

Source	DF	Type I SS	F Value	Pr > F
TRT	1	324.76125629	6.96	0.0114
TAG(TRT)	2	66.08742325	0.71	0.4981
TIME	1	134.93140622	2.89	0.0959
TIME*TRT	1	66.16641535	1.42	0.2400

Source	DF	Type III SS	F Value	Pr > F
TRT	1	71.72731961	1.54	0.2215
TAG(TRT)	2	65.33368060	0.70	0.5020
TIME	1	67.46067798	1.44	0.2355
TIME*TRT	1	66.16641535	1.42	0.2400

Tests of Hypotheses using the Type III MS for TAG(TRT) as an error term

Source	DF	Type III SS	F Value	Pr > F
TRT	1	71.72731961	2.20	0.2766

Dependent Variable: *Cooperia*

Source	DF	Type I SS	F Value	Pr > F
TRT	1	67.45457613	0.17	0.6848
TAG(TRT)	2	1868.99317420	2.31	0.1104
TIME	1	3603.88037562	8.92	0.0045
TIME*TRT	1	6499.86761584	16.08	0.0002

Source	DF	Type III SS	F Value	Pr > F
TRT	1	282.57146861	0.70	0.4074
TAG(TRT)	2	2728.59702384	3.38	0.0429
TIME	1	6989.40129594	17.29	0.0001
TIME*TRT	1	6499.86761584	16.08	0.0002

Tests of Hypotheses using the Type III MS for TAG(TRT) as an error term

Source	DF	Type III SS	F Value	Pr > F
TRT	1	282.57146861	0.21	0.6937

Dependent Variable: *Chabertia/Oesophagostomum*

Source	DF	Type I SS	F Value	Pr > F
TRT	1	431.77229788	6.94	0.0115
TAG(TRT)	2	426.22111367	3.42	0.0411
TIME	1	274.22912447	4.41	0.0414
TIME*TRT	1	123.20189260	1.98	0.1662

Source	DF	Type III SS	F Value	Pr > F
TRT	1	437.83414924	7.03	0.0109
TAG(TRT)	2	428.98425768	3.45	0.0403
TIME	1	141.03449064	2.27	0.1391
TIME*TRT	1	123.20189260	1.98	0.1662

Tests of Hypotheses using the Type III MS for TAG(TRT) as an error term

Source	DF	Type III SS	F Value	Pr > F
TRT	1	437.83414924	2.04	0.2893

Dependent Variable: ln(developmental success + 1) with DM as a covariate

Source	DF	Type I SS	F Value	Pr > F
TRT	1	0.78244390	0.78	0.3832
TAG(TRT)	2	13.35369585	6.63	0.0032
TIME	1	0.59368114	0.59	0.4470
TIME*TRT	1	1.57715843	1.57	0.2179
DM	1	18.24188804	18.11	0.0001

Source	DF	Type III SS	F Value	Pr > F
TRT	1	2.91520558	2.89	0.0965

TAG(TRT)	2	6.71970224	3.34	0.0455
TIME	1	0.88938660	0.88	0.3529
TIME*TRT	1	1.08293303	1.08	0.3059
DM	1	18.24188804	18.11	0.0001

Tests of Hypotheses using the Type III MS for TAG(TRT) as an error term

Source	DF	Type III SS	F Value	Pr > F
TRT	1	2.91520558	0.87	0.4499

Appendix 5a Recovering strongyle eggs from faeces

The method used for recovering eggs was essentially that employed by Hubert and Kerboeuf (1992). For all LDA applied in this thesis, the same method for egg recovery from faeces was used. The amount of faeces needed depended on the FEC of the sample. As 5000 – 8000 eggs were needed per microtitre plate (50 and 80 eggs/well, respectively), one would for instance need a minimum of 30 – 50 g of faeces if the FEC was 300 epg and 50 % of the eggs were expected to be lost during the recovery procedure described below.

METHOD:

1. Weigh out the required amount of faeces.
2. Soak faeces in water for 15 - 30 minutes at 4°C
3. Wash faeces through coarse sieve[1] into large plastic beaker[2], using tap water and a plastic teaspoon
4. Wash faecal suspension through 100-µm sieve[3] into another large plastic beaker, again using tap water
5. Wash suspension through a 20-µm sieve[4] until the water runs clear. The 20-µm sieve retains the eggs.
6. Wash eggs off 20-µm sieve and into a 50-ml Falcon tube[5], using a jet of water from a wash bottle[6]
7. Fill the Falcon tube to the 50-ml mark, mix and centrifuge[7] at max. 516 G[8] for 10 minutes. The eggs will then be in the sediment
8. Siphon off supernatant and suspend sediment in 20% aqueous $MgSO_4$[9]. Mix well and centrifuge at max. 516 G[10] for 5 minutes
9. Empty the supernatant into a 60-µm sieve[11], placed inside a 20-µm sieve, and wash thoroughly through the 60-µm sieve, using tap water, and collect eggs on the 20-µm sieve. Eggs should be cleaned to remove any $MgSO_4$, as even short time exposure will affect their viability.

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[1] coarse sieve (large tea strainer); aperture approximately 1 mm

[2] 2 L Nalgene® plastic beakers

[3] 100-µm (diameter = 10.2 cm) sieve made from hard plastic tubing with mesh glued on to one end

[4] 20-µm (diameter = 10.2 cm) sieve made from hard plastic tubing with mesh glued on to one end

[5] 50-ml Falcon plastic tubes, Becton Dickinson Labware, USA

[6] 500 ml Nalgene® wash bottle

[7] IEC Centra-8 Centrifuge, International Equipment Company, USA

[8] 1500 rpm; maximum centrifuge radius = 20.5 cm

[9] 20% aqueous $MgSO_4$, density=1.10; (100 g of Epsom Salts (Magnesium Sulphate) were dissolved in 500 ml of distilled water)

[10] 1500 rpm; maximum centrifuge radius = 20.5 cm

[11] 60-µm (diameter = 7.5 cm) sieve made from hard plastic tubing with mesh glued on to one end

10. Wash eggs off 20-µm sieve and into a 50-ml Falcon tube, using a jet of distilled water from a wash bottle.
11. Count 4 aliquots of 20µl and calculate the total number of eggs using the following formula: **(mean number of eggs/20µl) x 50 x volume(ml) = total number of eggs**
12. Adjust the concentration of eggs to approximately 1333 eggs/ml if 80 eggs/well are desired, and to approximately 833 eggs/ml if 50 (±15) eggs/well are desired

Appendix 5b Larval Development Assay – Control wells only

This Larval Development Assay was based on the method described by Hubert and Kerboeuf (1992) with respect to the nutrient medium used for culturing eggs to 3rd stage larvae, except that anthelmintics were not added to the wells before adding agar and nutrient medium. Furthermore only 20 wells per animal sample were used. A concentration of approximately 80 eggs per well was used.

The 96-well microtitre plates were incubated for 7 days at 27°C and eggs, 1st, 2nd and 3rd larval stages in each well were counted to calculate the percentage of eggs that had developed to 3rd stage larvae.

MATERIALS USED FOR THE ASSAY:

Nutritive medium (Yeast extract + Earles Balanced Salt Solution)

Yeast Extract

- 1 g of yeast extract[12] was added to 90 ml of 0.85% saline solution[13]. This mixture was sterilized by autoclaving and stored at –20°C until used.

Earles Balanced Salt Solution

- 'Earles Balanced Salt Solution', E 7510, Sigma Chemical Co. (stored in dark place)

To obtain the final mixture for the nutritive medium, Yeast extract and Earles Balanced Salt Solution were mixed 3:1 just prior to use in the assay.

E. coli suspension

- 15 mg of the lyophilized cells of *E. coli*[14] were added to 100 ml of distilled water. This mixture was autoclaved before use.

Amphotericin B solution

- 25 mg of Amphotericin B[15] were dissolved in 100 ml of distilled water.

Agar Matrix 2%

- 2 g of agar[16] were added to 100 ml of distilled water and heated in a microwave for 2 minutes at highest setting, with occasional mixing. The dissolved agar mixture was

[12] 'Spray dried autolyzed yeast extract', 100 g Y-1000, Sigma Cell Culture, Sigma Chemical Co.

[13] 'Saline tablets', Oxoid Unipath Ltd., Basingstoke, Hampshire, England (one tablet dissolved in 500 ml distilled water gives a concentration of 0.85% saline)

[14] 'Escherichia coli lyophilized cells of Strain W' (ATCC 9637), 1g EC-9637, Sigma Chemical Co.

[15] 'Amphotericin B – Solubilized', A-9525, Sigma Chemical Co.

[16] 'Bacto-Agar', Difco Laboratories, Detroit, Michigan, USA

then placed on a magnetic stirrer[17] on low heat until all agar had been dispensed into the wells of the 96-well microtitre plates

Preparation of plates

To all wells, 100 µl of warm 2% agar were added. The agar was allowed to cool and solidify at room temperature for a minimum of 30 minutes.

Preparation of cultures:

The preparation of cultures was carried out as described by Gill *et al.*, 1995. The procedure was as follows:

1. Mix 3 ml of egg suspension with 1 ml of nutritive medium, 1 ml of *E. coli* suspension and 90 µl of Amphotericin in a small glass beaker[18]. Mix well.
2. Using a multipipette[19], dispense 100 µl of the mixture on top of the solidified agar in each of the wells in a 96-well microtitre plate[20].
3. Place the plate in a humidity/incubator chamber[21], containing water, and incubate[22] at 27°C for 7 days.
4. After incubation, transfer the contents of each well to a scored glass slide by means of a pasteur pipette.
5. Add a drop of Lugol's Iodine, place a coverslip on top and count[23] the number of eggs, 1st, 2nd and 3rd stage larvae present in each well, using a compound microscope[24].

[17] Heidolph MR 1 magnetic stirrer

[18] Pyrex® glass beaker

[19] Eppendorf Multipette® Plus, Eppendorf-Netheler-Hinz GmbH, Germany

[20] 'Nunc' round-bottomed 96-well microtitre plates, volume of each well = 300 µl, Nunc, Denmark

[21] 'Nalgene humidity chamber' and 'Modular Incubator Chamber', Billups-Rothenberg, Del Mar, California (used to maintain high humidity level while incubating plates)

[22] Sanyo Incubator MIR 252, Sanyo Electric Co. Ltd., Japan

[23] 'Clay Adams' laboratory counter, Inc. N.Y.

[24] Olympus CH-2

Appendix 5c Procedure for measuring egg size and larval length

Eggs were obtained by means of a simple 'coverslip flotation method' in which 0.5 – 1.0 g of faeces was mixed with 10 ml of saturated NaCl. This mixture was poured through a coarse strainer (aperture approximately 1 mm) into a glass centrifuge tube and topped up with saturated NaCl until a convex meniscus was formed at the top of the tube. A glass coverslip was carefully placed on top and the tube was left to stand for at least 10 – 15 minutes. Then the coverslip was carefully lifted off, whilst keeping it horizontal, and placed on a glass slide.

Larvae were transferred from a Falcon tube on to a glass slide and a coverslip was placed on top.

METHOD:

1. Calibrate the equipment using a stage micrometer.
2. Measure eggs using a x40 objective on a compound microscope[25] and the 'Sigma Scan™ software[26], which is a digitizing system for making 2-dimensional measurements.
3. Measure the width and length of 50 eggs from each animal sample
4. Assuming that the volume of an egg is equivalent to that of an ellipsoid, egg volumes (V) are calculated using the following formula (Bronstein and Semendjajew, 1987):

$$V = \frac{4}{3}\pi \times \left(\frac{length}{2}\right) \times \left(\frac{width}{2}\right)^2.$$

5. Measure larvae using a x10 objective on a compound microscope and the 'Sigma Scan™' software.
6. Measure 100 3rd stage larvae per animal sample and the calculate the mean length.

[25] Olympus CH-2

[26] Sigma Scan™, Jandel Scientific, California; Digitizing tablet to make 2-dimensional measurements, Model JS-2; Ultima 1212-S (with LED cursor insert)

Appendix 5d Data from Chapter 5

O.c.= *Ostertagia circumcincta*

***Ostertagia* and Immunity**										
					Mean	Dev. Succ. @10°C		Dev. Succ. @20°C		
Tag	Trt	FEC 1	FEC 2	FEC 3	FEC	Culture 1	Culture 2	Culture 1	Culture 2	%D.M.
172	*O. c.*	300	150	100	183	54.6	43.8	37.3	149.1	45.7
208	*O. c.*	0	0	0	0					17.3
224	*O. c.*	150	200	200	183	96.4	135.8	120.1	83.9	44.4
280	*O. c.*	600	450	400	483	131.3	157.8	190.6	41.4	36.8
6610	*O. c.*	0	0	0	0					48.9
				Mean=	170	Mean=	103.3	Mean=	103.7	38.6
22	Control	350	250	500	367	95.6	82.5	163.7	228.4	40.4
50	Control	150	100	200	150	207.2	210.1	185.7	166.4	42.6
70	Control	150	100	250	167	201.3	150.0	57.7	158.4	46.2
212	Control	400	150	150	233	135.5	273.0	105.6	121.1	43.5
no tag	Control	100	50	100	83	313.0	213.3	211.1	330.5	47.1
				Mean=	200	Mean=	188.1	Mean=	172.9	43.9

Ostertagia circumcincta immunity										
Results of LDA										
O.c. group										
Tag	172		6610		280		208		224	
Well	L3	Dev.Succ	L3	Dev.Succ	L3	Dev.Succ	L3	Dev.Succ	L3	Dev.Succ
1	6	12.8	32	68.1	13	27.7	5	10.6	2	4.3
2	21	44.7	18	38.3	12	25.5	4	8.5	0	0.0
3	23	48.9	30	63.8	19	40.4	5	10.6	5	10.6
4	14	29.8	11	23.4	15	31.9	15	31.9	1	2.1
5	16	34.0	16	34.0	2	4.3	9	19.1	1	2.1
6	23	48.9	37	78.7	27	57.4	13	27.7	0	0.0
7	0	0.0	30	63.8	14	29.8	19	40.4	1	2.1
8	26	55.3	23	48.9	30	63.8	6	12.8	7	14.9
9	14	29.8	16	34.0	4	8.5	5	10.6	0	0.0
10	27	57.4	20	42.6	17	36.2	8	17.0	5	10.6
11	25	53.2	21	44.7	13	27.7	6	12.8	16	34.0
12	19	40.4	17	36.2	9	19.1	6	12.8	0	0.0
13	2	4.3	17	36.2	27	57.4	7	14.9	1	2.1
14	18	38.3	20	42.6	20	42.6	13	27.7	1	2.1
15	9	19.1	22	46.8	23	48.9	7	14.9	1	2.1
16	2	4.3	13	27.7	25	53.2	8	17.0	1	2.1
17	28	59.6	14	29.8	6	12.8	4	8.5	8	17.0
18	21	44.7	25	53.2	12	25.5	4	8.5	1	2.1
19	34	72.3	0	0.0	10	21.3	4	8.5	2	4.3
20	11	23.4	8	17.0	11	23.4	6	12.8	2	4.3
Mean=		36.1		41.5		32.9		16.4		5.9

Ostertagia circumcincta immunity																					
Results of LDA																					
Control group																					
Tag:	No tag				22				50				70				212				
Well	egg	L1/L2	L3	Dev.succ	egg	L1/L2	L3	Dev.succ	egg	L1/L2	L3	Dev.succ	egg	L1/L2	L3	Dev.succ	egg	L1/L2	L3	Dev.succ
1		6	66	91.7	8	2	0	0.0	2		0	0.0			24	100.0		7	41	85.4
2		8	78	90.7	15	1	0	0.0	1		0	0.0		2	25	92.6			0	0.0
3		0	0	0.0		5	52	91.2	2		0	0.0			53	100.0		8	11	57.9
4		6	90	93.8		3	74	96.1		6	0	0.0			42	100.0			84	100.0
5		5	84	94.4		1	71	98.6			0	0.0			38	100.0		4	89	95.7
6		2	87	97.8			60	100.0		2	43	95.6			34	100.0	1	11	18	60.0
7		2	77	97.5			82	100.0	1	5	17	73.9		2	18	90.0	1	5	19	76.0
8		1	54	98.2			72	100.0		1	29	96.7			0	0.0			58	100.0
9		5	80	94.1	14	1	0	0.0			94	100.0	6	4	0	0.0			123	100.0
10		5	67	93.1	7	7	0	0.0		12	26	68.4		1	21	95.5			0	0.0
11			84	100.0	21	1	1	4.3			112	100.0			48	100.0	1	6	0	0.0
12			0	0.0		6	4	40.0			97	100.0			54	100.0		2	34	94.4
13		9	70	88.6	6	1	0	0.0			0	0.0			43	100.0			56	100.0
14			0	0.0	2	2	2	33.3			0	0.0			56	100.0			103	100.0
15		5	61	92.4		6	3	33.3			0	0.0			59	100.0			100	100.0
16		2	74	97.4			75	100.0			70	100.0			40	100.0			28	100.0
17		4	83	95.4		2	42	95.5			4	100.0			45	100.0			107	100.0
18		1	89	98.9			57	100.0			90	100.0			54	100.0			86	100.0
19		2	61	96.8			57	100.0			117	100.0			63	100.0			92	100.0
20		16	15	48.4		3	25	89.3			1	100.0			0	0.0		1	1	50.0
		Mean=		78.4				59.1				56.7				83.9				76.0
		Std.Dev.=		35.5				44.9				48.3				36.3				36.4

Egg measurements (um)																				
O. circumcincta group											Control group									
Tag	172		208		224		280		6610		22		50		70		212		No tag	
	Length	Width	Length	Width	Length	Width	Length	Width	Length	Width	Length	Width	Length	Width	Length	Width	Length	Width	Length	Width
	85.6	49.6	84.3	47.7	94.1	50.6	87.9	54.5	83.5	46.2	92.7	47.1	91.2	51.2	82.6	53.6	91.8	46.6	85.4	49.8
	97.8	44.6	69.1	38.0	87.1	50.3	89.9	44.4	91.5	43.7	97.0	49.5	88.1	52.6	93.2	58.0	79.2	52.9	88.9	47.1
	94.9	49.4	65.0	39.9	86.9	52.2	97.4	50.4	81.9	42.3	90.0	48.5	89.4	49.6	85.0	48.0	84.6	49.5	87.7	42.9
	84.0	47.6	84.3	41.9	85.2	49.2	89.8	50.5	85.7	50.1	83.3	51.5	87.5	46.6	81.7	46.7	89.2	47.9	90.2	46.6
	78.2	48.6	85.1	44.8	95.3	51.1	85.3	50.9	87.7	43.0	85.8	48.1	90.6	51.0	88.0	48.6	92.0	51.8	84.6	50.5
	93.3	47.8	86.6	43.6	90.8	51.8	98.2	47.1	90.8	46.5	89.9	47.5	90.4	46.0	83.0	45.5	87.1	45.3	80.8	52.6
	89.6	48.1	76.4	46.2	92.5	50.9	90.5	52.8	93.1	45.8	90.1	46.3	95.0	49.0	78.4	46.3	97.3	47.3	89.2	49.2
	86.5	44.8	88.9	44.9	92.6	51.7	87.9	53.1	88.1	48.0	84.5	50.0	85.8	49.2	85.1	45.8	95.9	49.4	88.3	47.3
	85.4	47.6	88.3	43.1	83.0	56.2	94.9	46.9	85.8	42.2	90.7	46.6	93.6	49.8	84.3	47.6	91.6	49.2	83.5	47.7
	95.8	44.9	88.8	45.7	86.7	52.1	89.9	50.0	78.5	46.4	79.3	48.1	93.2	49.1	93.2	45.5	88.2	48.8	79.8	47.9
	86.2	45.5	82.8	47.0	72.1	39.4	86.0	49.5	81.6	43.1	81.1	50.2	92.7	47.8	90.2	48.9	87.3	48.3	86.6	52.1
	100.1	49.8	85.9	44.0	83.3	57.5	86.9	52.0	86.5	50.6	91.7	48.7	88.2	53.7	89.5	44.0	87.8	50.3	95.4	49.6
	98.1	46.3	86.2	43.8	99.9	47.8	92.2	53.7	87.3	44.0	89.5	47.5	88.2	52.3	82.3	46.9	92.4	50.4	82.2	47.4
	84.1	48.8	81.2	42.2	89.5	51.8	90.1	47.9	87.1	49.2	85.5	47.0	89.3	50.4	81.5	50.7	83.0	52.3	86.0	49.2
	99.7	45.8	84.5	49.5	101.8	50.6	90.4	50.8	93.6	52.0	86.1	49.1	89.0	47.5	80.1	50.2	81.0	53.8	89.9	43.6
	89.4	47.8	84.6	40.2	85.5	39.7	86.5	52.2	77.7	45.8	90.4	50.2	86.3	50.5	87.1	48.9	90.0	50.4	88.3	47.4
	91.2	44.7	88.4	44.4	89.2	46.8	92.3	51.6	101.4	52.4	90.3	51.0	92.9	48.7	75.8	53.5	91.8	49.1	83.7	46.6
	91.3	46.1	84.9	44.6	91.1	47.3	84.1	47.4	98.2	49.6	86.1	49.7	87.5	53.4	89.3	49.8	86.6	51.6	88.4	54.1
	93.5	48.7	75.7	36.1	92.6	45.5	84.5	51.7	80.5	49.0	89.2	52.6	91.0	44.9	88.5	46.2	95.5	49.9	89.0	50.5
	87.7	50.6	80.3	45.5	84.9	48.5	95.0	44.6	97.9	49.2	90.1	51.7	89.3	51.4	93.3	48.2	87.2	47.4	88.0	49.5
	84.4	52.4	90.9	41.4	88.9	46.4	86.7	44.2	83.4	48.2	90.5	45.4	77.5	53.1	88.7	44.4	88.2	45.7	82.3	49.8
	86.1	48.6	84.3	44.3	94.9	46.9	85.1	52.3	85.0	45.4	81.5	45.7	90.7	50.3	94.0	46.3	82.7	51.1	89.0	49.0
	93.2	49.3	99.5	41.1	97.8	47.0	90.2	46.7	85.0	46.5	86.1	48.7	90.3	47.8	82.8	48.5	90.8	51.2	84.2	48.6
	78.5	46.5	93.6	45.4	85.7	49.5	88.3	50.4	90.5	48.2	93.5	48.9	88.1	49.0	88.2	46.6	91.0	46.4	86.7	51.4
	87.4	49.1	85.7	44.4	85.4	47.3	89.3	47.4	83.5	43.4	88.3	51.9	90.8	47.3	84.8	48.8	92.3	51.1	91.9	46.5
	78.8	55.2	86.2	41.2	86.0	45.2	92.4	42.4	87.3	39.9	84.7	50.9	84.2	51.1	81.1	46.1	88.1	50.0	87.8	51.6
	99.5	48.5	88.1	47.7	84.8	50.2	94.7	47.0	85.5	44.7	94.4	47.5	79.0	50.0	88.6	42.8	93.3	51.3	91.8	47.8
	95.8	46.7	80.5	44.9	86.2	49.0	87.0	49.7	90.0	48.9	90.0	50.8	83.9	50.9	85.7	48.9	96.2	46.2	85.4	49.2
	88.3	50.6	87.9	42.9	87.2	47.4	83.6	47.7	85.8	43.8	76.6	54.4	95.7	48.0	87.0	49.8	85.0	45.0	91.8	53.3
	86.9	45.8	89.9	46.5	89.3	45.4	84.8	49.0	89.4	47.3	91.1	47.5	85.9	50.8	88.4	50.4	91.8	50.5	86.7	54.4
	87.8	47.8	88.7	46.0	96.9	48.1	94.2	46.4			86.1	50.6	73.3	50.6	91.0	52.9	91.6	44.6	80.0	46.9
	94.2	50.6	79.4	45.1	88.2	54.3	89.0	49.7			87.9	49.4	81.7	50.2	85.7	49.9	91.6	47.1	76.8	48.3
	89.9	47.8	88.6	44.0	97.7	48.9	101.9	45.0			85.2	53.3	91.5	50.0	77.7	52.2	94.4	51.1	82.7	53.2
	88.4	52.4	91.6	45.9	85.8	48.6	90.9	46.8			84.9	49.1	81.2	50.6	83.1	50.6	89.2	52.1	92.4	48.7
	86.3	55.8	82.6	44.9	87.1	47.6	93.7	46.6			81.8	49.6	92.0	45.9	75.3	48.6	92.8	48.9	78.1	50.0
	80.2	55.1	84.3	46.7	88.4	44.2	85.6	53.1			84.5	50.5	86.5	46.7	77.8	49.4	88.8	52.3	81.8	52.1
	95.5	53.5	85.6	43.4	93.0	48.9	91.3	47.1			87.6	50.5	87.8	45.3	86.7	51.0	90.2	49.3	97.9	47.5
	92.5	60.9	89.2	40.9	90.3	50.8	97.0	48.0			90.2	50.3	89.2	48.1	88.7	48.0	91.8	47.8	88.6	47.4
	80.5	45.6	81.4	46.1	90.2	48.0	82.9	53.3			82.2	51.4	88.7	45.8	82.7	49.2	89.9	47.0	94.6	45.7
	90.4	47.0	84.5	44.5	96.5	43.1	89.2	53.9			85.8	50.9	87.8	50.9	93.8	44.5	92.7	47.0	83.2	47.7
	83.5	45.1	86.6	44.0	90.2	44.9	81.2	51.3			92.9	49.6	84.4	50.4	72.4	47.6	83.5	51.7	80.5	50.5
	84.9	44.9	84.7	44.6	81.0	43.6	85.8	52.4			98.2	47.6	92.5	51.9	76.0	49.8	92.9	49.9	89.4	50.2
	72.4	47.7	79.6	48.4	88.0	49.0	89.7	52.4			80.4	43.6	84.7	46.1	83.7	47.4	86.5	51.1	85.8	52.2
	90.5	44.1	93.3	46.3	91.8	49.1	90.4	48.3			81.3	51.3	91.3	48.6	77.1	50.8	101.0	49.1	87.0	49.3
	84.3	47.7	82.7	44.8	86.5	52.8	91.9	47.4			88.9	48.2	98.8	45.5	88.7	50.1	90.6	48.1	91.9	47.9
	91.0	50.3	96.6	49.8	89.6	46.9	85.9	48.0			87.3	48.0	86.4	47.8	84.2	45.9	96.5	35.5	89.1	51.6
	86.4	48.0	83.7	48.2	95.4	45.1	91.9	48.1			86.3	47.7	85.5	49.5	93.7	50.2	90.7	53.7	73.3	46.3
	89.6	48.2	89.0	45.4	78.9	46.2	85.1	51.1			89.9	51.7	84.9	47.1	80.8	48.0	94.0	49.7	89.1	44.6
	76.4	47.7	85.6	48.5	92.4	47.8	89.7	52.3			95.5	49.8	87.5	49.8	87.8	43.4	97.0	50.0	84.6	53.0
	84.8	50.5	92.4	48.2	91.9	60.1	90.3	46.9			90.3	51.1	91.0	52.2					82.3	52.4
Mean=	88.4	48.6	85.4	44.6	89.4	48.7	89.6	49.3	87.5	46.5	87.7	49.3	88.2	49.3	85.1	48.5	90.3	49.1	86.5	49.2

Larval measurements (um)																
	Larvae cultured at 10°C								Larvae cultured at 20°C							
	Control group					*O. circumcincta* group			Control group					*O. circumcincta* group		
Tag	212	22	50	70	No tag	172	224	280	212	22	50	70	No tag	172	224	280
	836	749	897	812	835	830	850	795	912	829	884	880	877	786	881	796
	822	792	766	828	856	784	871	779	862	899	928	865	900	811	866	874
	838	792	859	806	847	758	836	822	835	917	881	864	879	735	906	870
	887	862	844	785	826	827	852	812	869	864	912	866	869	779	871	832
	868	860	787	780	824	845	833	827	828	927	890	873	862	834	831	878
	836	823	804	808	883	839	858	808	838	910	850	932	913	885	868	892
	866	806	791	838	893	865	797	809	885	886	907	863	867	858	879	753
	720	850	828	840	791	838	829	767	840	918	878	855	873	766	867	881
	850	802	908	857	712	774	845	800	871	889	843	873	873	830	775	851
	826	837	832	729	817	815	900	754	887	912	839	903	893	859	872	869
	855	799	849	749	823	756	819	848	818	935	876	708	748	831	886	876
	880	834	835	848	852	815	804	893	823	911	879	908	854	848	940	882
	857	745	848	828	810	770	848	804	804	899	863	821	848	845	928	864
	789	817	785	853	873	809	809	713	781	944	871	753	913	765	859	842
	866	870	782	860	821	771	843	819	822	754	918	875	864	788	889	858
	852	857	817	849	848	813	841	871	852	924	910	762	874	770	847	821
	831	892	840	818	824	815	855	741	874	819	858	953	868	769	926	940
	859	774	835	814	863	832	864	824	855	878	919	889	851	720	839	847
	783	838	840	860	853	782	812	810	853	782	879	877	878	890	825	849
	822	792	841	844	796	806	825	735	796	844	850	868	912	845	840	877
	851	824	872	861	911	868	848	828	876	744	831	868	926	875	872	784
	834	848	872	795	911	765	851	765	850	917	821	867	773	829	929	896
	822	877	813	822	848	804	868	863	859	874	953	897	797	761	856	836
	779	801	835	779	882	804	882	803	858	868	864	891	848	849	840	841
	802	851	803	834	827	816	837	835	857	861	885	792	874	778	909	856
	826	846	781	830	807	861	901	776	880	797	877	929	853	738	878	833
	839	819	840	871	861	820	768	695	817	789	821	818	759	761	843	867
	877	809	858	807	806	893	835	799	846	934	865	846	829	838	788	841
	865	884	825	845	852	792	832	818	814	823	921	864	825	852	845	853
	852	840	839	896	891	817	882	858	891	932	806	865	796	799	717	894
	849	864	764	853	857	860	854	831	830	899	887	896	883	841	897	870
	856	876	791	837	890	763	825	820	816	873	858	819	737	814	865	829
	736	837	835	851	844	797	829	800	870	942	856	896	912	828	793	799
	805	762	819	848	861	823	857	807	794	888	830	888	860	794	852	857
	831	869	845	839	791	838	799	802	766	836	876	840	861	728	780	874
	743	828	860	852	882	825	819	766	848	900	947	864	862	828	863	880
	770	885	874	789	864	790	809	813	829	847	895	868	907	850	893	864
	871	845	823	887	918	809	820	814	825	812	867	875	927	809	908	835
	822	875	832	823	840	714	909	816	809	910	1047	883	924	889	807	864
	900	924	843	787	901	824	794	846	795	938	861	877	860	816	868	892
	873	929	792	891	855	833	847	785	808	869	827	863	782	826	890	820
	900	830	784	827	876	797	780	778	872	861	851	824	846	762	832	879
	815	819	837	813	823	755	859	812	886	884	846	857	907	780	864	878
	851	882	823	811	906	755	818	834	805	853	901	938	864	769	852	883
	756	843	834	782	866	812	786	794	898	880	880	874	908	832	909	814
	864	845	816	792	857	797	816	798	883	852	906	863	860	832	761	811
	775	758	794	803	849	799	806	739	807	970	869	799	899	864	741	816
	785	825	858	800	840	847	853	825	893	804	881	749	885	788	922	836
	843	866	854	820	848	843	845	785	767	880	885	931	733	665	909	827

	788	834	822	827	837	821	858	766	857	929	932	932	891	704	798	779
	801	846	798	762	831	810	1006	776	843	919	886	848	892	841	816	867
	811	837	832	814	865	845	852	777	793	851	884	756	707	858	861	804
	849	856	846	775	821	863	867	798	741	877	913	859	820	771	942	800
	848	872	853	877	814	820	863	766	894	889	902	878	729	754	908	856
	818	933	857	866	858	799	891	773	732	863	882	883	893	734	896	859
	829	862	825	931	875	770	840	846	819	817	770	919	857	754	822	903
	793	805	847	811	824	799	824	788	832	848	924	850	918	860	853	855
	813	838	867	798	836	751	816	810	854	872	819	855	790	802	878	784
	856	828	860	800	907	796	857	843	866	877	835	881	882	800	930	864
	851	826	858	887	833	742	836	837	813	913	873	867	896	780	855	781
	807	836	807	810	836	787	851	802	851	884	851	888	917	791	819	812
	850	857	825	829	855	878	854	765	857	920	915	897	845	804	880	863
	840	881	845	865	865	778	872	857	833	878	864	866	918	868	883	850
	893	839	861	884	849	749	864	796	872	841	864	855	898	843	813	848
	815	780	844	782	807	886	834	818	857	854	863	878	856	821	829	760
	817	841	813	832	860	895	875	769	793	873	848	929	886	800	865	821
	851	863	836	907	907	794	887	793	866	938	826	864	802	853	810	820
	804	890	858	859	875	838	851	775	873	896	892	886	744	773	882	905
	844	827	855	852	795	809	906	839	821	913	965	830	896	802	818	2670
	814	783	848	837	783	760	788	790	885	883	849	820	883	877	829	853
	836	846	860	838	779	865	798	774	854	850	926	855	745	833	830	808
	839	881	824	842	823	735	883	761	778	879	919	867	895	804	837	897
	833	816	858	831	822	804	828	718	817	896	862	891	922	786	912	739
	822	882	818	725	804	857	874	847	783	893	912	859	872	916	875	794
	831	838	852	793	858	785	743	830	865	854	856	854	928	797	852	851
	820	799	785	785	764	866	907	742	792	890	824	736	846	746	818	858
	776	811	887	831	867	777	869	815	781	850	815	879	826	819	856	833
	846	843	847	898	871	845	851	778	777	831	879	883	961	860	908	850
	822	787	868	822	863	808	899	763	857	851	868	859	834	886	937	797
	834	771	840	825	885	777	828	789	866	785	916	859	894	832	886	796
	813	828	800	818	797	833	845	806	815	854	829	870	867	817	860	823
	789	831	837	806	845	793	911	799	827	914	806	851	954	864	937	849
	844	841	878	738	810	802	883	822	830	930	856	917	792	828	857	812
	811	864	770	846	869	784	869	834	903	836	896	921	908	690	731	752
	798	873	838	841	839	783	810	825	895	900	866	769	978	738	947	940
	855	852	868	770	839	738	831	749	844	858	929	929	919	806	799	851
	838	816	818	831	905	801	870	861	767	863	905	877	881	844	807	882
	807	855	840	844	814	778	867	753	768	856	780	819	918	797	861	838
	827	829	843	813	870	787	818	830	886	893	874	921	824	867	887	927
	846	783	850	860	684	795	791	831	801	857	830	756	899	785	815	769
	861	809	865	831	858	717	884	802	838	938	827	889	925	808	823	855
	788	834	851	793	875	784	857	795	876	861	942	850	865	790	894	924
	800	847	819	895	854	838	876	804	879	840	926	868	890	825	856	798
	844	787	781	779	778	847	856	791	850	914	910	866	771	890	858	900
	831	782	800	856	825	853	865	793	802	858	942	792	879	867	953	844
	840	826	844	864	834	740	878	856	836	871	812	910	872	778	874	834
	843	846	832	807	890	871	866	794	862	897	896	913	877	835	908	794
	839	830	793	804	822	789	876	839	857	878	806	887	888	802	869	821
	836	834	878	772	818	752	811	810	851	940	884	841	862	871	894	867
Mean	**829**	**835**	**834**	**826**	**843**	**807**	**847**	**801**	**838**	**877**	**876**	**863**	**864**	**811**	**861**	**863**
Mean Control group=						**Mean Oc. group=**			**Mean Control group=**					**Mean Oc. group=**		
			833					**818**				**863**				**845**

Larval Survival, *O.circumcincta*									
Culture temp= 20		Storage temp= 30							
		Week							
Tag	Trt	0	3	6	10	12	13	15	
22	Control	323	294	305	252	209	192	59	
22	Control	326	463		366	450	206	57	
50	Control	298	289	275	218	162	86	24	
50	Control	207	255		194	141	105	21	
70	Control	180	216	308	227	156	96	38	
70	Control	215	328		260	231	125	43	
212	Control	214	271	293	232	195	116	40	
212	Control	227	215		140	155	218	22	
No tag	Control	220	269	222	127	123	67	30	
No tag	Control	222	200		180	133	84	38	
172	*O. c.*	233	230	255	195	121	78	15	
172	*O. c.*	228	284		221	155	81	8	
224	*O. c.*	192	262	242	209	142	82	43	
224	*O. c.*	203	228		210	119	89	52	
280	*O. c.*	264	300	285	298	267	203	192	
280	*O. c.*	195	344		346	258	190	155	

Larval Survival, *O.circumcincta*																								
Culture temp= 10		Storage temp= 30																						
		Week																						
Tag	Trt	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14								
22	Control	200				149					111		67	61		11								
22	Control	195					206				157		65	61		14								
50	Control	158				201					124		79	61		11								
50	Control	169					143				120		61	67		14								
70	Control	146				182					100		86	74		8								
70	Control	173					159				127		106	69		7								
212	Control	167				160					146		82	57		15								
212	Control	207					201				133		86	61		5								
No tag	Control	208				149					151		100	73		25								
No tag	Control	190					136				112		68	76		12								
172	*O. c.*	121				107					44		32	15		3								
172	*O. c.*	108					91				21		21	20		3								
224	*O. c.*	170				204					72		55	50		9								
224	*O. c.*	135					169				94		56	65		8								
280	*O. c.*	207				235					98		47	35		2								
280	*O. c.*	193					142				114		57	23		3								

Larval Survival, *O.circumcincta*										
Culture temp= 20		Storage temp= 20								
		Week								
Tag	Trt	0	3	6	10	12	13	15	22	25
22	Control	336	409	390	319	383	282	330	337	260
22	Control	334	347		385	278	277		252	241
50	Control	235	298	334	275	241	243	220	237	245
50	Control	217	300		267	235	229		250	156
70	Control	290	279	291	298	244	280	230	216	184
70	Control	200	199		225	170	171		179	157
212	Control	240	239	187	157	159	127	114	130	121
212	Control	212	288		276	240	218		250	227
NT	Control	262	247	212	223	190	190	178	191	168
NT	Control	248	195		220	198	185		180	120
172	*O. c.*	269	254	229	241	182	190	180	174	157
172	*O. c.*	290	203		206	181	193		166	161
224	*O. c.*	220	246		160	173	160	147	95	76
224	*O. c.*	190	217	175	138	100	115		79	77
280	*O. c.*	218	286	298	289	183	166	180	150	78
280	*O. c.*	244	290		310	286	230		162	183

Larval Survival, *O.circumcincta*									
Culture temp= 10		Storage temp= 20							
		Week							
Tag	Trt	0	5	9	11	12	14	19	24
22	Control	231	246	193	147	171	170	188	139
22	Control	202	185	162	178	115		161	124
50	Control	217	188	155	169	155	168	145	127
50	Control	200	184	210	157	157		121	
70	Control	197	199	207	150	145	192	121	139
70	Control	173	179	184	161	117		131	128
212	Control	167	151	170	130	132	143	95	100
212	Control	218	167	176	165	152		116	76
NT	Control	141	122	112	67	133	121	116	67
NT	Control	195	50	60	120	44		40	45
172	*O. c.*	146	106	84	54	105	85	56	37
172	*O. c.*	131	101	43	122	54		48	35
224	*O. c.*	211	154	158	152	146	147	155	134
224	*O. c.*	155	164	123	159	108		125	125
280	*O. c.*	248	199	228	173	150	187	149	110
280	*O. c.*	211	194	170	178	145		80	82

Appendix 5e Statistical analysis – Chapter 5

Dependent Variable: FEC

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	1	51667.50000	51667.50000	3.65	0.0658
Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	51667.50000	51667.50000	3.65	0.0658

Dependent Variable: Developmental success in faecal cultures

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TEMP	1	412.92300	412.92300	0.09	0.7618
TRT	1	20179.24888	20179.24888	4.59	0.0418
TAG(TRT)	2	13692.59262	6846.29631	1.56	0.2300
TEMP*TRT	1	464.52675	464.52675	0.11	0.7478
Tests of Hypotheses using the Type III MS for TAG(TRT) as an error term					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	1.65934555	1.65934555	3.53	0.2011
TEMP	1	0.04880254	0.04880254	0.10	0.7779

Dependent Variable: Egg Volume

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	3702553592	3702553592	18.88	0.0001
TAG(TRT)	8	23218894514	2902361814	14.80	0.0001
Tests of Hypotheses using the Type III MS for TAG(TRT) as an error term					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	3702553592	3702553592	1.28	0.2914

Dependent Variable: Larval length

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	106457.6054	106457.6054	27.68	0.0001
TEMP	1	303626.4810	303626.4810	78.95	0.0001
TAG(TRT)	6	272460.4952	45410.0825	11.81	0.0001
TRT*TEMP	1	880.6377	880.6377	0.23	0.6323
Tests of Hypotheses using the Type III MS for TAG(TRT) as an error term					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	106457.6054	106457.6054	2.34	0.1766
TEMP	1	303626.4810	303626.4810	6.69	0.0414

Dependent Variable: %DM

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	1	2280.100000	2280.100000	0.09	0.7717
Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	2280.100000	2280.100000	0.09	0.7717

Dependent Variable: Developmental success in an LDA

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	1	98104.06990	98104.06990	93.01	0.0001
Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	98104.06990	98104.06990	93.01	0.0001

Dependent Variable: Larval survival in water

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	8640.4443	8640.4443	50.46	0.0001
WEEK*TRT	28	120018.4905	4286.3747	25.03	0.0001
CULTURE	1	4353.7202	4353.7202	25.42	0.0001
STORAGE	1	4098.9549	4098.9549	23.94	0.0001
TAG(TRT)	2	11262.1288	5631.0644	32.88	0.0001
CULTURE*STORAGE	1	4529.1680	4529.1680	26.45	0.0001
TRT*CULTURE	1	11.3970	11.3970	0.07	0.7967
TRT*STORAGE	1	359.5169	359.5169	2.10	0.1491
WEEK*TRT*CULTU*STORA	25	47043.4550	1881.7382	10.99	0.0001
Tests of Hypotheses using the Type III MS for TAG(TRT) as an error term					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	8640.444268	8640.444268	1.53	0.3411
CULTURE	1	4353.720167	4353.720167	0.77	0.4720
STORAGE	1	4098.954920	4098.954920	0.73	0.4834

Dependent Variable: Larval survival at 20°C

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	4902.91822	4902.91822	36.57	0.0001
WEEK*TRT	28	44626.01111	1593.78611	11.89	0.0001
CULTURE	1	595.85633	595.85633	4.44	0.0375
TAG(TRT)	2	7390.86478	3695.43239	27.56	0.0001
TRT*CULTURE	1	34.56133	34.56133	0.26	0.6128

Tests of Hypotheses using the Type III MS for TAG(TRT) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	4902.918222	4902.918222	1.33	0.3685
CULTURE	1	595.856333	595.856333	0.16	0.7269

Dependent Variable: Larval survival at 30°C

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	3673.6767	3673.6767	13.16	0.0005
WEEK*TRT	20	115142.5433	5757.1272	20.63	0.0001
CULTURE	1	4747.6920	4747.6920	17.01	0.0001
TAG(TRT)	2	4453.9612	2226.9806	7.98	0.0007
TRT*CULTURE	1	113.4907	113.4907	0.41	0.5255

Tests of Hypotheses using the Type III MS for TAG(TRT) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	3673.676674	3673.676674	1.65	0.3277
CULTURE	1	4747.692000	4747.692000	2.13	0.2817

Larval survival by treatment group

Control group

Dependent Variable: Larval survival

Source	DF	Type III SS	Mean Square	F Value	Pr > F
CULTURE	1	2613.07225	2613.07225	6.39	0.0126
STORAGE	1	4090.25108	4090.25108	10.00	0.0019
WEEK	14	68282.38246	4877.31303	11.93	0.0001

***O. circumcincta* group**

Dependent Variable: Larval survival

Source	DF	Type III SS	Mean Square	F Value	Pr > F
CULTURE	1	1924.25042	1924.25042	3.68	0.0591
STORAGE	1	653.08321	653.08321	1.25	0.2676
WEEK	14	51449.31783	3674.95127	7.02	0.0001

Appendix 6a Method for counting circulating eosinophils

This method was adapted from that described by Dawkins *et al.*, 1989. The only modification was to dilute whole blood 1:5 in 'Carpentiers Eosinophil Counting Solution' instead of 1:10 as described in the above reference. This modification was made to make the test more sensitive.

'Carpentiers Eosinophil Counting Solution':

- 1 ml of 2% aqueous Eosin Y[27]
- 1.5 ml of $CaCO_3$ saturated 40% formaldehyde.

Mix and make up to 50 ml with distilled water. Make up this solution just prior to use.

METHOD:

1. Collect blood in vacutainer tubes with sodium heparin added, to obtain whole blood
2. Add 800 µl of 'Carpentiers Eosinophil Counting Solution' to the required number of small tubes
3. Mix each blood sample well, take out a 200 µl subsample and pipette into the tubes containing 'Carpentiers Eosinophil Counting Solution'. Mix well using the pipette.
4. Leave for 30 minutes at room temperature to stain the eosinophils. Eosinophils stain orange-red
5. Use an 'Improved Neubauer Haemocytometer'[28] to count eosinophils. Count 4 large squares (area of each = 1 mm^2) each consisting of 16 small squares.
6. Volume counted = (4 x 1 mm^2) x 0.1 mm = 0.4 mm^3 = 0.4 x 10^{-3} ml
   Dilution factor = 5 (whole blood diluted 1:5 in 'Carpentiers Eosinophil Counting Solution')
7. As 1 cell counted = 1 cell per 0.4 x 10^{-3} ml = 2.5 cells per 0.001 ml = 2500 cells per ml and as dilution factor is 5, it follows that:
   1 cell counted = 5 x 2500 cells per ml = **12500 cells/ml** or **1.25 x 10^4 cells/ml**

[27] Sigma Chemical Co.., St. Louis, USA; C.I. 45380; Acid Red 87; $C_{20}H_6Br_4O_5Na_2$, FW 691.9, E-6003, Lot 75H2505

[28] 'Hausser Hy-Lite Ultraplane' and 'Weber England, B.S. 748'; 0.1 mm deep, 1/400 sq. mm

Appendix 6b Necropsy procedure

The method described below is that generally used for diagnostic worm counts in the Institute of Veterinary, Animal and Biomedical Sciences, Massey University. The procedure for taking out sections for histological examination is that previously used by Pomroy (1994).

METHOD:

1. Euthanase animals by injecting them intravenously with an overdose of sodium pentobarbitone
2. With animal lying on its left side, open up abdomen just ventral to the costae
3. Locate abomasum, place string ligatures at either end, and remove from carcass. Clean off as much of the mesentery as possible. Put abomasum in a labelled plastic bag.
4. Locate pylorus and place a string ligature just distal to this. Take out both the small and large intestines and place them on a plastic tray. Dissect the first 1.5 m free of the mesentery and place in a labelled plastic bag.
5. Take out 2 sections (2-3 cm's width) for histology and staple each on to a 4x6 cm piece of thin cardboard, with the mucosal surface upwards. Float one section in Neutral-buffered 10% Formalin (FA) and the other in Acetic acid-Formalin (IFAA) with the tissue facing downwards in the fixative. Transfer sections from IFAA to 70% Ethanol after 12 hours.
6. Dissect free the next 1.5 m of small intestine and place in labelled plastic bag. Again take out sections for histology and process as in 5..
7. Strip the mesentery of the rest of the small intestine until the ileo-caecal junction is reached. Place another ligature here, take out small intestine and put in labelled plastic bag.
8. Store recovered organs in freezer at –20 °C until further processing.
9. Cut out blocks from the histology sections, parallel to the length of the small intestine, and embed in paraffin wax.
10. Cut slices of three µm thickness and stain those fixed in FA with H&E (Appendix 6f) and Luna's method (Appendix 6h) and those fixed in IFAA with Toluidine Blue (Appendix 6g).

Appendix 6c Worm counting procedure – Small intestine

The method described is according to the one used at the Institute of Veterinary, Animal and Biomedical Sciences, Massey University.

METHOD:

1. Take small intestine out of freezer and thaw overnight.
2. Open the small intestine along its length using a pair of scissors with blunt ends.
3. Pull the opened intestine between the fingers to scrape the contents off the mucosa into a 10-litre bucket, under a trickle of water. The washed intestine is then digested according to the technique described in Appendix 6d.
4. Make up the contents of the bucket to 4 litres with water.
5. Mix the contents by cross-stirring vigorously. At the same time take out sub-samples of 400 ml (= 10% of the total volume). Transfer the two aliquots to jars for storage.
6. Add 10% Neutral-buffered formalin to one aliquot to obtain a final formalin concentration of 5%. This aliquot serves as a reserve.
7. The other 10% (400 ml) aliquot is poured from the jar into a 53µm-aperture large sieve and washed gently until the water runs clear. Material retained in the sieve is then counted.
8. Count worms in the entire volume of the sieved sample using a dissecting microscope.
9. Identify and count adult females and males, immature females and males and younger larval stages (4^{th} and 3^{rd} stage larvae).
10. Collect a minimum of 20 adult female worms and 20 adult male worms. Formalinise if necessary for later examination.
11. Multiply the number of worms counted (including L4s and L3s) by 10 to obtain the total worm burden.

Appendix 6d Pepsin digest technique

This method is used after the small intestine or abomasum has been washed and ensures that all adult worms and younger larval stages are removed from the mucosal surface and included in the total worm count.

The following mixture is used for the digestion of either one small intestine or one abomasum:

- 20 g of pepsin (70 FIP-U/g ; Riedel-de Haën, Seelze, Germany)
- 600 ml of distilled water
- 10 ml of concentrated HCl

METHOD:

1. Pour mixture into a large glass beaker containing either one small intestine or one abomasum
2. Incubate for 2 hours in a waterbath at 37°C.
3. After incubation pour digest fluid into a 10-liter plastic bucket, wash the small intestine or abomasum thoroughly under a trickle of water and make up contents to 4 liters.
4. Remove two 10% aliquots for counting. Formalinise one of these as a reserve sample, and count the other. Add the worm count from the digest fluid to that of the washings (Appendix 6c), to obtain a total worm count.

Appendix 6e Measuring adult worm length and counting eggs *in utero*

METHOD:

1. Place either adult female or male worms in a drop of lactophenol (see below), allow to clear and examine under a compound microscope using a x1 objective.
2. Measure the worm length using the 'Sigma Scan™' software (see Appendix 5c).
3. Measure 20 adult females and 20 adult males and calculate the mean length.
4. To count eggs *in-utero* of examine 20 adult female worms under a compound microscope using the x10 objective
5. Calculate mean value.

Lactophenol

Mix together:	pure phenol crystals	10 g
	Lactic acid	10 g
	Glycerol	20 g
	Distilled water	10 ml

Store in brown glass bottle or in dark place.

Appendix 6f Histology – Gill's haematoxylin and eosin (H&E)

This method was adapted from Gill *et al.* (1974).

Gill's haematoxylin:

Haematoxylin	4 g
Distilled water	700 ml
Ethylene glycol	250 ml
Sodium iodate	0.6 g
Aluminium sulphate	36 g
Acetic acid	50 ml

Mix in order given. Add acetic acid after all solids have dissolved. Maintain acid content by adding 1 drop of acetic acid per 100 ml of stain weekly (change monthly). Requires no differentiation. Can be used at once.

Alcoholic eosin

1% aqueous eosin (GI 45380)	100 ml
1% aqueous phloxine	10 ml
95% ethanol	880 ml
acetic acid	5 ml

Change this solution weekly.

METHOD:

1. Take to water
2. Gill's haematoylin for 3 minutes
3. Running water for 30 seconds
4. Scott's tap water for 30 seconds
5. Running water for 1 minute
6. Alcoholic eosin for 1 to 2 minutes
7. Running water for 30 seconds
8. Dehydrate briskly through alcohols without pause
9. Clear and mount

Appendix 6g Histology – Toluidine Blue (TB)

This method was adapted from Strobel *et al.* (1981).

Toluidine blue stain

toluidine blue (Gurr 29800)	0.5 g
0.5N hydrochloric acid	100 ml

Dissolve the toluidine blue in the hydrochloric acid.

METHOD:

1. Take to water
2. Stain in toluidine blue for 45 minutes
3. Wash in tap water for 7.5 minutes
4. Differentiate in 95% ethanol
5. Dehydrate quickly through alcohols
6. Clear and mount

Appendix 6h Histology – Luna's method for eosinophils

This method was adapted from Luna (1968).

STAINING REAGENTS

Weigert's haematoxylin:

solution A	haematoxylin	1 g
	95% ethanol	100 ml
solution B	ferric chloride, 29% aqueous	4 ml
	distilled water	95 ml
	concentrated HCl	1 ml

Add equal parts of solution A and B.

Biebrich's scarlet:

	Biebrich's scarlet	1 g
	distilled water	100 ml

Acid alcohol:

1% concentrated HCl in 70% ethanol

0.5% Lithium carbonate:

	lithium carbonate	0.5 g
	distilled water	100 ml

METHOD:

1. Mix 22.5 ml each of Weigert's haematoxylin solutions A and B and add 5 ml of Biebrich's scarlet
2. Stain sections for 5 minutes
3. Dip sections 10 times in acid alcohol to differentiate
4. Rinse in tap water to remove acid alcohol
5. Dip sections 7 times in lithium carbonate
6. Wash in running water for 2 minutes
7. Dehydrate through alcohols and mount

Appendix 6i Culturing larvae for infection doses

Larvae to be used for either trickle or challenge infection doses of *Trichostrongylus colubriformis* were cultured as follows (Massey Parasitology lab, pers.comm.):

1. Collected faeces is emptied into large plastic tray (approximately 30 x 50 cm)
2. Soak faeces in distilled water until softened
3. Mash faeces using plastic potato masher until consistency is even
4. Add vermiculite and mix well. Consistency should be moist but not wet or waterlogged.
5. Incubate faeces at 25 °C for 10 days, adding more water when necessary
6. Mix culture every second day to allow even access of oxygen
7. Recover larvae in large Baermann funnels as described in Appendix 2c

Appendix 6j Data from Chapter 6

Experiment 1

Trt 1 = steroid-treated group ; Trt 2 = non-steroid-treated group

FEC, circulating eosinophils and IgG1 levels

Week	Tag	Trt	FEC 1	FEC 2	Mean	Eos	TcL3	TcAd
0	1	1	0	0	0	2.5	0.313	0.3014
0	3	1	0	0	0	6.25	0.2305	0.2212
0	5	1	0	0	0	2.5	0.3081	0.5603
0	8	1	0	0	0	2.5	0.2413	0.3408
0	11	1	0	0	0	1.25	0.3598	0.3384
0	12	1	0	0	0	6.25	0.3724	0.5147
0	13	1	0	0	0	2.5	0.5609	0.3578
0	15	1	0	0	0	2.5	0.4081	0.3533
0	18	1	0	0	0	3.75	0.591	0.5209
0	2	2	0	0	0	10	0.7861	0.6806
0	4	2	0	0	0	5	0.7582	0.9336
0	6	2	0	0	0	17.5	0.5872	0.7131
0	7	2	0	0	0	12.5	0.5875	0.7663
0	9	2	0	0	0	6.25	0.9662	1.0383
0	10	2	0	0	0	7.5	0.7942	0.8237
0	14	2	0	0	0	23.75	0.6942	0.5005
0	16	2	0	0	0	2.5	0.4469	0.45
0	17	2	0	0	0	3.75	0.5494	0.589
1	1	1			.	2.5	0.2354	0.323
1	3	1			.	1.25	0.2369	0.2000
1	5	1			.	1.25	0.278	0.482
1	8	1			.	1.25	0.2834	0.2725
1	11	1			.	2.5	0.38	0.3055
1	12	1			.	2.5	0.3936	0.4607
1	13	1			.	1.25	0.4907	0.3003
1	15	1			.	1.25	0.47	0.382
1	18	1			.	6.25	0.6004	0.4023
1	2	2			.	5	0.9662	1.0853
1	4	2			.	1.25	0.7703	0.9345
1	6	2			.	3.75	0.5913	0.6853
1	7	2			.	1.25	0.5566	0.6367
1	9	2			.	1.25	0.9401	0.9407
1	10	2			.	7.5	0.8163	0.645
1	14	2			.	6.25	0.8155	0.5187
1	16	2			.	5	0.5454	0.4987
1	17	2			.	3.75	0.6607	0.65
2	1	1	0	0	0	7.5	.	.
2	3	1	0	0	0	1.25	.	.
2	5	1	0	0	0	1.25	.	.
2	8	1	0	0	0	7.5	.	.
2	11	1	0	0	0	13.75	.	.
2	12	1	0	0	0	1.25	.	.
2	13	1	0	0	0	1.25	.	.
2	15	1	0	0	0	2.5	.	.
2	18	1	0	0	0	1.25	.	.
2	2	2	0	0	0	5	.	.
2	4	2	0	0	0	2.5	.	.
2	6	2	0	0	0	8.75	.	.
2	7	2	0	0	0	11.25	.	.
2	9	2	0	0	0	1.25	.	.
2	10	2	0	0	0	3.75	.	.
2	14	2	0	0	0	8.75	.	.
2	16	2	0	0	0	2.5	.	.
2	17	2	0	0	0	1.25	.	.

FEC, circulating eosinophils and IgG1 levels								
Week	Tag	Trt	FEC 1	FEC 2	Mean	Eos	TcL3	TcAd
3	1	1	500	400	450	11.25	0.292	0.2593
3	3	1	400	350	375	5	0.2573	0.2177
3	5	1	400	350	375	2.5	0.2132	0.4148
3	8	1	300	550	425	7.5	0.2769	0.3303
3	11	1	500	350	425	10	0.2707	0.304
3	12	1	650	250	450	1.25	0.2614	0.3831
3	13	1	400	150	275	2.5	0.3898	0.2736
3	15	1	150	150	150	3.75	0.3386	0.315
3	18	1	150	150	150	1.25	0.4452	0.329
3	2	2	450	500	475	8.75	0.9948	1.0315
3	4	2	50	150	100	6.25	0.8903	0.9383
3	6	2	400	150	275	15	0.5944	0.7037
3	7	2	200	450	325	5	0.4819	0.5643
3	9	2	100	350	225	3.75	0.871	0.8492
3	10	2	100	650	375	7.5	0.7318	0.65
3	14	2	150	350	250	8.75	0.8128	0.5659
3	16	2	150	400	275	2.5	0.5445	0.4601
3	17	2	550	300	425	6.25	0.5895	0.6043
4	1	1	700	600	650	2.5	0.2354	0.2523
4	3	1	700	550	625	6.25	0.188	0.2039
4	5	1	700	650	675	1.25	0.2022	0.3914
4	8	1	950	750	850	1.25	0.3	0.3466
4	11	1	1000	900	950	5	0.2904	0.2475
4	12	1	450	750	600	1.25	0.285	0.3783
4	13	1	600	700	650	1.25	0.3862	0.2776
4	15	1	150	300	225	3.75	0.3118	0.282
4	18	1	200	250	225	1.25	0.4878	0.3762
4	2	2	350	400	375	5	0.9319	0.9595
4	4	2	350	400	375	1.25	0.88	0.8676
4	6	2	400	600	500	7.5	0.5615	0.6961
4	7	2	500	250	375	3.75	0.5418	0.5958
4	9	2	500	250	375	5	0.9258	0.8563
4	10	2	600	300	450	3.75	0.7395	0.6804
4	14	2	100	200	150	8.75	0.8275	0.6413
4	16	2	450	450	450	3.75	0.6004	0.4735
4	17	2	350	500	425	3.75	0.5993	0.6025
5	1	1	450	650	550	3.75	0.2414	0.245
5	3	1	1400	1700	1550	5	0.1902	0.1812
5	5	1	500	450	475	1.25	0.2121	0.3339
5	8	1	400	600	500	2.5	0.2196	0.2616
5	11	1	350	450	400	1.25	0.2072	0.2391
5	12	1	750	650	700	1.25	0.2359	0.3662
5	13	1	700	950	825	1.25	0.3064	0.2896
5	15	1	400	450	425	1.25	0.2708	0.295
5	18	1	500	450	475	2.5	0.3271	0.2991
5	2	2	200	250	225	12.5	0.9602	0.9903
5	4	2	400	650	525	1.25	0.9226	0.8532
5	6	2	300	150	225	8.75	0.5461	0.6124
5	7	2	100	200	150	11.25	0.4345	0.5161
5	9	2	950	550	750	2.5	0.824	0.8532
5	10	2	400	400	400	13.75	0.6136	0.6063
5	14	2	300	150	275	15	0.7815	0.6356
5	16	2	1000	550	775	3.75	0.6488	0.4931
5	17	2	1200	700	950	7.5	0.4882	0.5548

FEC, circulating eosinophils and IgG1 levels								
Week	Tag	Trt	FEC 1	FEC 2	Mean	Eos	TcL3	TcAd
6	1	1	1300	1050	1175	3.75	0.1977	0.3069
6	3	1	350	750	550	2.5	0.1508	0.1823
6	5	1	1200	900	1050	2.5	0.2879	0.2943
6	8	1	1150	950	1050	2.5	0.1916	0.2439
6	11	1	900	750	825	5	0.2012	0.2373
6	12	1	950	850	900	2.5	0.2447	0.3432
6	13	1	950	1400	1175	1.25	0.3091	0.2701
6	15	1	800	1000	900	1.25	0.6258	0.3357
6	18	1	700	1050	875	1.25	0.2905	0.2528
6	2	2	550	550	550	15	0.8974	1.0077
6	4	2	450	600	525	6.25	1.0061	0.9236
6	6	2	600	600	600	25	0.5209	0.6794
6	7	2	800	300	550	10	0.5147	0.5304
6	9	2	700	500	600	3.75	0.8327	0.7794
6	10	2	650	800	725	7.5	0.6001	0.5433
6	14	2	750	450	600	32.5	0.9449	0.6629
6	16	2	1200	650	925	5	0.6627	0.1365
6	17	2	950	650	800	2.5	0.5279	0.5579
7	1	1	1200	1400	1300	1.25	0.2584	0.2808
7	3	1	800	900	850	1.25	0.172	0.1993
7	5	1	500	500	500	3.75	0.1885	0.2583
7	8	1	1050	1150	1100	3.75	0.2181	0.2506
7	11	1	1400	1500	1450	2.5	0.2243	0.2148
7	12	1	500	700	600	1.25	0.2464	0.2885
7	13	1	1250	1350	1300	2.5	0.375	0.254
7	15	1	500	1000	750	2.5	0.226	0.2608
7	18	1	700	1000	850	1.25	0.228	0.3411
7	2	2	500	400	550	18.75	0.8615	0.9514
7	4	2	1100	1300	1200	18.75	0.9456	0.8468
7	6	2	850	850	850	11.25	0.521	0.6732
7	7	2	1000	800	900	7.5	0.4838	0.5763
7	9	2	500	500	500	10	0.8708	0.8026
7	10	2	950	1050	1000	8.75	0.6523	0.5678
7	14	2	1000	900	950	22.5	0.841	0.6398
7	16	2	500	700	600	1.25	0.5807	0.5592
7	17	2	600	700	650	6.25	0.578	0.5843
8	1	1	950	1150	1050	1.25	0.1633	0.2554
8	3	1	1750	1200	1475	1.25	0.1327	0.1991
8	5	1	950	1350	1150	2.5	0.1837	0.2536
8	8	1	1250	1550	1400	1.25	0.1857	0.236
8	11	1	1500	1200	1350	3.75	0.158	0.2149
8	12	1	1050	1250	1150	1.25	0.1853	0.2845
8	13	1	650	800	725	1.25	0.2217	0.2468
8	15	1	550	650	600	1.25	0.2175	0.2183
8	18	1	900	950	925	3.75	0.2856	0.3297
8	2	2	700	600	650	11.25	0.744	0.9065
8	4	2	400	500	450	27.5	0.9677	0.8667
8	6	2	450	450	450	8.75	0.4873	0.6547
8	7	2	500	500	500	15	0.45	0.5287
8	9	2	750	700	725	7.5	0.801	0.8014
8	10	2	750	600	675	12.5	0.5693	0.4943
8	14	2	250	150	200	42.5	0.863	0.6525
8	16	2	650	450	550	5	0.6804	0.5231
8	17	2	400	700	550	8.75	0.6842	0.5595
9	1	1	0	0	0	1.25	0.2053	0.2662
9	3	1	0	0	0	1.25	0.1889	0.1771
9	5	1	0	0	0	1.25	0.3268	0.2781
9	8	1	0	0	0	1.25	0.2236	0.2341
9	11	1	0	0	0	1.25	0.194	0.2053
9	12	1	0	0	0	1.25	0.243	0.2857
9	13	1	0	0	0	1.25	0.2546	0.2385
9	15	1	0	0	0	1.25	0.2417	0.2219
9	18	1	0	0	0	1.25	0.1967	0.3049
9	2	2	0	0	0	11.25	0.7679	0.8939
9	4	2	0	0	0	8.75	0.991	0.7987
9	6	2	0	0	0	8.75	0.4912	0.6685
9	7	2	0	0	0	11.25	0.6113	0.5754
9	9	2	0	0	0	10	0.8782	0.79
9	10	2	0	0	0	12.5	0.5559	0.4885
9	14	2	0	0	0	72.5	0.9344	0.6732
9	16	2	0	0	0	5	0.7015	0.5534
9	17	2	0	0	0	2.5	0.5999	0.5487

FEC, circulating eosinophils and IgG1 levels								
Week	Tag	Trt	FEC 1	FEC 2	Mean	Eos	TcL3	TcAd
10	1	1			.	3.75	0.1837	0.2585
10	3	1			.	1.25	0.1329	0.1807
10	5	1			.	1.25	0.1504	0.2158
10	8	1			.	1.25	0.1899	0.2223
10	11	1			.	1.25	0.1658	0.1972
10	12	1			.	2.5	0.1878	0.2424
10	13	1			.	1.25	0.1813	0.234
10	15	1			.	1.25	0.2036	0.1873
10	18	1			.	1.25	0.1813	0.2494
10	2	2			.	25	0.8189	0.7988
10	4	2			.	8.75	0.9319	0.6889
10	6	2			.	8.75	0.5944	0.5958
10	7	2			.	11.25	0.6843	0.4848
10	9	2			.	28.75	0.9137	0.7594
10	10	2			.	13.75	0.6095	0.5068
10	14	2			.	30	0.8931	0.5889
10	16	2			.	8.75	0.6823	0.4783
10	17	2			.	15	0.7005	0.4819
11	1	1			.	1.25	0.1666	0.1987
11	3	1			.	1.25	0.1555	0.1697
11	5	1			.	1.25	0.161	0.1726
11	8	1			.	1.25	0.2616	0.2119
11	11	1			.	1.25	0.1574	0.1883
11	12	1			.	1.25	0.1804	0.2412
11	13	1			.	1.25	0.1783	0.1876
11	15	1			.	1.25	0.207	0.1794
11	18	1			.	1.25	0.213	0.2122
11	2	2			.	26.25	0.9733	0.8139
11	4	2			.	8.75	0.7599	0.348
11	6	2			.	18.75	0.8151	0.5256
11	7	2			.	12.5	0.8604	0.4573
11	9	2			.	17.5	0.981	0.6542
11	10	2			.	12.5	0.7967	0.4402
11	14	2			.	15	0.8901	0.5299
11	16	2			.	11.25	0.7766	0.4095
11	17	2			.	11.25	0.7942	0.3945
12	1	1	400		400	1.25	0.1964	0.1758
12	3	1	350		350	1.25	0.1334	0.1407
12	5	1	50		50	1.25	0.1596	0.1581
12	8	1	350		350	1.25	0.2113	0.2151
12	11	1	300		300	1.25	0.1495	0.1678
12	12	1	400		400	1.25	0.1606	0.1996
12	13	1	450		450	1.25	0.1582	0.1667
12	15	1	0		0	1.25	0.1509	0.1632
12	18	1	300		300	1.25	0.1556	0.1744
12	2	2	100		100	6.25	0.9329	0.7921
12	4	2	0		0	6.25	0.9861	0.6624
12	6	2	100		100	20	0.7479	0.5286
12	7	2	150		150	6.25	0.7711	0.4697
12	9	2	0		0	7.5	0.9874	0.7155
12	10	2	250		250	5	0.6686	0.4174
12	14	2	0		0	13.75	0.8409	0.5252
12	16	2	50		50	6.25	0.7045	0.3589
12	17	2	200		200	5	0.7252	0.4019
13	1	1	750	750	750	1.25	0.1811	0.2081
13	3	1	900	550	725	1.25	0.1354	0.1442
13	5	1				0	.	.
13	8	1	1700	1400	1550	1.25	0.22069	0.2624
13	11	1	1150	500	825	1.25	0.22127	0.2085
13	12	1	850	1350	1100	1.25	0.18463	0.2407
13	13	1	900	1100	1000	1.25	0.20684	0.2159
13	15	1	900	750	825	2.5	0.15188	0.1825
13	18	1	550	600	575	1.25	0.17368	0.2678
13	2	2	300	350	325	1.25	0.8789	0.8329
13	4	2	100	0	50	23.75	1.0286	0.7441
13	6	2	250	500	375	8.75	0.62828	0.5531
13	7	2	100	150	125	21.25	0.81363	0.6689
13	9	2	0		0	8.75	0.82665	0.722
13	10	2	650	450	550	1.25	0.59206	0.443
13	14	2	150	200	175	18.75	0.80485	0.6352
13	16	2	450	750	600	3.75	0.63657	0.4666
13	17	2	600	350	475	1.25	0.6747	0.4814

Developmental Success in faecal cultures									
Trt 1= steroid-treated group					Trt 2= Non-steroid-treated group				
Tag	Trt	Culture	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 13
1	1	1	45.1	53.5	27.0	71.1	44.9	86.4	53.1
1	1	2	55.6	63.3	21.8	52.2	59.6	102.3	79.5
3	1	1	89.0	48.9	37.3	78.7	90.5	50.6	23.8
3	1	2	113.8	58.9	34.7	74.8	109.1	65.8	27.9
5	1	1	49.1	58.3	27.0	122.6	70.4	102.3	
5	1	2	57.0	46.8	42.5	149.4	48.5	96.9	
8	1	1	82.5	37.7	64.8	43.0	110.0	122.7	45.6
8	1	2	56.5	28.2	66.1	80.1	100.0	65.7	45.4
11	1	1	81.5	43.2	80.7	40.5	89.4	111.1	49.4
11	1	2	72.9	34.4	108.9	42.8	93.8	99.6	88.8
12	1	1	25.1	64.4	59.1	36.9	59.5	60.7	43.5
12	1	2	32.0	97.2	43.1	29.3	100.7	75.1	74.9
13	1	1	157.9	47.3	50.5	81.7	64.1	148.4	54.7
13	1	2	145.8	83.3	48.0	69.1	61.5	103.4	53.8
15	1	1	53.7	103.6	61.7	34.1	74.1	96.3	63.9
15	1	2	51.4	134.2	47.5	37.7	85.7	120.4	94.8
18	1	1	111.6	166.8	36.0	34.3	63.2	108.1	32.8
18	1	2	80.3	85.5	25.7	51.4	50.2	82.3	50.7
		Mean	75.6	69.7	49.0	62.8	76.4	94.3	55.2
2	2	1	69.4	68.3	161.2	121.3	81.3	110.5	65.6
2	2	2	74.2	59.3	107.6	79.9	99.0	79.3	65.0
4	2	1	149.0	118.7	91.1	91.3	48.2	69.9	146.8
4	2	2	154.2	137.3	53.1	57.1	35.9	69.8	149.5
6	2	1	104.6	99.4	105.1	90.2	63.8	71.6	68.3
6	2	2	83.5	101.2	84.5	58.3	59.6	119.1	79.2
7	2	1	48.8	23.0	52.8	59.5	70.0	37.4	17.6
7	2	2	53.8	11.3	55.1	74.5	85.2	35.3	13.7
9	2	1	115.7	79.7	28.0	63.4	93.9	37.1	43.8
9	2	2	81.2	64.2	22.3	78.6	60.7	28.4	69.9
10	2	1	59.4	115.5	88.1	39.5	74.7	90.9	22.9
10	2	2	48.1	67.1	86.1	19.5	67.1	88.3	30.5
14	2	1	42.4	130.7	37.4	24.3	49.9	41.5	81.0
14	2	2	49.5	172.2	38.4	32.4	25.3	106.7	64.8
16	2	1	190.4	107.6	110.2	32.2	83.5	115.3	18.5
16	2	2	176.2	125.9	71.9	23.9	78.4	82.5	52.7
17	2	1	49.1	53.7	29.9	46.3	67.6	77.7	18.5
17	2	2	56.4	57.7	30.5	9.7	54.2	93.7	52.7
		Mean	89.2	88.5	69.6	55.7	66.6	75.3	59.0

Worm counts, Male/Female-ratios, Worm lengths, *In utero* egg counts and histology															
Trt 1= steroid-treated group						Trt 2 = Non-steroid-treated group									
		Worms	Worms	Worm burden		Male/Female	Worm lengths		In utero egg counts	Histology					
TRT	Tag	*female*	*male*	W.B.	Establ.	M/F ratio	FWL	MWL	IUE	MMC 1	GL 1	EOS 1	MMC 2	GL 2	EOS 2
1	1	8170	2000	10170	50.85	0.24	7121	4313	23.6	62	0	91	104	0	122
1	3	8120	363	11800	59	0.04	7159	4381	22.2	79	0	133	66	0	195
1	5	6240	3780	10090	50.45	0.61	7460	5008	23.3	49	0	93	68	0	178
1	8	8450	3380	11880	59.4	0.40	7429	4740	27.2	72	0	130	72	0	225
1	11	8140	4460	12540	62.7	0.55	7418	4427	22.25	75	0	109	72	0	146
1	12	7210	2140	9350	46.75	0.30	7322	4788	26.45	65	0	70	96	0	157
1	13	7860	5200	13060	65.3	0.66	7722	4897	25.25	45	0	77	43	0	122
1	15	6150	1980	8150	40.75	0.32	7460	4544	22.2	53	0	86	66	0	120
1	18	6710	2720	9460	47.3	0.41	7409	5050	22.75	19	0	44	14	0	38
	Mean	7450	2891	10722	53.6	0.39	7389	4683	24	58	0	92	67	0	145
2	2	5050	1670	6720	33.6	0.33	5711	4432	16.2	193	0	55	115	0	54
2	4	3940	1270	5220	26.1	0.32	5488	4279	8.35	243	38	228	245	3	388
2	6	4090	1170	5270	26.35	0.29	5654	3854	15.55	142	14	267	107	3	324
2	7	2900	1680	4590	22.95	0.58	5793	4902	8.35	97	5	199	135	8	285
2	9	1100	270	1390	6.95	0.25	5384	3841	9.9	246	89	208	164	20	411
2	10	4080	700	4790	23.95	0.17	6598	4084	18.05	130	1	107	126	2	84
2	14	2800	490	3290	16.45	0.18	5635	3610	10.95	143	48	177	123	5	215
2	16	4440	1130	5570	27.85	0.25	5790	3912	15.4	209	3	44	154	3	103
2	17	5660	1890	7550	37.75	0.33	6580	4609	18	216	1	67	156	0	80
	Mean	3784	1141	4932	24.7	0.30	5848	4169	13	180	22	150	147	5	216

Female and male worm lengths and *in utero* egg counts																										
Trt 1 = Steroid-treated group																										
Tag																										
1	1	1	3	3	3	5	5	5	8	8	8	11	11	11	12	12	12	13	13	13	15	15	15	18	18	18
FWL	MWL	IUE	FWL	MWL	IUE	FWL	MWL	IUE	FWL	MWL	IUE	FWL	MWL	IUE	FWL	MWL	IUE	FWL	MWL	IUE	FWL	MWL	IUE	FWL	MWL	IUE
7382	4621	30	6617	3776	26	8120	4290	27	6836	5509	26	6709	4313	22	7096	4506	27	7465	4956	23	7790	4706	18	6709	4766	21
7535	3915	24	7177	4232	21	7898	4774	25	7149	5012	31	6997	4164	16	6773	4373	25	7435	4718	20	7334	4446	23	7081	5168	20
7338	4918	25	7101	3987	23	7557	5408	23	7160	4766	27	7065	4658	18	7023	5477	26	7606	5020	23	7281	4566	23	7779	6895	25
7085	4390	21	7358	4388	25	7410	5453	27	7140	4375	22	6985	4497	18	7343	5080	28	8053	5171	34	7478	4425	20	6555	5049	17
7180	4609	21	6508	4389	23	7340	4090	25	7740	4988	38	7467	4592	27	7512	4729	31	7985	4452	25	6881	3946	21	7277	4246	21
7870	4084	28	7204	3912	19	7169	5479	26	7485	5036	35	7229	4271	21	7290	4260	29	8029	4790	24	7773	4145	23	7657	5362	26
7101	4083	15	7274	4095	23	7533	5328	29	8403	5431	30	7946	3974	20	7644	4799	30	7284	4665	23	7377	4281	23	7836	5107	22
6307	4270	20	7036	4185	20	7057	4731	18	7707	4515	29	7496	4500	21	7399	5701	24	8283	4782	25	7747	5035	24	8038	4878	28
7161	3832	24	6248	3954	22	7322	4772	20	7032	4605	18	7792	4814	23	7334	5511	27	7593	5025	20	7013	3905	22	6463	4828	16
7264	4289	25	7548	3890	23	7268	4480	26	7290	4694	24	7798	4409	22	7776	5199	30	8085	4486	20	7711	4477	27	8029	5061	23
7113	4418	19	7186	4822	24	8537	4918	27	7847	4301	33	7425	4549	22	6419	4972	19	7965	5261	32	7434	4765	22	7396	4868	20
6712	4195	20	7068	4257	22	7178	5237	25	7147	4300	22	7182	4390	19	7896	4824	28	7863	5724	22	7577	4678	21	7661	4923	27
7347	4910	25	7129	4807	18	7427	4754	22	7528	4959	26	7956	4233	31	7975	5055	31	7527	5203	24	7571	5486	20	7237	5160	22
7040	4212	26	7188	4880	20	7296	5190	20	7681	4562	24	7370	4230	20	6879	4504	27	7801	4272	30	7267	4868	27	6846	5201	15
6773	3799	23	6919	4843	20	6953	4697	22	7377	4936	27	7490	4162	23	7146	4329	22	6979	5171	18	7772	3872	23	8450	4800	29
6912	4412	25	7736	4533	28	7848	5577	21	7172	4694	21	7317	4423	24	7524	4860	21	8021	4927	38	7460	4690	23	7787	4448	21
6883	3911	27	7688	5462	25	6721	5917	20	6943	4301	30	7145	4717	16	7511	4271	33	7557	5020	29	8209	5131	27	6853	4763	25
7186	4763	28	7433	4172	22	7687	4933	22	8030	4300	30	7646	4334	27	7038	4357	22	7243	4939	22	7286	4238	15	7538	4407	29
6981	4697	23	7917	4357	23	7479	5186	21	7532	4959	25	7641	4809	26	6667	4264	32	7927	4782	29	6895	4631	21	7277	4576	24
7244	3934	23	6837	4686	17	7408	4949	20	7390	4562	26	7697	4502	29	8193	4697	17	7732	4580	24	7350	4595	21	7718	6503	24

Female and male worm lengths and *in utero* egg counts																										
Trt 2 = Non-steroid-treated group																										
Tag																										
2	2	2	4	4	4	6	6	6	7	7	7	9	9	9	10	10	10	14	14	14	16	16	16	17	17	17
FWL	MWL	IUE	FWL	MWL	IUE	FWL	MWL	IUE	FWL	MWL	IUE	FWL	MWL	IUE	FWL	MWL	IUE	FWL	MWL	IUE	FWL	MWL	IUE	FWL	MWL	IUE
5382	4589	17	5950	4022	11	5893	3588	15	6321	5136	10	5215	3449	9	6558	4594	16	6211	3933	13	6492	3965	14	6486	4627	12
5385	4985	14	5168	4149	12	5699	3777	20	5678	5640	4	5011	4291	5	6531	4830	21	5365	3361	6	6118	4017	17	6694	4364	21
5775	3431	16	5605	4948	7	5536	3838	14	5947	5432	7	5708	4646	11	7010	3800	23	6267	3882	14	5640	4006	19	6577	5570	16
5690	3345	11	5268	4596	9	5361	4523	14	6218	4715	11	5301	3326	7	6503	4134	18	6164	3941	15	6071	3426	18	6595	4080	21
5488	4899	14	5572	4987	7	5524	3484	16	5655	5207	6	5627	4225	13	6573	3971	14	5070	3466	12	5587	3420	15	6680	5712	17
6038	4832	19	5659	3668	10	5633	3552	15	5201	4455	6	5941	3724	11	6252	4265	16	5283	3236	10	5395	4282	16	8871	4525	19
5793	3916	16	5869	4430	8	4855	3669	12	5816	5243	10	5196	2757	13	6423	5128	15	6180	3667	14	6170	3589	14	6183	5478	18
5326	4709	20	5750	4012	11	5688	4447	13	6203	5003	9	5558	3988	10	6392	3997	21	5212	3531	12	6010	4904	16	6665	4902	19
5885	4996	12	5578	3083	12	6308	4825	18	5809	4973	7	4678	4189	7	6382	4018	20	5711	3406	9	6008	3785	14	7120	4266	22
5244	4732	15	5343	4114	8	5301	3949	14	5730	5485	8	5193	4549	10	6727	3512	16	5566	3340	8	5315	3215	14	6189	5043	17
5976	4587	14	5563	3935	5	5676	3387	17	5833	5454	5	5552	4868	10	6824	3723	19	5150	4123	9	5872	3982	13	6907	4238	22
4868	4348	16	5937	4963	12	5874	3319	17	5331	4572	7	5959	3938	10	6794	3857	20	6213	3770	16	5554	4007	13	6724	4895	22
5860	4088	14	5197	4821	6	5398	4426	18	6471	4766	12	5480	3458	9	6656	3586	20	5711	3103	9	6155	4320	17	6939	4173	16
5922	4558	19	4677	5223	8	5662	3670	14	5940	3809	10	6033	3523	13	6714	3702	15	5650	3858	13	6752	4402	19	6775	3658	15
5689	4108	15	6078	3977	9	5953	4152	17	6404	4817	10	5525	3569	8	6906	4135	18	5247	4211	10	2201	3828	13	6441	4377	18
5911	3446	23	5425	4038	7	5199	4006	16	5535	5220	8	5389	3348	10	5914	3576	19	5973	3157	9	6426	4206	15	6013	4158	18
5760	4963	14	5150	4592	5	6067	2946	16	4880	4475	10	4144	4263	7	6151	4934	17	5188	4238	10	6220	3466	17	6632	5014	15
5845	4620	18	5397	3949	8	6090	3529	17	5316	4851	8	4957	3495	7	7393	3923	18	5636	3433	10	6673	4405	16	6647	3926	20
5844	4557	15	5408	4066	8	5760	4542	16	5844	4194	11	5378	4042	14	6513	3905	19	5623	3339	10	5269	3294	12	6501	4747	22
6540	4926	22	5166	4012	4	5593	3443	12	5723	4599	8	5831	3172	14	6749	4086	16	5273	3211	10	5869	3721	16	5952	4437	10

Mucosal mast cells (MMC), globule leukocytes (GL) and eosinophils (EOS)												
Trt 1 = Steroid-treated group							Trt 2 = Non-steroid-treated group					
Tag	Trt	Section	MMC	GL	EOS		Tag	Trt	Section	MMC	GL	EOS
1	1	1	62	0	91		2	2	1	193	0	55
1	1	2	104	0	122		2	2	2	115	0	54
3	1	1	79	0	133		4	2	1	243	38	228
3	1	2	66	0	195		4	2	2	245	3	388
5	1	1	49	0	93		6	2	1	142	14	267
5	1	2	68	0	178		6	2	2	107	3	324
8	1	1	72	0	130		7	2	1	97	5	199
8	1	2	72	0	225		7	2	2	135	8	285
11	1	1	75	0	109		9	2	1	246	89	208
11	1	2	72	0	146		9	2	2	164	20	411
12	1	1	65	0	70		10	2	1	130	1	107
12	1	2	96	0	157		10	2	2	126	2	84
13	1	1	45	0	77		14	2	1	143	48	177
13	1	2	43	0	122		14	2	2	123	5	215
15	1	1	53	0	86		16	2	1	209	3	44
15	1	2	66	0	120		16	2	2	154	3	103
18	1	1	19	0	44		17	2	1	216	1	67
18	1	2	14	0	38		17	2	2	156	0	80

Experiment 2

Infectivity of larvae from steroid-treated or non-steroid-treated group in experiment1					
Tag	Larvae from	Larvae from tag	Worm burden	Establ. %	M/F-ratio
18	Trt 1	1	8580	42.9	0.28
23	Trt 1	1	5480	27.4	0.11
26	Trt 1	1	9790	49.0	0.58
42	Trt 1	1	7700	38.5	0.21
9	Trt 1	8	9520	47.6	0.39
22	Trt 1	8	5430	27.2	0.12
29	Trt 1	8	9200	46.0	0.35
37	Trt 1	8	11550	57.8	0.47
10	Trt 1	11	9200	46.0	0.44
32	Trt 1	11	8870	44.4	0.32
39	Trt 1	11	8820	44.1	0.28
40	Trt 1	11	9090	45.5	0.44
1	Trt 1	12	8420	42.1	0.17
3	Trt 1	12	7750	38.8	0.21
35	Trt 1	12	7870	39.4	0.20
41	Trt 1	12	5590	28.0	0.17
12	Trt 1	18	9780	48.9	0.36
19	Trt 1	18	9800	49.0	0.34
20	Trt 1	18	9630	48.2	0.36
45	Trt 1	18	6840	34.2	0.10
4	Trt 2	2	8970	44.9	0.17
6	Trt 2	2	8150	40.8	0.15
24	Trt 2	2	8170	40.9	0.13
30	Trt 2	2	6080	30.4	0.23
5	Trt 2	6	7740	38.7	0.28
11	Trt 2	6	8580	42.9	0.15
14	Trt 2	6	11420	57.1	0.56
21	Trt 2	6	790	4.0	0.35
2	Trt 2	7	12420	62.1	0.30
8	Trt 2	7	7910	39.6	0.19
25	Trt 2	7	10930	54.7	0.20
44	Trt 2	7	4470	22.4	0.18
15	Trt 2	10	10210	51.1	0.42
28	Trt 2	10	9620	48.1	0.19
31	Trt 2	10	8460	42.3	0.26
34	Trt 2	10	10160	50.8	0.36
7	Trt 2	17	13420	67.1	0.60
16	Trt 2	17	7800	39.0	0.26
33	Trt 2	17	9220	46.1	0.41
43	Trt 2	17	7620	38.1	0.20

Appendix 6k Statistical Analysis – Chapter 6

Dependent Variable: FEC

Source	DF	Type III SS	F Value	Pr > F
TRT	1	1654595.9596	48.90	0.0001
WEEK	10	20266212.1212	59.90	0.0001
TAG(TRT)	16	1198573.2323	2.21	0.0066
TRT*WEEK	10	1393320.7071	4.12	0.0001

Tests of Hypotheses using the Type III MS for TAG(TRT) as an error term

Source	DF	Type III SS	F Value	Pr > F
TRT	1	1654595.95960	22.09	0.0002

WEEK=4

Source	DF	Type III SS	F Value	Pr > F
TRT	1	216701.388889	6.23	0.0238

WEEK=6

Source	DF	Type III SS	F Value	Pr > F
TRT	1	382812.500000	13.49	0.0021

WEEK=8

Source	DF	Type III SS	F Value	Pr > F
TRT	1	1430868.05556	24.94	0.0001

WEEK=12

Source	DF	Type III SS	F Value	Pr > F
TRT	1	170138.888889	10.23	0.0056

WEEK=13

Source	DF	Type III SS	F Value	Pr > F
TRT	1	568888.888889	8.06	0.0118

Dependent Variable: developmental success in faecal cultures

Source	DF	Type III SS	F Value	Pr > F
TRT	1	2017.2539709	2.04	0.1561
WEEK	6	14467.6800365	2.44	0.0299
TAG(TRT)	2	2279.9722511	1.15	0.3195
TRT*WEEK	6	5932.8594315	1.00	0.4292

Tests of Hypotheses using the Type III MS for TAG(TRT) as an error term

Source	DF	Type III SS	F Value	Pr > F
TRT	1	2017.25397087	1.77	0.3148

WEEK=8

Tests of Hypotheses using the Type III MS for TAG(TRT) as an error term

Source	DF	Type III SS	F Value	Pr > F
TRT	1	1635.59588272	3.13	0.0959

Dependent Variable: ln(eosinophils + 1)

Source	DF	Type III SS	F Value	Pr > F
TRT	1	73.41110598	365.12	0.0001
WEEK	13	8.33996291	3.19	0.0002
TAG(TRT)	16	20.55299822	6.39	0.0001
WEEK*TRT	13	17.91053529	6.85	0.0001

Tests of Hypotheses using the Type III MS for TAG(TRT) as an error term

Source	DF	Type III SS	F Value	Pr > F
TRT	1	73.41110598	57.15	0.0001

WEEK=0

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	2.9249930	2.9249930	10.60	0.0050

WEEK=4

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	1.0785112	1.0785112	5.31	0.0350

WEEK=5

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	4.2060997	4.2060997	13.28	0.0022

WEEK=6

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	5.4121751	5.4121751	15.48	0.0012

WEEK=7

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	6.8668409	6.8668409	22.65	0.0002

WEEK=8

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	11.289479	11.289479	45.80	0.0001

WEEK=9

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	11.857813	11.857813	35.34	0.0001

WEEK=10

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	14.939768	14.939768	96.86	0.0001

WEEK=11

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	16.385700	16.385700	340.95	0.0001

WEEK=12

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	8.1137140	8.1137140	88.67	0.0001

WEEK=13

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	6.6132982	6.6132982	11.67	0.0035

Dependent Variable: TCL3

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	13.967800	13.967800	2517.71	0.0001
WEEK	12	0.217443	0.018120	3.27	0.0003
TAG(TRT)	16	2.254419	0.140901	25.40	0.0001
WEEK*TRT	12	0.606579	0.050548	9.11	0.0001

Tests of Hypotheses using the Type III MS for TAG(TRT) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	13.967800	13.967800	99.13	0.0001

Dependent Variable: TCAD

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	8.6154057	8.6154057	2043.91	0.0001
WEEK	12	0.8521070	0.0710089	16.85	0.0001
TAG(TRT)	16	2.5738054	0.1608628	38.16	0.0001
WEEK*TRT	12	0.0600847	0.0050071	1.19	0.2942

Tests of Hypotheses using the Type III MS for TAG(TRT) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	8.6154057	8.6154057	53.56	0.0001

Dependent Variable: Mucosal Mast Cells

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	92416.000	92416.000	133.78	0.0001
SECTION	1	1248.444	1248.444	1.81	0.1965
TAG(TRT)	16	37257.556	2328.597	3.37	0.0087

Tests of Hypotheses using the Type III MS for TAG(TRT) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	92416.000	92416.000	39.69	0.0001

Dependent Variable: ln(Globule Leukocytes + 1)

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	27.770426	27.770426	81.96	0.0001
SECTION	1	1.375377	1.375377	4.06	0.0600
TAG(TRT)	16	23.846560	1.490410	4.40	0.0021

Tests of Hypotheses using the Type III MS for TAG(TRT) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	27.770426	27.770426	18.63	0.0005

Dependent variable: Tissue eosinophils

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	37377.78	37377.78	23.70	0.0001
SECTION	1	31329.00	31329.00	19.86	0.0003
TAG(TRT)	16	217559.78	13597.49	8.62	0.0001

Tests of Hypotheses using the Type III MS for TAG(TRT) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	37377.778	37377.778	2.75	0.1168

Section 1

Dependent Variable: Mucosal Mast Cells

Tests of Hypotheses using the Type III MS for TAG(TRT) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	67222.222	67222.222	41.98	0.0001

Dependent Variable: ln(Globule Leukocytes + 1)

Tests of Hypotheses using the Type III MS for TAG(TRT) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	20.753096	20.753096	16.01	0.0010

Dependent Variable: Tissue Eosinophils

Tests of Hypotheses using the Type III MS for TAG(TRT) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	14964.500	14964.500	3.88	0.0665

Section 2

Dependent Variable: Mucosal Mast Cells

Tests of Hypotheses using the Type III MS for TAG(TRT) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	29120.889	29120.889	23.95	0.0002

Dependent Variable: ln(Globule Leukocytes + 1)

Tests of Hypotheses using the Type III MS for TAG(TRT) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	8.3927070	8.3927070	17.92	0.0006

Dependent Variable: Tissue eosinophils

Tests of Hypotheses using the Type III MS for TAG(TRT) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	22826.722	22826.722	2.00	0.1760

Dependent variable: Worm burden

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	1	150858450	150858450	50.18	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	150858450	150858450	50.18	0.0001

Dependent variable: Establishment (%)

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	1	3771.4612	3771.4612	50.18	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	3771.4612	3771.4612	50.18	0.0001

Dependent variable: Male/Female-ratio

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	1	0.0382768	0.0382768	1.47	0.2432

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	0.0382768	0.0382768	1.47	0.2432

Dependent Variable: Female Worm Length

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	1	10685448	10685448	94.72	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	10685448	10685448	94.72	0.0001

Dependent Variable: Male Worm Length

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	1	1189318.9	1189318.9	9.43	0.0073

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	1189318.9	1189318.9	9.43	0.0073

Dependent variable: *in utero* egg counts

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	1	495.60014	495.60014	50.19	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	495.60014	495.60014	50.19	0.0001

Correlations

TRT = Non-steroid-treated group

Pearson Correlation Coefficients / Prob > |R| under Ho: Rho=0 / N = 9

	WORMS	GL1
WORMS	1.00000 0.0	-0.85028 0.0037
GL1	-0.85028 0.0037	1.00000 0.0

TRT = Non-steroid-treated group

	FWL	IUE
FWL	1.00000 0.0	0.72096 0.0284
IUE	0.72096 0.284	1.00000 0.0

EXPERIMENT 2

Dependent Variable: Worm burdens

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	260822	260822	0.05	0.8302
LARVAE(TRT)	8	27768890	3471111	0.62	0.7518

Tests of Hypotheses using the Type III MS for LARVAE(TRT) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	260822.50	260822.50	0.08	0.7909

Tests of Hypotheses using the Type III MS for REPS(TRT*LARVAE) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LARVAE(TRT)	8	27768890	3471111	0.62	0.7518

Dependent Variable: Male/Female-ratio

Tests of Hypotheses using the Type III MS for LARVAE(TRT) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	0.0026501	0.0026501	0.12	0.7427

Tests of Hypotheses using the Type III MS for REPS(LARVAE*TRT) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LARVAE(TRT)	8	0.1835006	0.0229376	1.40	0.2385

Appendix 7a The Larval Development Assay – without agar

LDA WITH CONTROL WELLS ONLY

An assay to test the development of eggs to 3rd stage larvae, when removed from the faecal environment, was also carried out. The procedure was as the one described for control wells in Appendix 5b, except that no agar was added. A concentration of approximately 50 (± 15) eggs per well was used.

Twenty wells were set up per animal sample. In each of these wells, the number of eggs, 1st, 2nd and 3rd stage larvae were counted and the percentage of 3rd stage larvae developed from the eggs was calculated.

Appendix 7b Post Mortem Procedure for collection of mucus

The procedure for recovering organs and sections for histology was as described in Appendix 6b, with the following modifications:

METHOD:

1. Dissect the duodenum free of the mesenterium until just distal of the bile duct entry, place string ligatures at either end of this section, remove and place in labelled plastic bag. Take out sections for histology and process as described in Appendix 6b.
2. Dissect free the next meter of small intestine for mucus recovery and store on ice until further processing. Take out sections for histology and process as described in Appendix 6b.
3. Dissect free the next meter of small intestine for mucus recovery and store on ice until further processing.

Appendix 7c Protocol for recovering and preparing intestinal mucus and contents

The procedure for collecting mucus was essentially that employed by Douch *et al.*, 1986, with some minor modifications (Modified method from Pomroy (1994) and Soe, pers.comm.).

Hank's balanced salt solution (Gibco BRL, Life Technologies) 1 liter of the medium had 0.35g of $NaHCO_3$ added to it before use. Keep this solution in fridge until use.
Contents = gut contents and gut flushing/washing (washed with Hanks' solution)
Mucus = gut mucosa surface scraping diluted with Hanks' solution

1. Carefully remove 2 x 100 cm small intestine as described in Appendix 7b. Store on ice until further processing.
2. Untie one end and empty contents into one 50-ml Falcon tube. Then pour 25 ml of Hanks' solution (kept at 4 °C) into one end of the intestine section using a small plastic funnel. Massage the length of intestine for one minute, flushing the fluid back and forth. Collect the flushing into the same tube as where the contents are.
3. Open the gut longitudinally and gently scrape the mucus off the mucosa and into a large glass petri dish, using the rounded edge of a plexi glass slide. Put the mucus scrapings in a 50-ml Falcon tube or smaller Falcon 10-ml centrifuge tube to measure volume. Dilute the mucus scrapings 1:1 with Hanks' solution.
4. Vortex this mixture for 1 minute and afterwards centrifuge at 2800 G[1] for 15 minutes.
5. Collect the supernatant into Eppendorf tubes and use for assay or in cryo tubes[2] to store at –70 °C.

[1] 3500 rpm; maximum centrifuge radius = 20.5 cm

[2] Nunc Cry Tube Vials, Nalge Nunc International, Denmark

Appendix 7d Modified Larval Development Assay – With intestinal mucus or contents

A larval development assay (LDA) was modified to test the effect of intestinal mucus or contents on the development of egg to 3rd stage larvae. No agar or anthelmintics were used, instead serial dilutions of either intestinal mucus or contents were added to and mixed with the egg solution, nutritive medium, *E. coli* suspension and amphotericin already added to each well. Approximately 50 (±15) eggs per well were used in this assay.

METHOD:

1. Mix 7.5 ml of egg suspension with 2.5 ml of nutritive medium, 2.5 ml of *E. coli* suspension and 150 µl of Amphotericin in a small glass beaker. Mix well.
2. Using an 8-channel multichannel pipette[3], dispense 100 µl of the mixture into each of the wells in a 96-well microtitre plate[4].
3. Add 250 µl of the test substance to 2 wells in lane 1, f. ex. Wells B1 and C1. A total of 3 testsubstances can be applied to one plate.

Row\Lane	1	2	3	4	5	6	7	8	9	10	11	12
A	Control	Control	Control	Control	Control	Control	Control	Control	Control	Control	Control	Control
B	a 1	a 2	a 3	a 4	a 5	a 6	a 7	a 8	a 9	a 10	a 11	a 12
C	a 1	a 2	a 3	a 4	a 5	a 6	a 7	a 8	a 9	a 10	a 11	a 12
D	b1	b2	b3	b4	b5	b6	b7	b8	b9	b10	b11	b12
E	b1	b2	b3	b4	b5	b6	b7	b8	b9	b10	b11	b12
F	c1	c2	c3	c4	c5	c6	c7	c8	c9	c10	c11	c12
G	c1	c2	c3	c4	c5	c6	c7	c8	c9	c10	c11	c12
H	Control	Control	Control	Control	Control	Control	Control	Control	Control	Control	Control	Control

Row\Lane	1	2	3	4	5	6	7	8	9	10	11	12
A	Control	Control	Control	Control	Control	Control	Control	Control	Control	Control	Control	Control
B	a13	a14	a15	a16	a17	a18	a19	a20	a21	a22	a23	a24
C	a13	a14	a15	a16	a17	a18	a19	a20	a21	a22	a23	a24
D	b13	b14	b15	b16	b17	b18	b19	b20	b21	b22	b23	b24
E	b13	b14	b15	b16	b17	b18	b19	b20	b21	b22	b23	b24
F	c13	c14	c15	c16	c17	c18	c19	c20	c21	c22	c23	c24
G	c13	c14	c15	c16	c17	c18	c19	c20	c21	c22	c23	c24
H	Control	Control	Control	Control	Control	Control	Control	Control	Control	Control	Control	Control

Layout for 96-well microtitre plates. Decreasing concentrations are listed as 'a1 – a24', 'b1 – b24' and 'c1 – c24' for 3 substances 'a', 'b' and 'c' that are to be tested in the assay. In rows A and H are control wells, where no test substances have been added.

[3] 'Jencons Sealpette 1200' Electronic Multichannel Pippettor, 8-ch., 50 – 1200 µl

[4] Falcon Microtest™ Tissue Culture Plate, 96-well, Flat bottom with Low Evaporation Lid, Product 35-3072, Becton Dickinson Labware

4. Using an 8-channel multichannel pipette aspirate and dispense into the same well 3 times to mix the test substance well with the contents of the well.
5. Aspirate and transfer 250 µl to wells in lane 2. Again mix well as mentioned above and continue in this manner until lane 8 of Plate 1. Transfer the 250 µl mixture to a new plate (Plate 2) and continue mixing in the same manner as for Plate 1. The dilution pattern is shown in Figure 1. Discard the 250 µl aspirated from lane 8 in Plate 2.
6. As the dilution factor is 250/350 = 0.71, the following series of dilutions given as %mucus/contents is then obtained: 100, 71, 51, 36, 26, 18, 13, 9, 6, 5, 3, 2, 1.6, 1.2, 1.0, 0.6, 0.4, 0.3, 0.2, 0.15, 0.1, 0.08, 0.05, 0.04
7. Place the plate in a humidity/incubator chamber, containing water, and incubate at 27°C for 7 days.
8. After incubation, transfer the contents of each well to a scored glass slide by means of a pasteur pipette.
9. Add a drop of Lugol's Iodine, place a coverslip on top and count the number of eggs, 1st, 2nd and 3rd stage larvae present in each well.
10. Count a minimum of three control wells. Use the average proportion of 3rd stage larvae developed in the control wells to adjust the proportion of 3rd stage larvae developed in the wells containing the serial dilutions of either intestinal or contents.
11. Plot the proportion of 3rd stage larvae (y-axis) against the log-transformed mucus or contents concentrations (x-axis). Calculate the LC_{50} (lethal concentration at which 50 % of larvae die) using a logistic regression and fitting[5] a sigmoid dose-response curve to the data.

[5] Curve-fitting software:GraphPad Prism[R] 3 for Windows, GraphPad Software Inc., USA

Appendix 7e Data from Chapter 7

Faecal Egg Counts and Developmental Success — **Trt 1 = Steroid-treated group, Trt 2 = Non-steroid-treated group**

Week 3

Tag	Trt	FEC	FEC	FEC	Mean	Dev.Succ. 1	2	3	Mean
2	1	250	150	250	217	11.7	98.7	102.9	71.1
9	1	300	300	200	267	79.3	85.8	61.0	75.3
11	1	450	400	450	433	20.4	23.3	23.1	22.2
12	1	450	500	350	433	71.0	63.9	75.4	70.1
18	1	500	350	750	533	22.0	5.0	11.4	12.8
19	1	200	300	300	267	89.3	145.4	95.8	110.1
				Mean	358			Mean	60.3
1	2	300	200	250	250	136.2	133.8	122.4	130.7
3	2	400	450	150	333	78.7	64.4	58.8	67.3
7	2	400	200	200	267	105.3	167.1	131.6	134.7
10	2	250	150	100	167	166.2	229.8	135.0	177.0
15	2	150	150	400	233	138.4	221.3	249.4	203.0
17	2	250	50	150	150	145.1	147.2	146.4	146.2
20	2	250	250	200	233	53.9	35.0	73.8	54.2
				Mean	233			Mean	130.5

Week 4

Tag	Group	FEC	FEC	FEC	Mean	Dev.Succ. 1	2	3	Mean
2	1	450	550	400	467	49.9	96.9	59.1	68.7
9	1	850	800	550	733	22.6	33.4	59.3	38.5
12	1	600	1200	1050	950	88.1	87.9	94.1	90.0
18	1	400	500	800	567	40.9	42.9	32.6	38.8
19	1	950	500	950	800	90.1	131.9	121.0	114.3
				Mean	703			Mean	70.1
1	2	150	150	350	217	24.6	65.1	8.5	32.7
3	2	800	950	1050	933	81.9	10.7	42.4	45.0
7	2	1000	700	800	833	5.5	14.5	2.2	7.4
10	2	800	1200	1000	1000	48.1	121.7	70.5	80.1
15	2	350	300	400	350	88.3	32.9	185.4	102.2
17	2	250	500	500	417	33.3	27.6	49.4	36.8
20	2	1200	750	1050	1000	12.2	38.2	24.2	24.9
				Mean	679			Mean	47.0

Week 5

Tag	Trt	FEC	FEC	FEC	Mean	Dev.Succ. 1	2	3	Mean
2	1	650	700	1000	783	133.7	120.9	105.9	120.2
9	1	550	950	1500	1000	137.3	129.3	124.3	130.3
12	1	1250	1400	1500	1383	57.5	42.3	51.1	50.3
18	1	1450	1400	1850	1567	18.3	22.8	33.9	25.0
19	1	1300	1550	1650	1500	30.5	24.0	48.3	34.3
				Mean	1247			Mean	72.0
1	2	1100	900	650	883	29.0	63.8	19.8	37.5
3	2	650	500	900	683	54.5	41.8	47.0	47.8
7	2	750	1000	1350	1033	41.7	47.2	69.0	52.6
10	2	1500	1400	1300	1400	64.1	80.7	75.8	73.6
15	2	1100	1000	1000	1033	105.1	79.4	85.2	89.9
17	2	1200	750	1650	1200	12.9	16.7	20.5	16.7
20	2	1100	950	850	967	80.3	93.9	102.3	92.2
				Mean	1029			Mean	58.6

Week 6

Tag	Group	FEC	FEC	FEC	Mean	Dev.Succ. 1	2	3	Mean
2	1	1000	1300	1050	1117	109.5	132.9	158.4	133.6
9	1	1250	1100	1600	1317	68.5	120.5	120.1	103.0
12	1	1850	1950	1200	1667	52.4	56.9	45.3	51.5
18	1	1750	1600	2050	1800	89.1	79.8	68.8	79.2
19	1	1400	1250	1400	1350	104.4	122.1	147.0	124.5
				Mean	1450			Mean	98.4
1	2	550	750	1200	833	52.8	24.1	23.4	33.5
3	2	850	1250	800	967	33.6	34.7	43.8	37.4
7	2	1400	1500	1300	1400	103.1	113.9	126.7	114.5
10	2	1100	1400	1400	1300	119.4	103.4	120.8	114.5
15	2	1250	950	1100	1100	97.9	18.4	128.3	81.6
17	2	300	450	700	483	54.6	138.4	116.3	103.1
20	2	1800	3000	1500	2100	26.1	6.1	25.8	19.3
				Mean	1169			Mean	72.0

Week 7

Tag	Trt	FEC	FEC	FEC	Mean	Dev.Succ. 1	2	3	Mean
2	1	950	550	450	650	100.8	29.6	42.0	57.5
9	1	1750	1750	1350	1617	98.4	75.5	70.9	81.6
12	1	1250	1500	1750	1500	7.7	11.9	12.5	10.7
18	1	1650	1200	2450	1767	137.4	115.5	125.4	126.1
19	1	900	1050	1050	1000	52.2	63.5	77.6	64.4
				Mean	1307			Mean	68.1
1	2	500	700	1450	883	80.2	81.5	84.5	82.1
3	2	1050	1100	1150	1100	86.3	104.6	106.3	99.1
7	2	900	1200	1200	1100	122.7	171.6	226.8	173.7
10	2	1000	1250	1150	1133	83.3	80.0	120.9	94.7
15	2	1500	2000	1550	1683	113.8	106.7	104.2	108.2
17	2	300	400	500	400	81.0	301.0	254.0	212.0
20	2	750	700	550	667	174.5	212.2	191.0	192.6
				Mean	995			Mean	137.5

Week 8

Tag	Group	FEC	FEC	FEC	Mean	Dev.Succ. 1	2	3	Mean
2	1	700	650	1100	817	113.4	132.6	97.1	114.4
9	1	1200	1350	1200	1250	154.5	166.8	166.7	162.7
12	1	900	1550	1500	1317	193.6	168.7	88.9	150.4
18	1	1100	1350	1850	1433	130.4	121.4	124.2	125.3
19	1	1200	1550	1100	1283	124.5	142.1	88.1	118.2
				Mean	1220			Mean	134.2
1	2	400	600	700	567	74.8	80.4	89.1	81.4
3	2	900	700	500	700	50.7	85.4	74.8	70.3
7	2	1000	1000	1000	1000	148.5	170.0	159.0	159.2
10	2	1200	1550	1400	1383	49.2	54.1	58.0	53.7
15	2	1500	950	1100	1183	112.3	103.7	87.0	101.0
17	2	800	800	850	817	156.0	158.8	165.8	160.2
20	2	1550	1550	850	1317	63.1	107.8	122.2	97.7
				Mean	995			Mean	103.4

Week 9

Tag	Trt	FEC	FEC	FEC	Mean	Dev.Succ. 1	2	3	Mean
2	1	1900	1600	1650	1717	36.0	92.8	118.5	82.4
9	1	1350	1550	1500	1467	63.0	85.9	92.2	80.4
12	1	1850	2050	2150	2017	80.1	26.1	160.7	89.0
18	1	1200	1800	2600	1867	80.0	45.2	53.7	59.6
19	1	1700	1300	2200	1733	85.4	130.7	72.1	96.1
				Mean	1760			Mean	81.5
1	2	1200	1450	1500	1383	41.6	74.3	91.2	69.0
3	2	900	700	1050	883	99.6	117.3	104.6	107.2
7	2	1550	1600	1200	1450	42.2	89.5	106.3	79.4
10	2	1850	1500	1500	1617	104.6	113.4	77.2	98.4
15	2	1550	1150	1050	1250	41.1	43.6	27.0	37.2
17	2	400	400	550	450	20.1	127.4	148.0	98.5
20	2	1450	1350	2050	1617	94.8	28.9	64.6	62.8
				Mean	1236			Mean	78.9

Week 10

Tag	Group	FEC	FEC	FEC	Mean	Dev.Succ. 1	2	3	Mean
2	1	1000	800	1050	950	34.6	48.0	36.6	39.8
9	1	1200	1250	1750	1400	95.3	161.9	23.8	93.7
12	1	2300	2200	3400	2633	72.9	68.6	12.6	51.4
18	1	2050	2350	1800	2067	100.6	103.3	111.2	105.0
19	1	2350	3000	3200	2850	23.6	47.3	34.5	35.1
				Mean	1980			Mean	65.0
1	2	200	400	400	333	71.5	93.0	92.5	85.7
3	2	600	300	350	417	36.7	77.6	50.6	55.0
7	2	650	1700	1200	1183	51.5	84.6	108.8	81.6
10	2	800	1250	1550	1200	119.9	125.0	106.6	117.2
15	2	1150	1150	850	1050	75.7	103.3	80.2	86.4
17	2	700	350	600	550	63.6	94.5	98.5	85.6
20	2	1150	850	1100	1033	72.7	83.6	75.3	77.2
				Mean	824			Mean	84.1

Week 11

Tag	Trt	FEC	FEC	FEC	Mean	Dev.Succ. 1	2	3	Mean
2	1	2150	2900	2450	2500	10.1	6.2	16.0	10.7
9	1	1700	2300	1700	1900	18.0	48.6	63.4	43.3
12	1	1700	1400	2000	1700	37.5	54.2	44.1	45.3
18	1	2150	2050	2150	2117	41.5	94.0	98.6	78.0
19	1	1600	2350	2000	1983	41.2	88.1	50.6	59.9
				Mean	2040			Mean	47.5
1	2	350	350	150	283	13.6	52.3	36.6	34.2
3	2	200	250	200	217	5.8	9.6	14.6	10.0
7	2	1700	1000	1850	1517	11.3	69.2	44.6	41.7
10	2	700	600	750	683	25.3	48.4	16.0	29.9
15	2	600	600	650	617	97.0	98.3	86.3	93.9
17	2	150	250	300	233	53.5	26.7	33.1	37.8
20	2	1400	1250	1650	1433	28.1	36.6	72.7	45.8
				Mean	712			Mean	41.9

Week 12

Tag	Group	FEC	FEC	FEC	Mean	Dev.Succ. 1	2	3	Mean
2	1	1300	800	1000	1033	66.2	72.2	36.7	58.3
9	1	2400	2450	2850	2567	32.6	34.8	19.6	29.0
12	1	1850	1800	1800	1817	7.0	18.8	13.6	13.2
18	1	2000	2100	2050	2050	142.1	117.1	158.6	139.3
19	1	2400	2750	2300	2483	50.2	38.7	15.6	34.8
				Mean	1990			Mean	54.9
1	2	100	50	100	83	116.6	115.4	96.5	109.5
3	2	50	50	0	33	221.4	305.7	282.5	269.9
7	2	100	300	350	250	23.3	34.5	3.0	20.3
10	2	750	500	900	717	96.3	75.8	95.9	89.3
15	2	200	50	300	183	87.8	90.4	92.0	90.1
17	2	300	1050	850	733	3.0	64.5	71.1	46.2
20	2	700	200	600	500	98.1	58.2	66.2	74.2
				Mean	357			Mean	99.9

Week 13

Tag	Trt	FEC	FEC	FEC	Mean	Dev.Succ. 1	2	3	Mean
2	1	2100	2400	2350	2283	43.6	68.5	46.0	52.7
9	1	3050	3550	3350	3317	19.3	27.6	23.2	23.4
				Mean	2800			Mean	38.0
1	2	50	0	50	33	37.1	25.2	50.5	37.6
7	2	200	100	200	167	7.7	3.3	9.9	7.0
15	2	150	150	50	117	462.0	244.1	363.7	356.6
20	2	900	950	400	750	12.5	15.7	12.0	13.4
				Mean	267			Mean	103.6

Week 14

Tag	Group	FEC	FEC	FEC	Mean	Dev.Succ. 1	2	3	Mean
2	1	1300	1700	1650	1550	4.7	33.1	18.3	18.7
9	1	1850	1750	1650	1750	65.4	11.8	18.9	32.0
				Mean	1650			Mean	25.4
1	2	0	50	0	17	64.7	89.1	5.7	53.2
7	2	100	100	0	67	2.9	0.0	7.1	3.3
15	2	0	0	0	0				
20	2	250	50	300	200	23.8	37.6	43.4	35.0
				Mean	71			Mean	30.5

Specific antibody levels

Trt 1 = Steroid-treated group | **Trt 2 = Non-steroid-treated group** | **Trt 3 = Control group**

Week	Tag	Trt	TcL3	TcAd	Week	Tag	Trt	TcL3	TcAd	Week	Tag	Trt	TcL3	TcAd
-1	2	1	0.66	0.27	4	2	1	0.20	0.22	9	4	3	0.52	0.21
-1	9	1	0.37	0.16	4	9	1	0.22	0.10	9	5	3	0.57	0.35
-1	11	1	0.49	0.16	4	12	1	0.43	0.13	9	6	3	0.28	0.14
-1	12	1	0.34	0.16	4	18	1	0.38	0.20	9	8	3	0.30	0.12
-1	14	1	0.48	0.21	4	19	1	0.34	0.13	9	13	3	0.43	0.12
-1	18	1	0.74	0.42	4	1	2	0.27	0.20	9	16	3	0.33	0.13
-1	19	1	0.81	0.29	4	3	2	0.75	0.54	10	2	1	0.18	0.13
-1	1	2	0.42	0.31	4	7	2	0.61	0.30	10	9	1	0.34	0.16
-1	3	2	1.11	0.54	4	10	2	0.62	0.32	10	12	1	0.26	0.11
-1	7	2	0.50	0.21	4	15	2	0.90	0.22	10	18	1	0.28	0.18
-1	10	2	0.47	0.13	4	17	2	0.68	0.27	10	19	1	0.23	0.09
-1	15	2	1.26	0.29	4	20	2	0.49	0.14	10	1	2	0.75	0.32
-1	17	2	0.93	0.44	4	4	3	0.41	0.14	10	3	2	1.43	0.73
-1	20	2	0.54	0.14	4	5	3	0.58	0.23	10	7	2	1.18	0.53
-1	4	3	0.69	0.19	4	6	3	0.25	0.11	10	10	2	0.60	0.20
-1	5	3	0.81	0.28	4	8	3	0.34	0.12	10	15	2	1.11	0.32
-1	6	3	0.51	0.24	4	13	3	0.78	0.18	10	17	2	1.36	0.76
-1	8	3	0.54	0.20	4	16	3	0.41	0.17	10	20	2	0.71	0.16
-1	13	3	0.94	0.25	5	2	1	0.20	0.17	10	4	3	0.45	0.17
-1	16	3	0.40	0.19	5	9	1	0.21	0.14	10	5	3	0.53	0.32
0	2	1	0.30	0.17	5	12	1	0.41	0.18	10	6	3	0.27	0.16
0	9	1	0.44	0.18	5	18	1	0.43	0.28	10	8	3	0.31	0.12
0	11	1	0.53	0.16	5	19	1	0.38	0.17	10	13	3	0.41	0.12
0	12	1	0.42	0.19	5	1	2	0.35	0.20	10	16	3	0.33	0.12
0	14	1	0.56	0.24	5	3	2	0.76	0.49	11	2	1	0.19	0.10
0	18	1	0.86	0.45	5	7	2	0.75	0.41	11	9	1	0.30	0.13
0	19	1	0.89	0.30	5	10	2	0.44	0.28	11	12	1	0.28	0.14
0	1	2	0.45	0.28	5	15	2	0.98	0.32	11	18	1	0.32	0.22
0	3	2	1.15	0.55	5	17	2	0.52	0.21	11	19	1	0.21	0.08
0	7	2	0.50	0.21	5	20	2	0.45	0.17	11	1	2	0.93	0.40
0	10	2	0.55	0.16	5	4	3	0.49	0.15	11	3	2	1.38	0.66
0	15	2	1.33	0.32	5	5	3	0.57	0.25	11	7	2	1.04	0.43
0	17	2	0.89	0.38	5	6	3	0.30	0.15	11	10	2	0.59	0.16
0	20	2	0.61	0.15	5	8	3	0.33	0.15	11	15	2	1.20	0.27
0	4	3	0.83	0.23	5	13	3	0.54	0.15	11	17	2	1.26	0.73
0	5	3	0.87	0.31	5	16	3	0.32	0.15	11	20	2	0.84	0.25
0	6	3	0.55	0.26	6	2	1	0.18	0.14	11	4	3	0.41	0.15
0	8	3	0.58	0.23	6	9	1	0.22	0.13	11	5	3	0.49	0.28
0	13	3	1.01	0.27	6	12	1	0.40	0.15	11	6	3	0.24	0.12
0	16	3	0.49	0.20	6	18	1	0.37	0.20	11	8	3	0.26	0.10
1	2	1	0.24	0.14	6	19	1	0.35	0.15	11	13	3	0.48	0.10
1	9	1	0.34	0.13	6	1	2	0.40	0.25	11	16	3	0.30	0.10
1	11	1	0.36	0.13	6	3	2	0.86	0.58	12	2	1	0.21	0.25
1	12	1	0.62	0.25	6	7	2	0.97	0.47	12	9	1	0.33	0.19
1	18	1	0.65	0.31	6	10	2	0.43	0.26	12	12	1	0.34	0.20
1	19	1	0.69	0.27	6	15	2	0.99	0.30	12	18	1	0.27	0.22
1	1	2	0.32	0.20	6	17	2	0.77	0.31	12	19	1	0.20	0.11
1	3	2	0.98	0.49	6	20	2	0.45	0.16	12	1	2	1.02	0.48
1	7	2	0.50	0.22	6	4	3	0.51	0.18	12	3	2	1.44	0.76
1	10	2	0.34	0.10	6	5	3	0.64	0.34	12	7	2	1.23	0.46
1	15	2	1.18	0.32	6	6	3	0.32	0.18	12	10	2	0.79	0.23
1	17	2	0.80	0.29	6	8	3	0.36	0.14	12	15	2	1.28	0.38
1	20	2	0.45	0.11	6	13	3	0.53	0.12	12	17	2	1.48	0.80
1	4	3	0.73	0.21	6	16	3	0.35	0.13	12	20	2	0.94	0.25
1	5	3	0.85	0.29	7	2	1	0.24	0.13	12	4	3	0.41	0.18
1	6	3	0.48	0.19	7	9	1	0.18	0.12	12	5	3	0.49	0.35
1	8	3	0.58	0.18	7	12	1	0.38	0.14	12	6	3	0.27	0.17
1	13	3	0.75	0.18	7	18	1	0.32	0.21	12	8	3	0.30	0.12
1	16	3	0.44	0.19	7	19	1	0.29	0.15	12	13	3	0.31	0.12
2	2	1	0.27	0.14	7	1	2	0.39	0.23	12	16	3	0.56	0.12
2	9	1	0.31	0.12	7	3	2	0.96	0.63	13	2	1	0.23	0.32
2	11	1	0.34	0.11	7	7	2	0.89	0.43	13	9	1	0.27	0.15
2	12	1	0.82	0.32	7	10	2	0.47	0.27	13	1	2	0.95	0.53
2	18	1	0.55	0.26	7	15	2	1.05	0.32	13	7	2	1.23	0.43
2	19	1	0.56	0.22	7	17	2	1.11	0.58	13	15	2	1.26	0.32
2	1	2	0.31	0.19	7	20	2	0.80	0.17	13	20	2	0.98	0.22
2	3	2	0.92	0.50	7	4	3	0.46	0.15	13	4	3	0.51	0.17
2	7	2	0.76	0.44	7	5	3	0.55	0.31	13	6	3	0.25	0.15
2	10	2	0.42	0.10	7	6	3	0.26	0.11	13	13	3	0.30	0.11
2	15	2	1.22	0.31	7	8	3	0.30	0.11	14	2	1	0.20	0.25
2	17	2	0.73	0.24	7	13	3	0.55	0.15	14	9	1	0.28	0.18
2	20	2	0.62	0.16	7	16	3	0.32	0.12	14	1	2	1.11	0.52
2	4	3	0.72	0.20	8	2	1	0.23	0.15	14	7	2	1.42	0.48
2	5	3	0.84	0.31	8	9	1	0.18	0.11	14	15	2	1.30	0.28
2	6	3	0.39	0.16	8	12	1	0.27	0.13	14	20	2	0.98	0.20
2	8	3	0.52	0.17	8	18	1	0.29	0.23	14	4	3	0.39	0.17
2	13	3	0.81	0.20	8	19	1	0.27	0.14	14	6	3	0.26	0.14
2	16	3	0.51	0.20	8	1	2	0.82	0.35	14	13	3	0.28	0.11
3	2	1	0.22	0.13	8	3	2	1.32	0.70	15	2	1	0.27	0.22
3	9	1	0.27	0.12	8	7	2	1.05	0.49	15	9	1	0.38	0.20
3	11	1	0.46	0.22	8	10	2	0.41	0.22	15	1	2	0.95	0.48
3	12	1	0.51	0.20	8	15	2	1.15	0.37	15	7	2	1.47	0.50
3	18	1	0.45	0.23	8	17	2	0.99	0.45	15	15	2	1.34	0.26
3	19	1	0.49	0.20	8	20	2	0.76	0.16	15	20	2	1.20	0.20
3	1	2	0.36	0.18	8	4	3	0.45	0.15	15	4	3	0.34	0.16
3	3	2	0.79	0.53	8	5	3	0.53	0.27	15	6	3	0.32	0.16
3	7	2	0.74	0.44	8	6	3	0.24	0.12	15	13	3	0.31	0.10
3	10	2	0.28	0.11	8	8	3	0.33	0.12					
3	15	2	1.10	0.34	8	13	3	0.54	0.15					
3	17	2	0.65	0.26	8	16	3	0.37	0.14					
3	20	2	0.44	0.15	9	2	1	0.20	0.13					
3	4	3	0.58	0.21	9	9	1	0.24	0.15					
3	5	3	0.64	0.26	9	12	1	0.24	0.12					
3	6	3	0.29	0.20	9	18	1	0.26	0.22					
3	8	3	0.40	0.17	9	19	1	0.25	0.10					
3	13	3	0.89	0.20	9	1	2	0.83	0.35					
3	16	3	0.45	0.20	9	3	2	1.30	0.71					
					9	7	2	1.09	0.51					
					9	10	2	0.41	0.21					
					9	15	2	1.09	0.32					
					9	17	2	1.07	0.63					
					9	20	2	0.58	0.15					

Worm Counts

Trt 1 = Steroid-treated group | Trt 2 = Non-steroid-treated group | Trt 3 = Control group

Tag	Trt	Killed	Female	Male	Immature	L4	L3	Total	M/F-ratio	%Establ.
12	1	Week 12	12070	5690		140	30	17930	0.47	26.7
18	1	Week 12	17120	5250		90	10	22470	0.31	33.4
19	1	Week 12	13140	7860	210	70		21280	0.60	31.7
3	2	Week 12	5470	3650		70		9190	0.67	13.7
10	2	Week 12	12050	6180		20		18250	0.51	27.2
17	2	Week 12	8760	5270	100	10		14140	0.60	21.0
5	3	Week 12	30			10		40		
8	3	Week 12						0		
16	3	Week 12						0		
2	1	Week 15	10300	8210	150	60		18720	0.80	22.3
9	1	Week 15	13240	7550	20			20810	0.57	24.8
1	2	Week 15	50	30			10	90	0.60	0.1
7	2	Week 15	1050	490	20			1560	0.47	1.9
15	2	Week 15	410	330	120			860	0.80	1.0
20	2	Week 15	4480	3240	90			7810	0.72	9.3
4	3	Week 15						0		
6	3	Week 15						0		
13	3	Week 15						0		

Female and Male Worm Lengths (mm)

	Steroid-treated group										Non-steroid-treated group									
Tag	2	2	9	9	12	12	18	18	19	19	3	3	7	7	10	10	15	15	17	17	20	20
	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male
1	7.15	5.50	6.34	6.19	7.62	4.77	6.62	5.76	5.88	5.66	5.01	4.16	6.25	4.51	6.28	5.36	5.22	5.16	5.52	5.15	5.37	5.13
2	6.42	6.30	7.05	5.59	7.21	4.46	6.89	5.40	5.64	5.13	5.66	5.71	5.16	5.83	6.22	5.25	5.21	4.96	5.37	5.61	5.14	5.52
3	6.15	5.67	7.29	6.38	7.16	5.24	6.66	5.02	6.21	6.32	5.36	5.96	5.56	4.91	6.31	4.62	6.13	4.99	6.16	4.46	5.61	5.16
4	6.27	4.54	6.35	5.16	7.00	6.46	7.24	4.72	6.72	5.97	6.14	5.46	4.95	4.26	5.75	5.24	6.36	4.43	5.37	5.57	6.30	5.54
5	6.71	5.23	6.19	6.08	6.90	6.21	6.41	5.16	5.91	5.64	5.94	5.49	5.31	5.98	5.99	4.56	5.79	3.69	5.62	5.33	4.81	5.47
6	6.95	5.07	6.66	6.43	6.89	5.43	6.66	4.46	7.21	5.78	6.06	4.55	5.40	5.26	6.14	5.52	5.97	4.11	6.00	5.59	5.55	4.66
7	5.94	5.24	6.74	5.40	7.52	6.79	7.26	5.01	7.00	5.19	5.87	5.90	5.46	4.72	6.44	4.60	5.27	5.05	5.83	5.64	6.19	5.07
8	6.17	4.50	6.43	6.43	6.34	4.73	7.03	4.73	7.31	5.40	5.69	5.38	5.54	5.49	5.88	5.23	5.62	5.57	5.54	6.11	5.69	5.94
9	6.15	5.49	7.08	5.02	6.14	6.94	7.63	4.71	7.02	5.75	5.01	5.00	5.02	4.92	6.32	4.42	5.95	5.28	5.75	4.47	5.12	5.33
10	5.99	4.97	7.02	5.64	6.71	5.36	6.74	4.24	6.34	5.93	5.99	5.00	5.52	4.28	5.54	4.82	5.35	4.89	5.10	5.49	5.00	4.97
11	5.95	5.07	6.76	5.62	6.71	6.41	6.75	6.10	6.84	4.81	5.05	5.89	5.05	4.61	5.22	4.84	5.40	4.84	4.66	5.22	5.98	5.80
12	6.14	5.47	7.11	6.16	6.44	5.74	6.59	3.83	7.03	5.78	5.19	5.16	5.61	4.94	6.14	3.86	5.87	4.25	6.19	5.33	5.80	5.81
13	6.30	4.99	6.95	5.27	6.05	4.79	7.64	5.23	7.79	5.34	4.60	5.23	5.97	4.86	5.91	4.40	6.12	4.71	4.60	5.20	4.99	5.73
14	6.14	5.57	6.35	5.69	7.02	5.76	5.94	5.13	6.25	6.26	6.43	5.47	6.31	4.58	5.99	5.76	6.20	5.19	5.95	5.90	6.12	5.27
15	6.45	6.35	7.21	6.26	6.46	4.81	7.40	4.71	7.20	4.73	5.66	5.22	5.62	5.57	5.97	5.04	5.57	5.69	5.51	4.53	4.69	5.77
16	6.31	5.01	6.54	5.13	7.16	5.70	7.16	8.77	6.50	4.57	6.06	5.16	5.32	5.29	5.81	3.67	5.74	5.50	6.00	4.78	5.81	5.20
17	6.51	5.23	6.57	5.65	6.40	6.54	6.36	5.65	6.12	6.76	5.99	5.11	5.21	4.78	5.28	5.34	5.26	5.40	6.07	5.79	6.00	5.43
18	6.53	5.53	6.07	5.63	7.36	5.04	7.19	6.44	6.90	5.95	4.12	4.57	5.18	5.68	6.39	3.93	5.65	4.75	6.12	5.26	5.32	6.36
19	6.02	5.56	5.94	5.24	6.96	5.59	6.70	6.20	7.10	5.70	5.08	5.16	5.20	4.48	5.46	4.75	5.76	4.81	4.91	4.44	6.37	6.09
20	6.16	4.14	6.35	5.02	8.24	4.97	6.34	4.25	7.00	5.25	6.45	5.15	5.85	5.86	5.27	5.75	5.56	5.40	5.82	5.26	5.92	5.35
Mean	6.32	5.26	6.85	5.73	6.82	5.59	6.87	5.19	6.70	5.61	5.58	5.24	5.46	5.05	5.90	4.88	5.71	4.95	5.63	5.27	5.80	5.46

***In utero* egg counts**

	Steroid-treated group					Non-steroid-treated group				
Tag	2	9	12	18	19	3	7	10	15	17	20
1	18	25	22	20	6	3	1	5	0	2	8
2	24	22	18	17	15	5	3	7	2	5	6
3	18	24	20	17	14	3	2	7	1	6	2
4	20	18	22	15	14	2	3	6	0	5	5
5	18	21	17	18	14	0	3	8	3	3	6
6	18	18	18	22	13	3	2	7	1	6	6
7	22	18	17	17	18	2	1	6	1	5	2
8	21	17	18	16	13	2	1	7	0	6	6
9	22	20	16	23	13	3	1	7	0	4	2
10	17	17	17	23	16	5	2	8	1	7	2
11	16	20	17	18	16	2	0	6	2	8	2
12	16	21	23	20	19	2	1	7	2	4	1
13	19	18	16	15	21	3	2	6	3	4	2
14	18	18	22	18	16	4	2	5	0	2	2
15	18	20	17	19	14	1	1	8	1	5	2
16	18	20	16	18	14	2	2	7	3	3	1
17	18	18	18	18	18	5	2	11	1	3	6
18	19	20	20	20	17	6	2	6	1	4	2
19	19	22	16	16	19	4	2	5	2	6	2
20	18	18	25	21	13	3	1	9	1	4	1
Mean	19	20	19	19	15	3	2	7	1	5	3

Histopathological findings in the small intestine (cells/mm2)

Trt 1 = Steroid-treated group | Trt 2 = Non-steroid-treated group | Trt 3 = Control group

Week	Tag	Trt	Section	MMC	GL	EOS	Week	Tag	Trt	Section	MMC	GL	EOS
12	12	1	1	22	0	17	15	2	1	1	25	0	31
12	12	1	2	33	0	38	15	2	1	2	40	0	26
12	18	1	1	45	0	28	15	9	1	1	52	0	23
12	18	1	2	46	0	47	15	9	1	2	69	0	33
12	19	1	1	11	0	7	15	1	2	1	227	193	19
12	19	1	2	19	0	27	15	1	2	2	206	78	65
12	3	2	1	220	115	1	15	7	2	1	180	265	82
12	3	2	2	140	263	58	15	7	2	2	187	160	217
12	10	2	1	119	22	221	15	15	2	1	161	382	1
12	10	2	2	140	21	218	15	15	2	2	245	223	239
12	17	2	1	131	146	245	15	20	2	1	82	1	131
12	17	2	2	138	87	155	15	20	2	2	100	1	140
12	5	3	1	93	8	90	15	4	3	1	101	0	97
12	5	3	2	162	14	123	15	4	3	2	135	1	113
12	8	3	1	85	0	85	15	6	3	1	78	0	41
12	8	3	2	123	1	216	15	6	3	2	105	0	32
12	16	3	1	243	2	85	15	13	3	1	174	10	28
12	16	3	2	156	1	94	15	13	3	2	179	17	45

Data from modified LDA with either mucus or contents added.

Values are representing the proportion of L3s at the various concentrations (conc.).

LC50 = LC_{50} ; R2 = R^2 = Goodness of fit for dose response curve

Conc. = proportion of mucus or contents in well					
Dilution	Conc	Dilution	Conc	Dilution	Conc
17	0.003	11	0.025	5	0.186
17	0.003	11	0.025	5	0.186
16	0.005	10	0.035	4	0.260
16	0.005	10	0.035	4	0.260
15	0.006	9	0.048	3	0.364
15	0.006	9	0.048	3	0.364
14	0.009	8	0.068	2	0.510
14	0.009	8	0.068	2	0.510
13	0.013	7	0.095	1	0.714
13	0.013	7	0.095	1	0.714
12	0.018	6	0.133		
12	0.018	6	0.133		

Week 12 **Test substance = mucus**

Egg source:			Tag 12						
Tag	12	18	19	3	10	17	5	8	16
LC50	0.23	0.13	0.15		0.19	0.19	0.06	0.06	0.07
R2	0.97	0.82	0.98		0.74	0.97	0.98	0.83	0.99
Conc	12	18	19	3	10	17	5	8	16
17									
17									
16									
16									
15									
15									
14							1.07	1.09	1.11
14							1.1	1.14	1.11
13							1.08	1.08	1.14
13							1.11	1.08	1.11
12		0.89	1				1.14	1.09	1.07
12		0.99	0.97				1.11	1.14	1.14
11	0.93	0.93	0.93				1.14	1.03	1.14
11	1.04	0.98	0.98				1.05	1.03	1.07
10	0.94	0.99	1.02	1	1	1.02	1.11	1.14	1.01
10	0.97	1.01	0.97	1.02	1.02	0.95	1.14	1.14	1.14
9	1	1.01	0.99	1	1.02	1.02	0.87	0	0.92
9	1.04	0.99	0.95	1	0.97	1.02	0.81	0.62	1.05
8		1	0.98	0.95	1.02	0.93	0.57	0.63	1.14
8		0.96	0.96	0.98	1	0.98	0.16	0.93	0.88
7	1.04	0.81	0.95	0.98	1.02	0.87	0	0	0
7	0.92	0.55	0.89	1.02	1.02	0.89	0	0	0
6	0.93	0.46	0.66	1.02	0.93	0.95			
6	0.98	0	0.87	1.02	0.94	0.96			
5	0.64	0.35	0	0.92	0.77	0.77	0	0	0
5	0.83	0.86	0.17	0.99	0.98	0.36	0	0	0
4	0.13	0		0.99	0	0			
4	0.45	0		0.94	0.2	0			
3	0	0	0	0.85	0	0	0	0	0
3	0	0	0	0.59	0	0	0	0	0
2				0.87	0	0			
2				0.77	0	0			
1	0	0	0	0.2	0.73	0	0	0	0
1	0	0	0	0.17	0.85	0	0	0	0

Egg source:			Tag 18						
Tag	12	18	19	3	10	17	5	8	16
LC50	0.1	0.06	0.11	0.2	0.18	0.13	0.07	0.03	0.08
R2	0.99	0.82	0.89	0.92	0.9	0.9	0.9	0.99	0.98
Conc	12	18	19	3	10	17	5	8	16
17							1.01		
17							1.06		
16	1.05	1.02	0.99	1.03	1.03	1.03	1.06	1	0.94
16	1.03	0.98	1.05	1	1.03	1.03	1.06	1.06	1.06
15	1.03	1.05	1	0.99	1.03	1	1.06	1.06	1.03
15	1.01	1.05	1.02	0.96	1.03	1.03	1	1	0.98
14	1.05	1.03	1	1.03	0.97	0.9	1.03	1.03	1.03
14	1.03	1.05	1.02	1.01	1.01	0.96	1.01	0.97	1.06
13	1.01	1.05	0.88	1.01	0.94	0.99	1	0.95	0.98
13	1.01	0.93	0.92	1.03	1.03	1	1.06	0.98	1.06
12	1.05	0.92	0.81	0.99	1.01	0.93	0.95	1.02	1.06
12	1.05	1.02	1.02	0.97	0.98	0.85	1.03	1.08	0.93
11	1.05	1.02	1.01	1.03	0.85	1	1.03	0.91	1.06
11	0.93	1.05	0.97	0.79	1.03	0.95	0.84	0.84	0.87
10	0.99	0.91	1	0.97	1.03	0.91	0.7	0.89	0.84
10	1.02	0.85	1.01	0.91	0.88	0.91	0.56	0.6	0.9
9	1.05	1	0.92	1.01	0.91	0.83	0.56	0.13	0.81
9	0.96	0.89	1	0.99	0.99	0.81	0.55	0.19	0.92
8		0.87	0.91	1	0.88	0.91	0.93	0.06	0.66
8		9	0.84	0.98	0.95	0.78	0.94	0.03	0.82
7	0.97	0.76	1.05	0.94	0.72	0.78	0	0	0
7	0.96	0.26	0.95	1.03	0.99	1.03	0.05	0	0.12
6	0.06	0	0	1	0.45	0	0		
6	0	0	0	0.99	0.89	0.78	0		
5	0.26	0.52	0.73	0.47	0.23	0	0	0	0
5	0	0	0	0.74	0.89	0.05	0	0	0
4				0.52	0	0	0		
4				0.23	0	0	0		
3	0	0	0	0.06	0	0.17	0	0	0
3	0	0	0	0.34	0	0.12	0	0	0
2	0						0	0	
2	0						0	0	
1	0	0	0	0.26	0	0	0	0	0
1	0	0.35	0	0	0	0.15	0	0	0

Egg source:			Tag 19						
Tag	12	18	19	3	10	17	5	8	16
LC50	0.12	0.09	0.11	0.35	0.2	0.131	0.06	0.04	0.05
R2	0.93	0.91	0.98	0.87	0.98	0.98	0.99	0.99	0.99
Conc	12	18	19	3	10	17	5	8	16
17									
17									
16									
16									
15							1.12	1.08	1.12
15							1.06	1.12	1.12
14	1	0.98	0.99			0.977	1.12	1.12	1.09
14	1.02	1.01	1.01			1.004	1.12	1.09	1.12
13	1.01	1.04	1.01	0.99	1.02	1.046	1.12	1.12	1.12
13	0.99	1.04	1.04	1.05	0.99	1.046	1.12	1.12	1.05
12	0.98	1.04	0.86	0.99	0.98	0.978	1.12	1.09	1.05
12	1	0.93	0.92	1.01	0.98	0.985	1.07	1.09	1
11	0.85	0.95	0.96	1.05	0.95	0.934	1.08	1.06	1.1
11	0.97	0.93	1.04	1.05	0.98	0.973	1.04	1.01	1.04
10	0.83	0.95	0.99	1.05	1	0.927	1.06	0.85	1.04
10	1.01	0.81	0.98	1.05	0.96	0.885	0.99	1	1.03
9	0.93	0.85	0.98	0.97	1.05	1.001	0.9	0.15	0.76
9	1.04	0.68	0.97	1.05	1.05	0.946	0.67	0.29	0.89
8	0.85	1.04	1.01	1.05	0.99	1.005	0.23	0	0.07
8	0.91	0.75	0.86	0.96	0.9	0.946	0.28	0	0.07
7	0.49	0.78	0.62	1.05	0.95	0.829	0	0	0
7	0.99	0.13	0.8	1.05	1.05	0.817	0	0	0
6	0.04	0	0	0.99	0.8	0.431	0	0	0
6	0.62	0	0.35	1.05	0.87	0.568	0	0	0
5	0	0	0	1.01	0.89	0.135	0	0	0
5	0	0.15	0	1.05	0.72	0.077	0	0	0
4				0.78	0	0.322			
4				0.8	0	0			
3	0	0	0	0.1	0	0	0	0	0
3	0	0	0	0.98	0.05	0	0	0	0
2				0.19	0	0			
2				0	0	0			
1	0	0	0	0.15	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0

Egg source:	Tag 3								
Tag	12	18	19	3	10	17	5	8	16
LC50	0.14	0.06	0.1		0.19	0.17	0.07	0.06	0.07
R2	0.99	0.98	0.99		0.93	0.94	0.99	0.95	0.99
Conc	12	18	19	3	10	17	5	8	16
17									
17									
16									
16									
15									
15									
14							1		
14							1.03		
13	1.03	1.03	1.03				1.03	1	0.99
13	1	1.01	1.03				1.03	1.03	1.03
12	0.99	0.97	1.03		1		1	1.03	1
12	1.03	1.03	1.03		1		0.99	1.03	1.03
11	1	1.03	1	0.99	0.98	0.99	1.03	1.03	1.03
11	1.03	1.01	1	1	0.99	1.02	0.98	1	1.03
10	1.03	0.99	1	0.98	1.02	1.02	1.03	1.03	1.03
10	0.97	1.03	1	1.02	1.02	1	0.98	0.97	1.03
9	0.99	0.92	1.03	1.02	1.02	0.98	0.99	0.75	1.03
9	1	0.81	1.03	1.02	1.02	0.98	0.97	0.59	1.03
8	0.96	0.64	0.98	1.02	1.02	1.02	0.73	0	0.68
8	1.03	0.26	0.99	1.02	1.02	1.02	0.68	0.82	0.87
7	1.03	0	0.62	1.02	1.02	1.02	0	0	0
7	0.8	0	0.68	0.99	1.02	1.02	0	0	0
6	0.68			0.98	0.86	0.95			
6	0.51			1.02	1.02	0.98			
5	0	0	0	0.94	0.91	0.64	0	0	0
5	0	0	0	0.95	0.1	0	0	0	0
4				0.94	0	0			
4				1.02	0	0			
3	0	0	0	1.02		0	0	0	0
3	0	0	0	0.68		0.2	0	0	0
2				0.93	0				
2				0.68	0				
1	0	0	0	0.41	0	0	0	0	0
1	0	0	0	0.51	0	0	0	0	0

Egg source:			Tag 10						
Tag	12	18	19	3	10	17	5	8	16
LC50	0.1	0.05	0.09	0.4	0.19	0.18	0.05	0.03	0.04
R2	0.88	0.99	0.96	0.96	0.93	0.92	0.98	0.98	0.99
Conc	12	18	19	3	10	17	5	8	16
17									
17									
16									
16									
15							1.04	1.04	1.04
15							0.96	1.01	0.99
14							0.97	1.04	0.97
14							0.93	1	1.04
13	1	1.02	1.02				1	1.01	1.02
13	0.95	0.98	1.02				1.01	1.01	1.04
12	1.02	1	1		1.02	0.99	1.04	0.97	1.01
12	1.02	1.02	0.95		1.02	1.02	0.96	0.94	0.98
11	1.02	1	1.02		1.02	0.99	0.96	0.96	0.96
11	0.95	0.95	1		0.98	0.99	1.04	0.64	0.96
10	1.02	0.99	0.99		1	0.99	0.92	0.56	0.85
10	1.02	1.02	0.99		1.02	1.02	0.99	0.52	0.8
9	1.02	0.91	1.02		0.95	1.02	0.81	0	0.1
9	1.02	0.74	0.95		1.02	0.96	0.64	0	0.31
8	0.93	0	0.82	0.92	0.88	0.87	0.35	0	0
8	0.93	0	0.88	0.97	0.82	0.96	0	0	0
7	0.38	0	0.15	0.99	0.82	0.73	0.23	0	0
7	0.68	0	0.68	1.02	0.89	0.97	0	0	0
6	0.31	0	0.15	0.97	0.79	0.79	0		
6	0.11	0	0	1.02	0.65	0.45	0		
5	0	0	0	0.99	0.83	0.51	0	0	0
5	0.73	0	0	0.96	0.22	0.68	0	0	0
4				0.96	0	0	0		
4				0.97	0	0	0		
3	0	0	0	1.02	0		0	0	0
3	0	0	0	0.88	0		0	0	0
2				0.27			0		
2				0.09			0		
1	0	0	0	0.25	0	0	0	0	0
1	0	0	0	0.11	0	0	0	0	0

Egg source:			Tag 17						
Tag	12	18	19	3	10	17	5	8	16
LC50	0.17	0.08	0.08	0.52	0.24	0.165	0.08	0.05	0.04
R2	0.77	0.98	0.99	0.9	0.82	0.99	0.89	0.95	0.99
Conc	12	18	19	3	10	17	5	8	16
17									
17									
16									
16									
15							1.06	0.99	1.04
15							1.1	0.99	1.08
14		1					1.05	1.1	1.08
14							1.06	1.06	1.03
13		1					1.1	1.08	1.07
13		1					1.07	1.02	1.04
12	1.02	0.99	1.02				1.05	1.08	1.08
12	0.88	0.95	1				1.03	1.08	1.08
11	1.02	0.93	0.98		1.02	1.016	0.99	1.08	1.03
11	1	0.99	0.96		1.02	1.001	1.1	1.07	1.04
10	1	0.94	0.99		0.99	0.997	0.37	0	0.03
10	0.96	0.98	1		0.97	1.016	0.85	0.76	0
9	1.02	0.76	0.76		0.94	1.018	0.92	0	0
9	0.97	0.99	0.99		0.99	1.016	0.98	0	0
8	0.95	0.06	0.06	1.02	0.94	1.018	0.3	0	0
8	0.89	0.3	0.3	0.95	0.99	0.968	0.27	0.11	0
7	0.81		0	0.97	0.97	0.947			
7	0.93		0	1.02	0.94	0.956			
6	0	0	0	1.02	0.88	0.046	0	0	0
6	0.83	0	0	1.02	0.25	0.313	0	0	0
5	0		0	0.97	0.97	0			
5	0		0	1.02	0.06	0			
4	0.74	0	0	1.02	0.1	0	0	0	0
4	0	0	0	0.98	0	0	0	0	0
3				0.82	0	0			
3				0.54	0	0			
2	0	0	0	0	0	0	0	0	0
2	0	0	0	0.41	0	0	0	0	0

Week 12 **Test substance = contents**

Egg source: Tag 12

Tag	12	18	19	3	10	17	5	8	16
LC50	0.08	0.06	0.07	0.05	0.04	0.06	0.05	0.03	0.02
R2	0.94	0.98	0.97	0.94	0.99	0.99	0.98	0.98	0.97

Test	Contents								
Conc	12	18	19	3	10	17	5	8	16
17									
17									
16				0.94			1.04	0.98	1.06
16				1.03			1	1.06	1.04
15				1.03	0.99		1.03	1.06	1.03
15				1	0.98		1	1.02	1
14	1.02	1.06	1.06	1	1.03	1.03	1	1.06	1.03
14	1.02	1	1.06	0.99	1.03	1.03	1.04	1.04	0.95
13	0.97	0.99	1.02	0.97	0.99	0.98	1.04	1.04	1
13	1.01	1.03	1	1	1.03	0.98	1.01	1.04	1.03
12	1.01	1.02	0.95	1.03	0.94	0.98	1	0.9	0.44
12	1.01	1.03	1	0.98	0.96	0.89	1.03	0.86	0.56
11	1.06	1.06	1.02	0.8	0.83	1	0.82	0.79	0.33
11	0.99	1	1.02	0.46	0.9	1.03	0.89	0.83	0.53
10	0.96	0.98	1.03	1.03	0.72	0.84	0.95	0.44	0
10	1.03	0.95	0.97	1.03	0.58	0.95	0.8	0	0
9	0.4	0.53	0.88	0.13	0.3	1	0.85	0	0
9	0.93	0.81	0.65	0	0.06	0.94	0.53	0	0
8	0.73	0.4	0.7			0.22	0		
8	0.79	0.7	0.75			0.09	0		
7	0.41	0	0	0	0	0	0	0	0
7	0.23	0	0	0	0	0	0	0	0
6	0.06								
6	0								
5	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0
4									
4									
3	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0
2									
2									
1	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0

Egg source: Tag 18

Tag	12	18	19	3	10	17	5	8	16
LC50	0.05	0.04	0.05	0.03	0.03	0.04	0.04	0.03	0.02
R2	0.98	0.93	0.99	0.85	0.97	0.96	0.99	0.91	0.94

Conc	12	18	19	3	10	17	5	8	16
17									1.17
17									1.17
16		1.09		0.99	1.05	1.02	1.17	1.12	1.14
16		1.09		1.02	1	1.02	1.11	1.17	1.12
15		1.07		0.98	1.05	1.02	1.17	1.17	1.17
15		1.06		1.02	1.05	1	1.14	1.17	1.17
14	1.09	1.03	1.07	1.02	1.02	1.02	1.17	1.17	1.17
14	1.07	1.08	1.04	0.87	1.02	1.05	1.17	1.14	1.09
13	1.04	1.02	1.01	1.03	0.95	1	1.05	1.17	1.17
13	1.07	1.09	1	0.98	1.05	1.05	1.13	1.17	1.17
12	1.05	1.07	1.09	1.05	1.02	0.96	1	1.12	0.9
12	1.04	1.07	1.09	1.05	0.98	0.98	1.08	1.17	1.17
11	0.98	1.06	1.09	0.87	0.85	0.99	0.97	1.06	0.17
11	1.02	1.07	1.09	0	0.89	1.05	1.03	1.17	0.7
10	1.02	0.93	1.09	0.57	0.15	0.86	1.17	1.17	0.58
10	0.93	0.08	1.09	0.81	0.7	0.63	1.06	0	0
9	0.34	0.16	0.22	0	0.07	0.12	0	0.19	0.11
9	0.55	0.5	0	0.12	0.15	0.7	0	0	0
8	0.46	0	0.04						
8	0	0	0						
7	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0
6									
6									
5	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0
4									
4									
3	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0
2									
2									
1	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0

Egg source: Tag 19

Tag	12	18	19	3	10	17	5	8	16
LC50	0.04	0.02	0.03	0.02	0.03	0.04	0.07	0.02	0.02
R2	0.99	0.75	0.93	0.99	0.98	0.99	0.87	0.99	0.99

Test	Contents								
Conc	12	18	19	3	10	17	5	8	16
17									1.05
17									1.05
16		1.07	1.07	1.07	1.07	1.02		1.05	1.05
16		1.04	1.02	1.07	1.07	1.07		1	1.01
15	1.07	1.02	1.04	1.01	1.07	1.04	1.05	1.05	1.05
15	1.07	1.03	1.07	1.07	1.07	1.07	1.05	1.05	1.05
14	1.07	1.07	1.07	1.07	1.07	1.04	1.02	0.96	1.05
14	1.07	1.03	1.07	1.07	1.07	1.07	1.05	1.05	1.02
13	1	1.07	1.03	1.03	1.04	1.07	1.05	1.05	1.02
13	1.07	1.04	1.07	1.07	1.03	1.07	1.05	1.02	1
12	1.07	0.98	0.97	0.77	1	1.04	0.98	0.98	0.45
12	1.02	1.01	1.01	0.84	1.07	0.95	1.05	0.94	0.66
11	1.07	0.93	0.78	0.36	0.67	1	0.99	0.12	0
11	1.04	0.57	0.49	0.27	0.94	0.87	0.98	0.42	0.12
10	1	0.86	0.1	0.05	0.44	0.73	0.35	0	0
10	0.73	0.51	0.44	0.09	0.62	0.57	0.61	0.05	0
9	0	0.08	0	0	0	0.08	0.94	0	0.1
9	0.14	0	0.56	0	0	0.08	0.81	0.07	0.05
8	0						0.6	0	
8	0						0.76	0	
7	0.05	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0
6									
6									
5	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0
4									
4									
3	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0
2									
2									
1	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0

Egg source: Tag 10

Tag	12	18	19	3	10	17	5	8	16
LC50	0.05	0.04	0.04	0.03	0.04	0.04	0.05	0.03	0.03
R2	0.99	0.99	0.99	0.99	0.99	0.94	0.89	0.99	0.99

Test	Contents								
Conc	12	18	19	3	10	17	5	8	16
17									
17									
16				1.02	0.94	1.02		1.01	1.01
16				0.99	1.02	1.02		1.01	1.01
15		1.03	0.99	1.02	1.02	1.02	0.95	1.01	1.01
15		1.03	1.03	1.02	1.02	1.02	1.01	1.01	1.01
14	1.03	0.99	1.03	0.98	0.98	1.02	1.01	0.99	1.01
14	1.03	1.03	1.03	0.99	0.99	1.02	0.97	1.01	0.97
13	0.99	1.03	1	0.98	0.98	1.02	1.01	0.99	1.01
13	1.03	1.03	1	1.02	1.02	1.02	1.01	1.01	1.01
12	0.99	0.98	0.98	1.02	0.96	1.02	0.96	0.99	1.01
12	0.98	1.03	0.99	0.97	0.99	0.96	1.01	0.93	1.01
11	0.99	0.97	0.97	0.71	0.71	0.73	1.01	0.97	0.83
11	1.01	0.99	0.99	0.94	0.94	0.93	0.97	0.83	0.96
10	0.98	1.03	0.93	0	0.78	0.84	0.17	0	0
10	1.03	0.87	0.99	0	0.8	0.51	0.9	0	0
9	0.59	0.21	0	0	0	0.79	0.63	0	0
9	0.31	0	0	0	0	0.17	0.72	0	0
8							0		
8							0		
7	0	0	0	0	0	0	0.2	0	0
7	0	0	0	0	0	0	0	0	0
6									
6									
5	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0
4									
4									
3	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0
2									

Egg source: Tag 17

Tag	12	18	19	3	10	17	5	8	16
LC50	0.14	0.05	0.05	0.04	0.05	0.06	0.07	0.04	0.02
R2	0.85	0.99	0.98	0.97	0.99	0.97	0.95	0.99	0.99

Test	Contents								
Conc	12	18	19	3	10	17	5	8	16
17									
17									
16									1.02
16									1.02
15				0.99				1.02	1.02
15				1.01				0.97	0.95
14	1.04	1.04	1	0.96	1.01	0.95		0.99	0.99
14	1.01	1.04	0.98	1.01	0.96	0.96		0.98	1
13	1.04	1.04	1.01	1.01	1.01	1.01	1	1	1
13	1	1.04	1.01	0.99	0.98	0.95	0.99	0.98	1.02
12	1.04	1.04	1.02	0.97	1.01	1.01	1.02	0.95	0.88
12	1.04	0.99	1.02	0.98	1.01	1.01	0.98	0.95	0.98
11	1.04	1.04	1.04	0.9	1.01	1.01	1.02	0.95	0.44
11	1.04	1.04	1.04	0.84	0.99	1.01	0.97	0.86	0.34
10	1.01	1.04	0.91	0.99	0.98	0.79	1.02	0.58	0.14
10	1.04	0.9	0.85	0.61	0.92	0.93	0.92	0.78	0.04
9	0.73	0.39	0.72	0.15	0.61	0.72	0.98	0.04	0
9	0.95	0.54	0.56	0.37	0.72	0.81	0.91	0.04	0
8	0.24	0.45	0.09		0.25	0.61	0.94	0	
8	0.79	0.32	0.42		0.25	0.25	0.29	0	
7	0.96	0	0	0	0	0.07	0.19	0	0
7	0.93	0.12	0	0	0	0	0.04	0	0
6	0.52	0					0		
6	0.45	0					0		
5	0.09	0.05	0	0	0	0	0	0	0
5	0.57	0	0	0	0	0	0	0	0
4	0								
4	0								
3	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0
2									

Week 15	Test substance = mucus																			
Egg source:			Tag 2							Egg source:			Tag 9							
Tag	2	9	1	7	15	20	4	6	13	Tag	2	9	1	7	15	20	4	6	13
LC50	0.08	0.05	0.05	0.04	0.02	0.03	0.07	0.04	0.03	LC50	0.14	0.06	0.07	0.03	0.02	0.02		0.05	
R2	0.92	0.94	0.89	0.95	0.99	0.96	0.97	0.99	0.96	R2	0.99	0.98	0.96	0.94	0.95	0.97		0.77	
Conc	2	9	1	7	15	20	4	6	13	Conc	2	9	1	7	15	20	4	6	13
17					1.02					17					1.02				
17					0.99					17					1.02				
16			1.01		1.02	1.02				16				1.02	1.02	1.02			1.01
16			0.98		0.96	1.02				16				1.02	0.99	1.02			1.01
15		1.04	1.04	0.97	1.02	0.99	0.96		1.01	15			0.98	1.02	1.02	1.02		0.95	0.98
15		0.99	1.01	0.98	1.02	1.02	1.01		0.7	15			1.01	1.02	1.02	1.02		0.95	0.98
14	1.04	1.04	0.96	1.02	1.02	1.02	1.01	1.01	1.01	14			1.01	0.94	1.02	0.96		0.96	1.01
14	1.04	1.04	0.98	0.97	1.02	0.99	1.01	0.98	1.01	14			1.01	1.02	0.71	1.02		1.01	1.01
13	0.95	1.04	0.94	0.96	0.98	0.84	1.01	0.9	0.97	13	1.03	1.03	1.01	1.02	0.93	0.95	0.97	0.95	1.01
13	0.96	1.04	1.01	1.02	1.02	0.96	0.87	1.01	1.01	13	1	1.01	1.01	1.02	0.94	1.02	0.96	1.01	1.01
12	0.88	0.82	0.62	0.96	0.76	0.94	0.95	0.93	1.01	12	0.99	0.97	0.97	1.02	0	0.97	1.01	0.89	1.01
12	0.99	0.96	0.97	0.85	0.51	0.98	1.01	0.91	0.97	12	1.03	1.03	1.01	0.97	0.34	0.97	0.84	0.92	1.01
11	1.04	1	1	0.92	0	0.51	0.96	1.01	0.91	11	1	1.03	1.01	0.68	0	0.51	1.01	0.5	0
11	0.97	0.96	1.04	0.9	0.25	0.79	0.94	0.93	0.71	11	1.03	1.01	1.01	1.02	0.34	0	0.98	0.95	0
10	0.94	1.04	0.58	0.95	0	0.38	0.91	0.59	0.27	10	1.03	0.99	0.86	0.2			0.86	0	0.4
10	0.94	0.84	0.62	0.34	0	0.68	0.97	0.71	0.65	10	0.97	1.03	0.96	0.61			1.01	0.86	0
9	1.04	0.76	0.42	0.11	0	0	0.79	0.1	0.13	9	0.99	0.92	0.87	0.51	0	0	1.01	0.81	0
9	0.69	0	0.62	0.25	0	0	0.76	0.08	0	9	1	0.81	0.55	0	0	0	1.01	1.01	0.5
8	0.9	0	0.78	0			0.79		0	8	0.96	0.64	0.46				0.96	0	
8	0.61	0.09	0	0			0.62		0.07	8	1.03	0.26	0.69				0.93	0	
7	0	0	0	0.09	0	0	0	0	0	7	1.03	0	0	0	0	0	0	0	0
7	0.52	0	0	0	0	0	0.05	0	0	7	0.8	0	0.2	0	0	0	1.01	0	0
6	0.37									6	0.68						1.01		
6	0.07									6	0.51						0.81		
5	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0
4										4									
4										4									
3	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0
2										2									
2										2									
1	0	0	0	0	0	0	0	0	0	1	0	0	0.2	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0

Week 15	Test substance = contents																			
Egg source:			Tag 2							Egg source:			Tag 9							
Tag	2	9	1	7	15	20	4	6	13	Tag	2	9	1	7	15	20	4	6	13
LC50	0.09	0.03	0.06	0.04	0.02	0.03	0.02	0.01	0.01	LC50	0.06	0.03	0.03	0.02	0.02	0.02	0.01	0.02	0.02
R2	0.99	0.96	0.99	0.96	0.98	0.94	0.98	0.99		R2	0.98	0.96	0.83	0.82	0.99	0.98	0.99	0.99	0.94
Conc	2	9	1	7	15	20	4	6	13	Conc	2	9	1	7	15	20	4	6	13
17							1.01	1.01	1.04	17				1.04	1.04	0.96	1.03	1.03	0.92
17							1.04	1.04	1.01	17				1.04	0.92	1.04	1.03	0.91	0.93
16				0.97	1.01	1.01	0.95	1.04	1.04	16		1.03	1.03	1.04	1.04	1.04	1.03	0.95	1.03
16				1.01	1.01	1.01	1.02	1	1	16		0.96	1.03	1.04	1.04	1.04	0.92	1.03	0.95
15		1.05		1.01	0.96	1.01	1.04	0.99	1	15		1.03	0.98	1.04	1.04	1.04	1.03	0.93	0.65
15		1.05		0.95	1.01	1.01	0.85	1.04	1.04	15		1.03	1.03	1.04	1.04	1.04	1.03	0.91	1.03
14		1	1.05	1.01	1.01	0.97	1.04	0.99	1.04	14	0.99	1.03	1.03	1.04	1.04	1.04	0.97	1.03	1.03
14		1.02	1.05	0.93	1.01	0.98	0.85	0.67	1.04	14	1.03	0.99	1	1.04	0.91	0.95	1.03	1.03	0.69
13		1.05	1.05	1.01	1.01	1.01	0.83	0.59	0.87	13	0.97	1.03	1.03	1.04	1.04	1.04	0.78	1.03	0.69
13		1.02	0.98	0.97	1.01	1.01	0.57	0.35	0.87	13	0.98	1.03	1.03	1.04	1.04	0.95	0.69	1.03	1.03
12	1.01	1	1.05	0.61	0.61	1.01	1.04	0	0.09	12	1	0.82	1.03	0	0	0.52	0	0	0
12	1.03	0.94	1.02	0.9	0.72	0.89	1.04	0	0.04	12	1.03	0.69	0.91	0.83	0	0.87	0	0	0.34
11	1.05	1.05	0.98	1.01	0.34	0.86	0.22	0	0	11	1.03	0.77	0.95	0.35	0	0	0	0	0
11	1.03	0.6	0.97	0.75	0	0.91	0.43	0	0	11	1.03	0.51	0.89	0.92	0	0.26	0	0	0
10	1.02	0.23	1.05	1.01		0.84				10	1.03	0.34	0	0					
10	1	0.24	1.05	0.79		0				10	0.84	0	0.51	0.42					
9	0.91	0	0.7	0	0	0	0	0	0	9	0.73	0	0	0	0	0	0	0	0
9	1.01	0	0.74	0.17	0	0	0	0	0	9	0.96	0	0.8	0	0	0	0	0	0
8	0.93	0.19	0.22	0						8	0.17	0							
8	0.97	0	0.3	0.04						8	0	0							
7	0.28	0	0	0						7	0	0					0	0	0
7	0.48	0.12	0.12	0						7	0.06	0					0	0	0
6	0			0						6									
6	0			0						6									
5	0	0	0	0.22	0	0	0	0	0	5	0	0	0	0	0	0	0	0	
5	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	
4				0						4									
4				0						4									
3										3							0	0	0
3										3							0	0	0
2										2									
2										2									
1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0

Appendix 7f Statistical Analysis – Chapter 7

Dependent Variable: FEC

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	2	42428031	21214016	391.68	0.0001
WEEK	16	41907464	2619217	48.36	0.0001
TAG(TRT)	17	4422683	260158	4.80	0.0001
WEEK*TRT	32	46950830	1467213	27.09	0.0001

Tests of Hypotheses using the Type III MS for TAG(TRT) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	2	42428031	21214016	81.54	0.0001

WEEK=9

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	801726.35	801726.35	6.33	0.0305

WEEK=10

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	3898937.6	3898937.6	11.35	0.0071

WEEK=11

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	5144528.2	5144528.2	23.47	0.0007

WEEK=12

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	7776495.8	7776495.8	38.17	0.0001

WEEK=13

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	8556474.1	8556474.1	40.02	0.0032

WEEK=14

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	3324321.3	3324321.3	298.05	0.0001

WEEK=15

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	13230000	13230000	590.05	0.0001

Dependent Variable: ln(developmental success + 1)

Source	DF	Type III SS	Mean Square	F Value	Pr > F
WEEK	11	16.298081	1.481644	3.06	0.0015
TRT	1	0.091291	0.091291	0.19	0.6653
TAG(TRT)	10	10.548151	1.054815	2.18	0.0256
WEEK*TRT	11	8.525140	0.775013	1.60	0.1112

Tests of Hypotheses using the Type III MS for TAG(TRT) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	0.0912910	0.0912910	0.09	0.7746

WEEK=3

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	1.6228837	1.6228837	3.99	0.0738

WEEK=7

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	2.4359933	2.4359933	3.91	0.0762

WEEK=8

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	0.5510839	0.5510839	5.64	0.0389

LDA - control wells only

Dependent Variable: developmental success in an LDA

Source	DF	Type III SS	Mean Square	F Value	Pr > F
WEEK	7	19092.302	2727.472	12.71	0.0001
TRT	1	4729.455	4729.455	22.05	0.0001
TAG(TRT)	10	9920.707	992.071	4.62	0.0001
WEEK*TRT	7	20940.961	2991.566	13.95	0.0001

Tests of Hypotheses using the Type III MS for TAG(TRT) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	4729.4545	4729.4545	4.77	0.0539

Dependent Variable: ln(TcL3 + 1)

Source	DF	Type III SS	Mean Square	F Value	Pr > F
WEEK	16	0.5256227	0.0328514	6.77	0.0001
TRT	2	4.1112999	2.0556499	423.67	0.0001
TAG(TRT)	17	2.5553410	0.1503142	30.98	0.0001
WEEK*TRT	32	1.8425249	0.0575789	11.87	0.0001

Tests of Hypotheses using the Type III MS for TAG(TRT) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	2	4.1112999	2.0556499	13.68	0.0003

By week

WEEK=4

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	2	0.1221929	0.0610964	4.45	0.0304

WEEK=5

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	2	0.1093266	0.0546633	4.55	0.0286

WEEK=6

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	2	0.1943779	0.0971890	6.99	0.0072

WEEK=7

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	2	0.3700977	0.1850489	12.98	0.0005

WEEK=8

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	2	0.5914439	0.2957219	22.18	0.0001

WEEK=9

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	2	0.5805758	0.2902879	19.30	0.0001

WEEK=10

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	2	0.7459687	0.3729843	27.43	0.0001

WEEK=11

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	2	0.8038932	0.4019466	38.35	0.0001

WEEK=12

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	2	1.0151037	0.5075519	56.70	0.0001

WEEK=13

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	2	0.4997227	0.2498614	40.62	0.0003

WEEK=14

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	2	0.6515073	0.3257537	62.07	0.0001

WEEK=15

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	2	0.6057013	0.3028506	52.63	0.0002

Dependent Variable: ln(TcAd + 1)

Source	DF	Type III SS	Mean Square	F Value	Pr > F
WEEK	16	0.0828908	0.0051807	2.37	0.0028
TRT	2	1.0046625	0.5023312	230.16	0.0001
TAG(TRT)	17	1.3243075	0.0779004	35.69	0.0001
WEEK*TRT	32	0.3305536	0.0103298	4.73	0.0001

Tests of Hypotheses using the Type III MS for TAG(TRT) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	2	1.0046625	0.5023312	6.45	0.0082

By week

WEEK=4

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	2	0.0424855	0.0212428	4.62	0.0274

WEEK=5

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	2	0.0406224	0.0203112	4.92	0.0227

WEEK=6

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	2	0.0722440	0.0361220	5.97	0.0124

WEEK=7

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	2	0.1248321	0.0624161	7.92	0.0045

WEEK=8

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	2	0.1373528	0.0686764	9.10	0.0026

WEEK=9

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	2	0.1496535	0.0748268	6.71	0.0083

WEEK=10

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	2	0.1828171	0.0914086	7.05	0.0070

WEEK=11

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	2	0.1867651	0.0933826	8.70	0.0031

WEEK=12

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	2	0.2004062	0.1002031	8.93	0.0028

Dependent Variable: Worm burden

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	698819641.7	232939880.6	20.26	0.0004
Error	8	91978650.0	11497331.3		
Corrected Total	11	790798291.7			

R-Square	C.V.	Root MSE	WORMS Mean
0.883689	26.57518	3390.771	12759.17

Source	DF	Type I SS	Mean Square	F Value	Pr > F
WEEK	1	237719008.3	237719008.3	20.68	0.0019
TRT	1	383499298.0	383499298.0	33.36	0.0004
WEEK*TRT	1	77601335.3	77601335.3	6.75	0.0317

Source	DF	Type III SS	Mean Square	F Value	Pr > F
WEEK	1	102921617.6	102921617.6	8.95	0.0173
TRT	1	43320614.0	43320614.0	3.77	0.0882
WEEK*TRT	1	77601335.3	77601335.3	6.75	0.0317

Worm counts
Week 12

Dependent Variable: Worm burden

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	1	67335000.00	67335000.00	5.16	0.0857

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	67335000.00	67335000.00	5.16	0.0857

Dependent Variable: Male/Female-ratio

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	1	0.02694204	0.02694204	1.93	0.2366

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	0.02694204	0.02694204	1.93	0.2366

Dependent Variable: Female worm length

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	1	1.79306667	1.79306667	96.23	0.0006

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	1.79306667	1.79306667	96.23	0.0006

Dependent Variable: Male worm length

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	1	0.17340000	0.17340000	3.20	0.1481

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	0.17340000	0.17340000	3.20	0.1481

Dependent Variable: *In utero* egg counts

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	1	240.6666667	240.6666667	51.57	0.0020

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	240.6666667	240.6666667	51.57	0.0020

Worm counts
Week 15

Dependent Variable: Worm burden

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	1	393765633.3	393765633.3	39.64	0.0033

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	393765633.3	393765633.3	39.64	0.0033

Dependent Variable: Male/Female-ratio

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	1	0.00163100	0.00163100	0.07	0.8023

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	0.00163100	0.00163100	0.07	0.8023

Dependent Variable: Female worm length

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	1	0.96123000	0.96123000	33.59	0.0102

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	0.96123000	0.96123000	33.59	0.0102

Dependent Variable: Male worm length

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	1	0.14283000	0.14283000	1.65	0.2893

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	0.14283000	0.14283000	1.65	0.2893

Dependent Variable: *In utero* egg counts

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	1	367.5000000	367.5000000	441.00	0.0002

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	367.5000000	367.5000000	441.00	0.0002

Histology

Dependent Variable: Mucosal mast cells (MMC)

Source	DF	Type I SS	Mean Square	F Value	Pr > F
WEEK	1	4900.000	4900.000	2.53	0.1229
TRT	2	94561.476	47280.738	24.37	0.0001
SECTION	1	841.000	841.000	0.43	0.5155
WEEK*TRT	2	2819.524	1409.762	0.73	0.4922

Source	DF	Type III SS	Mean Square	F Value	Pr > F
WEEK	1	734.827	734.827	0.38	0.5431
TRT	2	90556.456	45278.228	23.33	0.0001
SECTION	1	841.000	841.000	0.43	0.5155
WEEK*TRT	2	2819.524	1409.762	0.73	0.4922

Dependent Variable: Globule leukocytes (GL)

Source	DF	Type I SS	Mean Square	F Value	Pr > F
WEEK	1	11772.25	11772.25	2.11	0.1571
TRT	2	153601.31	76800.65	13.77	0.0001
SECTION	1	2131.36	2131.36	0.38	0.5413
WEEK*TRT	2	6045.20	3022.60	0.54	0.5875

Source	DF	Type III SS	Mean Square	F Value	Pr > F
WEEK	1	2821.00	2821.00	0.51	0.4827
TRT	2	149467.31	74733.66	13.40	0.0001
SECTION	1	2131.36	2131.36	0.38	0.5413
WEEK*TRT	2	6045.20	3022.60	0.54	0.5875

Dependent Variable: Eosinophils (EOS)

Source	DF	Type I SS	Mean Square	F Value	Pr > F
WEEK	1	4268.444	4268.444	1.12	0.2989
TRT	2	64371.364	32185.682	8.43	0.0013
SECTION	1	11881.000	11881.000	3.11	0.0882
WEEK*TRT	2	4450.998	2225.499	0.58	0.5645

Source	DF	Type III SS	Mean Square	F Value	Pr > F
WEEK	1	8332.827	8332.827	2.18	0.1503
TRT	2	59819.367	29909.683	7.84	0.0019
SECTION	1	11881.000	11881.000	3.11	0.0882
WEEK*TRT	2	4450.998	2225.499	0.58	0.5645

Week 12

Dependent Variable: MMC

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	2	98669.305	49334.652	26.81	0.0001
Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	2	98669.305	49334.652	26.81	0.0001

Dependent Variable: GL

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	2	161466.95	80733.47	15.32	0.0001
Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	2	161466.95	80733.47	15.32	0.0001

Dependent Variable: EOS

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	2	58695.539	29347.769	7.07	0.0028
Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	2	58695.539	29347.769	7.07	0.0028

Tukey's Studentized Range (HSD) Test for variable: MMC
Alpha= 0.05
Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	TRT
A	162.57	14	2
A			
A	136.17	12	3
B	36.20	10	1

Tukey's Studentized Range (HSD) Test for variable: GL

Tukey Grouping	Mean	N	TRT
A	139.79	14	2
B	4.50	12	3
B			
B	0.00	10	1

Tukey's Studentized Range (HSD) Test for variable: EOS

Tukey Grouping	Mean	N	TRT
A	128.00	14	2
A			
B A	87.42	12	3
B			
B	27.70	10	1

Week 15

```
Dependent Variable: MMC
Source                     DF     Type I SS  Mean Square   F Value     Pr > F
TRT                         2     98669.305    49334.652     26.81     0.0001
Source                     DF   Type III SS  Mean Square   F Value     Pr > F
TRT                         2     98669.305    49334.652     26.81     0.0001

Dependent Variable: GL
Source                     DF     Type I SS  Mean Square   F Value     Pr > F
TRT                         2     161466.95     80733.47     15.32     0.0001
Source                     DF   Type III SS  Mean Square   F Value     Pr > F
TRT                         2     161466.95     80733.47     15.32     0.0001

Dependent Variable: EOS
Source                     DF     Type I SS  Mean Square   F Value     Pr > F
TRT                         2     58695.539    29347.769      7.07     0.0028
Source                     DF   Type III SS  Mean Square   F Value     Pr > F
TRT                         2     58695.539    29347.769      7.07     0.0028

Tukey's Studentized Range (HSD) Test for variable: MMC
Tukey Grouping                  Mean       N  TRT
                  A            162.57      14  2
                  A
                  A            136.17      12  3

                  B             36.20      10  1

Tukey's Studentized Range (HSD) Test for variable: GL
Tukey Grouping                  Mean       N  TRT
                  A            139.79      14  2

                  B              4.50      12  3
                  B
                  B              0.00      10  1

Tukey's Studentized Range (HSD) Test for variable: EOS
Tukey Grouping                  Mean       N  TRT
                  A            128.00      14  2
                  A
          B       A             87.42      12  3
          B
          B                     27.70      10  1
```

Modified LDA with contents or mucus

```
Dependent Variable: LC50
                                  Sum of          Mean
Source                     DF     Squares        Square   F Value     Pr > F
Model                       9   0.3644139     0.0404904     12.44     0.0001
Error                      87   0.2830599     0.0032536
Corrected Total            96   0.6474738

                  R-Square          C.V.      Root MSE          LC50 Mean
                  0.562824      64.57060        0.0570             0.0883

Source                     DF     Type I SS  Mean Square   F Value     Pr > F
TSTSUBST                    1   0.1701619     0.1701619     52.30     0.0001
TSTTRT                      2   0.1367120     0.0683560     21.01     0.0001
EGGTRT                      1   0.0016610     0.0016610      0.51     0.4768
TSTTAG(TSTTRT)              3   0.0416643     0.0138881      4.27     0.0073
EGGTAG(EGGTRT)              2   0.0142147     0.0071073      2.18     0.1187

Source                     DF   Type III SS  Mean Square   F Value     Pr > F
TSTSUBST                    1   0.1888366     0.1888366     58.04     0.0001
TSTTRT                      2   0.0648451     0.0324226      9.97     0.0001
EGGTRT                      1   0.0088159     0.0088159      2.71     0.1034
TSTTAG(TSTTRT)              3   0.0410611     0.0136870      4.21     0.0079
EGGTAG(EGGTRT)              2   0.0142147     0.0071073      2.18     0.1187

Tests of Hypotheses using the Type III MS for TSTTAG(TSTTRT) as an error term
Source                     DF   Type III SS  Mean Square   F Value     Pr > F
TSTTRT                      2   0.0648451     0.0324226      2.37     0.2414

Tests of Hypotheses using the Type III MS for EGGTAG(EGGTRT) as an error term
Source                     DF   Type III SS  Mean Square   F Value     Pr > F
EGGTRT                      1   0.0088159     0.0088159      1.24     0.3813
```

By week and by test substance:

Week 12

TSTSUBST=Contents
Dependent Variable: LC50

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	0.0121378	0.0015172	7.18	0.0001
Error	36	0.0076114	0.0002114		
Corrected Total	44	0.0197492			

R-Square	C.V.	Root MSE	LC50 Mean
0.614598	33.57026	0.0145	0.0433

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TSTTRT	2	0.0023189	0.0011595	5.48	0.0083
EGGTRT	1	0.0007068	0.0007068	3.34	0.0758
TSTTAG(TSTTRT)	3	0.0049777	0.0016592	7.85	0.0004
EGGTAG(EGGTRT)	2	0.0041344	0.0020672	9.78	0.0004

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TSTTRT	2	0.0041617	0.0020808	9.84	0.0004
EGGTRT	1	0.0032001	0.0032001	15.14	0.0004
TSTTAG(TSTTRT)	3	0.0049777	0.0016592	7.85	0.0004
EGGTAG(EGGTRT)	2	0.0041344	0.0020672	9.78	0.0004

Tests of Hypotheses using the Type III MS for TSTTAG(TSTTRT) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TSTTRT	2	0.0041617	0.0020808	1.25	0.4019

Tests of Hypotheses using the Type III MS for EGGTAG(EGGTRT) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
EGGTRT	1	0.0032001	0.0032001	1.55	0.3395

TSTSUBST=Mucus
Dependent Variable: LC50

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	0.3705963	0.0463245	22.90	0.0001
Error	43	0.0869665	0.0020225		
Corrected Total	51	0.4575627			

R-Square	C.V.	Root MSE	LC50 Mean
0.809935	35.32748	0.0450	0.1273

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TSTTRT	2	0.2532820	0.1266410	62.62	0.0001
EGGTRT	1	0.0009556	0.0009556	0.47	0.4955
TSTTAG(TSTTRT)	3	0.1043138	0.0347713	17.19	0.0001
EGGTAG(EGGTRT)	2	0.0120449	0.0060224	2.98	0.0615

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TSTTRT	2	0.1748350	0.0874175	43.22	0.0001
EGGTRT	1	0.0093919	0.0093919	4.64	0.0368
TSTTAG(TSTTRT)	3	0.1055611	0.0351870	17.40	0.0001
EGGTAG(EGGTRT)	2	0.0120449	0.0060224	2.98	0.0615

Tests of Hypotheses using the Type III MS for TSTTAG(TSTTRT) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TSTTRT	2	0.1748350	0.0874175	2.48	0.2310

Tests of Hypotheses using the Type III MS for EGGTAG(EGGTRT) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
EGGTRT	1	0.0093919	0.0093919	1.56	0.3381

Week 15

TSTSUBST=Contents
Dependent Variable: LC50

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	0.0060224	0.0010037	12.15	0.0003
Error	11	0.0009087	0.0000826		
Corrected Total	17	0.0069311			

R-Square	C.V.	Root MSE	LC50 Mean
0.868890	30.49259	0.0091	0.0298

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TSTTRT	2	0.0027967	0.0013983	16.93	0.0004
TSTTAG(TSTTRT)	3	0.0026104	0.0008701	10.53	0.0015
EGGTAG(EGGTRT)	1	0.0006153	0.0006153	7.45	0.0196

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TSTTRT	2	0.0028637	0.0014318	17.33	0.0004
TSTTAG(TSTTRT)	3	0.0026104	0.0008701	10.53	0.0015
EGGTAG(EGGTRT)	1	0.0006153	0.0006153	7.45	0.0196

Tests of Hypotheses using the Type III MS for TSTTAG(TSTTRT) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TSTTRT	2	0.0028637	0.0014318	1.65	0.3293

TSTSUBST=Mucus
Dependent Variable: LC50

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	0.0112225	0.0018704	6.67	0.0063
Error	9	0.0025220	0.0002802		
Corrected Total	15	0.0137445			

R-Square	C.V.	Root MSE	LC50 Mean
0.816510	33.48624	0.0167	0.0500

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TSTTRT	2	0.0061810	0.0030905	11.03	0.0038
TSTTAG(TSTTRT)	3	0.0047722	0.0015907	5.68	0.0184
EGGTAG(EGGTRT)	1	0.0002693	0.0002693	0.96	0.3525

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TSTTRT	2	0.0041590	0.0020795	7.42	0.0125
TSTTAG(TSTTRT)	3	0.0047003	0.0015668	5.59	0.0192
EGGTAG(EGGTRT)	1	0.0002693	0.0002693	0.96	0.3525

Tests of Hypotheses using the Type III MS for TSTTAG(TSTTRT) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TSTTRT	2	0.0041590	0.0020795	1.33	0.3864

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