THE SELECTION OF SHEEP RESISTANT TO NEMATODE INFECTION

A thesis submitted in accordance with the requirements of the University of Liverpool for the Degree of Doctor of Philosophy.

by

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September 1995

DEDICATION

To my husband Chris, for his sacrifice, love and dedication and the memory of my grandparents, Mr and Mrs J Kelly who's love shall never be forgotten.

DECLARATION

The work presented in this thesis is my personal work which was carried out in the Department of Animal Husbandry at the University of Liverpool, Veterinary Field Station, Leahurst under the supervision of Mr. D.A.R. Davies and Professor M.J. Clarkson from October 1991 to October 1994, supported by a grant awarded by the Ministry of Agriculture.

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TABLE OF CONTENTS

ABSTRACT

A research programme was designed between October 1991 and October 1994 to investigate the possibility of selecting animals with increased resistance to gastrointestinal nematodes, and to develop a general evaluation procedure for a suitable selection programme.

In 1992 and 1993, Cambridge ewes, aged between 2 and 10 years, were used to investigate variability in faecal egg count (FEC) in the 4 weeks pre- to 4 weeks post-lambing.

The mean FEC was significantly higher in year 2, than in year 1 but there was a significant relationship between individual ewe counts in the two years. Two year old ewes had significantly higher counts than older ewes, in year 2 but litter size had no significant effect on egg output.

Lambs were faecal sampled during the summer months and a skewed distribution was seen in all 3 years. Faeces consistency was variable but there was no significant correlation between consistency of faeces and FEC. A significantly higher proportion of females than males had extremely low counts in groups of lambs in all 3 years (<200 FEC.). No effect of number of lambs reared was seen on FEC in all 3 years. In 1992 and 1993 there was no significant difference between sire groups. However, in 1994 there was a significant difference.

In Year 2, a sub-group of 64 of the lambs was placed indoors, given ivermectin and experimentally infected with either a single dose of 10,000 *O. circumcincta* infective larvae or 5 x 2,000 doses over a 10 day period.

In the experimental infections variation in FEC was reduced but still remained high and a skewed distribution was also seen as in natural infections. The highest correlations between one faecal sample and the overall mean of samples taken from 19 to 49 days post-infection occurred at 21 and at 27 days post-infection in single and trickle infection groups respectively.

Twenty-three of the above lambs were dosed with ivermectin and 2 weeks later placed on either a high or low concentrate diet and experimentally infected with 20,000 *O. circumcincta* larvae.

A significant correlation was seen between mean FEC and mean plasma pepsinogen concentrations. The effect of high or low concentrate diet on mean plasma pepsinogen concentrations was not significant but the effect of diet on mean individual FEC was significant. The numbers of adult *O. circumcincta* recovered at necropsy was significantly related to the mean FEC of each lamb but negatively correlated with the number of arrested larvae found.

Thirty-seven 12 month-old ram lambs were experimentally infected with approximately 20,000 infective larvae of *O. circumcincta* in order to compare infection rates with those of natural infections when they were lambs. A significant correlation was observed between the rams when they were experimentally infected at 12 months old and FEC of natural infections taken during the previous summer.

SUMMARY

Alternative strategies for nematode control in ruminants include breeding for resistance. In Northern Britain the most important species of nematode in sheep is *Ostertagia circumcincta,* although *Nematodirus battus* can cause scouring in young lambs in the spring..

A research programme was designed to investigate the possibility of selecting animals with increased resistance to this helminth, and to develop a general evaluation procedure for a suitable selection programme for resistance to nematodes.

The acceptance of parasite resistance into commercial breeding programmes will depend on the identification of methods of selection which are accurate, cost effective and repeatable. At present direct selection, using FEC seems to be the best method to fulfil the above criteria. However, measurements of other variables such as plasma pepsinogen concentrations, PCV's Live Weight Gain and eosinophil counts have been investigated.

In 1992 and 1993, 93 and 104 naturally infected Cambridge ewes, aged between 2 and 10 years, were used to investigate variability in FEC in the 4 weeks pre- and 4 weeks post-lambing period. The ewes were divided into two groups, one lambing in March (group 1) and the other lambing in April (group 2), and were housed 7 to 8 weeks prior to lambing.

In year 1, ewes were mated with Cambridge (3), Vendeen (1), Charollais (1), Suffolk (1) rams. In year 2 ewes were mated with Cambridge (8), Charollais (1), Texel (1) rams and in year 3 with 14 Cambridge rams.

The mean concentration of eggs was significantly higher in year 2 than in year 1, but there was a significant correlation ($r = 0.59$) between individual ewe counts in the two years. Actual values ranged from 38 to 1,370 in year 1 and 4 to 2,566 in year 2. These figures do not include 2 ewes in each year which had very high egg counts between 2,000 and 5,500 EPG during pregnancy and which had become so thin at one week post-lambing, it was thought necessary to administer an anthelmintic dose.

In year 2, two year old ewes had significantly higher egg counts and variability than older ewes, ($p < 0.05$) but litter size had no significant effect on egg output.

Litter size varied from 1 to 5 lambs and numbers reared from 1 to 3. Ewes and lambs were dosed with ivermectin at 6 weeks post-partum, i.e. circa three weeks post-turn-out. Thereafter in year 1, the lambs (177) were sampled on 3 occasions at a mean interval of 32 days from 14 to 22 weeks of age. In year 2, a faeces sample was taken from 208 lambs on two occasions within four days of each other at 16 weeks of age, at 30 days post-dosing with ivermectin. In year 3, 184 pure Cambridge lambs divided into 3 lambing groups were faecal sampled once pre-weaning when lambs varied from 14-16 weeks of age and twice post-weaning when aged 18-20 weeks; the latter samples were taken within 4 days of each other at 33-37 days post-weaning and drenching. In each year ,lambs were dosed with ivermectin post-sampling.

A skewed distribution was seen in 1992 with 29.5%, in 1993 42% and in 1994 with 49% of lambs having very low egg counts (<200) and with approx. 11% with high counts (>800) in 1992, 15.6% in 1993 and 8.7% in 1994.

Faeces consistency varied between a score of 1 to 5. There was a statistically significant relationship between faecal score and % dry matter, the regression coefficient being -0.635 ± 0.89 ($p<0.001$). There was however, no significant correlation between consistency of faeces and FEC in years 1, 2 and 3. No effect of faecal score was seen on live weight change in years 1, 2 and 3.

Some of the variation in FEC can be attributed to sex of the lamb, a significantly higher proportion ($p < 0.05$) of females than males had extremely low counts in all years (<200 EPG).

Analysis of variance tests however, demonstrated that there was no effect of sex on FEC of group 1 lambs in 1992, group 2 lambs in 1993, and group 2 and 3 lambs in 1994 although a significant effect was seen on group 2 lambs in 1992 ($p < 0.05$) group 1 lambs in 1993 ($p < 0.01$) and group 3 lambs in 1994 ($p < 0.05$).

In all 3 years there was no effect of number of lambs reared on lamb FEC.

In years 1 and 2, there was no overall significant effect of sire on FEC but differences were seen between individual sire groups. In year 3, however, a significant effect of sire ($p < 0.0001$) was seen on mean FEC.

In Year 2, a sub-group of 64 of the lambs sired by Cambridge rams was brought indoors, given ivermectin and experimentally infected with either a single dose of 10,000 *O. circumcincta* infective larvae or 5 x 2,000 doses over a 10 day period. This experimental infection was carried out in order to reduce phenotypic variation in FEC, due to grazing selectivity associated with unequal larval intake and to remove the effect of intercurrent infections which

influence the amount and dry matter content of faeces and also to relate output pattern to infection time and frequency. The lambs were blood sampled in order to measure plasma pepsinogen concentrations once pre-infection and then four times from 20 to 29 days post-infection. Faecal samples were taken twice weekly from 19 to 49 days post-infection. The lambs were weighed every week for 4 weeks.

In the experimental infections, variation in FEC was reduced but still remained high and a skewed distribution was also seen as in natural infections. No effect of FEC was seen on live weight change proberly due to the fact that only a small dose of larvae was given.

The highest correlations between one faecal sample and the overall mean of samples taken from 19 to 49 days post-infection occurred at 21 and 27 days post-infection in single and trickle infection groups, respectively.

Twenty-three of the above lambs were dosed with ivermectin and 2 weeks later placed on either a high (1200g/day) or low (750g/day) concentrate diet and experimentally infected with 20,000 *O. circumcincta* larvae. The lambs were divided into groups of 3 according to sex, male (M) or female (F), previous response to infection, high (H) or low (L) resistance and level of concentrate feeding, high (H) or low (L). Hay was available *ad libitum*. The lambs were faecal sampled twice weekly from 14 to 47 days post-infection and on 3 occasions blood samples were taken and plasma pepsinogen concentrations and eosinophil counts determined. Lambs were weighed weekly from 1 to 40 days post-infection. At 33 and 40 days post-infection, 6 and 8 lambs were sent to slaughter and their abomasums retained for examination. Adult and immature nematodes were recovered from the gut

contents and the abomasum digested in order to recover immature nematodes. The worms were counted as were the eggs in each female worm and any morphological difference noted.

A significant correlation ($r = 0.57$) was seen between mean FEC and mean plasma pepsinogen concentrations. The effect of high or low concentrate diet on mean plasma pepsinogen concentrations was not significant but the effect of diet on mean individual FEC was significant ($p < 0.05$). The association between eosinophil counts and FEC was significant with the two parameters correlating negatively ($r = -0.74$).

The numbers of adult *O. circumcincta* recovered at necropsy was significantly related to the mean FEC of each lamb ($r = 0.64$) but negatively correlated with the number of arrested larvae found ($r = -0.51$).

Seventeen parasite naive, experimentally reared lambs were first experimentally infected with a single dose of 10,000 *Ostertagia circumcincta* larvae when 9 weeks old and then again at 13½ weeks of age with a single dose of 20,000 larvae. The aims of the trial were to investigate if variation in response to a primary infection of *Ostertagia circumcincta* was apparent and if these differences correlated with those seen after a secondary challenge infection.

The correlation coefficient between the mean of the primary and the mean of the secondary infection was significant ($r=0.77$, $p< 0.001$). FEC were however, reduced suggesting some evidence of a self-cure reaction.

Thirty seven, 12 month old ram lambs were experimentally infected with approximately 20,000 infective larvae of *O. circumcincta* in order to compare

infection rates with those from infections acquired naturally when they were lambs. A significant correlation was observed between the FEC of rams when they were 12 months old and FEC of natural infections taken during the previous summer. Screening rams may thus be a more efficient method of selecting for resistant animals than screening progeny. Experimental infection of ram lambs may be a suitable method of identifying high resistant individuals.

ACKNOWLEDGEMENTS

I am grateful to the Ministry of Agriculture, Fisheries and Food who provided the financial support for this work.

I would like to express my sincere thanks to my supervisors, Mr. D. A. R. Davies for his advice, enthusiasm, guidance and friendship which I have greatly appreciated and I would also like to express my appreciation to Professor M. J. Clarkson for his advice, guidance and help.

I am indebted to Miss L. P. Turtle for her excellent technical assistance, especially with the plasma pepsinogen work which was performed with utmost professionalism. I am also extremely grateful for her continual encouragement and friendship.

I would also like to thank Mrs. A. W. Spence, Dr. T. L. J. Lawrence and Mrs. A. Tuson for their help and friendship over the last three years.

I would like to thank the Department of Veterinary Pathology for the use of their equipment especially Mr. M. Savage.

I would also like to thank Mr. N. Jones, Mr. R. Ellis and Mrs. A. Dennis, Animal Husbandry Farm, for their assistance with the animals.

I would also like to thank Dr. A. McKenzie and Dr. S. Pattinson, for their advice, friendship and encouragement.

I would like to thank Dr. R. Coop (Moredun Research Institute) for his advice and for providing the *Ostertagia circumcincta* larvae.

I am very grateful to my mother for her continual encouragement, advice, commitment, enthusiasm, faith and invaluable friendship. I would also like to thank my sisters for their encouragement and friendship.

I would especially like to thank my husband, Chris to whom I am indebted for his invaluable help, enthusiasm, encouragement, motivation, friendship and patience over the last few years, without which I would not have reached this far.

CHAPTER 1

LITERATURE REVIEW

1.1 Introduction

Sheep production is a major farming enterprise throughout the world. The main source of income for a sheep farmer in the UK is the sale of lambs for slaughter whereas in other areas of the world wool and milk production are the major outputs. Any factor which could adversely effect the productivity of the sheep farming system would cause a detrimental effect on the farm economy. As parasitic nematodes are capable of causing such an effect especially with the increasing frequency of anthelmintic resistant parasites, coupled with a consumer demand for chemical-free meat, alternative methods to chemotherapy for nematode control are being sought. Increasing host resistance to nematodes by genetic selection is one such alternative (Gray, 1991). There is well-documented evidence for genetic variation to gastro-intestinal nematodes both within and between breeds of sheep. However, better selection parameters to select for resistance to gastro-intestinal nematodes need to be employed in order to select young lambs quickly, economically and effectively.

The acceptance of parasite resistance into commercial breeding programmes will depend on the identification of methods of selection which are accurate, cost effective and repeatable. At present direct selection, using FEC seems to be the best method to fulfill the above criteria. However, measurements of other variables have been investigated.

1.2 Life cycle of trichostrongylid nematodes

Ostertagia circumcincta is the most prevalent nematode in the study therefore its lifecycle will be used as an example of gastro-intestinal nematodes as the other nematode lifecycles differ slightly.

The lifecycle of *Ostertagia circumcincta* follows the direct trichostrongylid pattern involving a single host.

Eggs are passed in the faeces of infected sheep. Development into first stage larvae (L_1) is influenced by temperature, moisture and oxygen tension (Soulsby, 1986). The first stage larvae feed on bacteria, and after a period of activity lasting about 12 hours, the L_1 stage enters a period of lethargy prior to the moult to the second stage larvae (L_2) which undergoes an incomplete moult to become the third stage larvae (L_3), which is the infective stage.

The infective larvae are distributed throughout the pasture by wind, rain and invertebrates such as earthworms and insects and also by fungal spores, (Bizzel and Ciordia, 1965).

Infective larvae are ingested by sheep grazing the contaminated pasture. Following ingestion, the third stage larvae exsheath. The third stage larvae penetrate the gut mucosa where they then moult to the fourth stage and finally the adult stage which starts to emerge from the mucosa. Development may be delayed at the 4^{th} larval stage as arrested larvae. The female worms lay eggs which can be observed in the faeces from 14 - 21 days post-infection (prepatent period), depending on the species.

1.3 Pathogenesis of gastrointestinal infection in sheep

The pathology caused by gastrointestinal nematodes varies depending on parasite species and location in the gastro-intestinal tract.. *Ostertagia. spp.* are associated with destruction of the abomasum's gastric glands, which are responsible for the production of hydrochloric acid. In their place, non-functional undifferentiated cells develop and eventually a thickened hyperplastic mucosa results. In heavy infections, an increase in pH is seen pepsinogen cannot be activated to pepsin and the digestion of proteins is reduced. There is also a loss of bacteriostatic action in the abomasum. An increased permeability of the abomasal epithelium results in a leakage of pepsinogen into the blood and a loss of plasma proteins into the gut lumen eventually leading to hypoalbuminaemia (Soulsby, 1986).

The effects of *Trichostrongylus* spp. and *Nematodirus* spp. are different. Both cause villous atrophy so reducing the area available for absorption of nutrients and fluids and in severe infections diarrhoea occurs.

In general, parasitic gastroenteritis causes inappetence which contributes to poor live weight gain and lowered production. The leakage of macromolecules into the gastrointestinal tract, extensive proliferation of epithelial cells, replaced by non-functional cells with imperfectly formed intercellular junctions (Murray, Jarrett and Jennings, 1970, and Murray, Miller, Sanford and Jarrett, 1971) causes a considerable strain on protein synthesis. This leads to increased requirements for erythrocyte and plasma protein synthesis at the expense of skeletal muscle protein. As a result, a loss in production is seen and in severe cases death occurs.

1.4 Treatment and control

Traditionally, anthelmintics have been used as a preventative measure (routinely treating pregnant ewes during the periparturient rise and young lambs) and also for the treatment of animals when clinical signs become apparent.

1.4.1 Treatment of pregnant ewes

As the periparturient rise is marked in ewes and is the most important source of infection for the lambs, it is of paramount importance that effective control of ewe nematode burdens is undertaken at this crucial period. It is generally thought best to dose ewes one month prior to partum (Urquhart, Armour and Duncan, 1987) with an anthelmintic which is effective against arrested larvae, such as certain benzimidazoles or avermectins in order to improve the condition of the ewe (Urquhart *et al*, 1987). Ewes at pasture will however, soon become re-infected through the ingestion of over-wintered larvae on the pasture. Thus it is advisable for an additional anthelmintic dose to be given 3 and 6 weeks post-partum.

1.4.2 Lambs turned out onto clean pasture

When the pasture has not been grazed for the previous 18 months ewes are the sole source of pasture contamination, from which the lambs may be infected. Boag and Thomas (1973) monitored FEC of lambs placed onto clean pasture (pasture previously only grazed by dosed ewes) and noticed that lambs had

extremely low worm burdens, of a mixed nematode infection, consisting mainly of *Ostertagia circumcincta.*

Alternate grazing of sheep with cattle coupled with anthelmintic treatment has been used successfully in Australia (Barger and Southcott, 1975). The success of such programmes is based upon the grazing of fields on an annual basis with each host, due to the relative non-susceptibility of cattle to sheep nematodes and *vice versa* (Urquhart *et al*, 1987). However, alternate grazing systems are not recommended when pastures are heavily contaminated with parasites capable of infecting both sheep and cattle, i.e. cattle have become susceptible to *Nematodirus battus* and sheep to *Cooperia oncophora.*

1.4.3 Lambs turned out onto dirty pasture

If non-contaminated pasture is unavailable, regular anthelmintic treatment (every 3 weeks, which is the prepatent period of the most important nematodes) should be given to lambs from 6 weeks of age and then until the overwintered larvae have decreased in number, i.e. mid-June.

1.5 Anthelmintic Resistance

In theory, correct prophylactic anthelmintic treatment offers the best method of control of gastrointestinal nematodes. However, since 1957 when Drudge, Leland and Wyant first reported anthelmintic resistance in *Haemonchus contortus* to phenothiazine, many accounts of anthelmintic resistance have been recorded involving benzimidazoles, levamisole and less frequently with the use of avermectins.(Waller, 1987; Jackson and Coop 1994) Benzimidazoles act by preventing the formation of microtubules in the parasites, which are responsible for transport of nutrients and waste products. Levamisole is a ganglion stimulant and causes paralysis to the parasites and avermectins are thought to act by stimulating the inhibitory neurotransmitter, gamma-aminobutyric acid. Le Jambre (1978) stated that nematode genotypes capable of resistance to anthelmintics are probably present in all populations of parasites at a very low frequency. Resistance is present when there is a greater frequency of individuals within a population able to tolerate higher doses of an anthelmintic than in a normal population of the worm species. Resistant genes are said to exist only in the heterozygous state and in the absence of anthelmintics are not selected for and therefore remain at a low frequency (Sykes, McFarlane and Familton, 1992). The authors also stated that the rate of development of resistance is dependent on the proportion of the parasite population which is on the pasture compared with that which is in the host. Jackson & Coop (1994) stated that anthelmintic resistance is a heritable characteristic with the offspring of resistant worms also carrying the gene(s) for resistance. If the larval population on the pasture is large, eggs from resistant worms in the host will be diluted by the susceptible population on the pasture which as yet has not been exposed to the anthelmintic and thus resistance will be slow to develop. However, when the population in the host

is large, no dilution of resistant eggs occurs and therefore resistance develops quicker. Thus, dosing animals at frequent intervals and 'the drench and move' method of parasite control, increases the likelihood of resistance.

Resistance to one anthelmintic may lead to side resistance to another anthelmintic with a similar mode of action or cross resistance to drugs with different modes of action. Multiple resistance is induced by multiple selection with anthelmintics in the same or different groups. A decrease in the frequency of resistant individuals can be observed after the removal of anthelmintic, however, Boorgsteede and Duyn (1989) stated that resistance will persist for many years even in the absence of the anthelmintic.

Resistance to anthelmintics is widespread especially in Australia, New Zealand, South America and South Africa and the constant need for regular drenching, due to the warm climate decreasing the development time of the parasites, has resulted in a dramatic increase in the incidence of resistant parasite populations (Waller, 1987). This fact, together with the massive total investment, in time and money required to produce new anthelmintics stresses the importance of preserving the effectiveness of the currently available anthelmintics (Windon, 1991a). Alternative control methods are being investigated which will decrease the need for anthelmintic use. One strategy, that of breeding for resistance to nematodes is therefore a high research priority for sheep producers (Gray, 1991).

1.6 Differences between breeds in resistance to parasitic infection

The existence of genetically controlled resistance to gastrointestinal nematodes between breeds of sheep has been known for many years.

Stewart, Miller and Douglas (1937) presented evidence that different breeds of sheep vary in susceptibility to *Ostertagia circumcincta.* The Romney breed of sheep which they used appeared uniformly resistant whereas, sheep such as Rambouillet and other British Breeds were both more susceptible, and more variable in their resistance. A few individuals within these breeds proved to be just as resistant as the Romneys: others were highly susceptible and others intermediate.

Stewart *et al* (1937) in a second experiment repeated the trial and found two Romney rams with progeny with high FEC. They concluded that susceptibility may be determined in part by genetic constitution or genotype.

Michel, (1969) stated that the development of resistance to *H. contortus* depends on the breed of sheep. British sheep became refractory after they had reached a certain age whereas Australian Merinos did not acquire this form of resistance even in adulthood.

Differences between Florida Native and Rambouillet breeds in susceptibility to *Haemonchus contortus* were reported by Loggins, Swanson and Koger (1966). Florida Native ewes and lambs were shown to have less eggs per gram of faeces than the Rambouillet sheep. The Rambouillet sheep, upon *post-mortem* examination contained four times as many *H. contortus* worms as the Florida Native sheep.

Radhakrishan, Bradley and Loggins (1972) experimentally infected nineteen Florida Native and eight Rambouillet lambs with larvae of *H. contortus*. They found significantly more eggs and a lower packed cell volume (PCV) in the Rambouillet lambs. At *post-mortem* examination fewer adult worms and more larvae were recovered from the Florida Native lambs and both the male and female adult worms recovered from the Florida Native lambs were shorter than those recovered from the Rambouillet lambs. It was concluded that an inherited factor was present which operated against the development of larvae to adult nematodes.

In the U.K. Scottish Blackface lambs were reported by Altaif and Dargie (1978) to be more resistant to infection with *H. contortus* than Finn Dorset lambs. However, Ross (1970) found that Dorset lambs seemed to be more resistant to infection with *Trichostrongylus axei* than were Scottish Blackface lambs.

Abbot, Parkins and Holmes (1985) noticed a difference in FEC's between Scottish Blackface lambs and Finn Dorset lambs, with Finn Dorsets which are more susceptible having decreased egg counts when fed a soya supplemented diet.

Wallace, Bairden, Duncan, Fishwick, Gill, Holmes, McKellar, Murray, Parkins and Stear (1995) noticed that Hampshire Down lambs when fed a basal diet had higher FEC's than those fed a supplemented diet.

Yadav (1987) investigated the difference in resistance of pure Welsh Mountain and Suffolk X Clun Forest lambs to a natural infection with trichostrongyle

larvae. He observed a similar initial egg count followed by a decline in the Welsh Mountain lambs but not the Suffolk X Clun Forest lambs.

Zajac, Herd and McClure (1988) found higher FEC in Dorset X Rambouillet pregnant ewes than in Florida Native pregnant ewes. Lambing Dorset X Rambouillet ewes showed a significant periparturient rise (PPR) whilst the FEC of periparturient Florida Natives did not differ from non-pregnant controls of that breed.

They then compared the PPR in ewes of three exotic breeds (Florida Native, Barbados Blackbelly and St. Croix) with that of North American domestic breed ewes (Rambouillet and Finn Dorset X Rambouillet). FEC were taken from ewes housed from late autumn to lambing and weaning. The exotic breeds showed no PPR whilst the domestic ewes showed a pronounced periparturient rise six to seven weeks after lambing. St. Croix X domestic ewes showed an intermediate PPR. It was concluded that a low or absent PPR is an important manifestation of breed resistance in exotic ewes and that this may be useful as a marker for selecting parasite-resistant sheep.

Scrivner (1967) showed that sires selected as genetically resistant to haemonchosis were also significantly more resistant to ostertagiasis. He examined the progeny from two breeds of rams, Targhee and Suffolk, and found resistance to experimental infections in the progeny of the Targhee ram.

1.7 Differences within breeds in resistance to parasitic infection

Differences in resistance have been seen within a number of sheep, in breeds such as Merino, Dorset, Romney and Corriedale to infections of a wide variety of gastrointestinal nematodes. The parasites include, *Haemonchus contortus* (Warwick, Berry, Turk and Morgan (1949), Whitlock (1958), Le Jambre (1978), Albers, Gray, Piper, Barker, Le Jambre and Barger (1987), Gray, Presson, Albers, LeJambre, Piper and Barker (1987)) *Trichostrongylus colubriformis* (Windon and Dineen (1984)) and to mixed infections with *H. contortus*, *T. colubriformis*, *Ostertagia circumcincta* and *Nematodirus* species.

Gregory, Miller and Stewart (1940) having analysed factors including genetic factors which influence *H. contortus* infections in sheep concluded that genetic constitution was the greatest single factor influencing the FEC under controlled environmental conditions. FEC were taken from Romney ewes. Ewes aged two years and eight months all from a single ram, which was described as transmitting high susceptibility to its progeny, showed a wide variety of susceptibility to *H. contortus*. Two ewes had extremely high FEC and two ewes had zero FEC. The other ewes of this group had intermediate counts. Ewes which were one year and eight months old were even more susceptible, and had high FEC, however, one ewe had a zero egg count and the difference between this ewe and the others was highly significant.

To eliminate the influence of age, the mean FEC for seven ewes aged two years eight months from the susceptible ram was compared with three two years eight months old progeny from the resistant ram. The difference between the two groups of progeny was highly significant.

Variation in host resistance to gastro-intestinal nematodes has also been observed in goats. Pomroy and Charleston (1989) noticed that nine month old Saanen goats artificially infected with 10,000 *Trichostrongylus colubriformis* larvae for 10 weeks had FEC ranging from 150-1150 EPG.

1.7.2 Suppressed resistance to gastro-intestinal nematodes in lactating animals

The phenomenon of 'spring rise', i.e. an increased faecal egg output during lactation in ewes was first described by Zawadowsky and Zvjaguintzer (1933) in Russia.

Dineen and Kelly (1972) attempted to find the nature of this immunological defect by looking at infections in lactating and nulliparous rats infected with *Nippostrongylus brasiliensis*.

When lactating rats were infected with 3,000 third-stage larvae much higher FEC and total worm burdens were seen than when nulliparous controls were infected with an equal number of larvae. When immune mesenteric lymph node cells obtained from nulliparous female donors were injected into the lactating females a decrease in FEC and total worm number was seen. The transfer of immune cells repaired the deficit in the rejection mechanism. Transfer of red cells from the lactating to the nulliparous female rats also resulted in decreased FEC and worm number, but to a lesser extent. Dineen and Kelly (1972) stated that potentially immune lymphoid cells were present in the mesenteric nodes of lactating females at the time that the rejection mechanism was severely impaired.

Earlier studies by Urquhart, Jarrett, Jennings, McIntyre and Mulligan (1966); Ogilivie and Hockley, (1968); Keller, (1970) have suggested that the rejection mechanism is bi-phasic. One phase is immunologically specific and the other, involves myeloid cells and the production and release of amines. Lactogenic hormones are capable of suppressing either or both these components either directly or indirectly. The results of Dineen and Kelly's experiment (1972) showed that the immune cells were functional in lactating recipients and transfer of immune cells repaired any deficiency in the rejection mechanism. Therefore it was concluded that there was no deficiency in the myeloid-amine component of the rejection mechanism.

The authors observed from their experiment three interesting points:-

a) potentially reactive cells are present in lactating donors.
b) effector cells can react in lactating recipients, but
c) the action of potentially reactive cells from lactating donors is substantially inhibited in lactating recipients.

Kelly and Dineen (1973) also investigated the suppressive effects of prolactin on the resistance of rats infected with *Nippostrongylus brasiliensis*.

Rats were administered prolactin during a primary infection with *N. brasiliensis*. Survival of the worms in the small intestine was prolonged. This observation therefore suggested that prolactin inhibits rejection of the parasite.

In order to investigate the immunological defect in prolactin treated rats, six groups of mice were infected with 2000 third-stage larvae of *N. brasiliensis*.

Group 1 - received no further treatment.

Group 2 - injected twice a day with prolactin throughout the course of the infection.

Group 3 - injected with 10^8 mesenteric lymph node cells (mLn cells) on the day they were infected.

Group 4 - as group 3 plus twice a day treatment with prolactin throughout the course of the infection.

Group 5 - injected with mLn cells, obtained from donor rats which had received twice daily injections of prolactin throughout the 15-day course of their immunising infection.

Group 6 - as Group 5 plus-cell recipients were treated twice daily with prolactin throughout the challenge infection.

Rats in all the groups, i.e. 1-6 were killed on day ten and total worm counts were taken at post-mortem.

The worm counts found, in ascending order, were,

Group 3	39
Group 5	307
Group 4	329
Group 6	439
Group 1	605
Group 2	662

Comparison of Groups 1 and 2 showed that prolactin did not affect the rate of establishment of infection. Comparison of the worm count of Group 1 which received no treatment after infection, with Group 3 which was injected with

mLn showed that the latter cells were highly effective in rejecting the parasite. However, when rats in Group 3 were treated with prolactin as in Group 4, the effect of the immune cells was seriously impaired. Group 5 animals which were injected with immune cells from prolactin treated donors had much lower worm counts than Group 1 animals indicating that cells from prolactin-treated donors eased the worm burden to some extent in untreated recipients but not as well as cells from untreated immune donors given to animals in Group 3. This difference between Group 5 animals which were untreated recipients of cells from prolactin treated donors was not statistically significant from Group 6 animals.

Connan (1968) observed a wide range in the number of overwintered larvae of *H. contortus* and *O. circumcincta* in ewes during the post-parturient rise.

The hormone prolactin is associated with the suppression in the host's immunity which is thus responsible for the periparturient rise in FEC. However, prolactin is not the sole factor involved and the mechanisms responsible remain unclear (Rahman and Collins, 1992). These authors observed that the rise in prolactin concentration started between 3 weeks and 1 week pre-partum in goats, and correlated significantly with the relation between prolactin levels and FEC, with a peak in FEC also occurring 3 - 1 weeks pre-partum. Fleming and Conrad (1989) observed that ovariectomized ewes treated with progesterone then prolactin during an artificial infection with *Haemonchus contortus* had higher worm burdens than those treated with prolactin only which in turn had higher FEC than those treated with progesterone only.

1.7.3 Differences in resistance to infection due to the sex of the host, i.e. oestrogen production

Hunninen (1935) observed that some days after parturition female mice became more resistant to *Hymenolepis nana* than males. Campbell and Melcher (1940); Sadun (1948, 1951) and Mathies (1959) demonstrated that oestrogen stimulates the resistance of mice to *Nematospiroides dubius* while testosterone has little or no effect. It was suggested that during the life-cycle of the worm it might be acted upon by two mechanisms of resistance controlled by oestrogen. The first mechanism is indirect and involves the laying down of connective tissue and the production of antibody, this mechanism effects the encysted larva which encysts in the gut wall. The second mechanism is the direct action on the adult parasite, of oestrogen carried in the blood and bile.
von Haam and Rosenfeld (1942) observed that the administration of oestrogen increased the resistance of both gonadectomized and normal male rabbits to *Pneumonococcus*, while testosterone had no effect.

1.7.4 The effect of age on selection

Gray (1991) studied selected and unselected sheep lines aged from up to 1 year old to 5&7 years of age. Sheep from the resistant line had lower FEC than unselected animals at all ages until 7 years old. However, seven year old resistant ewes had significantly lower FEC than ewes bred for susceptibility (486 v 6169, $p < 0.008$).

Differences in FEC due to age have also been seen in goats. Pomroy, Lambert and Betteridge (1986) observed that adult goats at pasture had lower egg

counts than one year old goats, thus suggesting that resistance had not yet developed fully in the younger animals.

Nematodiriasis caused by *Nematodirus battus* and *N. filicollis* has been described by many authors as being a disease of young lambs and adult animals, although resistant to infection are capable of perpetuating the infection on pasture (Gibson, 1963; Boag and Thomas, 1975'; Soulsby, 1986).

1.8 Direct and indirect selection for resistance

1.8.1 Direct selection

FEC as a measure of worm burdens have been successfully performed for many years. However, FEC can be influenced by a number of environmental and physiological factors such as abundance of larvae on pasture, dry matter of faeces, diet and faecal output and may not be a true representation of the infection rate (Windon, Gray and Woolaston, 1993). However, Eady & Woolaston (1992) stated that selection for resistance can be successfully performed in commercial breeding programmes on the basis of FEC and recommend how problems can be overcome or minimised.

1.8.2 Indirect selection for resistance (Phenotypic markers)

Windon *et al* (1993) described genetically correlated traits such as phenotypic markers (which are unaffected by environmental or physiological factors) as much more reliable indicators of resistance. Phenotypic markers identify a more precise measure of resistance to infection; predictive markers have a functional basis. However, at present no traits have been identified that would be more reliable than FEC as indicators of resistance.

1.8.3 Haemoglobin type

Over the years there has been tremendous interest in haemoglobin type as a marker for resistance to the blood sucking gut nematode *H. contortus*.

Jilek and Bradley (1969) noticed a difference in infection rates within the Florida Native breed of sheep. Sheep with haemoglobin type AA (HbAA) were infected less than HbAB and HbBB type lambs. However, Radharkrishan *et al* (1972) could not find a correlation between haemoglobin type of sheep and resistance against the parasite, *H. contortus*.

Evans and Whitlock (1964) also demonstrated that sheep which have haemoglobin type AA and also those which synthesise haemoglobin rapidly can withstand the pathogenic effects of *H. contortus* better than sheep which do not possess these characteristics. Sheep with haemoglobin HbAB seemed to be the most susceptible to infection whilst type HbBB were a little more resistant.

Evans, Blunt and Southcott (1963) noticed that Merino sheep with type A haemoglobin were less infected with *H. contortus* than sheep with haemoglobin type B. They suggested that this was due to the fact that at equivalent partial pressures more oxygen can be liberated from type B haemoglobin than from type A. Therefore *H. contortus* nematodes in sheep of type B are able to obtain more oxygen for respiration.

Evans *et al* (1963) discovered that sheep in areas where *H. contortus* was most prevalent were more likely to have haemoglobin type A. However, haemoglobin type has not been associated with level of resistance in other

infections such as scrapie, cysticercosis or echinococcis (Darcel and Avery 1960).

1.8.4 Sodium and potassium concentrations in the erythrocytes

In 1898, Abderhalden reported low potassium and high sodium concentrations in the erythrocytes of two resistant sheep within a flock infected with gastrointestinal nematodes.

Observed differences between the breeds, strains and sire groups suggested that many genes operate to alter the potassium ion concentration [K+] along with the major gene pairs, as suggested by Evans and King (1955). Evans working with Phillipson (1957) later showed that [K+] differences within cattle twin pairs were significantly less than those between pairs.

Kidwell, Bohman, Wade, Haverland and Hunter (1959) reported that differences in the potassium concentrations in the blood of sheep were not at all effected by sex, age or reproductive status. However, Evans and Blunt (1961) found rams to have a higher concentration of potassium in their blood and suggested that steroid hormones might modify the expression of the K gene. Castrated rams were shown to have a decrease in potassium concentrations.

1.8.5 Blood lymphocyte stimulation by parasite antigens

Riffkin and Dobson (1979) investigated the relationship between responses of sheep lymphocytes when controlled *invitro* with *Haemonchus contortus* antigens and the resistance of the sheep to infection with the parasite. They discovered that lymphocyte responsiveness to *H. contortus* develops early in the life of the sheep in the absence of the parasite possibly as a genetically controlled response to heterophile antigens. They also noticed that the inheritance of lymphocyte responsiveness to *H. contortus* between ewes and their lambs was significant only for L_3 larvae and not for the adult parasite antigen. This suggested that the trait may have evolved with respect to larval establishment rather than the development of the parasite. Also older sheep inhibited the development of more *H. contortus* at the early fourth stage than did lambs. Therefore inhibition of larval development was associated with the efficiency with which sheep mobilised their immune response to *H. contortus* antigens, which increases with age and is independent of the inherited level of immunological responsiveness to *H. contortus* antigens.

The level of response was positively correlated with resistance to subsequent primary, secondary and trickle infections by the parasite. In all the cases of infection the high responder (HR) group of sheep was found to have lower FEC and upon *post-mortem*, less worms in their guts than the low responder (LR) animals. These results confirmed that differences of lymphocyte responsiveness could be seen linked to the genetic structure of the sheep. The increase in immunological competence which resulted from challenge trickle infections was seen by Riffkin and Dobson (1979) to reinforce the difference in lymphocyte responsiveness between HR and LR sheep. This was said to be

reflected by the earlier onset of a more efficient 'self-cure' reaction in HR than LR sheep.

1.8.6 The relationship between ovine lymphocyte antigens and resistance and susceptibility to gastro-intestinal helminths

Outteridge, Windon and Dineen (1985) looked at lymphocyte antigens in high responder Merino sheep bred over three generations and selected for responsiveness to vaccination against *Trichostrongylus colubriformis*.

A particular antigen (SY1) was found to be present in high frequency on the lymphocytes of high responders (72.2%) and in a low frequency on the lymphocytes of low responder rams (21.9%). The SY2 antigen had a higher frequency in low responders 48.9% than high responders 25.2%. There was seen to be an association between the SY1 antigen and low FEC in random-bred sheep which had been vaccinated with irradiated larvae and challenged with normal larvae.

The results illustrated that the possession of SY1 antigen was more associated with a reduced FEC after vaccination than with primary challenge alone.

However, the SY1 and SY2 antigens were shown to have some degree of overlap between the high and low responder lambs for high responders the overlap was 25.3% for rams and 16.7 % for ewes and for the low responders the overlap was 8.2% for rams and 14% for ewes. It therefore seems that these animals need to be bred for more generations before a more complete separation may be seen.

Outteridge, Windon, Dineen and Smith (1986) looked at the sub-division of SY1 into SY1a and SY1b, which appeared to be alleles at the same locus.

Random bred and selected Merinos were used. Four generations of animals were available for experimental work, i.e. the three generations from the experiment undertaken in the previous year and an additional new generation.

SY1a and 1b were associated with low FEC and SY2 with high, as in the previous experiment.

A gradation in FEC was seen, animals with SY1a + 1b + 2 had the lowest FEC and the significance of this finding was not known. Those with combinations of 1a, 1b and 2 or 2 alone had intermediate egg counts. Whereas those sheep with none of these antigens had the highest egg counts.

However, Outteridge *et al* (1986) stated that although the above figure demonstrates that the SY1a + 1b was the best marker of the lymphocyte antigen combinations examined, it was shown statistically that this effect was mainly due to the high responder line.

Although SY1a + 1b sheep had generally lower F.E.C.s than either SY1a or 1b alone statistical analysis indicated that SY1a + 1b did not have additive effects.

Outteridge (1988) again looked at the effectiveness of the ovine lymphocyte antigen SY1 as a marker for acquired resistance to *Trichostrongylus colubriformis*.

The lambs used were unrelated to any used in the previous experiments. Sires and dams were selected on the basis of OLA type only and were not bred for high/low responsiveness or selected at random. The lambs bred were of predominantly one type. The lambs were tested by the same vaccination and challenge procedures as the high and low responders previously selected on the basis of FEC.

Eosinophil counts were taken and were generally higher in female than male lambs. A difference in eosinophil counts between SY1 and other OLA types was only seen at twenty-seven weeks, i.e. the last sample, however, the difference was not significant. This finding was in contrast to previous work (Windon and Dineen, 1984) where more eosinophils were found in high than low responder lambs. They described this fact as being due to a failure to select for myeloid cell responses when selection is based on OLA type alone.

The wool growth of the lambs was measured by a dye-bonding technique (Windon and Dineen, 1984). The live weights of lambs from all sire groups were also measured weekly. No differences in any of these two parameters was seen between lambs of OLA SY1 and any other marker, i.e. there was no penalty in lambs bred for the marker.

From looking at the FEC of the lambs it could be seen that lambs with OLA SY1 had half the number of eggs per gram of faeces than other ovine lymphocyte antigens.

There was a difference in FEC in sire groups, these differences were increased when comparisons were made for the presence or absence of SY1a and/or SY1b for males ($P<0.001$) and females ($P<0.02$).

During the eleven weeks after challenge the parasitic infection differed greatly in male and female lambs. In males the average FEC for lambs with SY1a and/or 1b dropped until nine weeks while lambs with other OLA types had slightly increased FEC.
However, at eleven weeks the FEC for SY1a and/or 1b males rose and the FEC for other OLA types decreased slightly, therefore the ,mean FEC were not significantly different at this stage. This was said to be due to a diminution in the immune response at eleven weeks in SY1a and/or 1b positive males.

For females the differences were most obvious at seven and nine weeks and again there was a decrease in the difference between the two groups at eleven weeks. The responses of both kinds of females, i.e. SY1a and/or 1b and the other types ran parallel. Windon and Dineen, (1984) suggested that this fact was due to most females of all types, unlike males, responding to vaccination and challenge with *T. colubriformis*.

This smaller difference observed between groups of females compared with males was said to be responsible for the lack of significant sire and lymphocyte antigen effect for the presence or absence of SY1a and/or 1b. Analysis of variance revealed a significant sire and antigen effect for males.

Outteridge (1988) concluded that the resistance to *T. colubriformis* could be improved by use of the genetic marker in the stud Merino system.

Douch and Outteridge (1989) investigated the relationship between ovine lymphocyte antigen and parasitological and production parameters in Romney sheep.

The criteria used in order to measure resistance were FEC, plasma pepsinogen levels, live weight and weight gains.

Two flocks of New Zealand Romney sheep were looked at in relation to resistance to nematode parasites.
Flock 1 was an open breeding flock with rams selected on the basis of parasitological and productivity criteria. Whilst flock 2 was a closed flock with breeding animals selected for high body weight or high growth rate under parasite challenge in the absence of anthelmintic treatment. The ovine lymphocyte antigen SY1a + 1b was found exclusively in low egg count (LEC) sheep in both flocks. This fact was especially true of flock 1. At each fortnightly sampling during the primary challenge flock 1 sheep with SY1a + 1b had lower FEC than sheep with any other OLA.

SY6 occurred significantly more frequently in sheep with high FEC.

Sheep with SY16 ovine lymphocyte antigens were significantly heavier than those without the antigen. Gautschi, Gaillard, Schwander and Lazary (1986) described a similar association in pigs. A reduced weight gain was seen in flock 1 prior to initial drenching in OLA SY1b and SY1a + 1b. However, Dough and Outteridge (1989) needed to be convinced by looking at other flocks in order to decide whether the trait was consistent.

Plasma pepsinogen concentrations were lowest in ewes of flock 1 having few SY2 or SY3 OLAs. Only a very few sheep had SY2 or SY3 antigens and such a result could not be confirmed on such a small number of sheep. However, low FEC were not generally associated with low pepsinogen levels.

The Romney flock had a low frequency of some lymphocyte antigens (e.g. SY1a, 2, 3, 4, 5, 9) compared with the previously tested Merino sheep. Therefore a correct analysis of the relationship between lymphocyte antigens and FEC or body weight based on results obtained from the Merino flock could not be made. However, in Merinos SY2, 3, 5, 9 have been associated with animals susceptible to nematodes after vaccination. In addition the fact that SY1a + 1b was associated with low FEC in both breeds pointed to the same mechanism of immunity and the same influence of the OLA system on immune responsiveness to parasites in both Romney and Merino breeds.

Douch and Outteridge (1989) described the associations between OLA type and resistance to nematodes as reflecting the function of the linked genes, i.e. genes linked to the Major Histocompatibility Complex (MHC) rather than immune response genes which are part of the complex itself.

It was concluded that although the OLA SY1a + 1b are present only in low frequency they may still be useful as a predictive marker for resistance of Romney sheep to nematodes.

However, work carried out by Cooper, van Oorschot, Piper and Le Jambre (1989) on *Haemonchus contortus* infection in Merinos, described a lack of evidence of linkage between the OLA region and genes for susceptibility and resistance in six rams. It was therefore concluded that a larger analysis would be necessary before the hypothesis that the OLA region affects susceptibility to *H. contortus* could be dismissed.

1.8.7 Circulating complement-fixing antibodies

Windon and Dineen (1981) looked at the effects of both sire and dam responsiveness to vaccination with irradiated *T. colubriformis* larvae on the responsiveness of their F^1 progeny lambs. The sires and dams were mated on the basis of responder X responder and non-responder X non-responder. The progeny were first vaccinated with irradiated larvae (20,000) at eight and twelve weeks of age. They were then treated with anthelmintic at sixteen weeks and later challenged with infective larvae (again 20,000) a week later.

After taking FEC from both sets of progeny it could be seen that responder matings produced fewer eggs than progeny from non-responders. There was also a higher egg count in ram than ewe lambs. It was noted that the higher the egg count the less circulating complement-fixing antibodies to the larvae could be found in the animal's blood. Therefore ewe lambs from responder matings had the highest serum antibody levels i.e., they were most resistant. It was concluded that the response to vaccination at an early age is genetically determined and the response of progeny is most vigorously expressed when both the sire and the dam have been selected.

1.8.8 Lymphoblastic and IgA containing cells

Smith, Jackson, Graham, Jackson and Williams (1987) investigated physiological and parasitological differences between naive and previously infected one year old Grey face cross Suffolk lambs infected daily with 2000 *Ostertagia circumcincta* larvae. The previously infected lambs were either given a repeated or interrupted dose.

Many eggs were seen in the faeces of the naive lambs and many worms in the abomasal washings. However, the previously infected lambs, showed no evidence of eggs in their faeces. Only a low number of worms could be seen in the gut of previously infected interrupted challenged lambs and even fewer in the previously infected ,continuously challenged lambs.

The previously infected lambs showed some degree of resistance to nematode infections. Previously infected lambs which were continuously infected with larvae showed a higher resistance than the interrupted challenge lambs. This was said to be due to the synthesis of IgA, i.e. IgA synthesis appeared to be directly maintained by the continued input of larvae. IgA is said to either cause parasitic stunting or/and be involved in worm exclusion mechanisms.

Naive lambs were shown to have a continuous decline in the albumin content of their lymph, this was said to be due to the prolonged lymph drainage due to the high numbers of developing and mature *Ostertagia circumcincta* present in susceptible sheep (A fall in lymph albumin is often associated with ostertagiasis) (An increase in pepsinogen was seen with a decrease in lymph albumin). It was also know that *Haemonchus contortus* and *Trichostrongylus*

colubriformis alter ovine gastrointestinal motility, thereby reducing lymph flow. IgA cell output increased slightly, with day to day variation in the naive lambs. An increase in lymphoblast output and a decrease in IgA could also be seen.

The previously infected lambs showed a different pattern of IgA cell and lymphoblast output, a more rapid, simultaneous increase was seen which was largely confined to a few days at the start of each challenge period. As mentioned earlier an increase in IgA was also seen in the previously infected lambs.

Concentration of pepsinogen increased much more rapidly in the previously infected lambs than the naive lambs. As with the IgA response, the pepsinogen content of the lymph of the immune sheep remained elevated only if larval intake was maintained.

1.8.9 Eosinophil Responses

Dawkins, Windon, Outteridge and Dineen (1988) investigated the differences in eosinophil responses in sheep bred for high and low responsiveness to *Trichostrongylus colubriformis.* The experiment investigated the numbers of circulating antibodies after vaccination and challenge infections. Many studies have associated eosinophilia with rapid expulsion which therefore suggests a contributing factor to host resistance. (Rothwell and Dineen, 1972, 1973; Dawkins, Carroll and Grove 1992). There is also evidence for an effector cell function which results in parasitic cell damage. (Goetzl and Austen, 1977; Kazura and Grove 1978; Olson and Schiller, 1978; Handlinger and Rothwell,

1981). In addition eosinophilia has been proven to be under genetic control (Vadas, 1982; Wakelin and Donachie, 1983a).

Lambs from two different breeding systems were used, 1G4A and 1G4B. 1G4A lambs were bred by artificial insemination procedures carried out within the closed high and low responder flocks. Five male and five female lambs were picked at random for the test. 1G4B lambs were bred by conventional mating of selected dams and sires from high and low responder flocks.

In addition randomly-bred unselected lambs were used in the experiment as controls, (1G4A; six males and six females: 1G4B; three males and three females). All the lambs were reared and maintained under worm-free conditions in pens and weaned at eleven weeks of age.

On inspection FEC of 1G4A lambs confirmed that the differences between high and low responder lines were highly significant ($p<0.001$). However, no difference in FEC due to sex of the animal was apparent.

1G4B lambs were also shown to portray significant differences in FEC between high and low responders ($p<0.001$). A significant difference in FEC could be seen between sexes in high responder lambs, i.e. female lambs were observed to be less infected than males.

The vaccinated random bred lambs had intermediate FEC between high and low responders while unvaccinated randoms had FEC higher than low responders.

Analysis of 1G4A high and low responder lambs demonstrated that the difference between the two groups were nearly significant at eleven weeks and thereafter were significant at the 5% level or greater.

Random bred control lambs were observed to have circulating eosinophil numbers which declined to a base level over the first seven weeks of life. Vaccination did not induce eosinophilia and after challenge infection eosinophil numbers increased in both unvaccinated and vaccinated random bred groups.

The correlation between eggs per gram of faeces and eosinophilia for 1G4A lambs was low in the pre-vaccination period (1-7 weeks of age) but increased in the negative direction over time. Randomly bred vaccinated animals when included tended to reduce the observed correlations.

IG4B lambs showed a significant eosinophilia in high responder lambs. No eosinophilia was observed in the low responder lambs or the untreated random control animals.

All 1G4B lambs, i.e. high and low responders and random-bred animals except vaccinated lambs showed a significant negative correlation between EPG and eosinophilia.

The results from 1G4A lambs confirmed a strong and direct relationship between high responders and eosinophilia. Vaccination procedures and challenge infection induced an increased circulating eosinophil number in the high responder animals, in contrast to the extremely low eosinophil levels of the low responder lambs. Random bred lambs showed a wide variation in

eosinophil numbers between individual animals. This observation displayed the spectrum of the host responsiveness in the population.

Gregg, Dineen, Rothwell and Kelly (1978) reported that eosinophilia developed after challenge infection in an unselected group of lambs. This observation therefore explained the fact that no eosinophil response was detected in random bred lambs after either the primary or secondary vaccination procedures but only after challenge infection.

It has previously been stated (Vadas, 1982; Wakelin and Donachie, 1983a; Sewell and Vadas, 1983) that the magnitude of the eosinophil response is under genetic control of the host. Gregg *et al* (1978) concluded that resistant animals have significantly more eosinophils than susceptible animals. Handlinger and Rothwell, (1981) also witnessed this when looking at guinea pigs infected with *T. colubriformis*. However, Dawkins *et al* (1988) concluded that in their present study there was no evidence to suggest that high eosinophil counts would be a reliable predictive marker for resistance to *T. colubriformis* prior to infection as pre-vaccination correlations were low and not significant.

Windon *et al* (1993) noticed that circulating eosinophils were elevated in high responder lambs and stated that they were associated with a development of resistance to *T. colubriformis.* As this elevation was seen in a flock selected for *Trichostrongylus* resistance only a weak association was observed with tissue eosinophilia. However, tissue eosinophilia was associated with resistance in infections with *H. contortus.* They concluded that these differences in immunological mechanisms were due to their relative importance to these different nematode species.

Stear, Bairden, Bishop, Duncan, Karimi, McKellar and Murray (1995) observed that eosinophil responses were higher and peaked slightly earlier in sheep with high faecal egg counts.

1.8.9 Globule Leucocyte counts in duodenal tissues

Dineen, Gregg, Windon, Donald and Kelly (1977) vaccinated colostrum fed and colostrum deprived lambs at three months of age (weaning) with irradiated larvae of *T. colubriformis* and then challenged the lambs with infective larvae at 17-20 or 37-40 weeks of age. They found no significant difference between the worm counts of these groups after challenge. It was therefore concluded that the failure of lambs generally to respond as well as adult sheep to vaccination was not due to 'feedback' inhibition of the immune response by the action of maternal antibody.

The lambs could be divided into two groups, i.e. 'responders' and 'non-responders'. The results suggested that genetically-determined factors played an important role in the responsiveness of lambs to vaccination.

The more resistant lambs seemed to have higher counts of globule leucocytes in their duodenal tissues. Responder animals were shown to have many globule leucocytes in the sections of duodenum tested, whereas non-responder animals possessed a negligible amount of leucocytes.

Dineen *et al* (1977) suggested that either globule leucocytes are involved in the resistance mechanism, or more likely they are by-products of cellular events involved in resistance. The latter is probably true as they failed to obtain very strong negative correlations in responder groups between leucocyte and worm counts.

Jones, Windon, Steel and Outteridge (1990) described globule leucocytes as end products of immune responses and that they release histamine and leukotrienes into intestinal tissue.

1.8.10 The inhibitory factor in gastro-intestinal mucus

Kimambo and MacRae (1988) measured the inhibitory factor in gastrointestinal mucus of sheep which were resistant to *T. colubriformis*.

The samples of the inhibitory factor for *in-vitro* measurement were obtained from nine Suffolk X Finn Dorset wether lambs. Five of the lambs had been dosed daily for thirty four weeks with 2500 *T. colubriformis* infective larvae. The lambs were then untreated for twenty four weeks before being re-challenged for ten weeks.

The remaining four lambs were pair-fed with four of the five infected lambs, but were kept worm free throughout the experiment.

An extremely high number of exsheathed *T. colubriformis* larvae formed into coils and stopped moving when they were treated with mucus from the small intestine of re-infected sheep. Only a few showed a similar reaction when they were treated with mucus from the small intestine of control sheep. This was also true with mucus from the abomasum.

This inhibitory effect of the mucus accounted for the inability of the infective larvae to establish in the resistant sheep either during the last twenty weeks (Kimambo, MacRae and Dewey, 1988a) or during the re-challenge infection (Kimambo, MacRae, Walter, Watt and Coop, 1988b). These observations

were similar to those experienced when rodents were infected with nematodes. Mucus plays an important role in the expulsion of adult worms and inhibits the establishment of the larvae (Carrol, Mayrhofer, Dawkins and Grove, 1984). Other workers have found that the establishment of infective larvae is prevented by mucus in ruminants (Chiejina and Sewell, 1974). Douch, Harrison, Buchanan and Greer (1983) observed that LMI (larval migration inhibitory) activity in mucus from intestines of sheep with low FEC was higher than that from sheep producing many eggs or from sheep raised parasite free.

Kimambo and MacRae (1988) also noticed LMI activity in mucus from the abomasum of resistant infected sheep (0.73 compared with 0.06 in control sheep) and suggested that inhibitory activity is also present in areas which are not effected by *T. colubriformis* but by other parasites, e.g. *Ostertagia* in the abomasum. The mucus from the small intestine of infected resistant animals was shown to have some LMI activity against *Ostertagia ostertagi* and *Cooperia oncophora* larvae in addition to *T. colubriformis* larvae. This observation was similar to that of Dineen *et al* (1977) who reported that the mechanism responsible for parasite elimination was triggered into action by a specific antigen marker but was non-specific in action; thus sheep resistant to *T. colubriformis* were also resistant to a mixed infection.

Kimambo and MacRae (1988) also saw that mucus from control sheep was showed to possess a greater LMI activity against infective larvae of *O. ostertagi* and *C. oncophora* than against *T. colubriformis* larvae. They suggested that this observation would explain why these predominantly cattle parasites, do not infect sheep to a great extent.

Ileal digesta was collected from the infected and control animals before infection, after 6, 12, 18, 32 weeks of continuous daily dosing with 2500 *T. colubriformis* larvae, after the 20 weeks rest period and during week 5 of the subsequent re-challenge infection (Kimambo *et al* 1988a, b).

The LMI activities were similar in the infected and control groups at the beginning of the experiment (0.35 and 0.31). However, during the period of infection the LMI activity in the ileal digesta from the infected group increased with time, reaching highest activity at week 18 of infection. Differences between infected and control animals were significant throughout this period.

1.9 The mechanism of resistance to parasitic infection

1.9.1 Single and major or polygenic gene action

Brindley and Dobson (1981) looked at the selection of resistant and susceptible populations of mice to infections with the gastrointestinal nematode *Nematospiroides dubius* and noted that the liability of parental mice to *N. dubius* infection correlated positively with that of their progeny. They concluded that the susceptibility of mice to the parasite *N. dubius* was under genetic control of several genes and that the heratibility of the trait was 0.45. However, when selection was extended to many generations, it was clear that selection for liability progressed more quickly than that for refractiveness. However, Brindley and Dobson (1981) also observed that female mice were less susceptible to infection than males and suggested that this was probably due to sex-linkage of a single gene controlling the trait. However, Dobson (1961 and 1964) during his previous work suggested that the greater liability of males was due to the differences in the murine environment controlled by sex hormones. Increased liability of female mice to *N. dubius* infection was seen to correlate positively with progeny litter size. This was said to be due to linkage of genes controlling these two parameters.

However, Sitepu and Dobson (1982) undertook a similar experiment and their aim was to establish colonies of mice which could be distinguished as susceptible (S) or resistant (R) to challenge infection with *N. dubius*. The difference in FEC between S and R became progressively greater with each selection. The variance of the mean egg output decreased more rapidly with selection in S compared with R line mice, especially after a challenge infection. Brindley and Dobson also saw this in their 1981 experiment and

concluded from both experiments that a dominant gene was acting to make mice refractory to primary and resistant to secondary infections with *N. dubius*.

Wakelin (1975b) investigated *Trichuris muris* infections and showed that resistance in some mice and a few other laboratory animals is controlled by a simple dominant autosomal gene. He suggested that the immune response is under direct genetic control and a single gene may be involved. Wakelin, (1975a) previously noted that the susceptibility of mice to *T. muris* was due to genetically determined differences in the ability of animals to bring about the immune expulsion of the parasite, and was independent of the size of the infection experienced. The time taken to achieve expulsion was specific for some strains of mice and rapid expulsion was the result of the presence of a dominant gene.

Whitlock and Madsen (1958) stated that natural resistance in sheep to trichostrongyloides was controlled by a dominant gene.

Windon, *et al* (1993) suggested that a major gene was responsible for resistance observed in one particular ram's progeny (Golden Ram), however, he mentioned that it was not able to be genetically proven when genetic analysis was performed on first and backcross generations. He suggested that selecting for resistance is thought to be due to a number of genes with heritabilities of 20% - 30%.

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1.9.2 Specific and general resistance to parasitic infection

As grazing sheep encounter mixed populations of parasites it would be sensible to select for heterologous resistance or for resistance to one species which would also have a heightened resistance against other species of parasites. Windon, (1990) stated that it would be expected that resistance would be conferred against the heterologous species if the mechanisms responsible for resistance to the homologous species also played an important role.

Windon, (1990) observed that high responder lambs selected for resistance against *Trichostrongylus colubriformis* were resistant to a wide selection of gastro-intestinal nematodes. However, third generation progeny, bred for resistance to *Trichostrongylus colubriformis* were only slightly resistant against infection with *Haemonchus contortus.* Similarly infecting the *Haemonchus contortus* resistant flock with *Trichostrongylus colubriformis* was 64% less effective than infecting with only *Haemonchus contortus* (Woolaston, Barger & Piper, 1990). They concluded that genetic selection to gastro-intestinal nematodes is more effective against a single species of nematode than a variety of species.

Dineen *et al* (1977), looked at the role of immunologically specific and non-specific components of resistance in cross-protection to intestinal nematodes in a flock of forty, six to eight months old Merinos.

A substantial level of protection (81%) was given when irradiated *T. colubriformis* were used to vaccinate against challenge with normal infective larvae of the same species. The level of protection (34%) was substantially

reduced against challenge with a closely related species *T. vitrinus* and no significant protection was evident against single species challenge with a generically unrelated nematode, *Nematodirus spathiger*. However, vaccination with *T. colubriformis* gave 98-100% protection against adult worms of all three species of nematodes in multiple challenge.

The total adult worm counts for *T. colubriformis* in the vaccinated control animals were significantly lower in multiple species than in single species infection. However, in multiple and single infections of *T. vitrinus* no significant difference could be seen. Dineen *et al* (1977), suggested that *T. colubriformis* was disadvantaged by the presence of the other species during multiple infection.

Fourth stage larvae of *N. spathiger* were recovered from all single, multiple challenge vaccinated, and unvaccinated sheep infected with this species. As the larvae were present thirty three days after infection it was obvious that they were arrested during development. This was not due to vaccination as no significant difference could be seen between vaccinated and unvaccinated groups. However, the absence of *N. spathiger* adults indicated that at some stage of development after the fourth stage larvae the parasite becomes susceptible to vaccination provoked in the lambs by multiple challenge.

The fact that no mature *N. spathiger* worms were found after vaccinating animals challenged with all three nematode types suggested a high level of protection was seen but the fact that a reduction in the species was not seen after single species challenge suggested the involvement of non-specific protection. Dineen *et al* (1977), noted that although relevant antigenic relationships between *Nematodirus* and *Trichostrongylus* probably did not

exist, a substantial level of non-specific protection could be expressed in vaccinated animals provided that the appropriate antigenic stimulus was produced by concurrent *Trichostrongylus spp* infections. The workers therefore suggested that the terminal effectors of resistance are immunologically non-specific.

However, Dineen *et al* (1977), also recognised a second possibility for protection only in the multiple-species challenge, i.e. low-level specific cross reactivity, undetectable in single-species challenge may be expressed in multiple-species challenge due to an additive effect of the antigenic stimulus due to the presence of *T. colubriformis*, the homologous species.

Adams, Anderson and Windon (1989a) examined cross-immunity in sixteen month old worm free Merino wethers between *Haemonchus contortus* and *Trichostrongylus colubriformis* in sheep vaccinated with irradiated larvae of either species.

Vaccination with *T. colubriformis* larvae was responsible for some protection against challenge infection with *T. colubriformis*. However, vaccination with *H. contortus* irradiated larvae did not cross-protect against *T. colubriformis*.

Vaccination with *H. contortus* larvae gave protection against *H. contortus* infection but vaccination with *T. colubriformis* gave no significant protection against challenge infection with *H. contortus*.

For both nematode species, the values for FEC in the cross-vaccinated and challenged sheep fell between those for the homologously vaccinated and challenged sheep and the infection controls. Therefore these results showed

that acquired immunity due to irradiated larvae protects only against the species used for vaccination. The results showed a ninety percent reduction in FEC from sheep vaccinated and infected with *H. contortus* and a seventy eight percent reduction in sheep vaccinated and infected with *T. colubriformis*.

1.9.3 Innate and acquired resistance to parasitic infection

Brindley and Dobson (1983) looked at innate and acquired immunity in mice after infection with *Nematospiroides dubius*.

The mean number of adult worms recovered from the treated nine groups showed significant variation. Overall the groups treated with sera from once infected mice (IMS) harboured fewer parasites than those treated with sera from naive mice (NMS) and the control groups. The control groups contained equal amounts of worms.

Serum from infected refractory (R), random bred (Rd) and liable (L) mice conferred the same low levels of passive protection on all mice against *N. dubius*.

A significant variation was seen in FEC between the six groups of mice ten days after infection. The FEC of R, Rd and L IMS were less than NMS and the controls. There was no variation in FEC between the three control groups. In addition, there was no significant difference between R, Rd and L NMS groups ten days after infection.

Brindley and Dobson (1983) concluded that there were no differences between the protective efficacy of IMS from any of the R, Rd, or L donor mice in two recipient strains. In addition, the serum anti *N. dubius* antibody titres from the three lines of donors did not vary with selection over eleven generations.

When the recipients were treated with IMS from all three selected lines of infected donors a limited passive protective immunisation was seen, especially

in one particular strain (the C3H strain). The low level of passive protective immunity coupled with failure to differentiate between R and L mice indicated strongly to Brindley and Dobson (1983) that selection had separated mice which differed in innate rather than acquired immunity to *N. dubius*. However, they realised that this fact did not prove that the donor R and L mice had the same degree of protective immunity. The R and L mice used in Brindley and Dobson's experiment showed differences in their FEC without differences between their titres of anti-*N. dubius* serum antibody after primary infection. Thus, the R and L mice expressed differences in their innate rather than in their acquired immunity to primary infections with *N. dubius*. They also noticed that multiple infections with *N. dubius* stimulated greater protective immunity accompanied by higher anti-*N. dubius* serum antibody titres in R than in L mice, thus indicating to Brindley and Dobson (1983) that the genetic mechanisms controlling innate and acquired protective immunity were linked.

1.10 Mechanisms which may be responsible for variation in resistance.

1.10.1 Worm establishment

Gordon (1948) saw a dramatic decrease in FEC of *Haemonchus contortus* occurring simultaneously in nearly all the sheep of many flocks in one area. This was later described by Stewart (1950) to be due to massive reinfection, i.e. once the new 'population' of worms had become established a rapid expulsion of the previous infective population occurred. However, this did not occur with populations of the nematode *Trichostrongylus colubriformis,* Stewart (1953) described worm expulsion as an allergic response.

Barger, Le Jambre and Davies (1985) investigated how populations of *H. contortus* were regulated in animals exposed to continuous infections.

Sheep were infected with one of four different doses of infective larvae. The sheep were infected three times a week for fifteen weeks. Then at intervals of three weeks sheep from each of the four groups were administered radio labelled larvae and then killed in order to measure the establishment of incoming larvae during the continuing infection.

Barger *et al* (1985) found that in the groups receiving the highest larvae doses per week, i.e. 2400 and 4800 larvae, worm numbers rapidly declined and by the end of the experiment had lower worm burdens than those administered 600 and 1200 larvae per week. This showed that the number of worms lost per unit time is related to the rate of larval intake and it is also known to be associated with the duration of the host's previous experience of infection. Forty-five per cent of larvae administered became established at week seven

6% at week ten and at week thirteen an insignificant number were established. The proportion of incoming larvae arrested increased as the infection progressed. Barger *et al* (1985) concluded that *H. contortus* numbers were regulated by the host's development of resistance to infection, the host's previous experience and by a loss of establishment of worms as the infection progressed.

Seaton, Jackson, Smith and Angus (1989) infected three groups of six-month old lambs daily with 1000 third stage larvae of *Trichostrongylus vitrinus* for four, eight and twelve weeks. After each period , one of those groups consisting of five animals and a group of four worm-free controls were challenged with three consecutive daily doses of 1000 third stage radiolabelled larvae. These lambs were killed ten days after the first dose, and their worm burdens examined. After four weeks of continuous infection partial immunity to the establishment of challenge was apparent and by eight and twelve weeks there was almost total resistance to incoming worms.

1.10.2 The role of Th lymphocytes

Windon *et al* (1993) stated that T helper (Th) or CD4 lymphocytes are of paramount importance in the resistance to gastro-intestinal nematodes. Gill, Watson & Brandon, (1993) stated that the cells were of major importance in resistance to *Haemonchus contortus,* as lambs became susceptible after depletion with monoclonal antibodies. Haig, Windon, Blackie, Brown and Smith (1989) demonstrated that CD4 cells were activated during *Haemonchus contortus* infection. Also *in vitro* proliferative responses to parasite antigen in these cells were blocked by monoclonal antibodies directed against ovine MHC class II determinants. Th lymphocytes produce cytokines which control

and regulate the immune response. In rodents cytokines produced by Th2 lymphocytes are responsible for the induction of IgE, mastocytosis and eosinophilia which are associated with resistance in lambs (Gill, Gray and Watson, 1991; Windon, 1991b). Susceptibility was associated with a Th1 response (Else & Grencis, 1991).

1.10.3 The effect of nutrition on infection

Downey (1966) looked at the effect of cobalt levels on sheep infected with the gastro-intestinal nematode *Trichostrongylus axei.*

The test animals were reared artificially under worm-free conditions and fed on a diet low in cobalt. At the age of eleven weeks the lambs were divided into groups, half were fed a cobalt supplemented diet and the other half kept on the low cobalt diet. Five weeks later one group of lambs fed on the cobalt supplement diet and one group fed on the very low cobalt diet were infected with 51,000 infective larvae over a period of time.

The two groups fed on cobalt deficient diets, i.e., the worm free and worm infested groups showed a similar clinical appearance of cobalt deficiency up until six weeks after the introduction of infection.

The cobalt deficiency was responsible for a reduction in growth. Infection in cobalt deficient lambs caused no further decrease in weight, whilst cobalt fed lambs when infected were seen to lose weight rapidly. However, cobalt fed non-infected lambs showed an increase in live weight gain over the experimental period.

Downey (1965) described one experiment with *Haemonchus contortus* and cobalt states.

The lambs on a low cobalt diet and infected with the nematode had initially lower FEC and less anaemia than those infected lambs receiving cobalt supplement. Downey (1965) stated that a low cobalt status in the host had an unfavourable effect on the parasites. Downey concluded that *T. axei* was similarly susceptible to the cobalt status of the host.

The results suggested an interaction between cobalt deficiency and the initial stages of parasite attack. Since however, the lambs in the two cobalt-deficient groups were gaining little or no weight during much of the experimental period differences in weight change due to infection comparable with those seen in cobalt sufficient lambs were not expected. A number of lamb deaths occurred as the infection and deficiency progressed. Downey was therefore unable to state whether the effects of infection and cobalt deficiency were additive.

Downey (1966) published a further paper describing the relationship between *Ostertagia circumcincta* infestation and cobalt status in lambs.

The author looked at the effect of prolonged worm infestation on serum vitamin B_{12}.

Blood samples of infected, cobalt fed lambs showed depressed vitamin B_{12} concentrations. This according to Downey was probably due to a number of factors including an increased excretion of vitamin B_{12} via the faeces in the form of diarrhoea.

The cobalt deprived infected lambs showed a greater decline in haemoglobin concentrations than the infected lambs which were given cobalt and a more pronounced retardation of weight gain than the cobalt/infected and the cobalt deprived/non infected groups. Downey (1966) also described evidence of lambs having a low cobalt intake harbouring more parasites than lambs receiving adequate cobalt. Therefore Downey concluded that the effect of infection and a low cobalt diet seemed to be additive in this particular experiment.

The previous results described cobalt supplementation as enhancing fecundity and the pathogenicity of worms such as *T. axei* and *H. contortus*. The author therefore assumed that the minute doses of cobalt given at the beginning of infection to the cobalt deprived/infected lambs enabled the worms to reproduce normally. These lambs now were more susceptible to infection due to their low cobalt intake as compared with lambs in the cobalt/infected group.

Downey (1966) also suggested that *O. circumcincta* may differ from *H. contortus* and *T. axei* in being insusceptible to cobalt status of the host.

Abbot, Parkins and Holmes (1985) noticed a difference in FEC's between Scottish Blackface lambs and Finn Dorset lambs, with Finn Dorsets which are more susceptible having decreased egg counts when fed a soya supplemented diet.

Wallace, Bairden, Duncan, Fishwick, Gill, Holmes, McKellar, Murray, Parkins and Stear (1995) noticed that Hampshire Down lambs when fed a basal diet had higher FEC's than those fed a supplemented diet. Lambs

supplemented with fishmeal showed improved resistance to *Trichostrongylus colubriformis* (van Houtert, Barger & Steel, 1992), and lambs supplemented with meat and bone meal and soyabean meal also showed improved resistance to *Trichostrongylus colubriformis* (Kambara, McFarlane, Abell, McAnulty and Sykes, 1993).

Bang, Familton, and Sykes, (1990) looked at the effect of copper oxide wire particle treatment on establishment of *Trichostrongylus colubriformis, Ostertagia circumcincta* and ,*Haemonchus contortus* in lambs. Five grams of uniform size copper oxide wire particles were given orally five days before infection with 20,000 *Trichostrongylus colubriformis,* larvae 20,000 *Ostertagia circumcincta* larvae or 3000 *Haemonchus contortus* larvae The animals were slaughtered 21 days after the last infective dose. Parasite burdens in the lambs treated with copper oxide wire particles were reduced by 96% in the case of *Haemonchus contortus* and 56% in the case of *Ostertagia circumcincta* compared with burdens in controls. There was however, no significant effect of copper on the establishment of *Trichostrongylus colubriformis.*

Coop & Field, (1983) looked the effect of phosphorus intake on growth rate, food intake and quality of the skeleton of growing lambs infected with *Trichostrongylus vitrinus.* The lambs were dosed daily for twelve weeks with 2500 larvae and given two levels of phosphorus intake; 2.75 gP/kg dry matter and 1.88 gP/kg dry matter. Both low phosphorus and *T. vitrinus* infection reduced dry matter intake and growth rate and the combined effect was additive

Suttle, Knox, Jackson, Coop and Angus (1992) noticed that after the addition of molybdenum to the diet of lambs exposed for four weeks to a trickle infection of *T. vitrinus*, the number and size of the worms was reduced. These effects were particularly obvious in female worms from female lambs. Worms from molybdenum treated lambs contained less proteinase enzyme activity and secreted less proteinases in culture irrespective of the sex of the host. However, pathogenicity was not affected. They concluded that molybdenum may be toxic to the parasite and may also aid in the process of larval rejection.

Abbott, Parkins and Holmes (1988) investigated the effect of giving high or low protein diets to three month old lambs and infecting them with a trickle infection of *H. contortus* (200 larvae given three times a week). Clinical signs of infections became evident only in the low protein diet group. The severe anaemia, hypoproteinaemia and reduced survival were coupled with high egg counts and extremely high worm burdens. The lambs fed on a low protein diet did not develop a resistance to further infection as did the high protein group. Live weight gain was not effected by infection in high protein lambs but decreased significantly in a low protein lambs. However, nitrogen retention, iron absorption and digestive efficiency were not effected in either group although loss of appetite was observed in the low protein lambs.

Abbott and Holmes (1990) investigated the influence of dietary protein on the response of sheep to vaccination with irradiated *Haemonchus contortus* larvae. Twenty lambs were fed diets containing either 169g CP kg^{-1} DM or 88g CP kg^{-1} DM from 7 months of age. Five lambs fed either diet were vaccinated with irradiated larvae and then the remaining lambs were administrated 10,000 infective larvae. FEC, packed cell volumes and red cell counts were performed in order to monitor infection rates. The results demonstrated that there was a

difference between the vaccinated and challenge controls within a dietary group. However, there was no difference between the two dietary groups.

Coop, Huntley and Smith, (1995) looked at the effect of dietary protein supplementation on the development of immunity to *Ostertagia circumcincta* in thirty four-and-a-half-month-old growing lambs. Half the lambs (groups 1 & 2) were fitted with abomasal catheters and infected daily with 2000 *O. circumcincta* L3 larvae for eight weeks. Half those lambs(group 1) received 45 g of crude protein day^{-1} as a continuous infusion into the abomasum from weeks 1-8. At week nine, both groups of lambs, together with eight naïve controls, were treated with an anthelmintic and challenged a week later with 50,000 larvae and killed ten days later. A further six worm-free lambs were used as controls. All lambs were given a complete ruminant ration (167 g crude protein kg^{-1}) ad libitum. The cumulative liveweight gains of both the trickle-infected groups was less than that of the controls. The mean FEC's were lower in group 1 but total worm burdens were similar for all three groups.

1.10.4 The effect of infection on nutrition

MacRae (1993) stated that clinical and subclinical intestinal parasitism is characterized by impaired production with poor growth rates in young animals and loss of body weight in older animals.

Abomasal nematodes

Sykes *et al* (1992) looked at the effect of infection on nutrition and observed that *O. circumcincta* adversely effects gut pH which in turn effects solubility and absorption of nutrients in subsequent sections of the tract whereas other abomasal dwellers, e.g. *T. axei*, have much less intimate contact with tissues therefore there is a less of an effect on pH.

Intestinal nematodes

Kimambo *et al* (1988a) observed the effect of prolonged subclinical infection with *T. colubriformis* on the performance and nitrogen metabolism of growing, 5 month old lambs.

They noticed that the infection caused a decreasing growth rate from week 6 to 13 of dosing with infective larvae. This was said to be due to inappetence and the disruption in the nitrogen digestion and metabolism of the lambs. However, the effect was reduced as the experiment progressed. A reduction in the coefficient of digestion of ^{35}S microbial protein in infected lambs however, continued passed week 19.

There is conflicting evidence on the question of whether sheep which have developed resistance to the intestinal nematode *Trichostrongylus colubriformis* suffer any nutritional penalty when re-challenged with the parasite.

Steel, Jones and Symons, (1980) and Kimambo *et al* (1988b) demonstrated that continuous challenge of lambs which were showing resistance to establishment of *T. colubriformis* had no effect on parameters such as feed intake, nutrient digestibility, growth rate, wool growth, nitrogen retention, plasma leakage into the gut and fractional catabolic rate of albumin. Wagland, Steel, Windon and Dineen (1984) also found little effect on growth rate or wool growth rate when lambs immunised with irradiated larvae were challenged with infective *T. colubriformis* larvae. However, other workers have reported problems when resistant sheep have been challenged with infective larvae. Workers such as Barger (1973) and Barger and Southcott (1975) reported a reduced wool growth but with little associated effect on body weight gain, Yakoob, Holmes and Armour (1983b) observed increased plasma pepsinogen level, fractional catabolism of albumin and plasma leakage into the gut of resistant non-lactating ewes when they were challenged with a multiple infection, i.e. of mixed parasites. Anderson (1973) also witnessed increases in plasma pepsinogen concentration and reductions in wool growth when sheep were grazing on a low contaminated pasture.

Kimambo *et al* (1988a) conducted an experiment to examine a number of nutritional and immunological parameters of re-challenged resistant sheep. The workers attempted to simulate the situation where sheep are exposed to a contaminated pasture, after a worm-free winter. The sheep were challenged daily with *T. colubriformis* larvae for ten weeks after a prolonged, six months

parasite-free period. This type of re-challenge was unlike the experiments mentioned above as those animals were re-challenged immediately after the primary infection for only short periods of time.

Kimambo *et al* (1988a) used eight, twenty-one month old Suffolk X Finn Dorset sheep. Half had been dosed previously with 2500 *T. colubriformis* larvae daily for thirty-four weeks, followed by a twenty-four week parasite free period.

These sheep were then again dosed daily with 2500 larvae for ten weeks. The other four sheep had been kept parasite free and pair-fed with the four infected sheep throughout the experimental period.

Plasma-N leakage into the gut by use of $CrCl_3$ injected intravenously was assessed continuously over the first twenty days of dosing and again during weeks seven and eight of dosing.

Digestion of ^{35}[S]-labelled microbial protein within the small intestine was measured during weeks four and five. Digesta N flow to the terminal ileum was measured during weeks five and six. In addition faeces and urine were collected for five days during week four of dosing in order to determine N retention in the control and infected sheep.

The results showed that re-challenge infection did not cause any decrease in appetite or live-weight gain, and there was no significant difference between the control and re-challenge sheep in ration digestibilities, N retention, N flow at the terminal ileum or digestibility of ^{35}[S]-labelled microbial protein from

the small intestine. However, re-challenge did cause some increased plasma-N leakage.

These results agreed with those on lambs immunised with irradiated larvae and then infected with *T. colubriformis* (Steel *et al* 1982; Wagland *et al,* 1984) and on resistant ewes infected with a multiple infection of mixed parasites (Yakoob *et al,* 1983b). Kimambo *et al* (1988a) suggested that these observations were due to damage to the mucosa caused by the burrowing of infective larvae or that an immune reaction may have been caused by the presence of the parasites in the gut, which increased the permeability of the intestinal mucosa to macro molecules, probably by a hyper-sensitive reaction caused by IgE-mediated mast-cell degranulation.

The workers further suggested that if plasma leakage was associated with an inflammatory response, i.e., increased vascular permeability from hyper sensitivity in the gut mucosa, then Kimambo *et al* (1988) stated that their experiment indicated that previously resistant sheep respond to challenge infection much more rapidly than naive lambs experiencing primary infection. Barker (1973) infected naive lambs with infective larvae and noticed that a twelve day period elapsed before plasma leakage occurred. Steel *et al* (1980) also noticed a delay, i.e. plasma leakage did not occur until after week four of continuous dosing.

1.10.5 Grazing behaviour

Twin lambs only have two thirds of the milk supply available to single lambs. Therefore twin lambs increase their herbage consumption and whilst outdoors graze and consume more herbage than single lambs by as early as week four. This increased herbage intake by twin lambs increases rapidly from around week six to seven of lactation (Gibb and Treacher, 1980; Gibb, Treacher and Shanmugalingam, 1981). As twin lambs graze more than single lambs they are more likely to pick up larvae on the pasture. Therefore lambs which 'survive' this initial infection have a greater chance of becoming resistant to future infections than do lambs which are less likely to pick up the helminths from the pasture at an early age. Thus some lambs born in multiple births may have a better chance of developing resistance to helminth infections.

The main aims of the experiments described in the thesis were to evaluate the extent to which variation to nematode infection occurs; both in Cambridge ewes during the periparturient rise period and in growing Cambridge and Cambridge cross lambs. The research programme was designed to investigate the possibility of selecting animals with increased resistance to *Ostertagia circumcincta*, and to develop a general evaluation procedure for a suitable selection programme for resistance to nematodes.

Exploiting variation in resistance to nematode infection in sheep breeding populations is a possible method for the control of gastrointestinal nematodes. Phenotypic variation was measured by taking faecal samples on which FEC were performed and blood samples on which plasma pepsinogen concentrations were determined.

CHAPTER 2

VARIATION IN NEMATODE INFECTION RATES IN EWES DURING THE PERIPARTURIENT RISE

2.1 Introduction

Nematode infection with a number of different parasite genera is predominantly associated with lambs during their first grazing season. However, gastrointestinal nematode infections are also seen in ewes in the weeks around partum (periparturient rise). The epidemiological consequences of this infection in ewes are two-fold (1) Survival of the parasitic stages within the pregnant host during environmental stress is ensured resulting in (2) exposure of infective stages of the parasite to the susceptible lambs (Fleming and Conrad, 1989).

The periparturient rise is said to be due to a number of factors primarily, hormonal immunosuppression, antigenic stimulus, nutrition and season, (Urquhart and Armour, 1973). It is thought by most authors that the rise in FEC reflects a fall in the immune status of the host due to endocrinological changes associated with reproduction and lactation. However, a less severe increase in faecal egg production has been seen in non-pregnant ewes and castrated rams (Dobson, 1964; Grossman, 1989) which is attributable to seasonal, i.e. diurnal changes. The maturation of arrested larvae thus is considered to be di-phasic; firstly a hypothalamic-pituitary mediated stimulus which is dependent on season and then a stimulation by the pregnancy hormone prolactin which results in a full maturation of the larval population, (Gibbs, 1967). Connan (1968) observed a wide range in the number of overwintered larvae of *H. contortus* and *O. circumcincta* in ewes during the post-parturient rise.

The inclusion of parasite resistance as a selection criterion/objective in commercial breeding programmes will depend on the development of recording methods which are accurate, cost effective and repeatable. At present recording of FEC seems to be the best method to fulfill the above criteria.

The main aim of the experiments described in this chapter was to evaluate the extent to which FEC varied in ewes during the periparturient period as part of a research programme designed to investigate the possibility of selecting animals with increased resistance to nematodes.

2.2 Materials and Method

The experiments were conducted in the Department of Veterinary Clinical Science and Animal Husbandry, University of Liverpool over three years from 1991 to 1994.

Experimental Animals

In 1992 (year 1) and 1993 (year 2) 93 and 104 respectively, naturally infected Cambridge ewes, aged between 2 and 10 years, were investigated. The ewes were lambed in two groups each year. In 1992 group 1 consisted of 34 mature ewes and 10 shearling ewes and the second group 38 mature ewes and 11 shearling ewes. In 1993 group 1 consisted of 51 mature ewes and 1 shearling ewe and the second group 33 mature ewes and 19 shearling ewes. The ewes of each group were synchronised using progesterone sponges (Upjohn Limited) and mated at the second oestrus, in both years group 1 was mated in mid October and group 2 in early November. So group 1 lambed in early March and group 2 at the beginning of April. In year 1 the ewes of both groups were mated with one of three Cambridge or a Vendeen or a Charollais or a Suffolk ram. In year 2 ewes were mated with one of eight Cambridge or a Charollais or a Texel ram.

The ewes were housed and brought in from contaminated pasture previously by lambs and ewes that year. The ewes were winter sheared 7 to 8 weeks prior to lambing and remained inside until 5 weeks *post-partum*. Ewes were vaccinated with HeptovacP (Hoechst), a multivalent clostridial vaccine at 2-3 weeks *pre-partum*. At housing, hay (CP = 9.22 %, M.E = 9.33) only was fed

ad-libitum. From 6 weeks pre-partum a complete diet (CP = 11.1 % ,i.e.111g/kg DM) for pregnancy needs was also provided and fed at increasing amounts until parturition.

Parasitological Techniques

Faecal samples were taken and FEC were recorded in both years and groups initially at 8 weeks pre-lambing. Samples were then taken weekly from 4 weeks pre to 4 weeks post-partum in year 1 and from 4 weeks pre to 3 weeks post-partum in year 2. FEC were also recorded within 24 hours of lambing. Faeces were collected from the rectum and egg counts were performed using the Modified McMaster technique (MAFF Manual of Veterinary Parasitological Laboratory Techniques, 418). Three grams of faeces were added to 42 ml of saturated saline and ground in a pestle and mortar to make a solution. The faeces solution was placed in a McMaster slide with a Pasteur pipette and two replicates (both chambers of the slide) were counted and the mean of the two calculated. Using this method of counting both chambers it was possible to measure 50 eggs per gramme intervals.

In 1992 (year 1), third stage larvae were identified in order to observe the most prevalent nematode species present. Third stage larvae were cultivated from nematode eggs by incubating faecal samples taken 3-2 weeks *pre-partum* from 8 heavily infected ewes at 27 ^{o}C for 7 days. After this incubation period larvae were recovered from faeces by the Baermann method (MAFF Manual of Veterinary Parasitological Laboratory Techniques, 418). The larvae were identified under a compound microscope, using a X 10.0 objective (X 100 magnification).

Haematological Techniques

In 1993 blood samples were obtained from 50 ewes in group 1 by jugular venepuncture at 3 weeks *pre-partum*, within one week of *partum* and at 2 weeks *post-partum*, two samples were taken on each occasion. One sample was collected in a vacutainer containing heparin to enable plasma to be obtained which was then used to measure plasma pepsinogen levels and a second sample collected in a heparinised container was used to estimate packed red blood cell volumes.

Plasma from blood in the heparinised tubes was separated from the cellular component of the blood by centrifugation for the measurement of plasma pepsinogen concentrations by a modified version of the method of Edwards (1969) (see Appendix 4) and concentrations were expressed as (Mm tyrosine/ min/ 37 ^{o}C) .

Packed cell volumes (PCV's) were determined by using microhaematocrit apparatus (Hawksley, Sussex England) and were expressed as percentage of haematocrit in a volume of blood.

Statistical Analysis

Statistics were undertaken by the use of the computer package Statistical Analysis System (SAS). Data of FEC was found to have a significant skewed distribution and was transformed into log form (x + 1) before analysis. The means presented for FEC are therefore geometric means unless otherwise stated and the variability of each mean figure is described by its percentage

standard deviation obtained by taking the antilog of the log form standard deviation, and by 95% confidence limits of the mean. Statistical analysis performed included t-tests, analysis of variance, GLM, least significant difference tests and chi-square tests.

2.3 Results

Nematode eggs were identified as trichostrongylid (including *Nematodirus spp.*), *Trichuris ovis* and *Strongyloides papillosus*. The trichostrongylid eggs (excluding *Nematodirus spp.*) were then cultivated into larvae for identification purposes.

Cultivation of larvae

Cultivation of trichostrongylid third stage larvae from nematode eggs in faecal samples by the Baermann technique demonstrated that 81% of the eggs hatched were of the *Ostertagia* genus (Table 2.1). (i.e. *Ostertagia circumcincta* which is the most prevalent ovine gastrointestinal nematode in Great Britain). It appears in ewes during the periparturient rise after a period of hypobiosis in the lumen of gastric glands of the abomasum. Other nematode larvae found included Trichostronylus spp. and Cooperia spp..

Table 2.1 : Cultivation of third stage larvae

Ewe	Larvae Recovered	% Prevalence of all Nematode Eggs	% Prevalence of Larvae Recovered Used to Estimate EPG	EPG
0472	*Trichostrongylus spp.* *Ostertagia spp.* *Cooperia spp.* *Strongyloides papillosus* *Trichuris ovis*	7 80 7 4 2	10 80 10	1350
0238	*Ostertagia spp.*	100	100	300
6073	*Ostertagia spp.* *Trichostrongylus spp.*	90 10	90 10	850
8222	*Trichostrongylus spp.* *Ostertagia spp.* *Cooperia spp.* *Strongyloides papillosus*	20 60 13 7	20 70 10	1400
0305	*Trichostrongylus spp.* *Ostertagia spp.*	30 70	30 70	1100
0304	*Trichostrongylus spp.* *Ostertagia spp.* *Cooperia spp.* *Strongyloides papillosus* *Trichuris ovis* *Nematodirus spp.*	15 70 5 3 2 5	15 80 5	2650
0320	*Trichostrongylus spp.* *Ostertagia spp.* *Cooperia spp.* *Nematodirus spp.*	10 70 10 10	10 80 10	1200
0489	*Trichostrongylus spp.* *Ostertagia spp.* *Cooperia spp.* *Strongyloides papillosus* *Trichuris ovis*	15 70 7 5 3	20 75 5	1000

Faecal Egg Counts (FEC)

The pattern of trichostrongylid egg counts from 4 weeks *pre* to 4 weeks *post-partum* was not uniform from year to year as shown in Table 2.3 and Figures. 2.1 and 2.2. Some differences appeared in individual group patterns in year 1, group 1 ewes reached a peak at *partum* and group 2 ewes peaked 3 weeks *pre-partum.* In 1993, year 2, group 1 ewes reached a peak 1 week prior to partum and group 2 ewes at *partum* and then 2 weeks *post-partum.*

The mean egg counts of the 9 samples taken in year 1, in the period 4 weeks pre to 4 weeks *post-partum* were significantly lower ($p < 0.05$) than the 8 samples taken in year 2, over the 4 weeks pre to 3 weeks post-partum period as shown in Table 2.2. These figures do not include those of 2 ewes in each year which had very high egg counts, between 2,000 and 5,500 EPG, during pregnancy and which had become so thin at one week post-lambing, (with egg counts between 1,900 and 6,000 FEC) it was thought necessary to administer an anthelmintic dose.

Table 2.2 : Variation in FEC of ewes in both years 1 and 2

Year	n	Mean	%S.D.	confidence limits	mean sample range
1992	90	279	1.97	244-320	36-1390
1993	101	442	2.10	381-512	13-2745

Table 2.2a : Mean of faecal samples *pre-partum*, *post-partum* and the overall mean

	Week			
Ewes		*Pre-Partum*	*Post-Partum*	Overall Mean
Group 1 1992	Mean	219	193	284
	n	42	39	44
	%S.D.	3.19	4.09	2.18
	conf limits	154-310	124-299	224-355
Group 2 1992	Mean	305	154	276
	n	45	45	49
	%S.D.	2.06	3.50	1.80
	conf limits	248-377	107-222	234-324
Group 1 1993	Mean	434	375	456
	n	49	47	51
	%S.D.	2.32	2.12	1.86
	conf limits	339-537	303-465	376-531
Group 2 1993	Mean	335	403	427
	n	52	46	50
	%S.D.	3.24	2.77	2.37
	conf limits	243-460	304-546	339-537

No overall effect of group was seen on FEC and the majority of mean FEC for both groups in each year were not significantly different (Tables 2.2b and 2.2c). Year to year sample means were however, significantly different except 3 and 2 weeks pre-partum and an overall effect of year was seen on FEC ($p < 0.05$)as shown in Table 2.2d. This data was analysed by performing GLM and LSD tests.

Table 2.2b : Significance of differences of means of samples between groups in year 1

Week	4	3	2	1	P	1	2	3	4
Sig.	0.0001	n.s.	n.s.	0.05	0.0001	0.02	n.s.	n.s.	n.s.

Pre-partum	*Post-partum*
n.s.	n.s.

Table 2.2c : Significance of differences of means of samples between groups in year 2

Week	4	3	2	1	P	1	2	3
Sig.	n.s.	0.001	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Pre-partum	*Post-partum*
n.s.	n.s.

Table 2.2d : Significance of differences of means of samples between years

Week	4	3	2	1	P	1	2	3
Sig.	0.0001	n.s.	n.s.	0.0001	0.003	0.005	0.05	0.001

Pre-partum	*Post-partum*
0.0001	0.001

Table 2.3 : Means of faecal samples from 4 weeks pre- to 4 weeks post-partum.

		Week								
Ewes		4	3	2	1	P	1	2	3	4
Group	Mean	5	149	157	196	248	115	110	38	43
1	n	43	43	42	43	44	44	44	41	42
1992	%S.D.	9.59	7.63	11.18	6.50	5.62	12.87	13.84	15.89	15.91
	conf limits	2-9	81-274	75-321	111-344	149-413	54-245	50-238	16-89	19-98
Group	Mean	153	184	64	74	34	24	52	15	72
2	n	49	49	47	46	40	47	48	46	48
1992	%S.D.	8.07	10.2	13.78	11.43	18.20	21.93	14.31	16.90	10.73
	conf limits	85-275	96-353	30-134	37-151	14-84	10-58	24-110	7-34	37-142
Group	Mean	358	372	280	407	401	280	233	251	
1	n	51	51	51	50	50	49	48	47	
1993	%S.D.	2.40	3.14	4.46	4.4	3.08	5.07	6.11	5.41	
	conf limits	274-450	257-489	181-415	262-605	291-544	178-433	140-386	155-407	
Group	Mean	288	87	200	279	309	250	335	288	
2	n	52	52	52	52	50	49	48	46	
1993	%S.D.	5.74	17.10	6.98	6.04	6.79	7.74	4.6	5.83	
	conf limits	179-463	40-187	29-339	171-455	182-525	141-445	217-510	174-479	

Figure 2.1 : Ewes, 1992

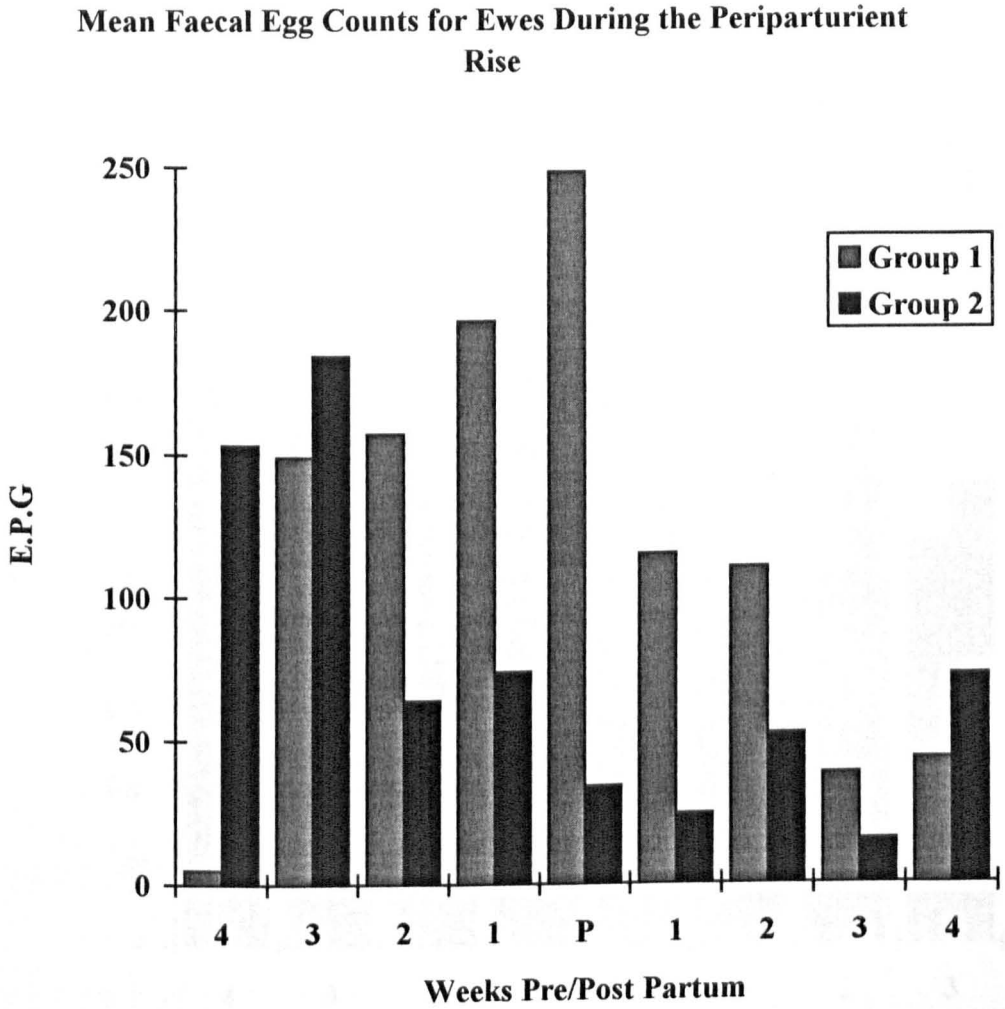
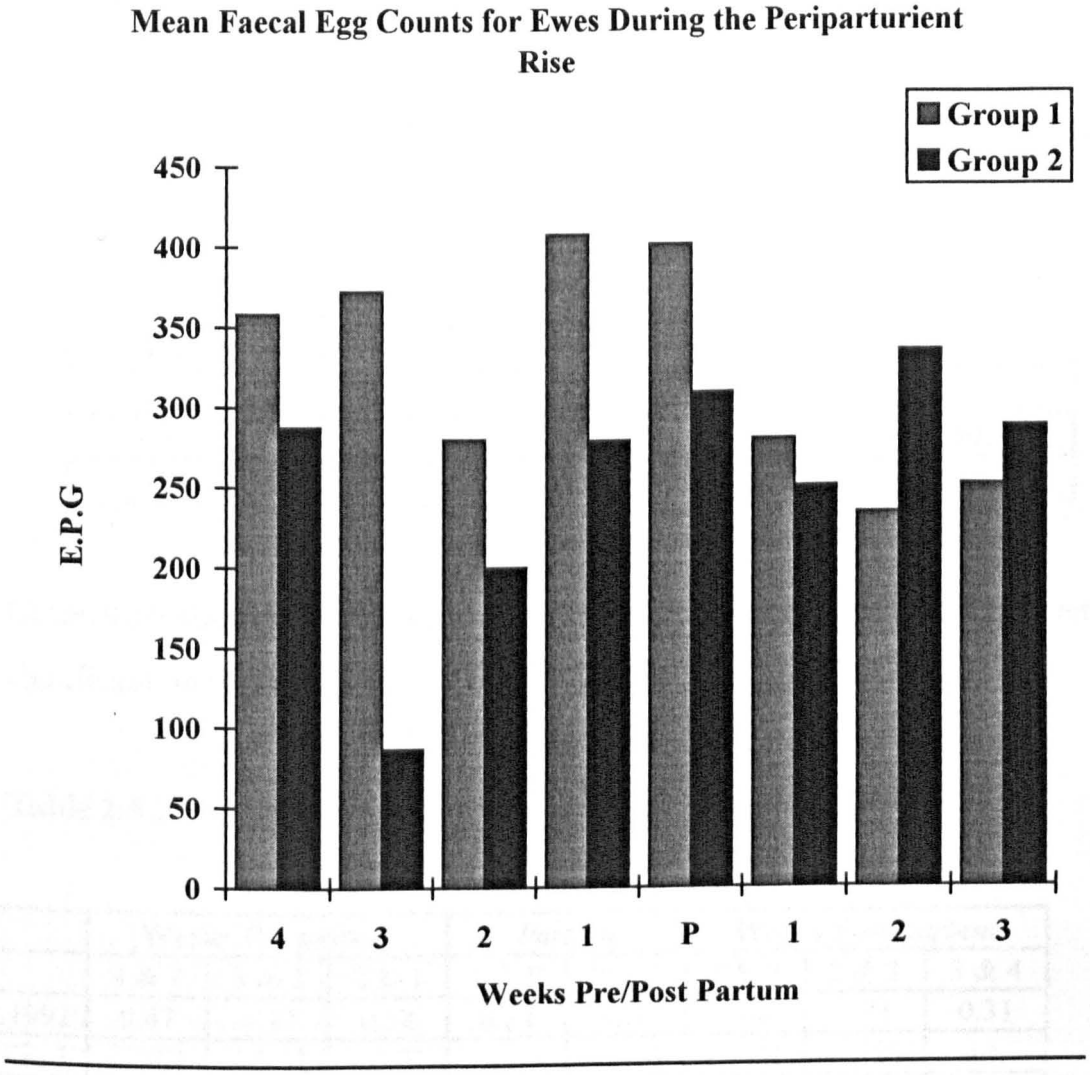


Figure 2.2 : Ewes, 1993



There was a significant correlation between individual ewe counts recorded in both years (r = 0.59).

The relationships between *pre* and *post-partum* consecutive sampling periods, and between individual samples and the overall mean are quantified by the correlation coefficients shown in Tables 2.4, 2.5 and 2.6.

Table 2.4 : Correlation coefficients of pre- compared with post-partum faecal samples

Year	n	Correlation	Significance
1992 Gp 1	37	0.35	< 0.05
1992 Gp 2	33	0.44	< 0.05
1993 Gp 1	39	0.85	< 0.001
1993 Gp 2	46	0.71	< 0.001

Correlation coefficients between mean pre- and mean post-partum FEC were significant in both years.

Table 2.5 : Correlation coefficients for consecutive faecal samples

	Weeks *Pre-partum*			***Partum***		**Weeks *Post-partum***		
	4 & 3	3 & 2	2 & 1	1 & P	P & 1	1 & 2	2 & 3	3 & 4
1992	0.47	0.37	0.58	0.28	0.70	0.48	0.71	0.31
Gp 1	< 0.01	< 0.02	< 0.01	n.s.	< 0.01	< 0.01	< 0.01	n.s.
1992	0.14	0.56	0.10	0.02	0.48	0.33	0.13	0.52
Gp2	n.s.	< 0.01	n.s.	n.s.	< 0.01	< 0.05	n.s.	< 0.01
1993	0.76	0.60	0.67	0.50	0.53	0.55	0.27	
Gp 1	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	n.s.	
1993	0.75	0.53	0.83	0.63	0.78	0.64	0.71	
Gp 2	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	

Correlation coefficients for consecutive samples in year 1 for group 1 were non-significant between 1 week pre-partum and partum and between 3 - 4 weeks *post-partum,* however, other correlation coefficients were significant. The fluctuating correlation coefficients of group 2 ewes from 2 weeks *pre-partum* to *partum* and also between 2 and 3 weeks *post-partum* indicate a variable production pattern of eggs/g for this group. In 1993 with the exception of group 1 ewes in weeks 2 & 3 post-partum all the correlations were significant ($p < 0.01$).

Table 2.6 : Correlation coefficients for individual faecal samples compared with the overall mean

	Weeks *Pre-partum*					Weeks *Post-partum*			
	4	3	2	1	P	1	2	3	4
1992	0.23	0.56	0.61	0.54	0.67	0.64	0.67	0.56	0.52
Gp 1	<0.05	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
1992	0.45	0.62	0.68	0.51	0.48	0.49	0.61	0.31	0.48
Gp2	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
1993	0.69	0.76	0.75	0.70	0.56	0.50	0.42	0.26	
Gp 1	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	n.s.	
1993	0.86	0.84	0.63	0.68	0.79	0.89	0.81	0.82	
Gp 2	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	

Correlation Coefficients of samples compared with the overall mean in 1992 for both groups , 1 and 2 were all significant ($p < 0.01$), and ($p < 0.05$) for 4 weeks *pre-partum* in group 1 ewes. Correlation coefficients were even higher in year 2 especially for group 2 ewes. However, the correlation between the faecal sample taken at 4 weeks *post-partum* and the overall mean was non significant.

Faecal samples taken at eight weeks *pre-partum* showed trichostrongylid eggs were present in the faeces of some ewes in both years 1 and 2. In the latter

year significantly higher numbers were present and a significantly smaller proportion of ewes were recorded as having a zero egg count. Thus the variability from sheep to sheep was extremely large as shown in Table 2.7.

Table 2.7 : FEC at 8 weeks *pre-partum*

	n	Mean	% S.D.	conf limits	Range	% with 0 FEC
1992 Group 1	38	3	10.67	2-7	0 - 800	69%
1992 Group 2	46	2	7.35	1-4	0 - 1250	78%
1993 Group 1	37	310	4.10	189-497	0 - 3846	3%
1993 Group 2	50	175	7.51	101-307	0 - 3100	12%

The correlations between 8 weeks *pre-partum* and the overall mean of 4 weeks *pre-partum* to 4 weeks *post-partum* and the *pre-* and *post* means are shown in Table 2.8.

Table 2.8 : Correlations between 8 weeks *pre-partum* and the overall mean and the *pre-* and *post* means

	Pre-partum	***Post-partum***	**Overall Mean**
1992	0.12	0.18	0.13
Gp 1	n.s.	n.s.	n.s.
1992	-0.01	0.36	0.06
Gp2	n.s.	$p < 0.02$	n.s.
1993	0.66	0.21	0.60
Gp 1	$p < 0.001$	n.s.	$p < 0.001$
1993	0.44	0.32	0.41
Gp 2	$p < 0.005$	$p < 0.05$	$p < 0.005$

Effect of Age of Ewe on Nematode Infection Rate

Overall two year old ewes in year 1 had significantly higher egg counts and greater variability than older ewes, ($p < 0.005$) (Table 2.9), with 71.4% of 2 year old ewes having higher mean FEC than the mean FEC for older ewes. However, only 50% of 2 year old ewes in year 2 had higher FEC than the mean FEC of older ewes and the differences were not significant.

Table 2.9 : Effect of ewe age on FEC

	Week									
Ewes		4	3	2	1	P	1	2	3	4	Mean
2 Year Olds 1992	Mean	57	311	183	226	153	71	214	66	206	432
	n	20	20	20	21	20	21	21	21	20	21
	% S.D.	11.92	8.31	11.81	7.52	14.49	22.79	10.81	16.60	7.03	1.89
	conf limits	19-168	122-785	62-539	95-535	47-494	19-269	78-589	20-229	89-483	330-568
Older Ewes 1992	Mean	25	141	81	97	84	47	54	17	39	245
	n	72	72	69	68	64	70	71	66	70	72
	% S.D.	17.30	8.86	13.00	9.59	12.33	17.94	14.41	15.87	13.58	1.90
	conf limits	13-48	85-233	44-148	57-167	45-154	24-92	29-101	9-33	21-72	212-285
2 Year Olds 1993	Mean	268	97	296	427	342	548	593	324		451
	n	21	21	21	21	20	20	19	18		20
	% S.D.	5.58	15.85	4.50	2.37	5.11	2.04	2.29	5.56		1.86
	conf limits	129-558	30-315	156-560	292-617	166-693	402-752	406-851	147-716		349-655
Older Ewes 1992	Mean	331	200	209	311	355	220	232	257		428
	n	80	80	81	80	80	78	77	75		80
	% S.D.	3.69	8.51	16.05	6.14	4.77	7.31	5.94	5.62		2.14
	conf limits	249-441	125-321	149-308	209-460	251-499	142-341	156-344	174-380		366-508

Effect of Litter Size on Nematode Infection Rate

Litter size did not significantly affect ewe trichostrongyle egg output in year 1 or year 2 (Table 2.10).

Table 2.10 : Effect of litter size on mean FEC of ewes

		Litter size					
Year		1	2	3	4	5	Total
1	mean	313	276	276	229	589	279
	n	18	28	31	14	2	93
	%S.D.	2.58	1.82	1.87	1.65	2.47	1.97
	conf limits	203-484	220-344	221-344	176-297	.	244-320
2	mean	325	417	506	470	470	442
	n	12	21	32	26	9	101
	%S.D.	1.72	2.86	2.06	1.78	1.96	2.10
	conf limits	237-443	264-656	391-661	374-587	240-577	381-512
Total		318	336	393	386	492	364

Plasma Pepsinogen Concentrations

The mean *pre-*, *post-partum* and *partum* plasma pepsinogen concentration of group 1 ewes for year 2 are presented in Table 2.11. The concentrations increased substantially from *pre-* to *post-partum* and *pre-* and *post* figures were equally variable. The relationship between mean plasma pepsinogen concentrations and FEC was significant ($p < 0.01$) $r = 0.54$. Correlations between consecutive samples were non significant ($r = 0.04$ and $r = 0.30$). The relationship between mean *pre-partum* FEC and *pre-partum* plasma pepsinogen levels was non significant ($r = -0.22$) as was the relationship between *partum* FEC and *partum* plasma pepsinogen levels ($r = -0.22$).

However, mean *post-partum* FEC and *post-partum* plasma pepsinogen levels did correlate significantly ($p < 0.01$) $r = 0.54$.

Table 2.11 : Mean *pre-*, *post-partum* and *partum* plasma pepsinogen concentrations of group 1 ewes, year 2

	n	Mean	S.D.	C.V.
Pre-	50	883	571.4	64.7
Partum	42	2461	2031.4	82.5
Post	43	3310	2233.9	67.5

Packed Cell Volumes

The mean packed cell volumes of blood samples taken at parturition and *post-partum* are presented in Table 2.12. The two samples correlated significantly($p< 0.001$) $r = 0.70$. A negative correlation was seen between mean PCV and mean FEC ($p < 0.05$) $r = -0.43$. A high negative correlation was seen *pre-partum* between PCV and FEC ($p < 0.025$) $r = -0.51$. However, *post-partum* a non-significant correlation was seen. Plasma pepsinogen concentrations or PCV's, were not affected by ewe age or litter size.

Table 2.12 : Mean packed cell volumes for *partum* and *post-partum*

	n	Mean	S.D.	C.V.
Partum	23	32	5.6	17.5
Post	17	32	3.8	11.9

The relationship between mean plasma pepsinogen concentrations and PCV's was negative and non-significant $r = -0.25$.

2.4 Discussion

Ostertagia circumcincta was found to be the most prevalent nematode in the area as described by Yadav (1987). It is described by many authors (Boag and Thomas, 1971, 1973, 1977; Thomas and Boag, 1973; Reid and Armour, 1972, 1975a; Waller and Thomas, 1978a; Urquhart and Armour, 1973) to be the most prevalent nematode in Britain.

The results of the present study show that there was certainly a large amount of variability in trichostrongyle infection rates demonstrated by plasma pepsinogen concentrations, PCV's and primarily FEC. Some ewes produced faeces with extremely low FEC's with egg counts of zero whilst others had infection rates so high (in excess of 2,000 EPG) that they had to be treated with an anthelmintic immediately *post-partum* for welfare reasons. These ewes with extremely high egg counts which had very watery faeces, probably had even higher burdens than was represented by the counts because the dry matter of the faecal material was considerably reduced. However, high FEC could not always be considered to be associated with scouring, because ewes with high counts often had pelleted faeces when being fed dried food.

Ewes in group 1, year 1 reached a peak of FEC at partum and ewes in group 2 three weeks pre-partum. Thus indicating that a substantial decrease in the immunity of the ewes in group 2 was apparent earlier in the periparturient period than in group 1. However, in 1993 FEC in both groups 1 and 2 reached a peak within a few days of each sample date, i.e. 1 - 0 week pre-partum. Variation in the time of peak FEC is suggestive of different timing of decreased immunity. Connan (1968) observed a wide range in the number of overwintered larvae of *H. contortus* and *O. circumcincta* in ewes during the

post-parturient rise. The hormone prolactin is associated with the suppression in the host's immunity which is thus responsible for the periparturient rise in FEC. However, prolactin is not the sole factor involved and the mechanisms responsible remain unclear (Rahman and Collins, 1992). These authors observed that the rise in prolactin concentration started between 3 weeks and 1 week *pre-partum* in goats, and correlated significantly with the relation between prolactin levels and FEC, with a peak in FEC also occurring 3 - 1 weeks pre-partum. Fleming and Conrad (1989) observed that ovariectomized ewes treated with progesterone then prolactin during an artificial infection with *Haemonchus contortus* had higher worm burdens than those treated with prolactin only which in turn had higher FEC than those treated with progesterone only.

Faecal samples taken at 8 weeks *pre-partum* of many ewes showed trichostrongylid eggs to be present in the faeces of 26.5% of ewes in year 1, and 92.7% of ewes in year 2 with a few ewes having FEC over 1000 in both groups 1 and 2. Ewes lambing in year 2 had mean FEC of 310 for group 1, and 175 for group 2. This observation contrasts with most other reports (Soulsby, 1987; Urquhart and Armour. 1973), Zajac, Herd and McClure (1988) and Rahmans and Collins (1992) which stated that increase in parasite ova is usually from 2-3 weeks prior to partum. The difference in timing of faecal egg production remains unclear but may be due to diet quality or concurrent infections such as Chlamydia which is known to peak at about eight weeks *pre-partum.*

The significantly higher trichostrongylid egg counts in 2 year old ewes recorded in year 1 contrasts with Sykes, McFarlane, and Familton, (1992). who reported maturity of resistance to be achieved at 2 years of age.

Variability of egg counts indicates that only a proportion of 2 year old ewes were highly susceptible. Other authors have described the establishment of resistance at different ages. Barger (1988) stated that lambs of 8 months of age were able to develop a good protection against *Trichostrongylus colubriformis* and *Ostertagia circumcincta.* Six month old lambs, after 8 weeks of daily dosing with 1,000 L_3 *Trichostrongylus vitrinus* showed almost total resistance to larval challenge (Seaton, Jackson, Smith and Angus, 1989). The present result may differ from previous observations due to factors such as, difference in breed, diet, nutritional status etc.

There was no difference between infection rates in 3 year old and older ewes. However, Woolaston (1992) observed no variation in FEC in Merino ewes aged between 2-7 years. This lack of difference between infection rates in older ewes may be due to a point mentioned by Gray (1991) who stated that as the ewe ages the differences between egg output in resistant ewes and susceptible ewes disappears, i.e. the resistant selected ewes at 7 years of age became less resistant than at 5 - 12 months of age and susceptible ewes became less susceptible.

The absence of an effect of litter size in both years contrasts to that observed by Woolaston (1992) and Courtney, Parker and Herd, (1986) who noticed that ewes with twins had higher counts than those with singles and suggested that elevated levels of prolactin observed in ewes with multiple births contributed to the decreased immunity.

Correlation coefficients of samples compared with the overall mean in 1992 for both groups, 1 and 2 were all significant ($p < 0.01$), and ($p < 0.05$) for 4

weeks *pre-partum* in group 1 ewes. Thus any sample taken from 4 weeks *pre-* to 5 weeks *post-partum* would provide a reasonably accurate estimate of FEC. It would thus be possible to assess ewes at four weeks *pre-partum.* Correlation coefficients were even higher in year 2 for group 2 ewes with 'r' estimates in most cases < 0.8.

The significant correlations between plasma pepsinogen concentrations, packed cell volumes and FEC indicate that all three methods are useful parameters for the selection of resistant animals. FEC represent level of adult worm fecundity, i.e. eggs produced by female worms, however, the amount of abomasal damage caused by the emergence of maturing parasites is demonstrated by plasma pepsinogen concentrations. It is known that the increase of pepsinogen in the blood during ostertagiasis is a consequence of leakage from the lumen of the abomasum due to an increased permeability of the gut mucosa when the immature worms emerge from the gastric glands. Extremely high plasma pepsinogen concentrations in excess of 3.5 Units were observed, however, even though a significant correlation was observed between FEC and plasma pepsinogen concentrations the concentrations were not a true representation of FEC. This observation is in accordance with several studies which have demonstrated that older animals previously exposed to natural (Anderson, 1973) and experimental (Barger and Southcott, 1975) trichostrongyle infections, have increased plasma pepsinogen concentrations coupled with loss in production whilst exhibiting zero or extremely low worm egg counts. Yakoob, Holmes and Armour (1983b) demonstrated that a previously naturally infected ewe which had then been artificially infected with *O. circumcincta* had a FEC of only 300 EPG but an elevated plasma pepsinogen level of 1.2 Units of tyrosine. However, an unchallenged previously naturally infected ewe had an egg count of 500 but a

low plasma pepsinogen level of only 0.5 Units. Yakoob *et a l*(1983b) suggested that the elevated plasma pepsinogen concentrations in parasite infected adult ewes which had been previously infected for many years were due to an increased immune reaction induced by the ingested larvae, i.e. a hypersensitive self-cure reaction. The author concluded that with immune animals when parasitic burdens cannot be detected by FEC alone measurements of plasma pepsinogen and pasture larval counts could be performed.

Packed cell volumes correlated significantly ($p < 0.05$) with FEC although packed cell volume (PCV) is a parameter used more frequently to observe pathogenicity in blood feeding nematodes such as *Haemonchus contortus* which cause marked haemorrhage through wounds in the abomasal mucosa, resulting in anaemia. Infection with *Ostertagia spp.* is associated with morphological and functional destruction of the gastric glands of the abomasum. However, such an effect may be explained by the fact that after prolonged infection with non haematophagous nematodes anaemia may be seen, (Soulsby, 1986) and that this anaemia is due to a deficiency in the amino acids required for haemoglobin synthesis.

CHAPTER 3

VARIATION IN RESISTANCE OF GROWING CAMBRIDGE AND CAMBRIDGE CROSS LAMBS TO NATURALLY ACQUIRED GASTROINTESTINAL NEMATODE INFECTIONS

3.1 Introduction

The existence in lambs of genetic variability in infection rates of gastrointestinal nematodes has been well documented with species such as *Haemonchus contortus* and *Trichostrongylus colubriformis* in America and Australia. Gray, Presson, Albers, Le Jambre, Piper and Barker, (1987) suggested that the variation within a breed is just as great as variation between breeds. There is however, only limited published information available on between-strain and flock to flock variation compared with that of between-breeds and Windon, Gray and Woolaston (1993) suggested it is this source that appears the most potentially promising for genetic improvement and heritability values of 0.2-0.4 have been observed indicating considerable possibilities for improving resistance by genetic selection.

In the U.K, a number of studies have been carried out on resistance to *Ostertagia circumcincta* infections. Altaif and Dargie (1978) demonstrated that Scottish Blackface sheep were more resistant than Finn Dorsets. Yadav (1987) found that Welsh Mountain lambs had lower FEC than Clun Forest lambs when infected with *Ostertagia circumcincta.* Altaif and Dargie (1978) also reported that within Scottish Blackface populations, sheep having haemoglobin A were more resistant to nematode infections than those with haemoglobin B.

Non-genetic factors also contribute to variation in nematode infection rate. Many studies have shown that male animals are less resistant than female animals. Hunninen (1935) observed that female mice were more resistant than males to *Hymenolepis nana.*

In sheep, males after puberty have been shown to be more susceptible than females to experimental infections of *Oesophagostomum columbianum* (Dobson, 1964; Bawden, 1969) *Trichostrongylus colubriformis* (Windon and Dineen, 1981) and *Haemonchus contortus* (Adams, 1988). The removal of the ovaries from female lambs increased *Oesophagostomum columbianum* numbers and reduced the differences due to lamb sex (Dobson, 1964).

Some authors (Gibb and Treacher, 1980; Gibb, Treacher and Shanmugalingam, 1981) observed the effect of the number of lambs being reared by the ewe on FEC of lambs. Twin lambs graze more than single lambs because they have less milk available to them from their dams. They are thus likely to ingest more larvae from the pasture and to be immunologically 'primed' by the summer months and have a greater degree of resistance to subsequent nematode infections than single lambs.

In the light of the above findings an investigation to quantify the within population differences in the resistance of lambs to gastrointestinal nematodes principally *O. circumcincta* was carried out in 3 consecutive years on lambs being reared in field conditions.

3.2 Materials and Methods

Experimental animals

In 1992 (year 1), 177 and 1993 (year 2), 208 Cambridge and Cambridge cross lambs born in March (group 1) and April (group 2) were used to investigate variability of FEC during the summer months. Also in 1994 (year 3), 184 pure Cambridge lambs divided into 3 lambing groups born March, April (early) April (late) were used to investigate variability of FEC during the summer months. Mating was synchronised using progesterone sponges so each group of lambs was born within a 7 day period. All lambs were born indoors and were housed for 3 to 5 weeks on a deep litter straw bedding floor free of strongylate parasites. Litter size varied from 1 to 5 lambs. The number of lambs suckled was generally reduced at 5 days to a maximum of 3 so ewes reared 1, 2 or 3 lambs although one ewe reared 4 and another 5 lambs in 1993. Lambs were weighed and individually tagged 12-24 hours *post-partum.* The lambs were also weighed at 8, 14 and 22-24 weeks of age. Ewes and lambs were dosed with ivermectin (dose rate 2.5 ml per 10 kg body weight) at 6-8 weeks post-partum, i.e. circa three weeks after turn out on to pasture and then at an average interval of 32 days. In year 1 lambs were turned out onto clean pasture which had not been grazed by lambs or ewes during the previous 2 years and remained there until 10 weeks of age when they were placed onto infected pasture which had been grazed by ewes and lambs during the previous year and where they remained throughout the experiment. Both groups 1 and 2 in year 2 and groups 2 and 3 in year 3, were turned out on to clean pasture (which had not been grazed by ewes and lambs in the previous years) until weaning at 16 weeks of age. Group 1 lambs in year 3 were turned out on to a pasture which had not been grazed by ewes and lambs the previous autumn

and remained there until weaned at 16 weeks of age. They were then moved to a potentially more contaminated pasture grazed by ewes in the last stages of pregnancy in late April and early May.

Sires and dams

In year 2 a few of the ram lambs to use as sires the following year were assessed on the basis of faecal egg production and were selected as either high or low responders.

The dams of the lambs were faecal sampled during the periparturient rise as mentioned in the previous Chapter.

Parasitological techniques

In year 1, (1993) a small random group of 10 lambs was faecal sampled initially at 21 and then at 28 days post-drenching. Egg counts were absent from all samples at 21 days and counts were generally low at 28 days, some individuals had faeces with counts above 500 EPG. A 32 day sampling interval was thus chosen to allow the identification of animals with variable counts and at the same time to avoid the build up of worm burdens with consequential pathogenic effects. The lambs were faecal sampled on 3 occasions between 14 and 23 weeks of age; one sample was taken pre-weaning and two post-weaning. Samples in each case were taken at 32 days post-drenching with Ivermectin. In year 2, lambs were faecal sampled and drenched with Ivermectin at weaning at 16 weeks of age. A faeces sample was taken from

each lamb at 32 and 36 days post-drenching. In year 3, lambs were faecal sampled once pre-weaning when lambs varied from 14-16 weeks of age and twice post-weaning when aged 18-20 weeks the latter samples were taken within 4 days of each other at 33-37 days post-weaning and drenching.

Trichostrongylidae eggs in faeces were detected by the modified McMaster technique and expressed as eggs per gramme (EPG) of faeces. In each year faecal samples were scored for dry matter content on a scale which varied from 1 (very watery faeces) to 5 (hard pelleted faeces). In year 1, the dry matter of 25 faecal samples was determined by drying in a hot air oven at 80 ^{o}C for 48 hours and the dry matter content was then related to the score.

Pasture larval counts

Herbage samples were taken from the pasture grazed by group 1 lambs in year 2 on 5th June and again on the 27th July when newly weaned lambs were moved on to the field. In year 3, samples were taken on the 7th July 3 and 5 weeks pre-faecal sampling of groups 1 and 2 respectively. In each case grass samples from 400 loci within the paddock were collected, bulked and processed by the method described in MAFF Technical Parasitology Handbook (418).

Statistical Analysis

Statistics were undertaken by the use of the computer package Statistical Analysis System (SAS). Data of FEC was found to have a significant skewed distribution and was transformed into log form (log10 x+1)before analysis. The means presented for FEC are therefore geometric means unless otherwise stated and the variability of each mean figure is described by its percentage standard deviation obtained by taking the antilog of the log form standard deviation, and by 95% confidence limits of the mean.

3.3 Results

Pasture larval count

Pasture larval counts identified *Ostertagia* as the most prevalent nematode genus present. In year 2, 1993 the pasture larval count in larvae per kg dried herbage on the pasture grazed by group 1 lambs on 5/6/93 (7 weeks pre- faecal sampling) was 50 and on 22/7/93 (at faecal sampling), 945. In 1994, a pasture larval count was carried out on 7/7/94(3 and 5 weeks pre- faecal sampling respectively) on pasture grazed by both group 1 and 2 lambs, larvae per kg dried herbage were 427 and 252, respectively.

Faecal Egg Counts (FEC)

The post-weaning counts in years 1, 2 and 3 are shown in Table 3.1. A large amount of within group variation in egg numbers was observed in all groups in the three years of sampling. In each group some lambs had a zero count whilst others had counts of 3000 EPG, well above the considered danger level.

In each year the mean FEC was calculated for each lamb from the figures used to compile Table 3.1a. Figures 3.1 to 3.4 (are derived from the non transformed data and these means are shown in Table 3.1b). indicate the frequency distribution of lambs in terms of these mean egg counts in each of the 3 years of study. These distributions show an obvious non normal frequency in all 3 years and were found to be significantly skewed ($p < 0.01$). The data was thus transformed to log form and the statistical analysis carried out on the transformed data.

Table 3.1a : Geometric means of FEC for all groups of lambs in years 1992, 1993 and 1994

		1992		1993		1994		
		Group 1	Group 2	Group 1	Group 2	Group 1	Group 2	Group 3
FEC 1	Mean	69	196	225	254	110	15	300
	n	74	87	87	68	90	50	48
	% S.D.	10.6	8.5	2.5	4.3	6.7	14.9	4.00
	conf limits	40-119	128-296	185-272	178-355	74-162	7-32	202-452
FEC 2	Mean	260	50	254	363	152	16	316
	n	50	86	82	68	90	50	48
	% S.D.	5.5	15.9	2.6	3.4	3.7	13.5	4.2
	conf limits	160-412	29-86	205-308	272-485	116-198	8-33	207-483
FEC	Mean	284	214	214	283	157	21	339
	n	48	70	96	90	90	51	47
MEAN	% S.D.	3.1	5.9	2.6	4.1	3.4	12.6	3.8
	conf limits	205-388	151-303	177-258	211-377	123-204	10-43	228-503

Table 3.1b : *Arithmetic means of FEC for all groups of lambs in years 1992, 1993 and 1994*

		1992		1993		1994		
		Group 1	Group 2	Group 1	Group 2	Group 1	Group 2	Group 3
FEC 1	Mean	263	601	281	457	245	145	594
	Range	0-1850	0-3100	0-1650	0-2100	0-750	0-1000	0-2750
FEC 2	Mean	552	314	365	588	233	167	637
	Range	0-2250	0-2350	0-2200	0-3100	0-1000	0-1350	0-3000
FEC MEAN	Mean	467	477	311	487	239	156	621
	Range	0-1117	0-3167	0-1875	0-2150	0-950	0-1050	0-2625

Figure 3.1 : Proportion of lambs in successive FEC bands, 1992-Year 1 (FEC values are the mean of 2 samples)

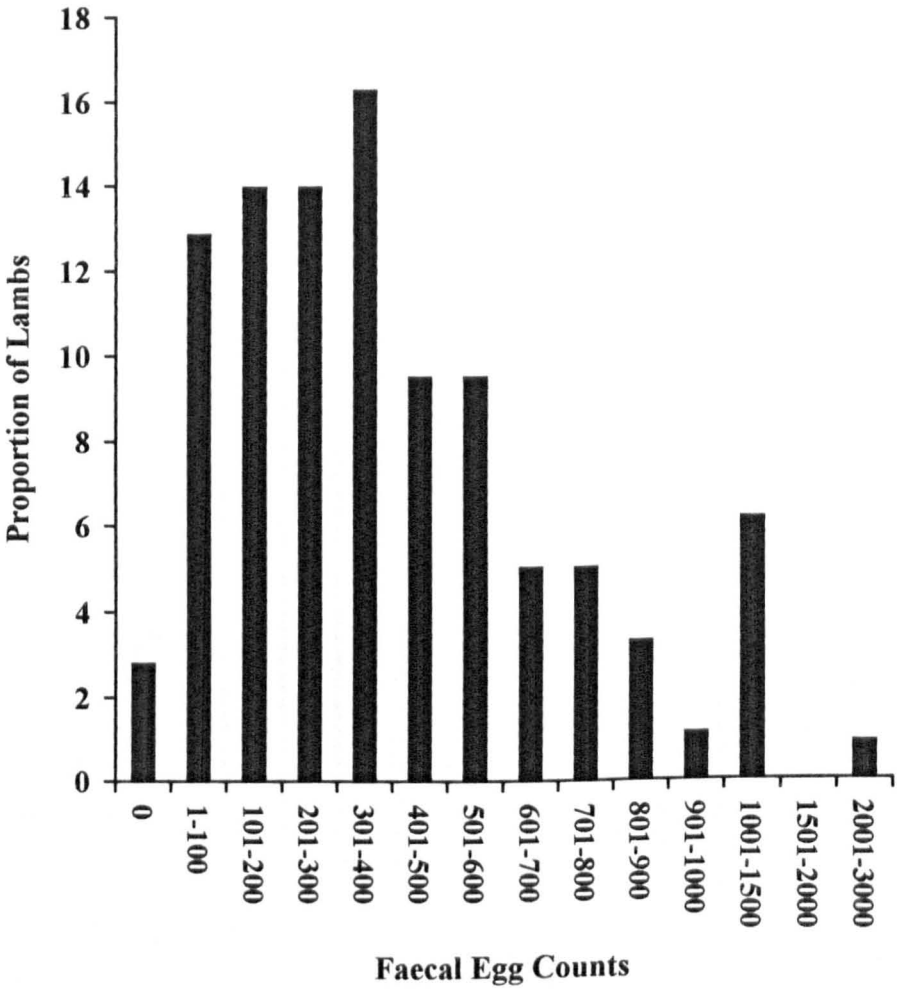


Figure 3.2 : Proportion of lambs in successive FEC bands, 1993-Year 2 (FEC values are the mean of 2 samples)

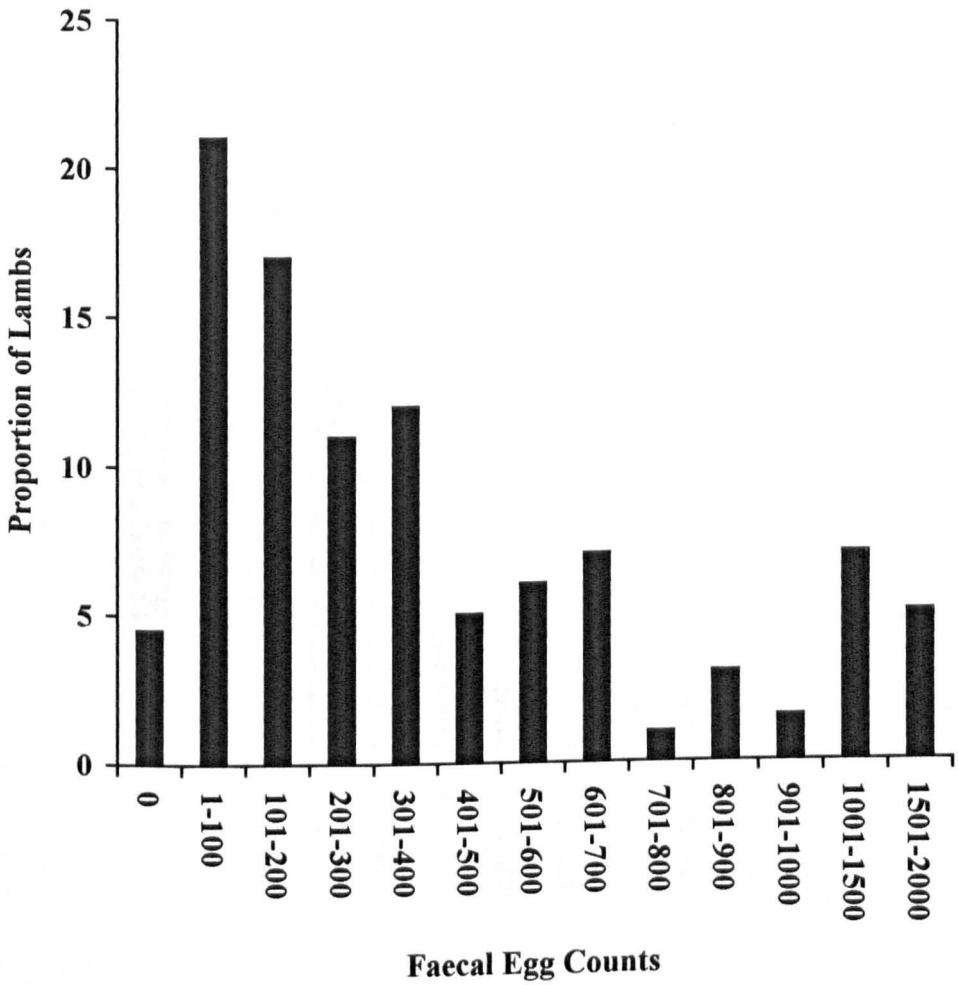
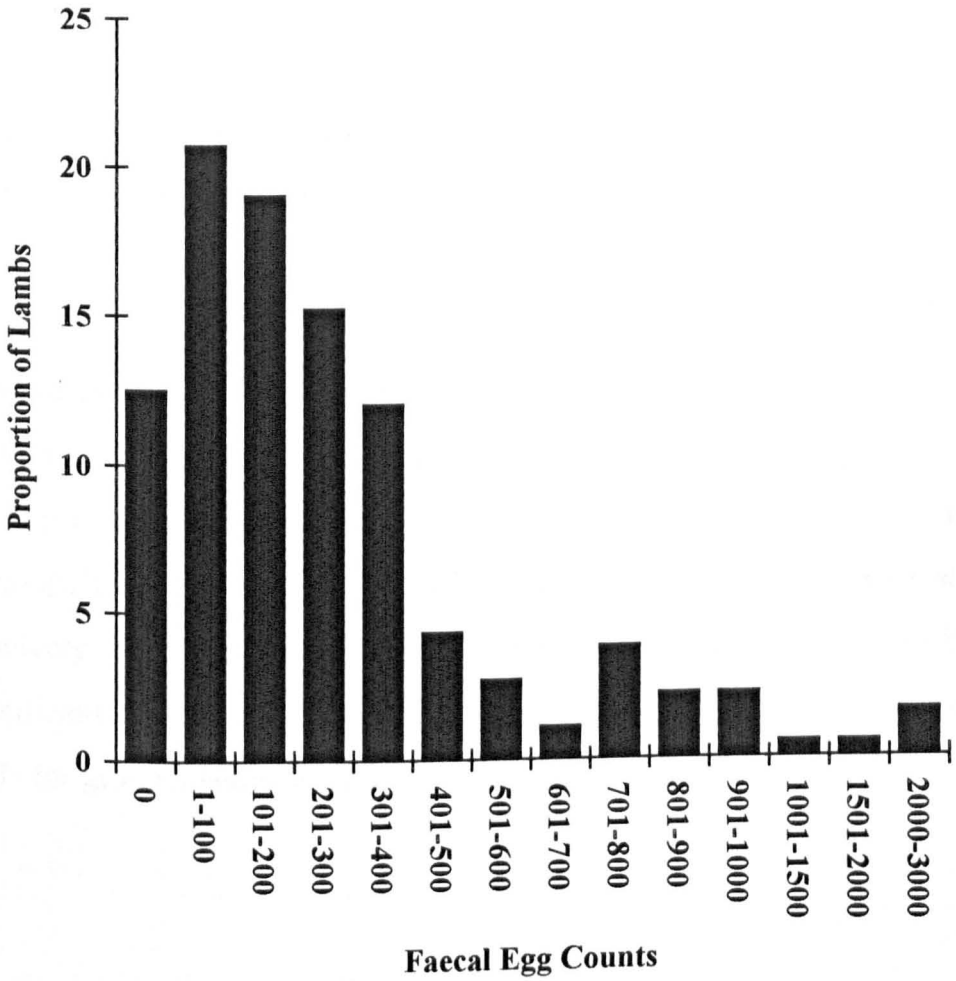


Figure 3.3 : Proportion of lambs in successive FEC bands, 1994-Year 3 (FEC values are the mean of 2 samples)



Correlation coefficients between consecutive faecal sample egg counts and between individual counts and the overall mean count

In year 1 (1992), the correlation for group 1 lambs between post-weaning consecutive sampling dates was low (Figure 3.4). However, in the second group of animals in this first year, both groups in year 2, and in all 3 groups in year 3 (see Table 3.2) correlations were highly significant ($p < 0.001$). Figures 3.5.- 3.10 show a graphical representation of the situation.

Correlations between all samples taken and the overall group means were also highly significant ($p < 0.001$) as shown in Table 3.2 and Figures 3.11-3.17. Pre- and post- weaning correlations were mainly non significant (Table 3.2) for all years 1, 2 and 3 i.e. 0.17 for group 1, year 1 lambs 0.11 and 0.06 for year 2 groups 1 and 2, respectively and 0.13 and 0.01 for year 3 groups 1 and 2, respectively. However, the correlation between pre- and post- weaning FEC was significant, i.e. 0.35 ($p<0.01$) for group 1 lambs in year 1 and 0.61 ($p<0.001$) for group 3 lambs in year 3.

Table 3.2 : Correlation coefficient matrix showing r values for pre- and post, consecutive FEC and each FEC and the overall mean

	Pre- & Post	1 & 2	1 & mean	2 & mean
1992 Group 1	0.17 n.s.	0.18 n.s.	0.57 $p < 0.01$	0.80 $p < 0.01$
1992 Group 2	0.35 $p < 0.01$	0.38 $p < 0.01$	0.78 $p < 0.01$	0.73 $p < 0.01$
1993 Group 1	0.11 n.s.	0.65 $p < 0.01$	0.88 $p < 0.01$	0.94 $p < 0.01$
1993 Group 2	0.06 n.s.	0.64 $p < 0.01$	0.87 $p < 0.01$	0.94 $p < 0.01$
1994 Group 1	0.13 n.s.	0.83 $p < 0.001$	0.97 $p < 0.001$	0.94 $p < 0.001$
1994 Group 2	0.01 n.s.	0.87 $p < 0.001$	0.96 $p < 0.001$	0.98 $p < 0.001$
1994 Group 3	0.61 $p < 0.001$	0.72 $p < 0.001$	0.93 $p < 0.001$	0.92 $p < 0.001$

Figure 3.4 : Graph showing the relationship between faecal samples 1 and 2 of year 1, group 1 lambs

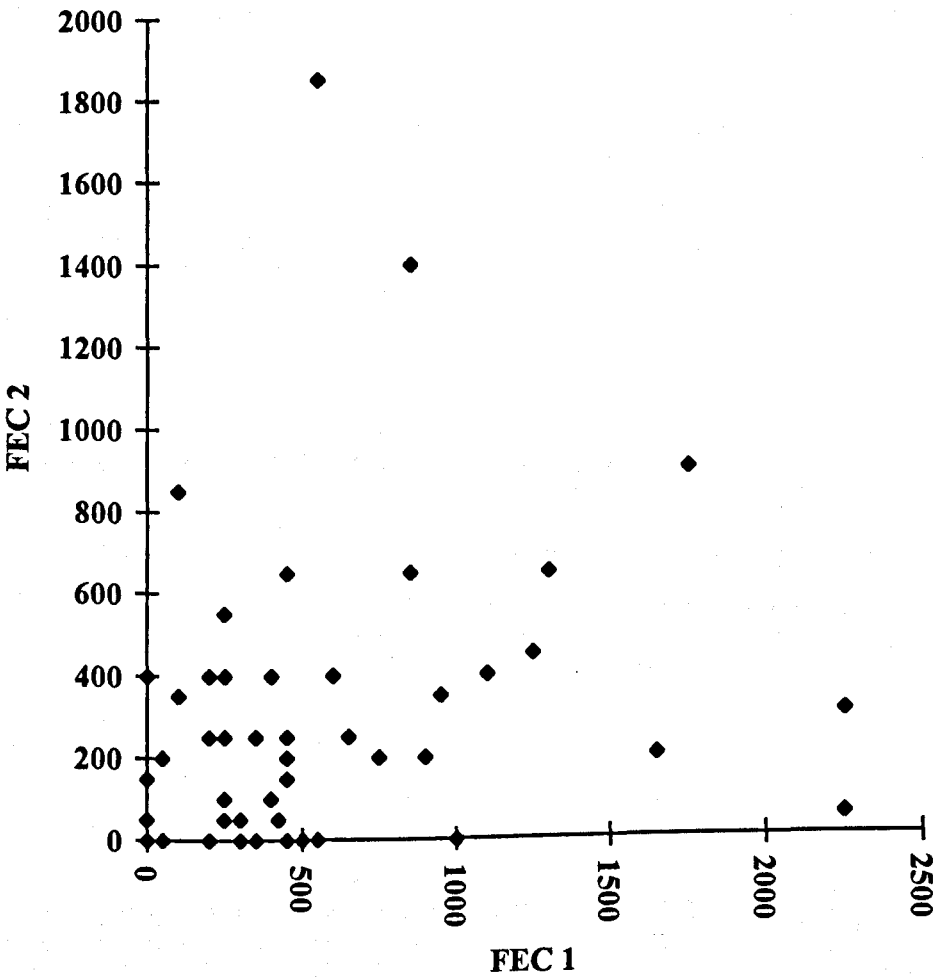


Figure 3.5 : Graph showing the relationship between faecal samples 1 and 2 of year 1, group 2 lambs

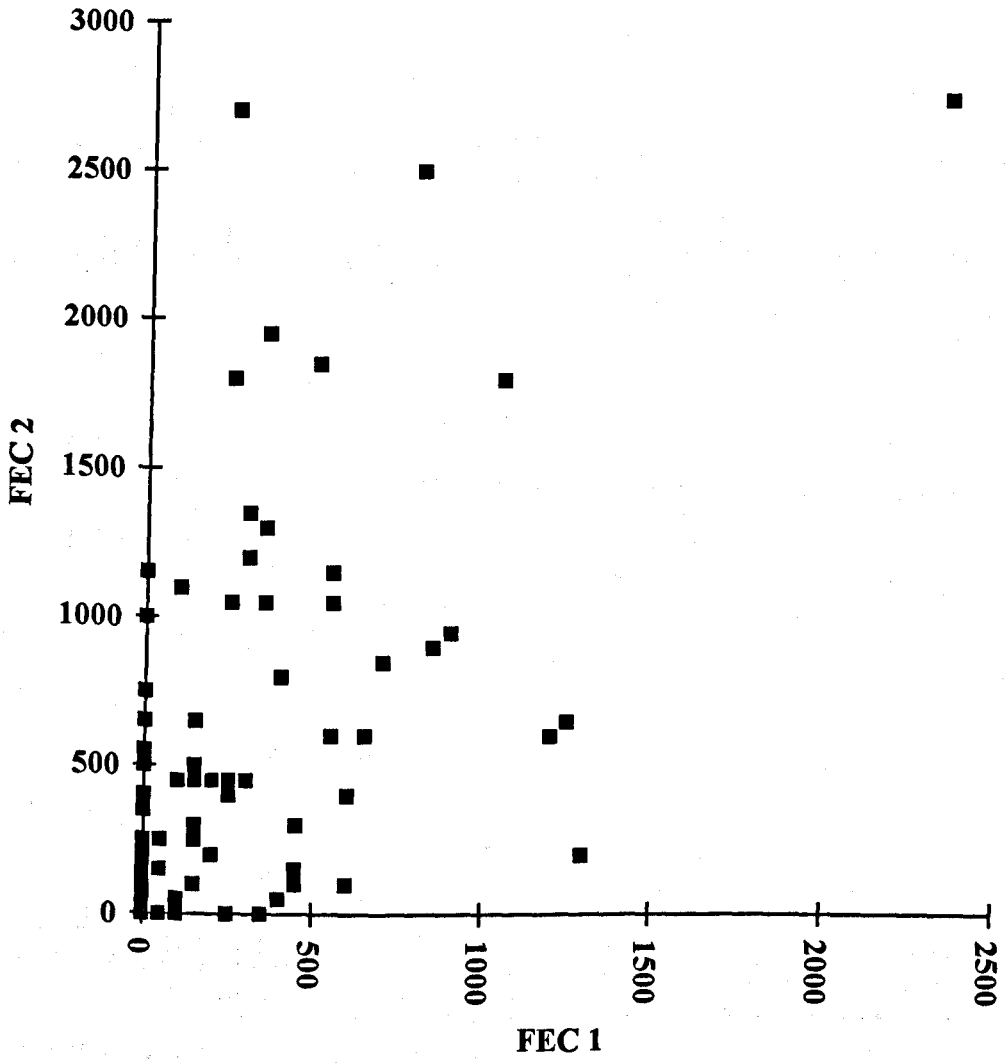


Figure 3.6 : Graph showing the relationship between faecal samples 1 and 2 of year 2, group 1 lambs

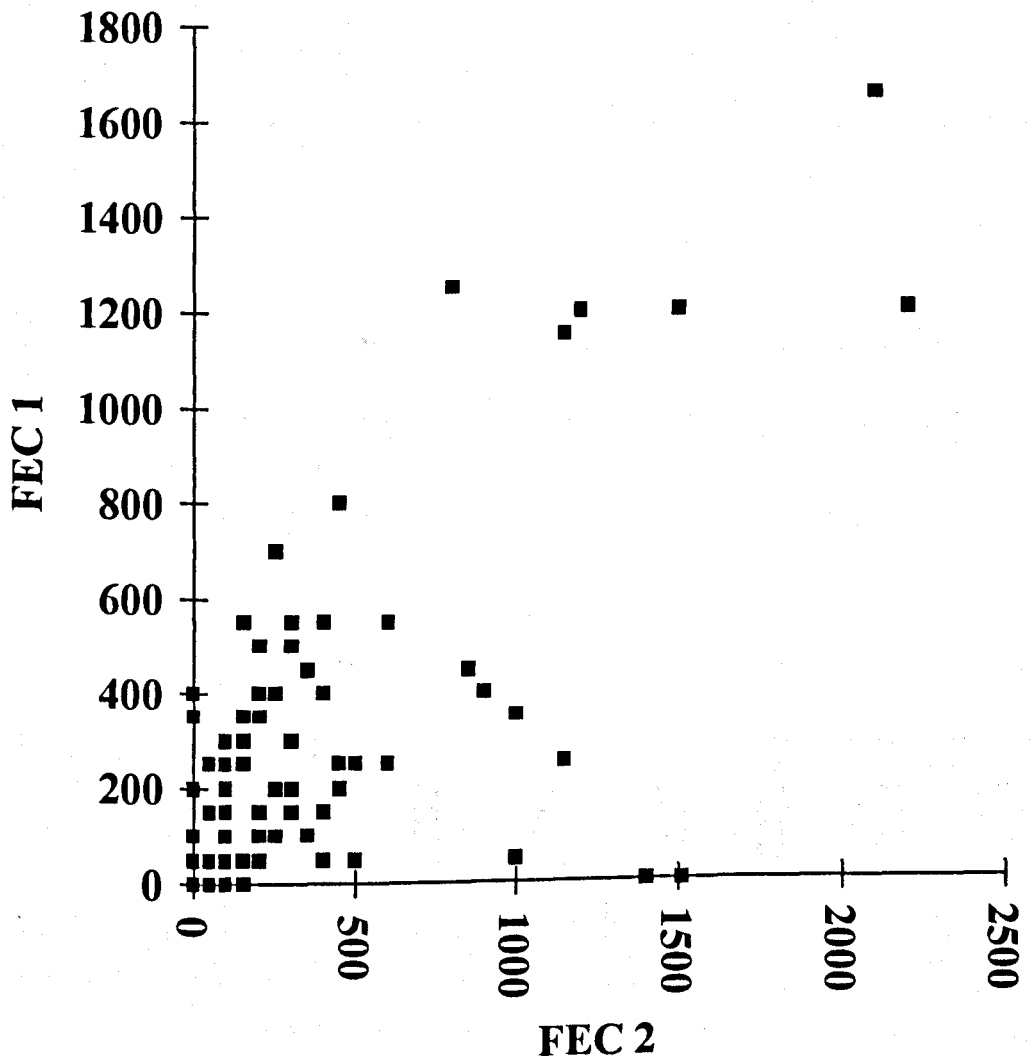


Figure 3.7 : Graph showing the relationship between faecal samples 1 and 2 of year 2, group 2 lambs

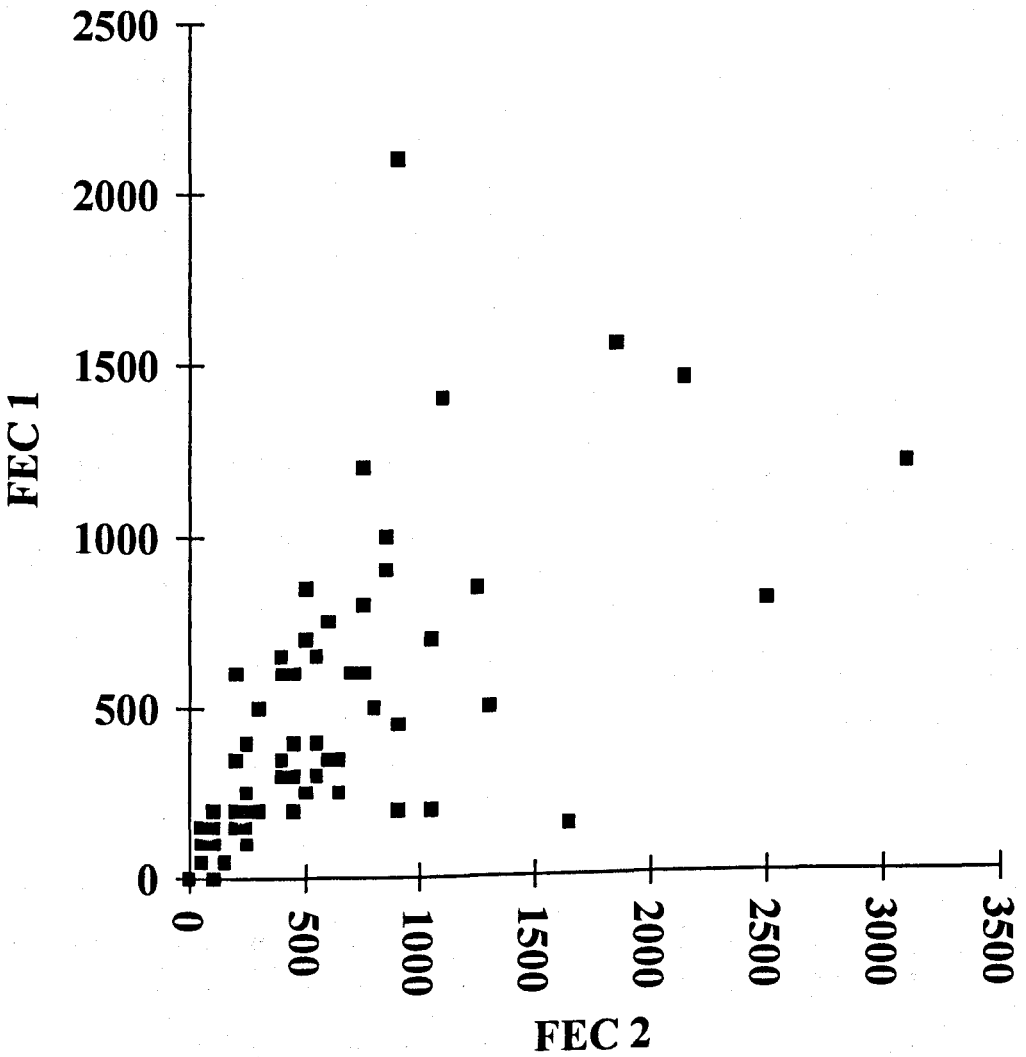


Figure 3.8 : Graph showing the relationship between faecal samples 1 and 2 of year 3, group 1 lambs

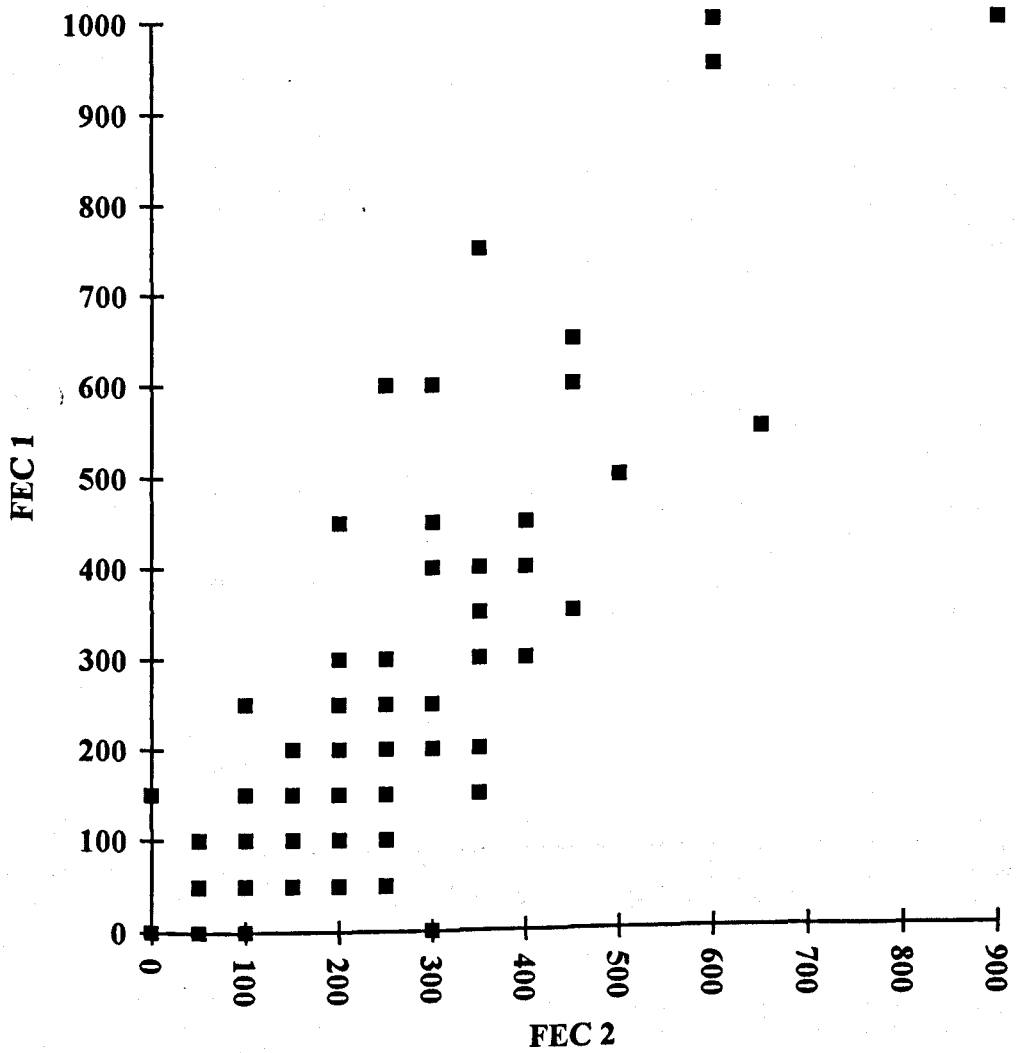


Figure 3.9 : Graph showing the relationship between faecal samples 1 and 2 of year 3, group 2 lambs

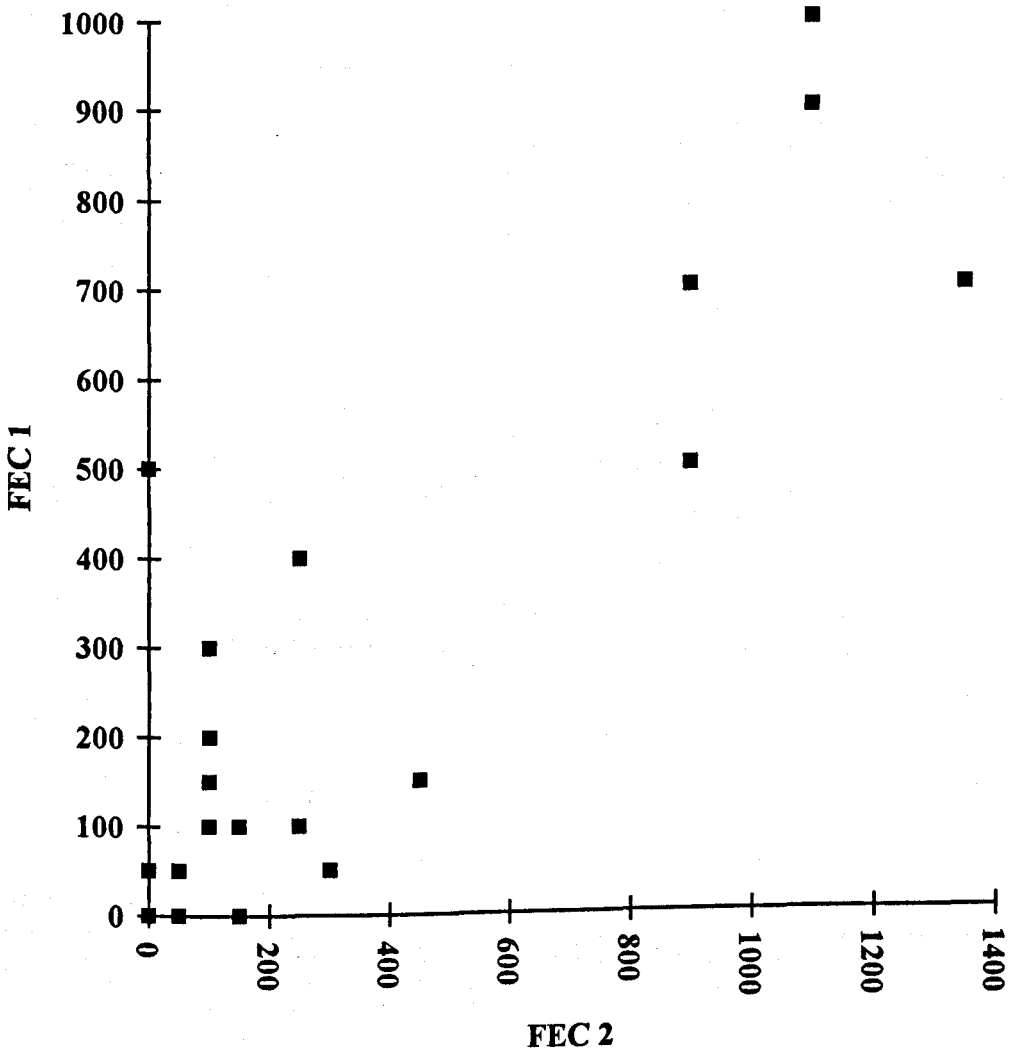


Figure 3.10 : Graph showing the relationship between faecal samples 1 and 2 of year 3, group 3 lambs

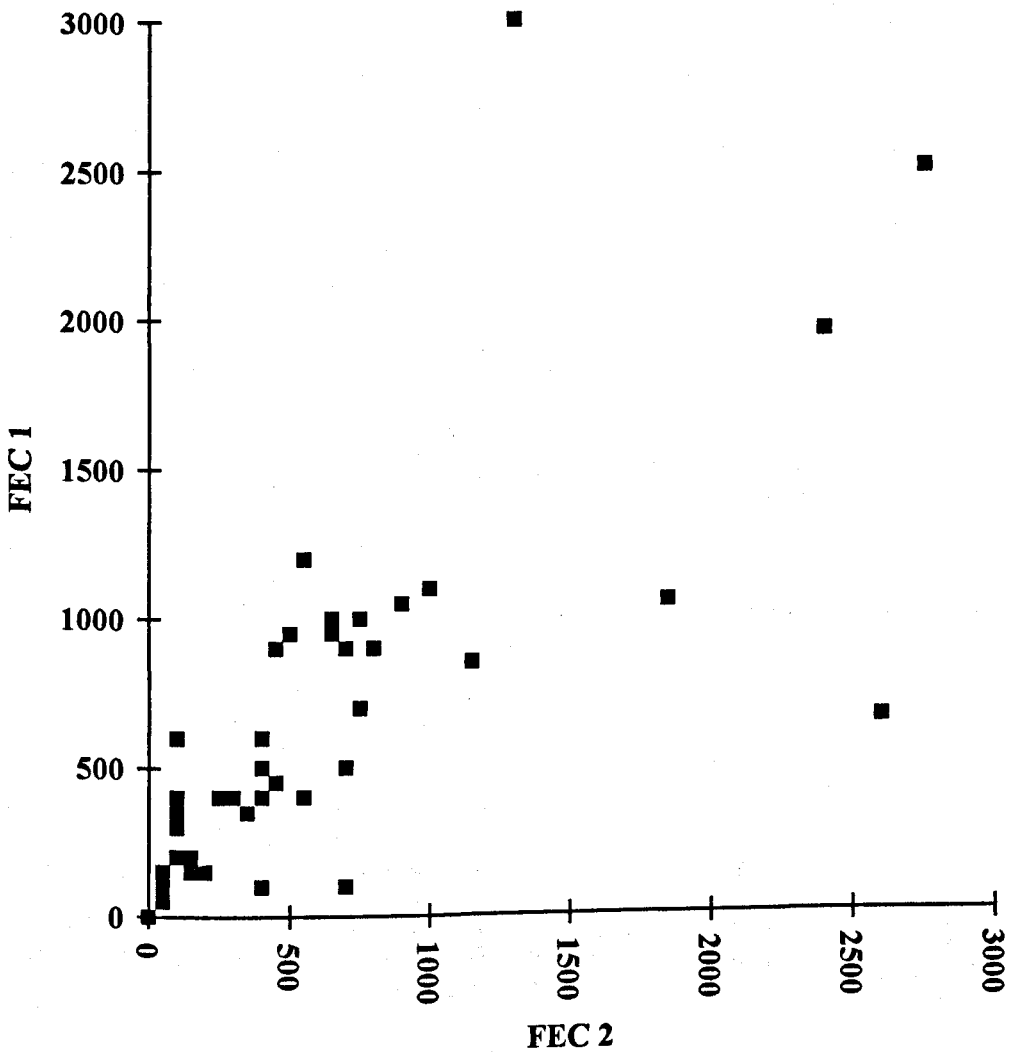


Figure 3.11 : Graph showing the relationship between faecal samples 1 and 2 and the overall mean of year 1, group 1 lambs

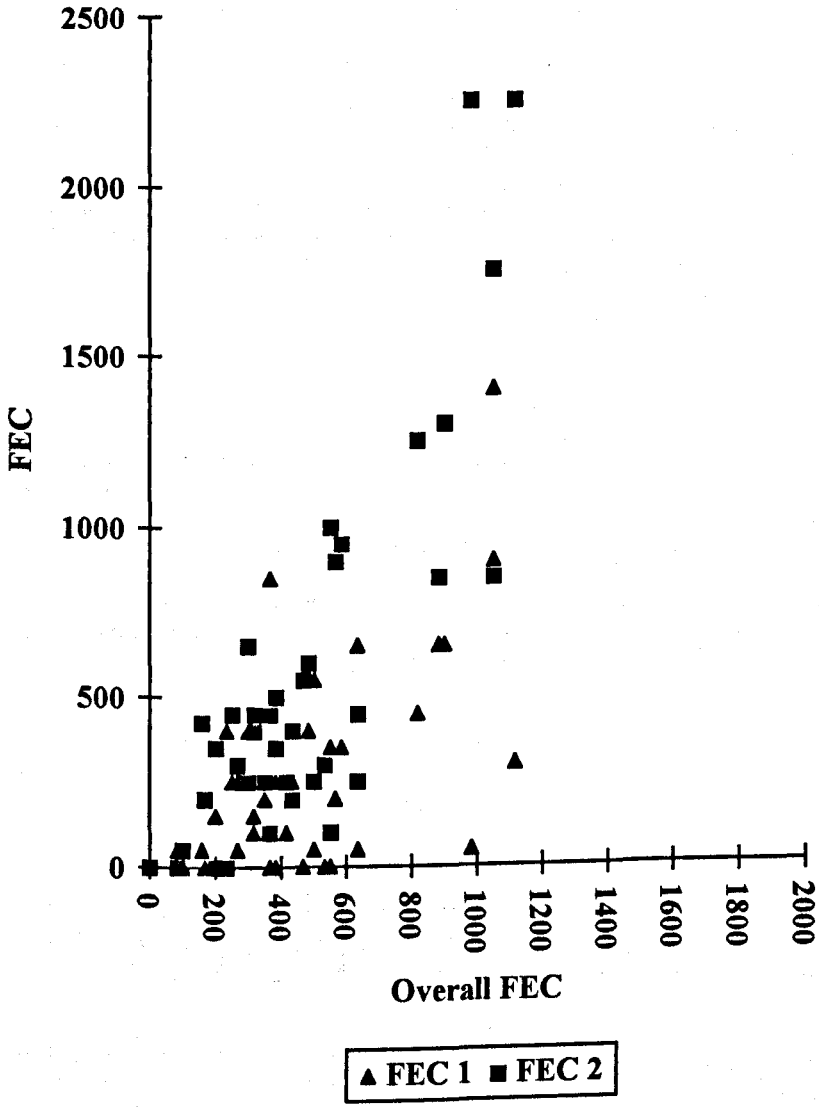


Figure 3.12 : Graph showing the relationship between faecal samples 1 and 2 and the overall mean of year 1, group 2 lambs

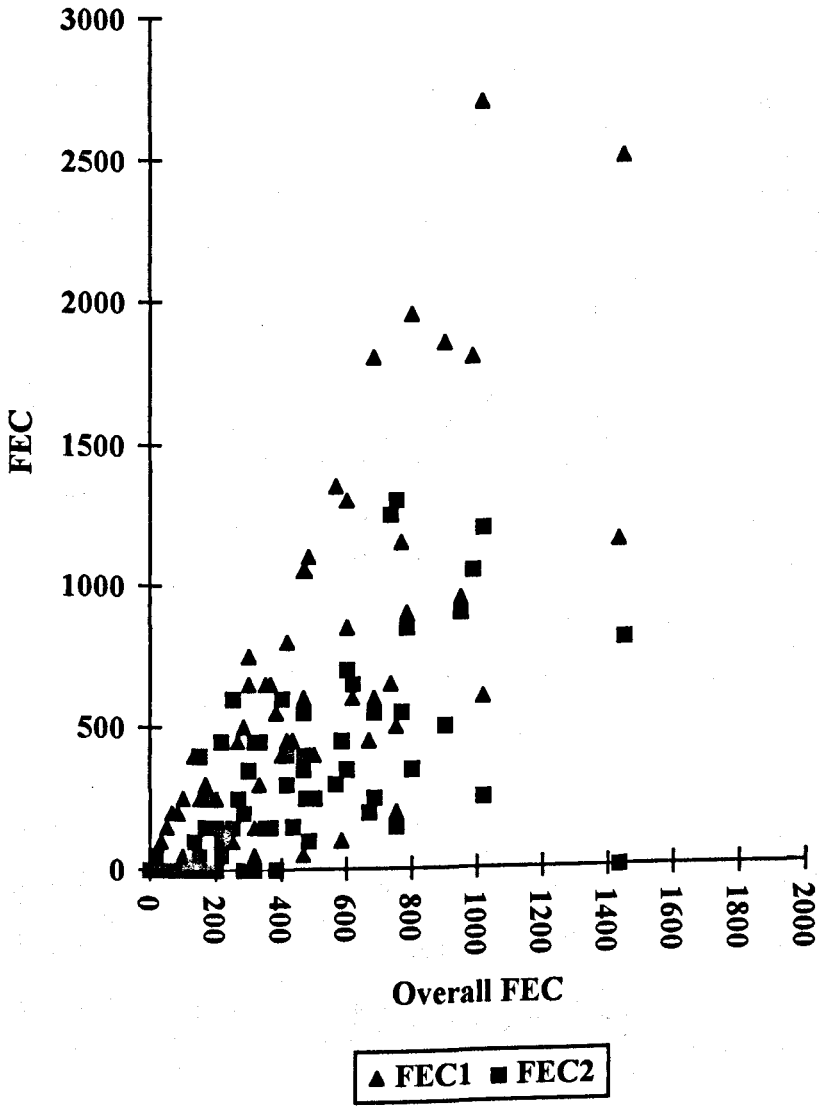


Figure 3.13 : Graph showing the relationship between faecal samples 1 and 2 and the overall mean of year 2, group 1 lambs

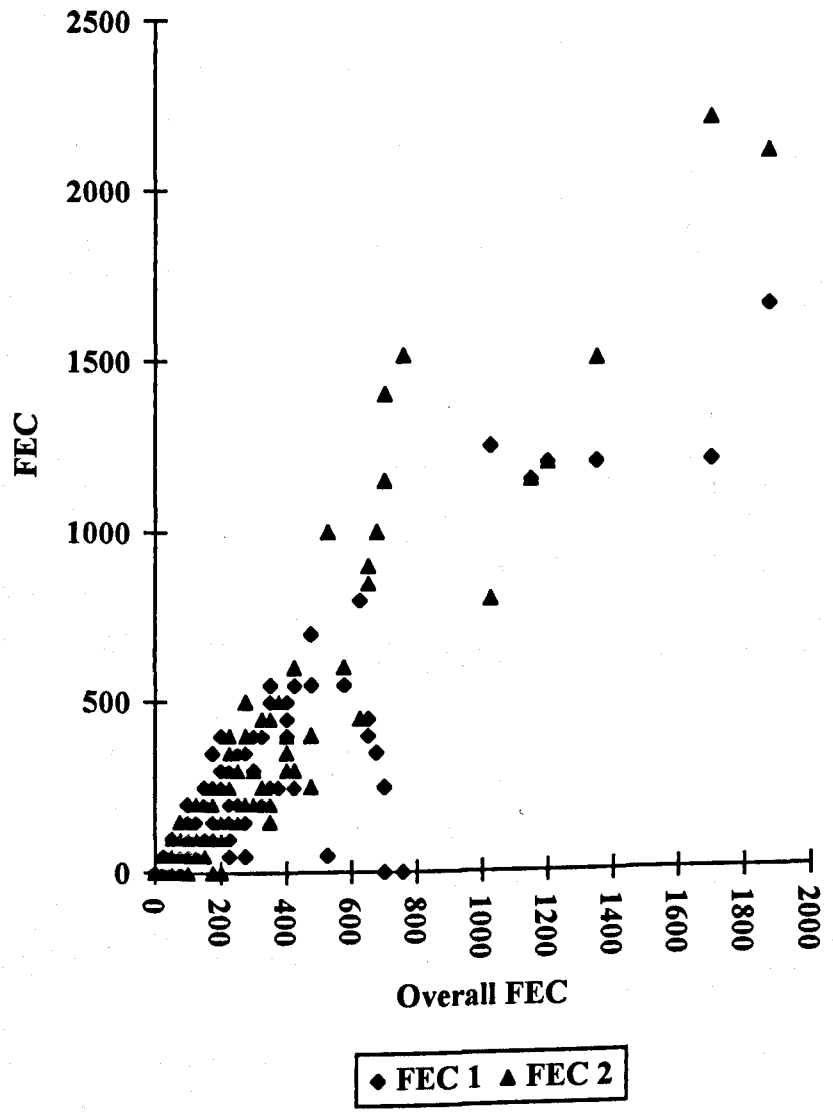


Figure 3.14 : Graph showing the relationship between faecal samples 1 and 2 and the overall mean of year 2, group 2 lambs

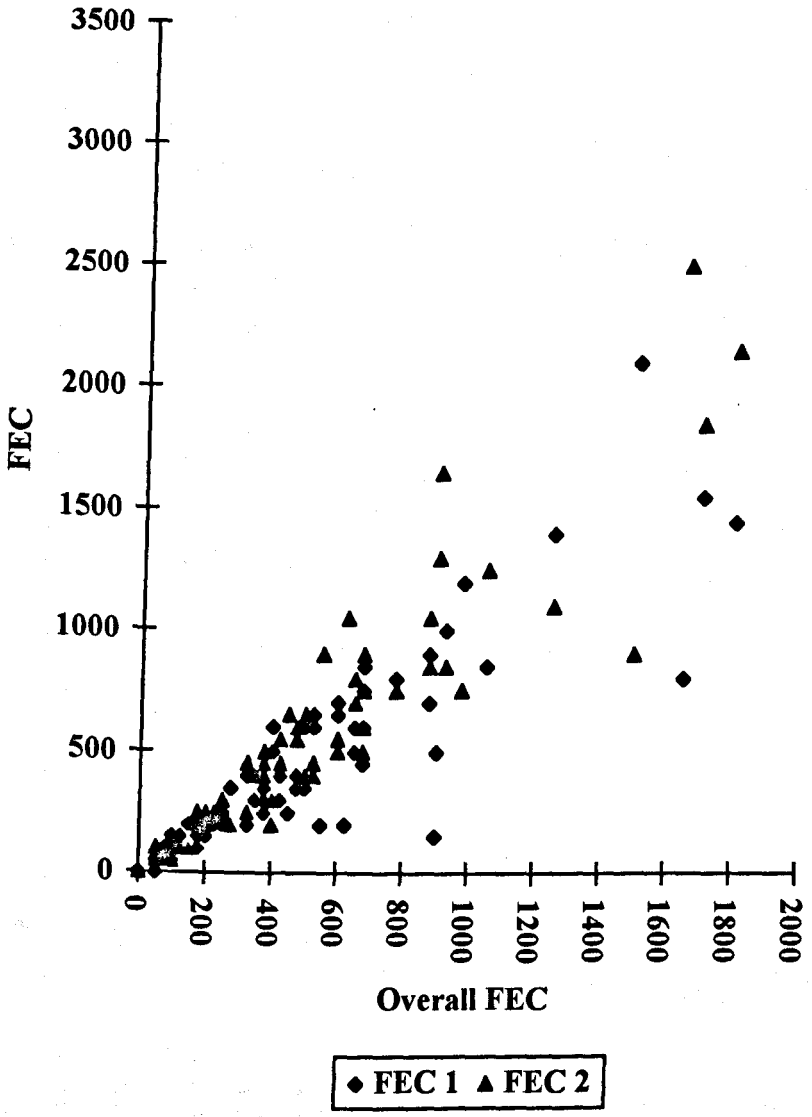


Figure 3.15 : Graph showing the relationship between faecal samples 1 and 2 and the overall mean of year 3, group 1 lambs

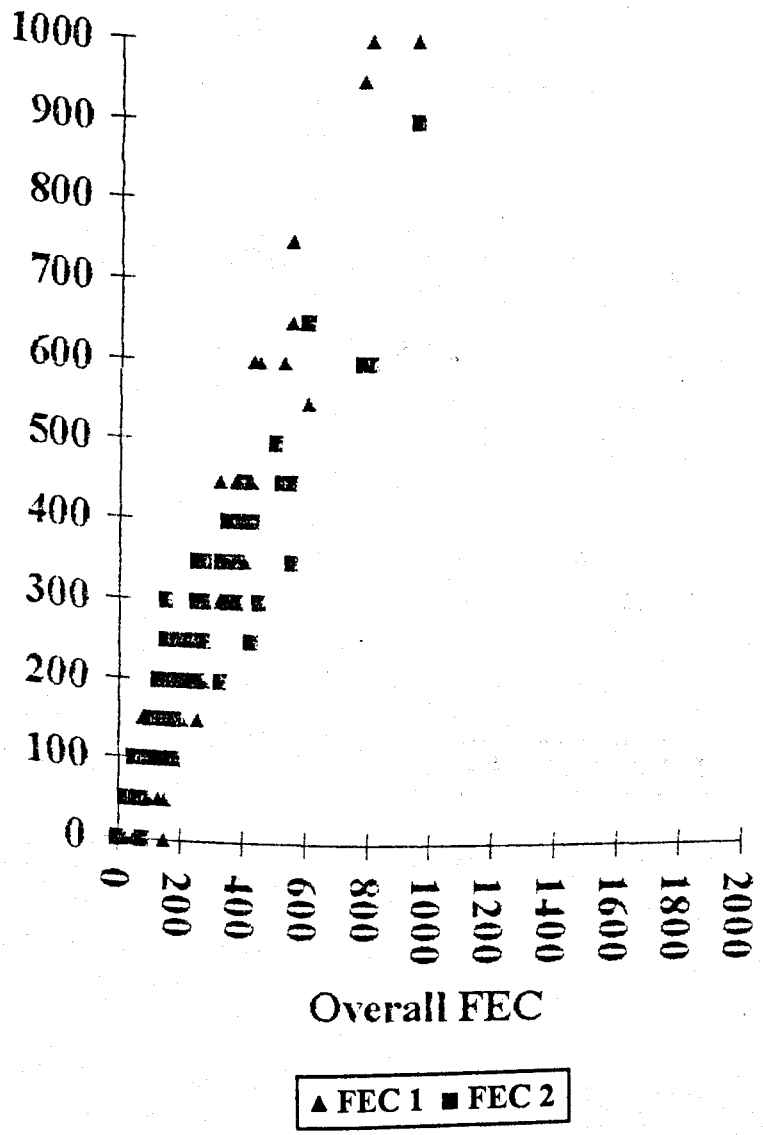


Figure 3.16 : Graph showing the relationship between faecal samples 1 and 2 and the overall mean of year 3, group 2 lambs

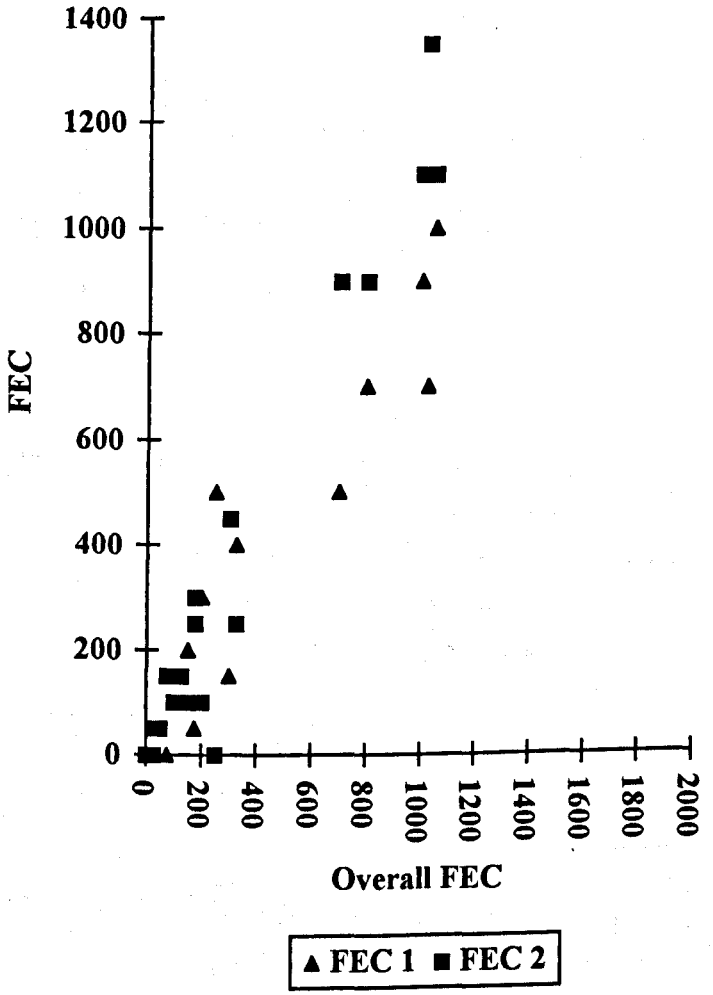
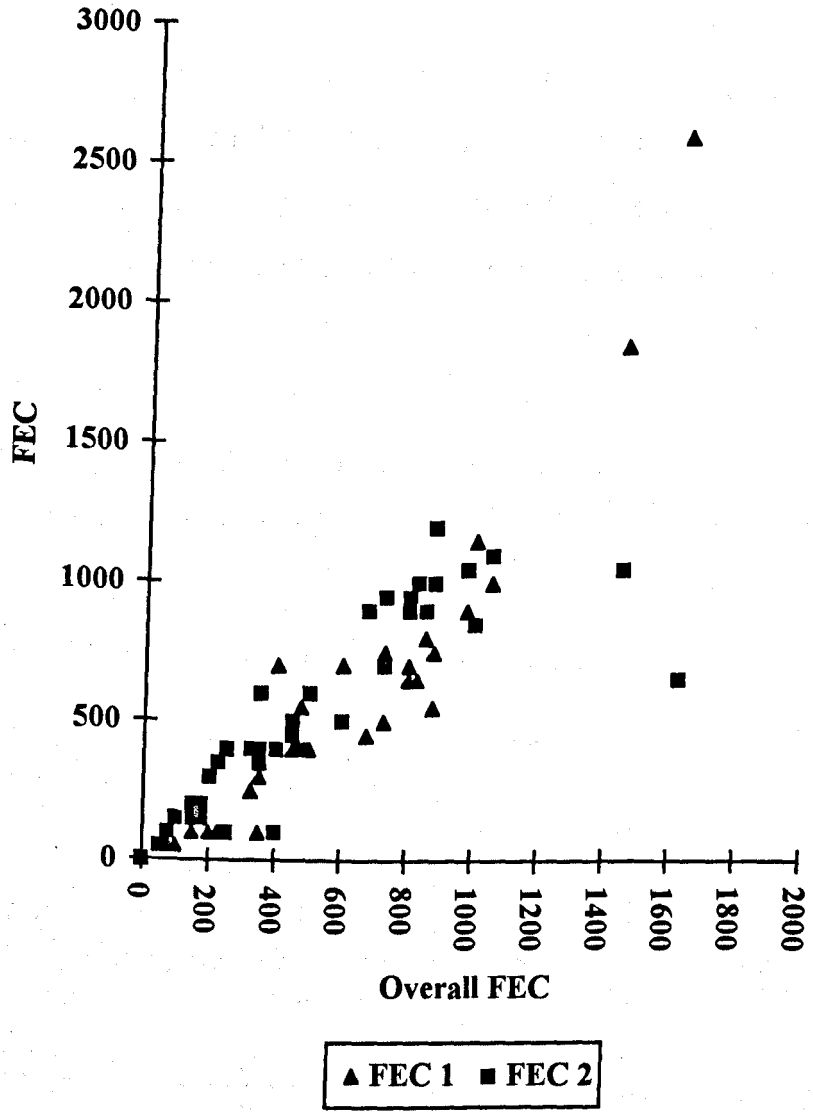


Figure 3.17 : Graph showing the relationship between faecal samples 1 and 2 and the overall mean of year 3, group 3 lambs



Factors affecting faecal egg count (FEC)

Faeces consistency

The faecal dry matter score varied between 1 and 5. In year 1 there was a statistically significant relationship between faecal score and % dry matter, the regression coefficient being -0.635 ± 0.89 (Figure 3.18) ($p<0.0005$). There was however, no significant correlation between consistency of faeces and FEC (Figure 3.19). Table 3.3 shows the dry matter content of lambs faeces and its faecal score. (Each percentage faecal dry matter scores is derived from the mean of samples from 3 lambs) No effect of faecal score was seen on live weight change in years 1, 2 and 3.

Table 3.3 : Dry matter content of lambs faeces

Faecal Score	% Dry Matter
1.0	22.2
1.5	31.1
2.0	50.0
2.5	75.0
3.0	-
3.5	78.0
4.0	80.0
4.5	91.3
5.0	93.0

Figure 3.18 : The relationship between faecal score and percentage dry matter of faeces (Year 1)

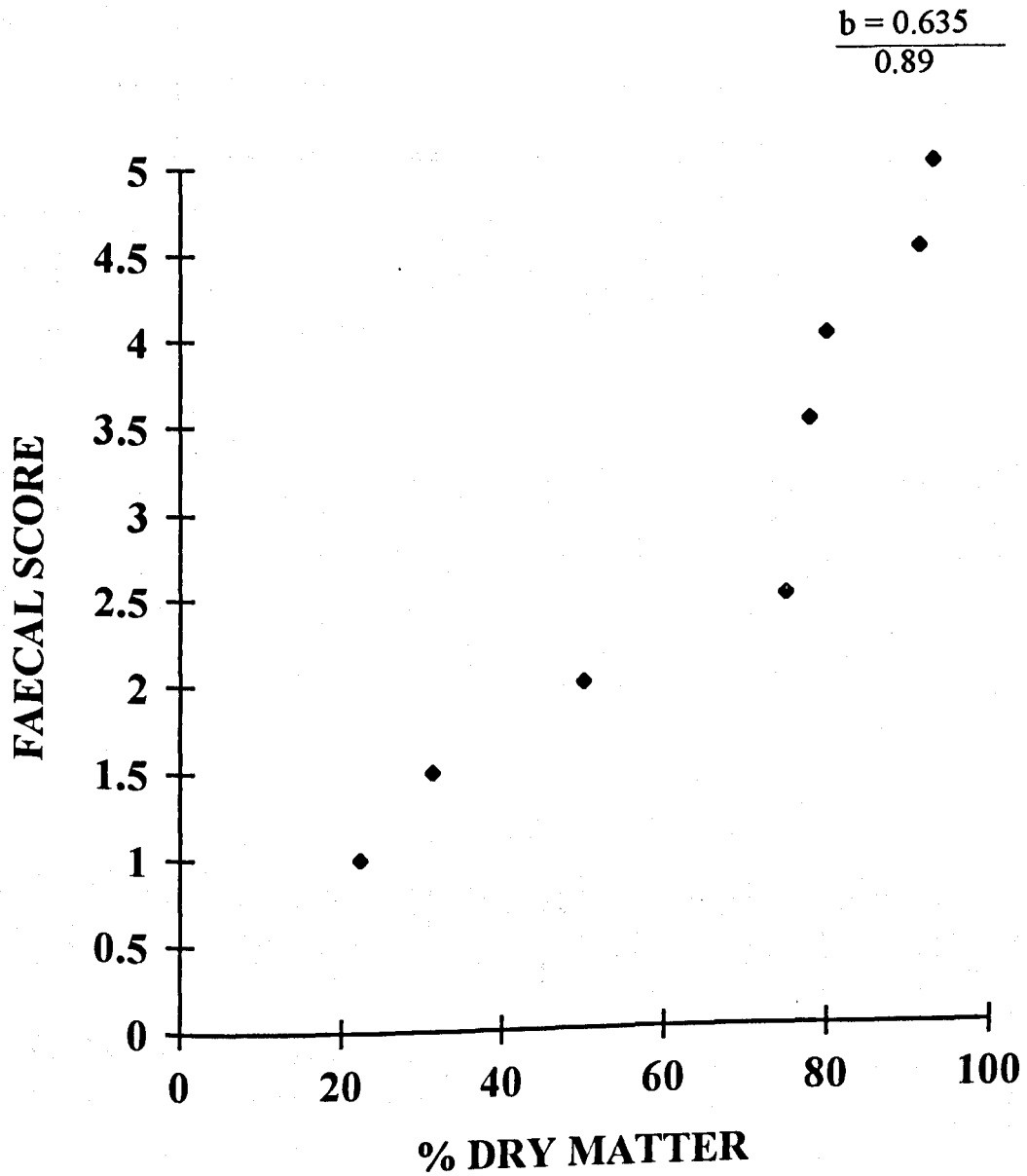
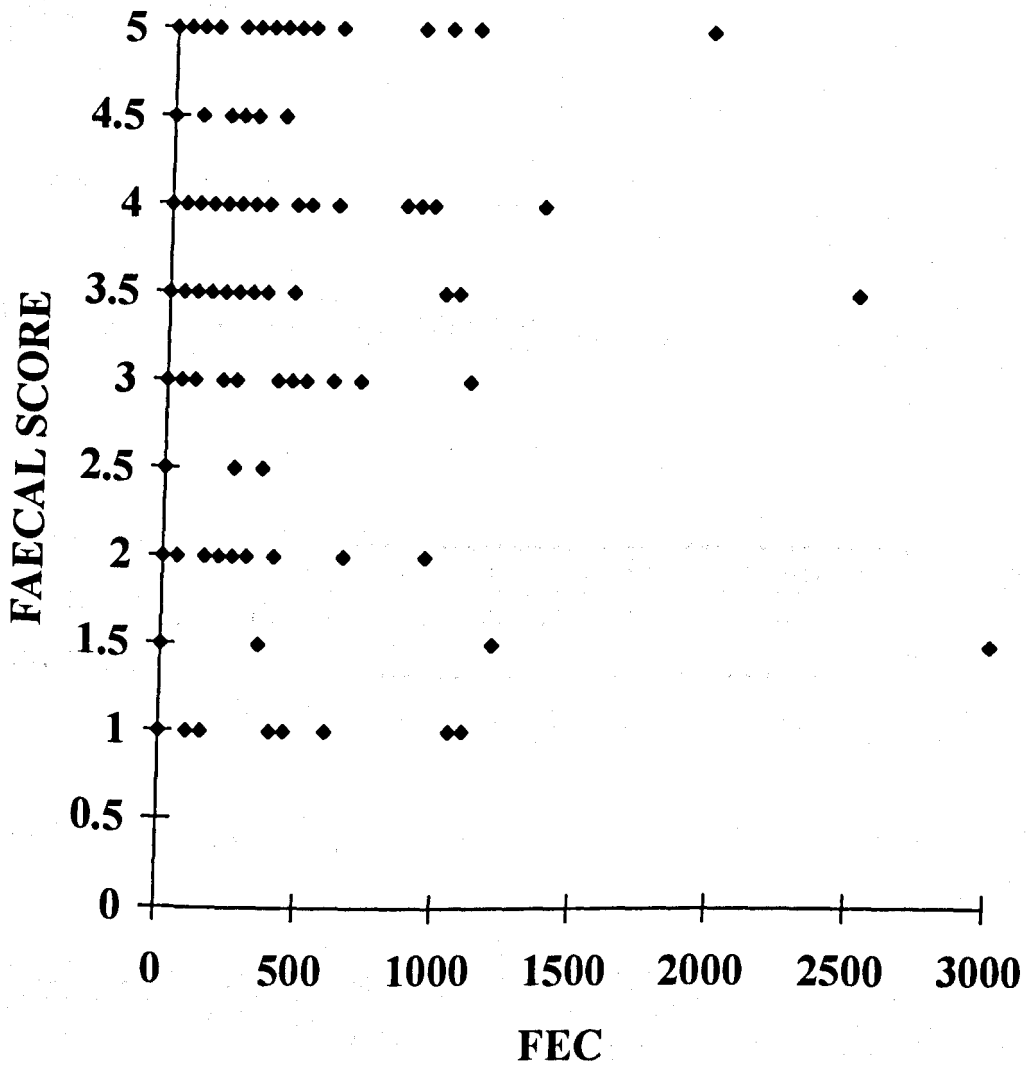


Figure 3.19 : The relationship between faecal score and FEC of 1994 lambs



Factors affecting FEC of 1994 lambs adjusted according to FEC/kg dry matter (results and analysis on fresh faecal material appear in subsequent sections of the chapter)

Effect of sex of lamb on FEC

There was no effect of sex of lamb on FEC (Table 3.4)

Table 3.4 :The mean FEC for male and female lambs in year 3

Sex	n	Mean	% S.D.	conf limits	significance
Male	83	125	10.00	76-209	n.s
Female	78	105	13.18	59-188	

Effect of number of lambs reared on FEC

No effect of number reared was observed on FEC (Table 3.5).

Table 3.5: Effect of litter size reared on mean FEC of lambs

Litter Size				
Year		1	2	3
1	Mean	65	246	99
	n	34	49	77
	% S.D.	14.93	7.24	15.06
	conf limits	25-166	139-434	19-512

Effect of sire on FEC

Significant effects of sire and group ($p < 0.0001$) were seen on mean FEC when Cambridge ram 14 had progeny with considerably higher egg counts ($p < 0.005$) than the others.

Table 3.6: Effect of sire on FEC

Sire	n	Mean	% S.D.	conf limits
Camb 1	12	131	15.14	2-73
Camb 2	10	13	16.60	1-171
Camb 3	10	221	15.78	29-1661
Camb 4	9	238	2.68	110-517
Camb 5	17	31	19.05	7-141
Camb 6	16	219	1.23	214-225
Camb 7	2	272	3.63	.
Camb 8	11	63	8.32	15-269
Camb 9	13	182	5.18	67-497
Camb 10	18	200	6.92	76-526
Camb 11	8	501	1.55	372-676
Camb 12	7	307	4.10	78-1208
Camb 13	10	63	22.86	9-427
Camb 14	14	822	3.63	375-1741

Significantly different progeny groups in terms of FEC ($p < 0.05$). Least significant different comparisons showed the following.

Camb 14 > Camb 1
Camb 14 > Camb 2
Camb 14 > Camb 3
Camb 14 > Camb 5
Camb 14 > Camb 8
Camb 14 > Camb 10
Camb 14 > Camb 13
Camb 5 > Camb 1
Camb 5 > Camb 2
Camb 5 > Camb 13
Camb 5 > Camb 12

Camb 13 > Camb 1
Camb 13 > Camb 2
Camb 12 > Camb 1
Camb 12 > Camb 3
Camb 11 > Camb 1
Camb 11 > Camb 2
Camb 11 > Camb 8
Camb 11 > Camb 5
Camb 4 > Camb 1
Camb 4 > Camb 2
Camb 4 > Camb 3
Camb 4 > Camb 5

Camb 10 > Camb 1
Camb 10 > Camb 2
Camb 10 > Camb 3
Camb 10 > Camb 5
Camb 9 > Camb 1
Camb 9 > Camb 2
Camb 9 > Camb 3
Camb 9 > Camb 5
Camb 6 > Camb 5
Camb 6 > Camb 3
Camb 3 > Camb 1
Camb 3 > Camb 13
Camb 2 > Camb 12

Effect of sex of lamb on FEC

Some of the variation in FEC can be attributed to sex of the lamb a significantly higher proportion ($p < 0.05$) of females had extremely low counts in all years (<200 EPG) (Table 3.7).

Analysis of variance tests however, demonstrated that there was no effect of sex on FEC of group 1 lambs in 1992, group 2 lambs in 1993, and group 2 and 3 lambs in 1994 although a significant affect was seen on group 2 lambs in 1992 ($p < 0.05$) group 1 lambs in 1993 ($p < 0.01$) and group 3 lambs in 1994 ($p < 0.05$) . The mean FEC for male and female lambs in years 1,2 and 3 are shown in Table 3.8.

Table 3.7 : Percentage of male and female lambs in 3 categories of FEC

		Eggs Per Gramme of Faeces		
	Sex	<200	200-800	>800
1992	Male	21.6	64.9	13.5
	Female	43.1	51. 4	5.5
1993	Male	33.9	47.5	18.6
	Female	49.6	40.0	10.4
1994	Male	45.3	48.4	6.3
	Female	59.1	30.7	10.2

Table 3.8 : The mean FEC for male and female lambs in years 1,2 and 3

	Sex	n	Geometric Mean	% S.D.	conf limits	significance
1992 Group 1	Male	13	391	1.92	264-574	n.s
	Female	35	252	3.87	157-402	
1992 Group 2	Male	29	401	4.06	237-668	$p < 0.05$
	Female	46	143	6.53	83-253	
1993 Group 1	Male	47	275	2.57	209-364	$p < 0.01$
	Female	43	166	2.44	126-218	
1993 Group 2	Male	49	271	3.33	191-380	n.s
	Female	39	303	5.13	178-513	
1994 Group 1	Male	48	183	2.96	133-250	n.s
	Female	42	131	3.99	101-227	
1994 Group 2	Male	26	54	9.44	22-132	n.s
	Female	24	6	10.11	2-16	
1994 Group 3	Male	24	224	4.42	121-415	$p < 0.05$
	Female	21	489	2.91	94-2679	

Effect of number of lambs reared on FEC of lambs

In all 3 years of the study no effect of number reared was observed on FEC (Table 3.9).

Table 3.9: Effect of number of lambs reared on mean FEC of lambs

Number of Lambs Reared per Ewe				
Year		1	2	3
1	Mean	283	270	200
	n	16	40	42
	% S.D.	3.31	2.75	2.02
	conf limits	148-536	194-373	166-240
2	Mean	89	180	275
	n	8	67	108
	% S.D.	9.30	4.05	4.18
	conf limits	13-605	132-251	241-315
3	Mean	98	188	81
	n	36	61	85
	% S.D.	6.70	5.57	8.69
	conf limits	51-187	120-289	51-130

Effect of sire on FEC

The mean FEC for sire groups in years 1,2 and 3 are shown in Tables 3.10, 3.11, and 3.12 and Figures 3.20, 3.21 and 3.22. In years 1 and 2 there was no overall significant effect of sire on FEC but there was variation between individual sire groups. In addition no sire X group interaction was observed but in both years a group effect on FEC was observed. ($p < 0.01$) with group 1 lambs in year 1 and group 2 lambs in year 2 having higher FEC than groups 2 and 1 respectively. In year 3 significant effects of sire and group ($p < 0.0001$) were seen on mean FEC for groups 2 and 3 when Cambridge ram 14 had progeny with considerably higher egg counts ($p < 0.005$) than the other

Cambridge rams. However,, no sire effect was observed on group 1 lambs in 1994.

Table 3.10 : FEC of sire groups 1992

Sire	n	Mean	% S.D.	conf limits
Camb 1	9	158	2.09	98-256
Camb 2	32	280	3.81	176-445
Camb 3	25	203	3.48	125-332
Camb 4	33	222	4.15	136-361
Charollais	36	77	7.60	40-149
Vendeen	26	420	3.29	266-663
Suffolk	18	278	5.19	130-595

Significantly different progeny groups in terms of FEC ($p < 0.05$). Least significant different comparisons showed the following.

Vendeen> Cambridge 1

Vendeen> Cambridge 2

Vendeen> Cambridge 3

Vendeen> Cambridge 4

Vendeen> Charollais

Figure 3.20 : Graph showing the FEC of different sire groups of year 1 lambs

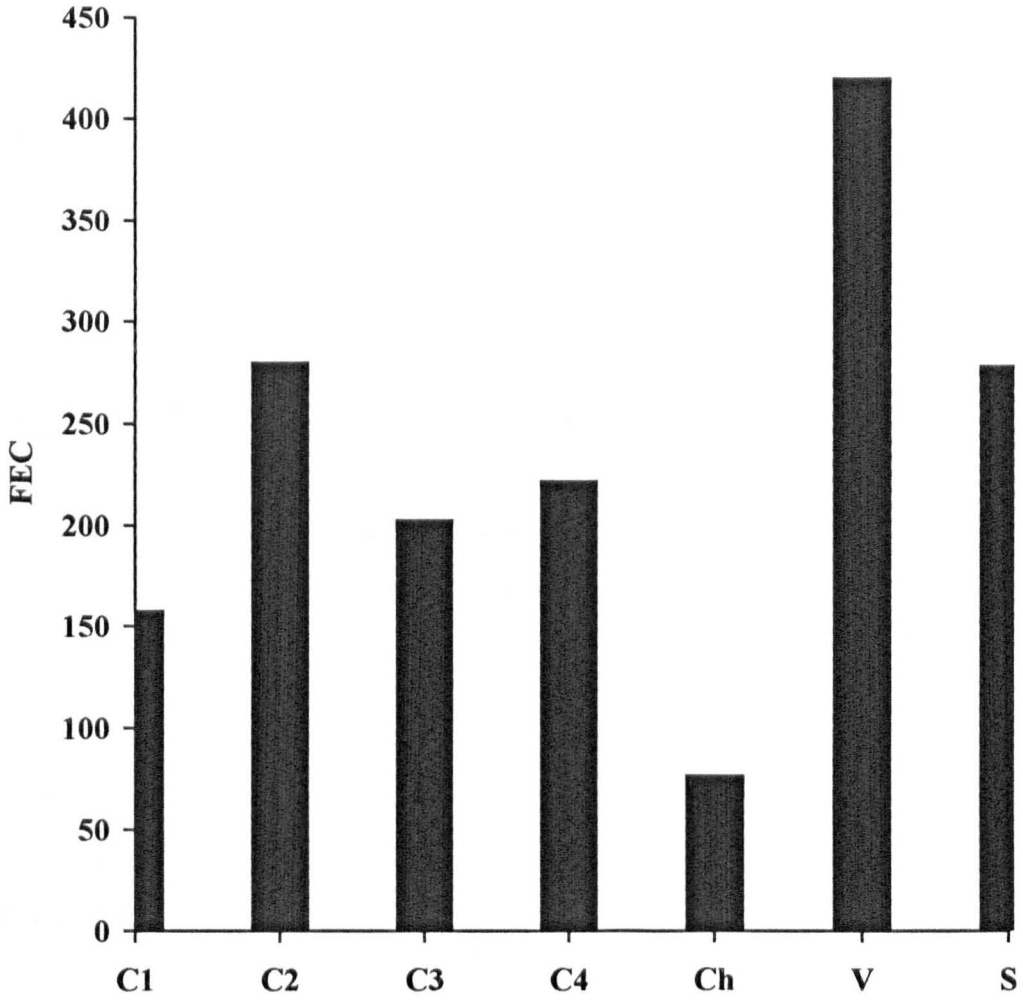


Table 3.11 : FEC of sire groups for year 2, 1993 lambs

Sire	n	Mean	% S.D.	conf limits
Camb 1	15	198	2.43	136-293
Camb 2	17	152	2.51	98-235
Camb 3	14	112	8.96	36-353
Camb 4	23	370	2.54	255-541
Camb 5	15	235	2.47	167-369
Camb 6	22	265	4.68	138-501
Camb 7	16	316	2.81	190-525
Camb 8	5	109	17.72	9-1367
Camb 9	2	653	1.27	.
Camb 10	9	445	2.96	220-906
Charollais	24	193	4.41	108-352
Texel	22	115	8.07	48-275

Significantly different progeny groups in terms of FEC ($p < 0.05$). Least significant different comparisons showed the following.

Camb 10 > Texel

Camb 4 > Texel

Camb 7 > Texel

Camb 4 > Camb 3

Camb 7 > Camb 3

Camb 10 > Camb 3

Figure 3.21 : Graph showing the FEC of different sire groups of year 2, 1993 lambs

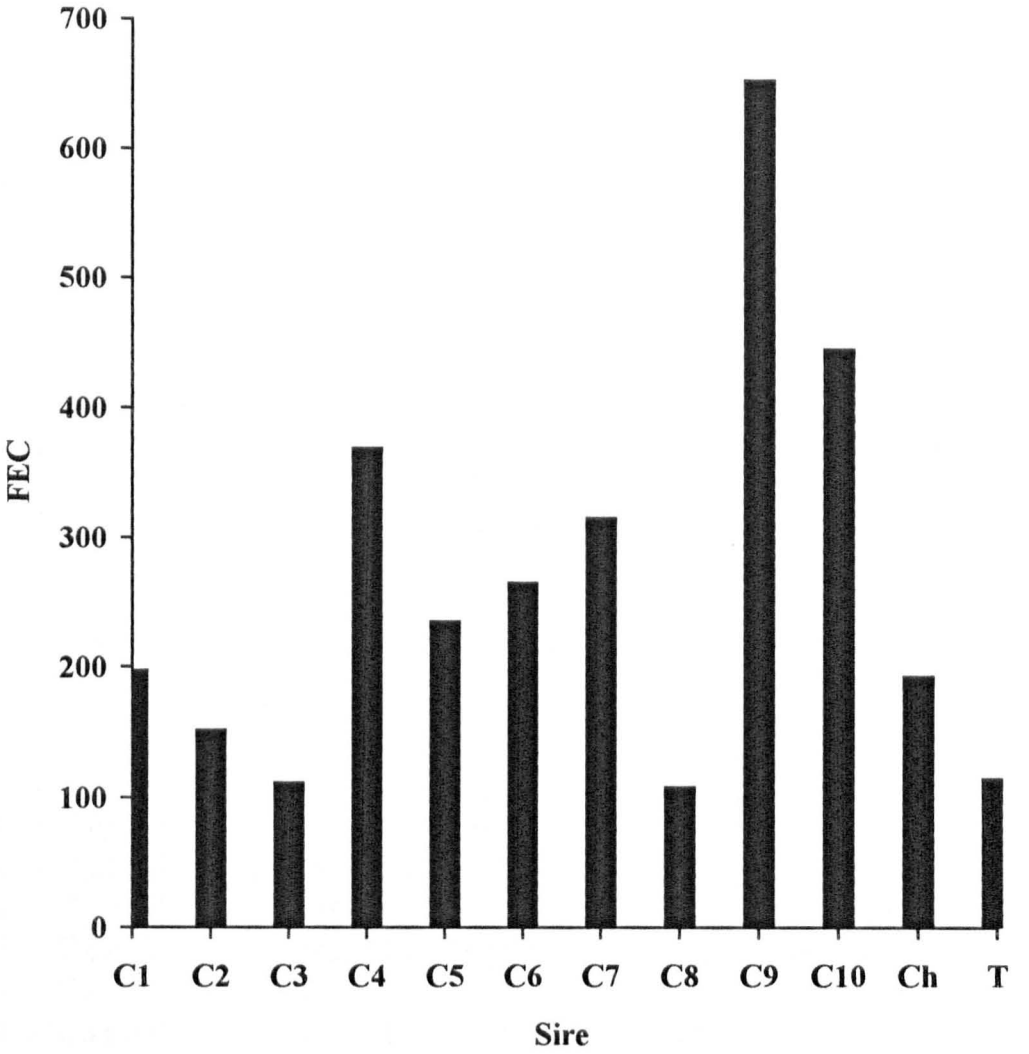


Table 3.12 : FEC of sire groups for year 3, 1994 lambs

Sire	n	Mean	% S.D.	conf limits
Camb 1	16	13	11.14	4-42
Camb 2	11	14	14.66	5-69
Camb 3	11	49	7.91	14-166
Camb 4	10	122	2.87	64-237
Camb 5	20	68	10.12	25-185
Camb 6	19	189	1.91	143-255
Camb 7	2	106	1.63	.
Camb 8	14	76	7.36	27-216
Camb 9	14	166	5.00	71-385
Camb 10	20	224	2.73	144-348
Camb 11	8	264	2.33	147-472
Camb 12	7	210	2.90	96-460
Camb 13	15	158	10.16	49-511
Camb 14	14	714	2.64	427-1175

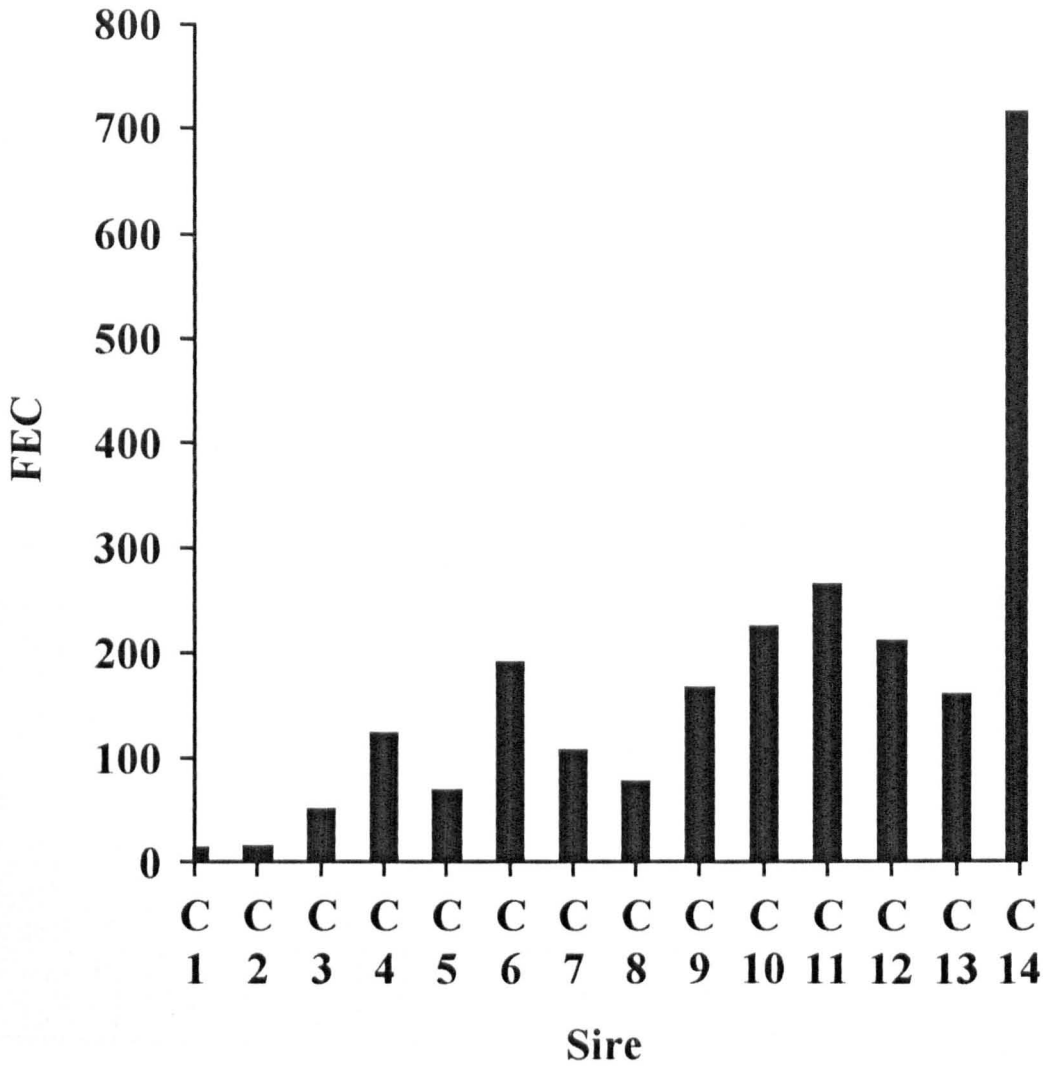
Significantly different progeny groups in terms of FEC ($p < 0.05$). Least significant different comparisons showed the following.

Camb 14 > Camb 1
Camb 14 > Camb 2
Camb 14 > Camb 3
Camb 14 > Camb 4
Camb 14 > Camb 5
Camb 14 > Camb 6
Camb 14 > Camb 8
Camb 14 > Camb 10
Camb 14 > Camb 13
Camb 5 > Camb 1
Camb 5 > Camb 2

Camb 13 > Camb 1
Camb 13 > Camb 2
Camb 12 > Camb 1
Camb 12 > Camb 1
Camb 11 > Camb 1
Camb 11 > Camb 2
Camb 11 > Camb 3
Camb 11 > Camb 5
Camb 8 > Camb 1
Camb 8 > Camb 2
Camb 4 > Camb 1
Camb 4 > Camb 2

Camb 10 > Camb 1
Camb 10 > Camb 2
Camb 10 > Camb 3
Camb 10 > Camb 5
Camb 10 > Camb 8
Camb 9 > Camb 1
Camb 9 > Camb 2
Camb 9 > Camb 5
Camb 6 > Camb 1
Camb 6 > Camb 2
Camb 6 > Camb 3
Camb 3 > Camb 1

Figure 3.22 : Graph showing the FEC of different sire groups of year 3, 1994 lambs



The relationship between FEC of sires when they were growing lambs and the FEC of their progeny

There was some suggestion that there was a relationship between FEC of sires when they were growing lambs and the FEC of their progeny $r = 0.50$ ($p < 0.10$) (Table 3.13).

Table 3.13 : FEC of sires when growing lambs and their sire groups for 1994 lambs

Sire	Progeny Group FEC	Sire FEC
Camb 2	14	175
Camb 3	49	175
Camb 4	122	300
Camb 5	68	125
Camb 6	189	200
Camb 8	76	550
Camb 9	166	100
Camb 10	224	175
Camb 11	264	625
Camb 12	210	1900
Camb 13	158	1400
Camb 14	714	1900

Effect of dam on lamb FEC

There was no significant correlation between dam FEC during the periparturient rise and that of their lambs in both years 1 and 2, $r = 0.11$ and 0.06 respectively .

Live weight change

In all 3 years, lambs were weighed at weaning and 6 weeks later, i.e. at the time of faecal sampling. The growth rates (g/day) from weaning to the last faecal sample, 5 -6 weeks post-weaning, for each lamb group in years 1, 2 and 3 are shown in Table 3.14. High coefficients of variation were observed and low growth rates as would be expected in newly weaned parasitized sheep. However, in all years 1, 2, and 3 non significant correlations were observed between cumulative weight change and mean FEC (Table 3.14 and Figures 3.23, 3.24 and 3.25).

Table 3.14 : Mean, standard deviation and coefficient of variation estimates for growth rate (g/day) over the six week period from weaning to faecal sampling

	Mean (g/d)	S.D.	C.V.	correlations between LWG and FEC
1992 Group 1	78.6	69.05	87.88	-0.047
1992 Group 2	95.2	71.40	75.00	-0.052
1993 Group 1	66.7	42.86	64.29	0.20
1994 Group 1	152.4	53.57	35.16	0.01
1994 Group 2	50.0	100.70	201.43	0.08
1994 Group 3	85.7	39.29	45.83	-0.34

Figure 3.23 : Graph showing the relationship between mean FEC and live weight gain (LWG) of lambs in year 1 over a 6 week period

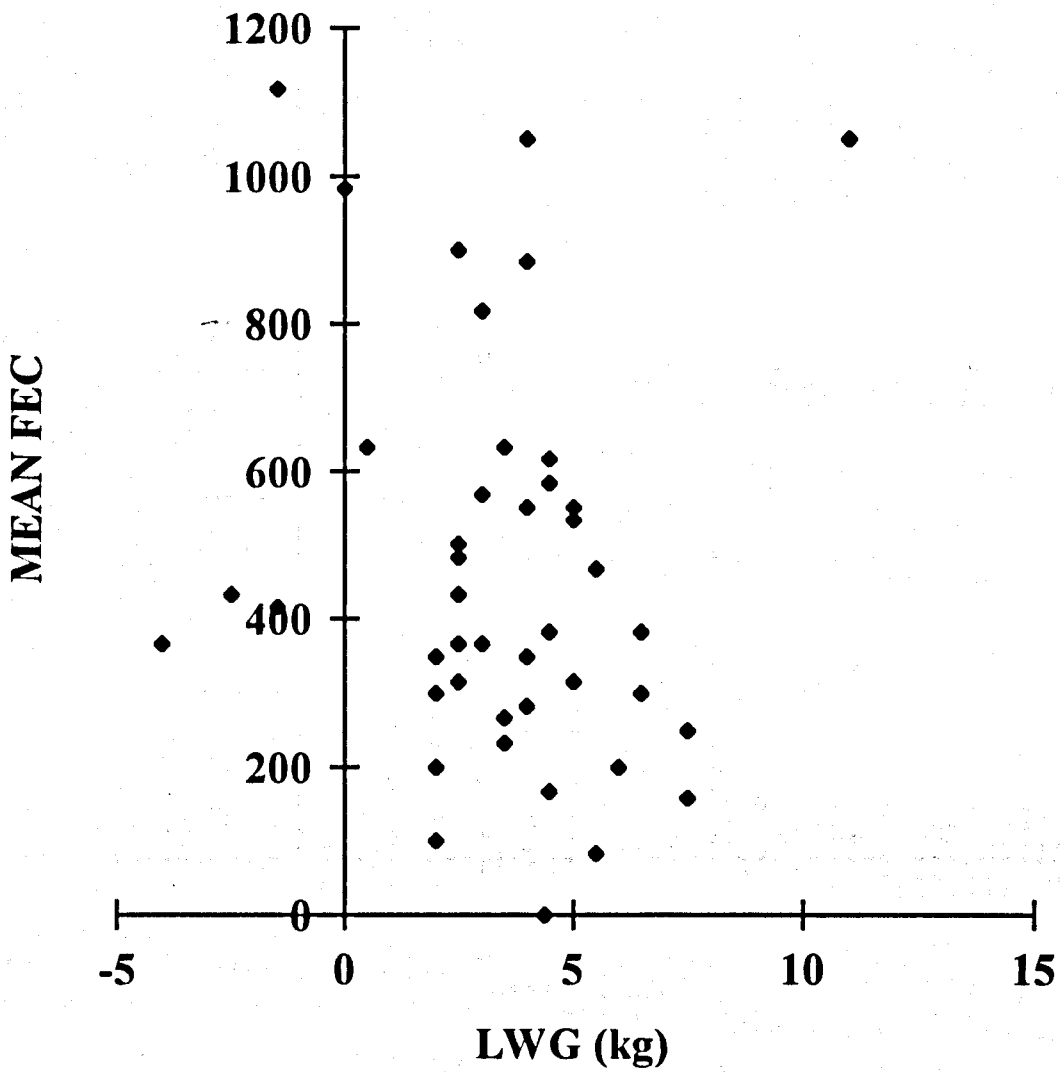


Figure 3.24 : Graph showing the relationship between mean FEC and LWG of lambs in year 2 over a 6 week period

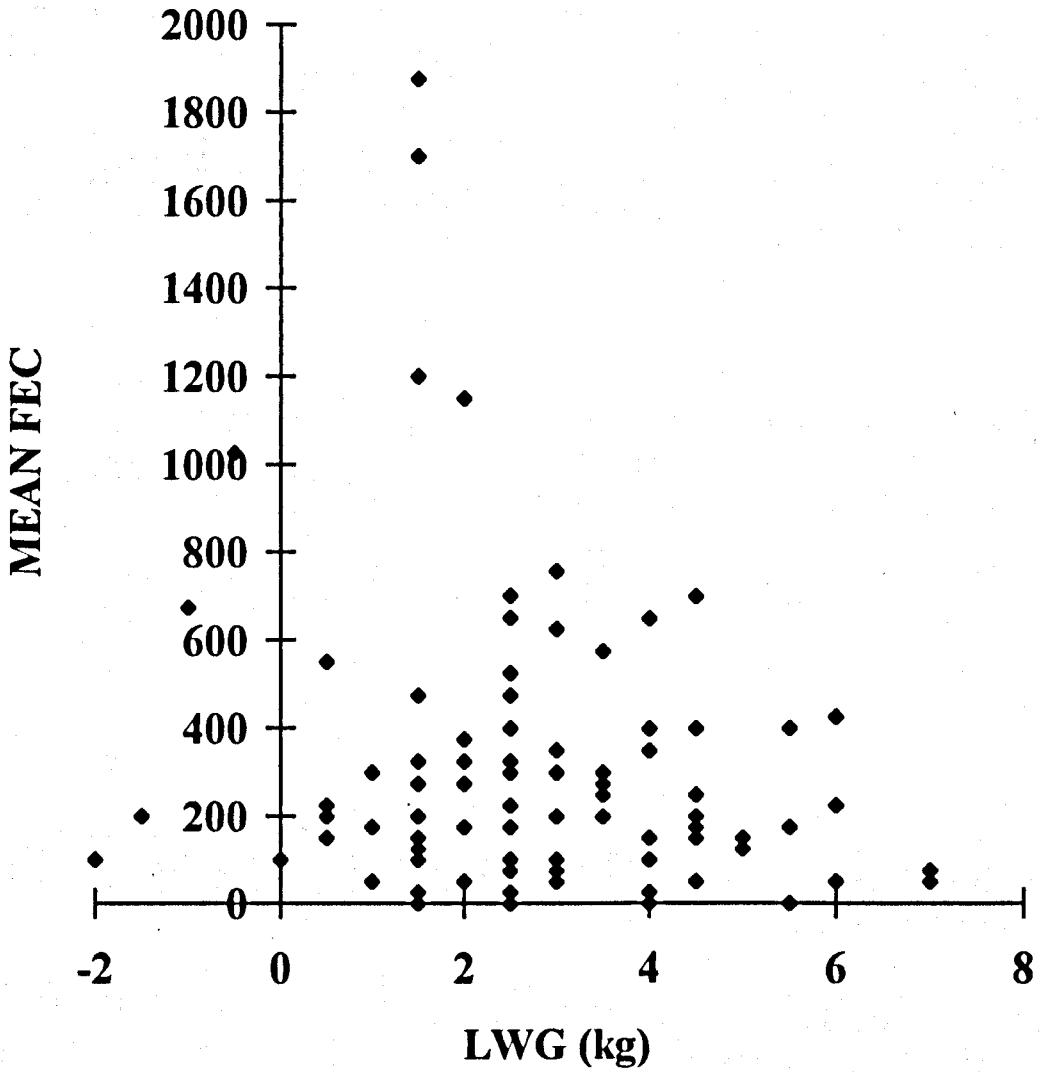
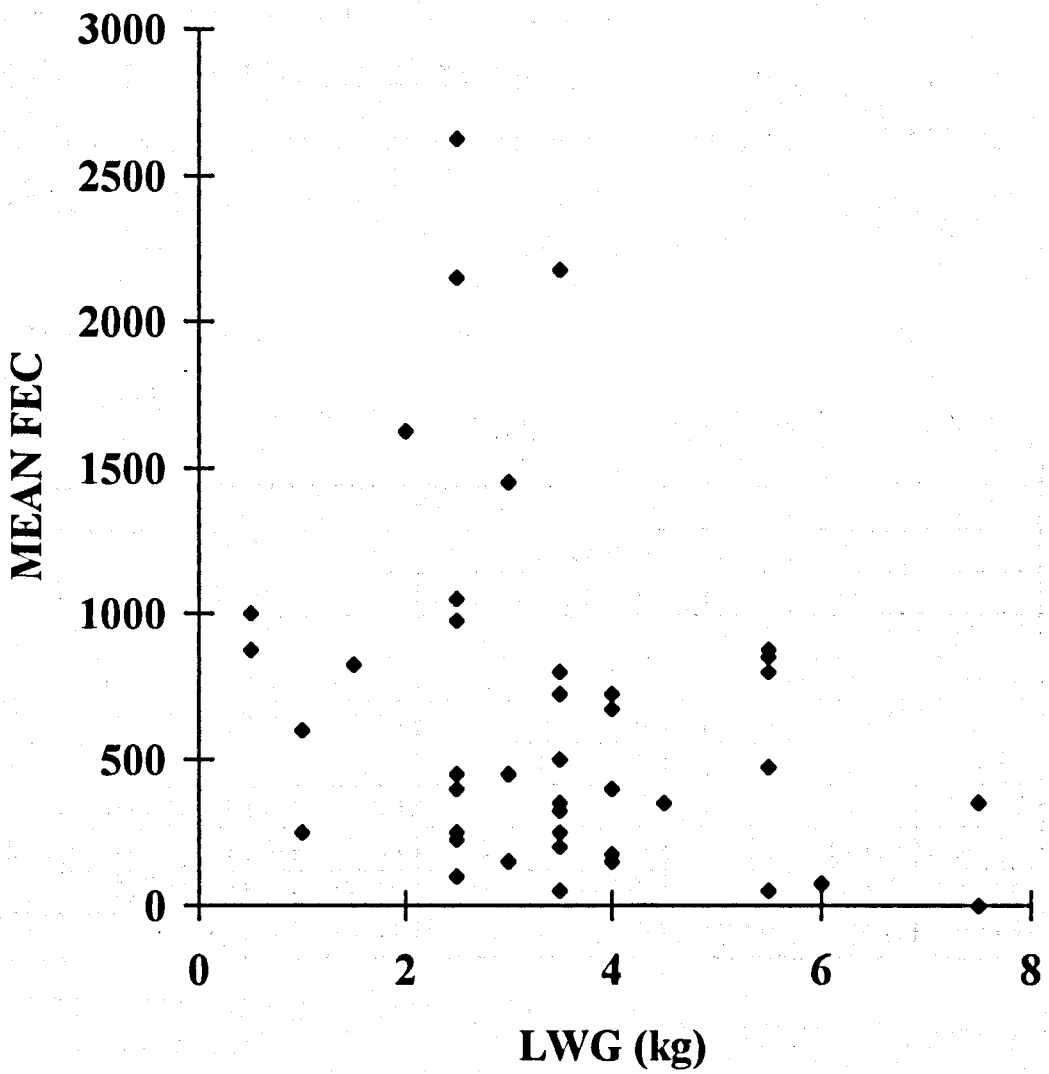


Figure 3.25 : Graph showing the relationship between mean FEC and LWG of lambs in year 3 over a 6 week period



In year 1 (1992), a significant effect of sire was seen on the mean live weight gain ($p < 0.05$) (Table 3.15 and Figure 3.27) but no effect of sex of lamb was seen on the mean live weight gain.

In 1993, an effect of sire ($p < 0.01$) but not sex was seen on mean live weight gain (Table 3.16 and Figure 3.28). In 1994, both sex ($p<0.02$) and sire ($p<0.01$) significantly affected live weight gain of lambs (Table 3.17 and Figure 3.29).

Table 3.15 : Effect of sex and sire on live weight gain (g/d) of year 1, lambs 1992.

Sire	Sex of Lamb							
	Male			Female			Both sexes	
	n	Mean	s.e.	n	Mean	s.e.	Mean	s.e.
Camb 1	3	99.3	26.05	3	89.3	3.44	93.6	13.13
Camb 2	11	80.1	18.06	21	70.7	22.55	73.9	20.74
Camb 3	8	72.9	27.36	11	57.9	14.76	62.8	38.50
Charollais	13	93.8	36.76	10	114.2	17.18	98.7	24.38
Camb 4	10	115.4	20.00	13	97.6	18.75	105.5	21.14
Suffolk	8	171.2	67.65	12	91.5	19.54	121.0	34.20
Vendeen	7	105.5	18.38	9	33.1	24.41	64.7	25.50
Mean		102.2	29.81		77.9	17.80	87.6	7.93

Figure 3.27 : Graph showing the LWG of different sire groups of year 1, 1992 lambs

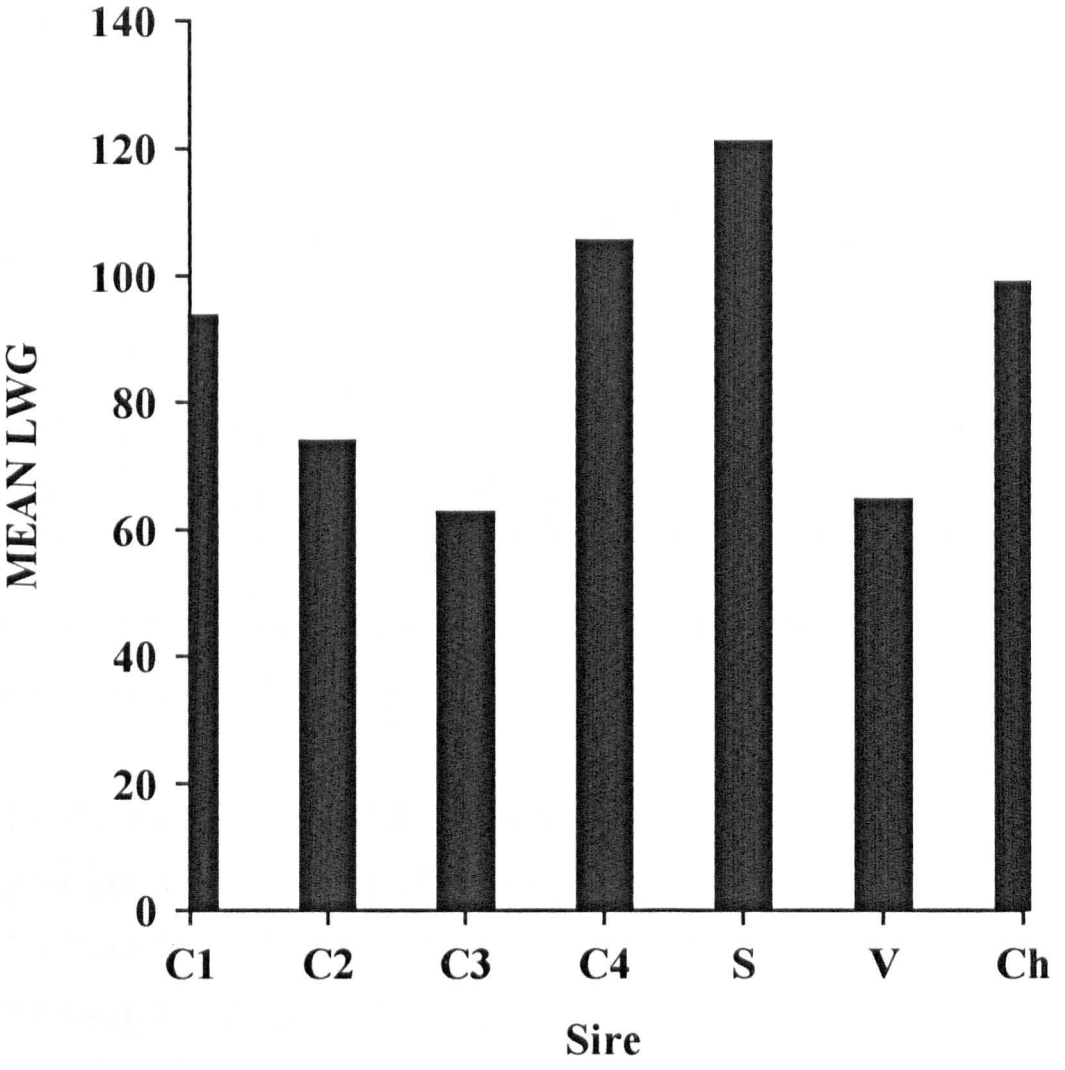


Table 3.16 : Effect of sex and sire on live weight gain of year 2 lambs, 1993

Sire	Sex of Lamb							
	Male			Female			Both sexes	
	n	Mean	s.e.	n	Mean	s.e.	Mean	s.e.
Camb 6	4	50.6	15.60	8	59.5	12.72	56.5	13.68
Camb 4	7	28.8	17.37	2	29.8	77.46	29.0	30.72
Camb 7	10	57.1	14.53	6	87.4	19.14	68.6	16.26
Camb 5	2	95.2	33.40	1	83.8	-	91.2	14.31
Camb 1	2	59.5	0	5	85.7	8.72	78.2	6.23
Charollais	2	23.8	11.96	6	61.4	14.19	52.0	13.64
Camb 3	6	77.4	12.15	5	71.4	11.29	74.7	11.75
Camb 2	8	78.8	19.77	6	61.4	7.19	71.3	14.38
Texel	2	136.9	5.90	5	69.0	10.20	88.4	8.97
Mean		62.9	15.42		68.5	14.79	66.5	4.46

Differences between LWG of sire groups ($p < 0.05$) Least significant different comparisons showed the following.

Camb 1 > Camb 4

Camb 2 > Camb 4

Camb 3 > Camb 4

Texel > Camb 4

Camb 5 > Camb 4

Camb 7 > Camb 4

Figure 3.28 : Graph showing the LWG of different sire groups of year 2, 1993 lambs

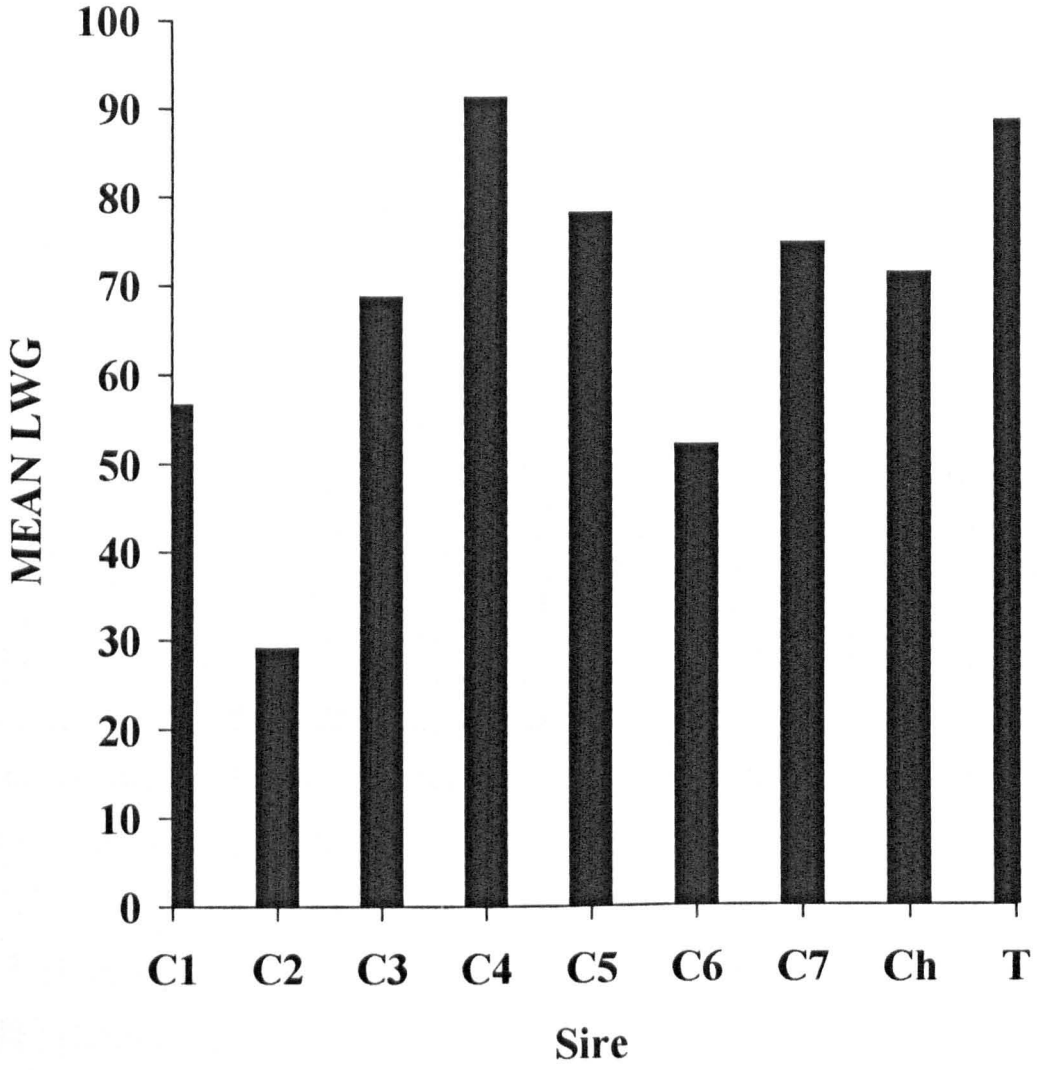


Table 3.17 : Effect of sex and sire on live weight gain (g/d) of year 3 lambs, 1994

Sire	Sex of Lamb							
	Male			Female			Both sexes	
	n	Mean	s.e.	n	Mean	s.e.	Mean	s.e.
Camb 1	7	86.1	22.44	7	78.3	34.23	82.1	19.60
Camb 2	5	121.4	11.48	6	45.7	9.43	80.0	14.85
Camb 3	9	150.0	19.44	2	131.0	13.46	147.1	16.64
Camb 4	4	157.7	8.93	6	115.0	15.84	132.1	11.98
Camb 5	9	147.6	37.86	8	108.6	35.82	129.5	22.16
Camb 6	7	166.7	11.52	1	107.1	.	130.2	10.56
Camb 7	1	71.4	.	1	83.3	.	77.4	5.91
Camb 8	8	190.5	10.90	6	123.8	20.36	164.8	3.92
Camb 9	9	148.1	11.42	5	123.8	15.31	139.5	9.36
Camb 10	11	145.0	15.29	9	107.1	22.43	128.1	10.97
Camb 11	4	132.6	36.54	4	125.0	11.18	131.0	19.43
Camb 12	2	119.0	59.70	6	79.3	14.64	81.3	16.83
Camb 13	7	78.3	20.92	8	52.1	8.17	64.3	7.44
Camb 14	6	69.5	21.38	7	73.1	15.63	71.4	12.40
Mean		134.2	6.76		94.26	5.43		

Differences between LWG of sire groups ($p < 0.05$). Least significant different comparisons showed the following.

Camb 7 > Camb 8

Camb 13 >Camb 8

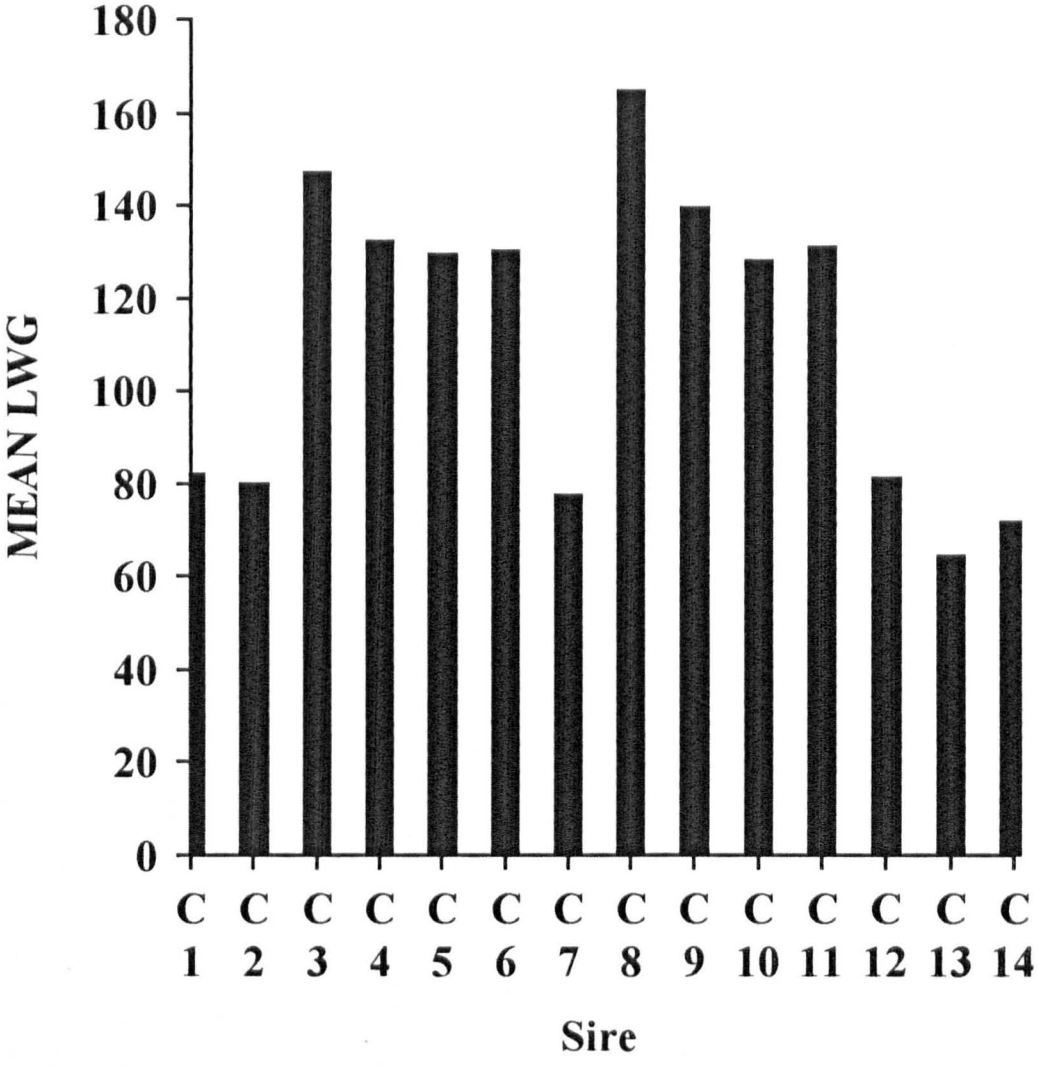
Camb 12 >Camb 8

Camb 14 >Camb 8

Camb 1 > Camb 8

Camb 2 > Camb 8

Figure 3.29 : Graph showing the LWG of different sire groups of year 3, group 1 lambs



Effect of faeces consistency on live weight gain

Faeces consistency varied between 1 to 5 but no effect of faecal score was seen on live weight gain in years 1, 2 and 3.

3.4 Discussion

In order for lambs to be assessed as high or low faecal egg producers larvae either have to be experimentally administered or ingested naturally in field conditions. In year 2, pasture larval counts found on herbage which group 1 lambs were initially turned out onto were low. Such a result would be expected as lambs or ewes had not grazed the pasture during the previous 2 years. However, only a very few samples were taken and such a low number would not be a true indication of level of pasture contamination. In order to infect the lambs to monitor faecal egg production the lambs were moved post-weaning (14-16 weeks of age) and placed on to infected pasture and a pasture larval sample was taken 5 weeks later. Pasture larval counts were extremely high. One and five days later lambs were faecal sampled and a wide variation in FEC was observed between samples at each date. Pasture larval levels in year 3, 1994 were not high, probably due to the fact that samples were taken 1 week post-weaning when lambs were placed onto the pasture. Thomas and Boag (1972), however, stated that even negligible levels of larval contamination should not be neglected as a source of significant infection to young lambs. Perhaps if faecal samples were taken later in the year allowing pasture larval burdens to accumulate, FEC of lambs would be higher. Thomas and Boag (1972), however, noticed higher FEC in August and September in lambs which had been turned out onto clean pasture in April than those lambs which had been placed on contaminated pasture. The authors explained their findings as prior exposure earlier in the summer resulting in the stimulation of an immune response in the lambs on contaminated pasture which subsequently limited the second rise in egg count.

Once lambs have been exposed to infective larvae and are displaying faecal egg production, in order to select phenotypically resistant lambs from those which are susceptible, it is necessary to have variation in performance.

The natural infection system produced at 32-35 days post-dosing a wide variation in FEC. In all three years, some lambs had FEC of less than 50 EPG, the minimum number of eggs which are detectable with the modified McMaster technique whilst other lambs displayed egg counts in excess of 2000 EPG which were associated with clinical signs of helminthiasis in some but not all cases. The 32-35 day period would seem to be necessary to allow variation in FEC to occur whilst avoiding excess damage and performance loss except in very susceptible animals.

The statistically significant skewed frequency distribution seen in all years 1, 2 and 3 with 29.5%, 42% and 49% of lambs respectively having less than 200 EPG and a long tail of susceptible animals has been described also by many authors. Lunn and Northop-Clewes (1993) described gastrointestinal helminth infections as having a highly skewed distribution pattern, and suggested that 20% of the population could carry 80% of the worms. In year 1, 11% had high counts (>800); comparable figures for year 2 and year 3 were 15.6% and 8.7% respectively. Wakelin and Blackwell (1988) stated that irrespective of host or parasite species, most hosts harbour few parasites and a few harbour large numbers of parasites and that heterogeneity in host exposure to infection occurs because of one or a combination of two or more of the following

factors; behavioural, social or demographic, previous challenge, genetic trait or nutritional status.

The high variability in infection rates suggests that selection of animals resistant to nematode infections could be worthwhile. However, because of the skewed distribution, much of the variation is linked to the long tail of susceptible animals so that apart from the percentage of animals with high counts selection within the remaining population may be difficult.

The accuracy of techniques for selection criteria for resistant animals is extremely important. The measure of worm burden by performing nematode egg counts on lambs' faeces is universally thought of as the accepted technique to assess infection rates. Factors such as time and frequency of sampling and dry matter of faeces all effect FEC and therefore have to be kept as consistent as possible.

Time and frequency of faecal sampling was investigated by taking FEC at different stages in the lambs' development (pre-weaning and at post-weaning) and at different intervals. Taking faecal samples pre-weaning could indicate resistant lambs early in their development (pre- 14-16 weeks of age) and early infection may allow lambs to be primed sooner. All correlations between pre-weaning and post-weaning were however,, mainly non significant for all 3 years (group 2 lambs in year 1 and group 3 lambs in year 3 being the exception) a fact which may be due to milk production of the ewe and creep feed availability. Pre-weaning sampling was thus not a very reliable indicator of lamb infection rates.

In year 2, faecal samples taken 21 days post-weaning and dosing were all zero. At 28 days post-dosing, counts were either zero or very low which was probably due to the residual effect of the anthelmintic. In year 2 at 32 and 36 days post-dosing, the means were not significantly different, i.e. 225 and 254 for group 1 and 254 and 363 for group 2. In year 3 at 35 and 39 days post-dosing with an anthelmintic, means were also not significantly different, i.e. 110 and 152 for group 1, 15 and 16 for group 2 and 300 and 316 for group 3. These means thus indicate that perhaps taking only one faecal sample would be sufficient in order to determine which lambs are phenotypically resistant and susceptible.

A number of factors could be responsible for correlations of consecutive sampling dates not being significant for group 1 lambs in year 1, in contrast to the significant relationships observed between consecutive sampling dates for group 2 year 1 and both groups 1 and 2 in year 2 all groups 1, 2 and 3 in year 3. The sampling interval was 30 days in year 1 and the 2 samples were thus taken when the lambs were different ages and the FEC represented different infections. Samples taken in years 2 (32 and 36) and 3 (35 and 39 post-dosing) displayed the effect of day to day variation in FEC.

In order to maximise the efficiency of selecting resistant and susceptible animals on the basis of FEC non-genetic factors which could effect FEC such as consistency of faeces, sex of lamb and litter size reared should be considered.

Faecal consistency varied amongst lambs from 1 (watery faeces) to 5 (pelleted faeces). There was however, no significant correlation between consistency of faeces and FEC in all years 1, 2 and 3. Some lambs with an extremely high FEC which would normally be associated with clinical signs of helminthiasis produced extremely hard pelleted faeces This non-significant correlation could have been due to a number of factors. Dry matter of faeces can be reduced by additional factors other than helminth burdens such as intercurrent infections (with coccidia) and lush pastures which could cause within group variability to be a problem (Sykes, 1995). Faeces may thus be watery and no nematode eggs be detected if the cause is non-parasitic and even in heavy worm burdens faeces with a low dry matter can be unrepresentative of the level of infection. Soulsby (1986) suggests that 2g of normal faeces be taken as a sample, 2.5g of soft faeces, 3g of a medium-soft stool not formed into pellets, 5g of a pultaceous stool and 7g of a watery stool. If FEC was expressed as EPG/g DM faecal samples a score of 1 should be increased by a factor of 4.20, a score of 2 by 1.86 and 4 by a factor of 1.16. Adjusting FEC figures in terms of dry matter increased egg counts but made no difference to the concluding results of the study.

Non-genetic factors such as sex of lamb need to be considered when describing animals as resistant or susceptible in order to prevent confusing or biasing results when selecting resistant animals. More female than male lambs had lower infection rates (< 200 EPG) in all years 1, 2 and 3. Windon (personal communication, 1993) also observed a higher proportion of female than male lambs with low infection rates when experimentally infected with *Trichostrongylus colubriformis*. An effect of lamb sex on mean FEC was

observed for group 2 lambs, year 1, group 1 lambs year 2 and group 3 lambs in year 3. All lambs were aged between 16-22 weeks of age when faecal sampled and thus not all animals would have reached puberty at time of sampling which would explain why some groups of lambs displayed no effect of gender on FEC. Many authors (Woolaston, Barger and Piper 1990; Knight and Vegors, 1972; Albers, Gray, Piper, Barker, Le Jambre and Barger, 1987) have noticed during pre-puberty no difference in mean FEC due to lamb sex. Courtney, Parker and Herd, (1985a) however, described a sex effect after puberty with experimental infections of *Haemonchus contortus* in St. Croix, Florida Native, Barbados Blackbelly and domestic crossbred lambs. Ewe lambs had significantly lower infection rates than ram lambs. Yazwinski, Goode, Moncol, Morgan and Linnerud (1981) whilst investigating *Haemonchus contortus* resistance in Barbados Blackbelly sheep noted a sex difference with females being more resistant than males, however, a breed X sex difference was not found. Herd, Queen and Majewski (1992) noted that bulls had significantly higher FEC than steers and heifers, but no significant difference was observed between steers or heifers and those with anabolic implants. They concluded that it is necessary to distribute sexes equally between groups when undertaking research trials with animals of mixed sexes as in the present study and recommend that grazing difference between males and females may be responsible for differences in nematode infection rates as mortalities have been observed amongst bulls, due to severe helminthiasis, when they have grazed with heifers of the same age and breed which appeared to be unaffected. Hogarth-Scott (1969) demonstrated that homocytropic IgG1a increased markedly in response to a parasitic infection and suggested that the capacity to produce IgGIa was linked to the X chromosome, therefore

accounting for the greater production in ewes than in rams. The author also suggested that host androgens are involved. Campbell and Melcher (1940) showed that castration followed by the administration of oestrogen to male rats, increased their resistance to *Cystercus crassicollis*, whereas androgens administered to the speyed female seemed to have the opposite effect. Grossman (1989) noticed a higher number of immunoglobulins in females than males and that cell-mediated immune responses were more active. These cell-mediated immune responses were stimulated by oestrogens, whilst androgens had the opposite effect. Grossman (1989) suggested that an increased immunity of females over males allows them to cope with increased environmental challenges brought about by the stress of reproduction.

The absence of an effect of number of lambs reared in all years contrasts to that observed by other authors. Twin and triplet lambs have less milk supply available to them than single lambs. Therefore twins and triplets increase their herbage consumption and whilst outdoors graze and consume more herbage than single lambs. As these lambs graze more than single lambs they are more likely to pick up larvae on the pasture. Therefore lambs which 'survive' this initial infection have a greater chance of becoming resistant to future infections than do lambs which are less likely to pick up the helminths from the pasture at an early age. Thus some lambs born in multiple births if they survive the initial exposure to gastrointestinal nematodes may have a better chance of developing resistance to helminth infections (Gibb and Treacher, 1980; Gibb, Treacher and Shanmugalingam, 1981).

FEC of lambs from different sire groups were investigated in order to see if differences in FEC were genetically based , lambs in each sire group were either full or half sibs. In years 1 and 2, although no overall ram effect was significant individual ram comparisons were found to be significantly different In year 1, the Vendeen X Cambridge lambs had lower FEC than any other lambs. In year 2, one particular Cambridge ram produced progeny with significantly lower FEC than any other ram, however, there was no significant breed effect. In year 3, a significant sire effect on FEC was observed ($p < 0.01$) indicating a genetic basis for resistance. The heritability of resistance based on half-sib groups was first recorded by Whitlock (1955) and Whitlock and Madsen (1958) who observed that the progeny of one ram was noticeably less susceptible to natural infection with *Haemonchus contortus* than lambs sired by other rams. The authors concluded that this within-breed difference could be to a single dominant gene.

Gray, Presson, Albers LeJambre, Piper and Barker, (1987) used 613 lambs from 40 sire groups and demonstrated the heritability of resistance to be higher (29%) than that of growth (18%). Albers, Burgess, Adams, Baker, Le Jambre and Piper (1984) investigated mean FEC and noticed that the resistant group had a much lower FEC than the susceptible lambs and concluded that the resistant sire must have been homozygous for a rare gene with dominant action and major effect. Windon (1991) suggested that the immunological response to vaccination with irradiated L_3 of *Trichostrongylus colubriformis* of lambs was under genetic control and selected high responder (resistant) and low responder (susceptible) rams to vaccination with irradiated larvae. The correlation between sire FEC when growing lambs and their progeny groups

for 1994 lambs was approaching significance ($r=0.50$, $p < 0.1$) thus suggesting that by selecting lambs with low FEC it may be possible to produce resistant progeny.

There was no significant differences in faecal egg production of lambs of different dams in both years 1 and 2. However, this is in contrast with the findings of Windon and Dineen (1981) who were able to select for resistant lambs based on FEC of their dams. They demonstrated that when both sire and dam had been selected for resistance or susceptibility to experimental infections with infective larvae that there were highly significant differences in the FEC of the progeny than if only the ram had been selected for resistance or susceptibility. However, in the present study ewes were monitored for FEC when they were adult females during the periparturient period, not when they were growing lambs which is suggested by Windon and Dineen (1981) as the time to distinguish resistant and susceptible animals. Thus selection for resistant animals conveys better results if both parents are placed in the selection programme on the basis of their performance in previous challenge infections when lambs.

Live weight gains in terms of g/d were low and were influenced by the post-weaning low grass quality and the fact that concentrates were not fed in order to encourage consumption of infective pasture. The absence of a significant relationship in the post-weaning period between cumulative liveweight gain and mean FEC contrasts with the findings of many authors (Urquhart, Armour and Duncan, 1987; Soulsby, 1986; MacRae, 1993; Sykes and Coop, 1976.) Fox (1993), however, stated that liveweight gain may not be a good indicator

of parasite burden because although a decrease in weight gain is seen due to a reduction in skeletal growth, anorexia, alterations in protein metabolism, depressed activity of some intestinal enzymes and diarrhoea an increased water intake and retention is seen thus causing some weight gain. Thus the composition of weight gain may be different and only the short term effect of live weight gain is therefore an adequate system to evaluate resistance. Other factors such as age at weaning and the genetic ability to grow effects variability from lamb to lamb thus decreasing the effectiveness of LWG as an indicator of FEC.

A non-significant correlation was observed between weight gain and faecal score in all years 1, 2 and 3. In contrast Urquhart *et al* (1987) and Soulsby, (1986) suggested that scouring is generally taken to affect liveweight gain. This observation made in the present study was probably due to the fact that in many lambs the diarrhoea did not persist and was therefore insufficient to affect liveweight gain. A non-significant correlation was observed between faecal score and FEC. Factors other than parasitic infection are also described as causing scouring, e.g. lush pastures or intercurrent infections which are suggested by the positive response of scouring lambs to sulphonamide drugs. Most lambs with watery faeces brought on or enhanced by nematode infestation were very small therefore liveweight gains for these animals were negligible.

Concluding remarks

There is evidence to suggest that substantial variation occurs in faecal egg production in naturally infected grazing lambs. By devising a selection programme it is conceivable that this type of infection could be used as a way of screening large numbers of lambs in order to select high and low egg producing animals. One faecal sample taken at about 32 days-post dosing with anthelmintic seems a feasible way of undertaking the first step in the selection process. However, in order to gain a more precise representation of infection rate, experimental infections indoors where time and frequency of infection and control of intercurrent infections can be carefully monitored would probably be a more effective way of selecting animals after the initial screening process.

CHAPTER 4

VARIATION IN INFECTION RATES OF LAMBS EXPERIMENTALLY INFECTED WITH *OSTERTAGIA CIRCUMCINCTA*

4.1 Introduction

Experimental infections allow a reduction in phenotypic variation in FEC (Eady and Woolaston, 1992), because they remove differences between lambs due to unequal larval intake associated with grazing selectivity and reduce the effect of intercurrent infections which influence the amount and dry matter content of faeces. Following experimental infections, it is also possible to relate output pattern to time and frequency of infection.

Eady and Woolaston (1992) stated that if the amount and success of prior exposure of lambs to infective larvae is doubtful, then experimental infections should be given to prime the animal's immune system as this type of infection offers greater control in terms of type, level and duration of infection. However, resistance to natural infection represents the characteristic to be altered by selection and the authors state that the type of experimental infection used will depend upon grazing management and the preference of the breeder.

There are two possible types of experimental infections, single in which the prescribed dose of infective larvae is given in a single dose and trickle infections in which the infection is given in more than one dose.

Single infections are simple to administer, less laborious and faecal egg output is easily related to infection rate. Several authors have investigated the relationship between experimental infections and output of eggs in the faeces. Hong, Michel and Lancaster (1986) infected lambs with a single dose of *Ostertagia circumcincta* larvae and noted that a constant proportion of the worms was lost per unit time and that the size of the burden was approximately proportional to the rate of infection. The latter point was also

observed by Gibson and Everett (1978) who infected 4½ month old lambs with single infections of 50, 250 or 2,500 *O. circumcincta.*

Although single infections are easy to administer trickle infections mimic more closely natural conditions. This is important because faecal egg output has been shown to be affected by subsequent incoming numbers of larvae. When Hong, Michel and Lancaster (1987) infected lambs daily with either 250, 500 or a 1,000 *O. circumcincta* infective larvae for 140 days worm burdens were related to the infection rate and it was suggested that the number of adult worms lost in the faeces was related to the number present in the abomasum. It was noticed that in the trickle infections, the number of worms declined from a peak at 40 days which suggested that either fewer worms became established as time progressed or the rate of loss was rapid due to subsequent incoming larvae in the trickle infections. In contrast, when Jackson and Christie (1979) infected 4-month-old worm free lambs five times per week with 100, 320 or 4,000 *O. circumcincta* larvae for 20 weeks, they observed the mean FEC of the 3 groups to be similar.

In the following trial single and trickle infection methods were used to investigate how egg counts of individual lambs kept indoors and experimentally infected compared with those made when the same lambs were assessed at a younger age, earlier in field conditions. Such a trial was considered would have particular relevance to the development of selective breeding techniques in which the objective would be to avoid the irregularities associated with field infections.

4.2 Materials and Methods

Experimental Design

A 2 x 4 x 2 x 2 factorially designed experiment was carried out in year 2 using a sub-group of 64 of the lambs sampled in the field that year. The lambs were entire males (M) or females (F), were sired by 4 Cambridge rams, and had either high (H) or low (L) FEC in the field assessment. They were housed at 28 weeks of age and given Ivermectin. Two weeks later they were experimentally infected with either a single dose of 10,000 *O. circumcincta* infective larvae designated the single infection (S) treatment or 5 doses of 2,000 *O. circumcincta* larvae, at 2 day intervals over a 10 day period designated the trickle infection (T) treatment. A control group of 36 lambs of both sexes of similar breed was housed, and given Ivermectin at the same time but were not infected.

The lambs were separated into male and female groups and fed 300g of concentrate lamb pellets (BOCM Pauls) per day; hay also was available ad libitum. The lambs were weighed once weekly from 0 to 45 days post-infection. Faecal samples were taken twice weekly from 19 to 49 days post-infection, and FEC were recorded by the Modified McMaster technique as described previously. A blood sample was taken pre-infection and then on four occasions from 20 to 29 days post-infection in order to measure plasma pepsinogen concentrations.

The Infective Larvae

The infective larvae were obtained in the following way. Four naive donor lambs which had been experimentally reared and were worm-free, were each infected with 25,000 third stage larvae, obtained from the Moredun Research Institute. Eggs were found in the faeces of 3 of the lambs at day 19 post-infection and day 22 of the remaining lamb. Faeces were collected daily from day 19 from 3 lambs and from day 22 from the remaining lamb by placing the lambs in metabolism cages. The faeces were then mixed with peat and water until a crumbly consistency was obtained and placed in air-tight boxes in a room maintained at 27^{o}C for 7 days. The faeces were then placed in a Baermann apparatus which was filled with water for 24 hours and the larvae were subsequently collected in the water. The majority of faecal matter was removed from the larval suspension, by placing it on filter paper in a Buchner funnel. The filter paper containing some faecal matter and the larvae was dried and placed inversely in a Baermann apparatus filled with water. After 24 hours, the larvae migrated into the water from the filter paper containing the faecal matter. The clean water containing the larvae was then collected. In subsequent years this method of larval culture was replaced by another method obtained from the Moredun Research Institute, as described below.

TRICHOSTRONGYLE LARVAL CULTURES

(obtained from the Moredun Research Institute)

Materials :

Faeces from a monospecifically infected donor lamb

Culture trays (400 x 200 x 75 mm)

Polythene bags (500 x 300) 500 gauge

22°C incubator

1.00 mm stainless steel sieve

High wet strength paper (Cleanaroll Ltd.)

Filter holder (50 mm diameter 100 mm long)

Rubber band

250 ml beakers

250 ml culture flasks

100 ml and 10 ml volumetric flasks

200-1000 µl finnpipette and tips

Eel worm slide

Compound microscope with mechanical stage

Stereo microscope

Procedure :

1. The faeces were placed in the culture trays at a maximum depth of 30 mm and incubated inside a loosely sealed polythene bag, to enable some exchange of air, at 22°C for ten days.

2. Following incubation the sample was flooded with warm tapwater (22°C) and left to soak for 1 hour. Coarse faecal material was then separated from the fluid using the 1 mm sieve. Following a 2 hour sedimentation at 4°C the volume was reduced and the sample then cleaned of fine faecal debris by Baermannisation using the high wet strength paper. The sample was poured through the paper held on the filter holder by a rubber band, the paper was used to temporarily restrain the larvae which are inactive following the cold incubation. The filter holder was then immersed in warm tapwater (22°C), and the larvae migrated through the filter.

3. The clean larval suspension was then labelled and stored at 4°C until the larvae were required

4. The larvae were counted by re-suspending the culture taking a 1 ml subsample which was placed in a 10 ml volumetric flask. Three 1 ml subsamples were then taken and using a microscope slide and a stereo microscope the numbers of larvae per volumetric flask were then counted. The 1:10 dilution made represented the numbers of larvae per ml of original culture. The total number of larvae were counted and the concentrations adjusted for the required larval dose.

6. The dose was administered with a 10 ml syringe with a short (3 cm) plastic extension fitted over the nozzle.

Statistical Analysis

Statistics were performed using the computer package SAS as mentioned previously.

All Faecal Egg Count data was transformed to log form (log10 x+1) (unless otherwise stated). Presented means are therefore geometric means obtained by taking the antilog of the log form mean. Least square analysis and analysis of variance were performed on the log form data.

4.3 Results

Faecal Egg Counts (FEC)

Effect of method of administrating the infective dose

The trichostrongyle FEC for the single and trickle infection groups for each sampling date are shown in Figure 4.1. The pattern of egg output was different between the two groups. Lambs infected with a single dose had high initial FEC at approx. 19-25 days post-infection. Trickle infected lambs increased to a peak at 29 days post-infection. Following peak FEC, in both groups, there was a rapid decrease over the following 7-10 days but thereafter the decline was much more gradual and at 49 days post-infection, many lambs were still producing faeces with moderate counts. The FEC of single and trickle infected lambs at 42 and 49 days post-infection were not significantly different ($p < 0.1$).

A significant effect of infective dose was observed on overall mean individual lamb faecal egg production over the 21 day faecal sampling period ($p < 0.05$), with those lambs infected with a single dose of larvae having overall higher FEC than lambs infected with trickle doses. (Table 4.1). This difference was mainly because of the substantially higher counts in the early stages (19-21 days post-infection) of the lambs infected with a single dose and the relatively small and mainly non significantly different increased counts of the trickle infected lambs in the late stages. Lambs infected with a trickle dose displayed higher faecal egg output than those lambs infected with a single dose only at 38 days post-infection (Table 4.2 and Figure 4.2).

Table 4.1 : Effect of infective dose on faecal egg production

Dose	Mean	n	% S.D.	conf limits
Single	180	32	1.39	160-203
Trickle	146	32	1.52	125-169

Table 4.2 : Significance of differences of means of samples between the two different methods of administering the infective dose.

Days post-infection	19	21	23	27	30	33	38	42	49
Sig.	0.002.	0.01	n.s.	n.s.	n.s.	n.s.	0.05	n.s.	n.s.

In the experimental infections, variation in FEC was reduced compared with natural infections. The percentage standard deviations and 95% confidence limits were 1.39, 160-203 and 1.52, 125-169 for single and trickle infection groups compared with 2.43, 340-645 and 3.61, 200-519, respectively for the same lambs in field conditions. However, variation was still high, especially in the group infected with trickle infections, and a skewed distribution was seen as in natural infections with a high proportion of lambs having mean infection rates below 200 EPG (Figure 4.3).

Figure 4.1 : FEC of lambs infected with either a 10,000 single dose or a 5 x 2,000 trickle doses of *O. circumcincta.*(non logform)

Within figure numbers = Coefficient of Variation

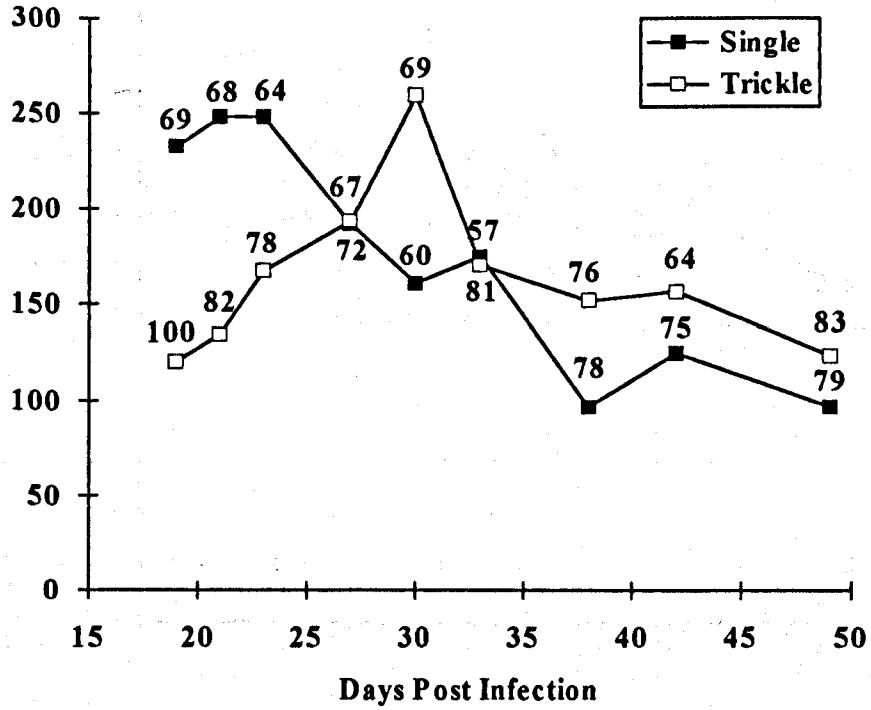


Figure 4.2 : FEC of lambs infected with either a 10,000 single dose or a 5 x 2,000 trickle doses of *O. circumcincta* (geometric means)

Within figure numbers = Percentage Standard Deviations

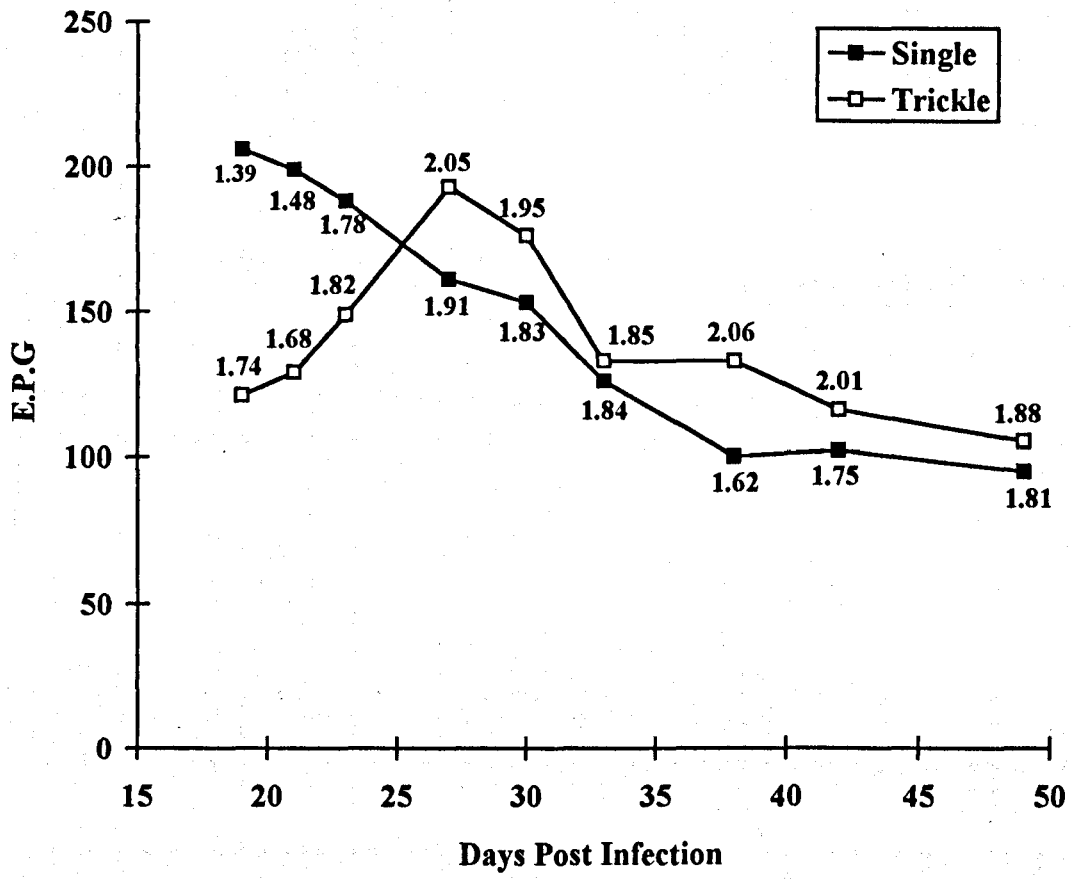
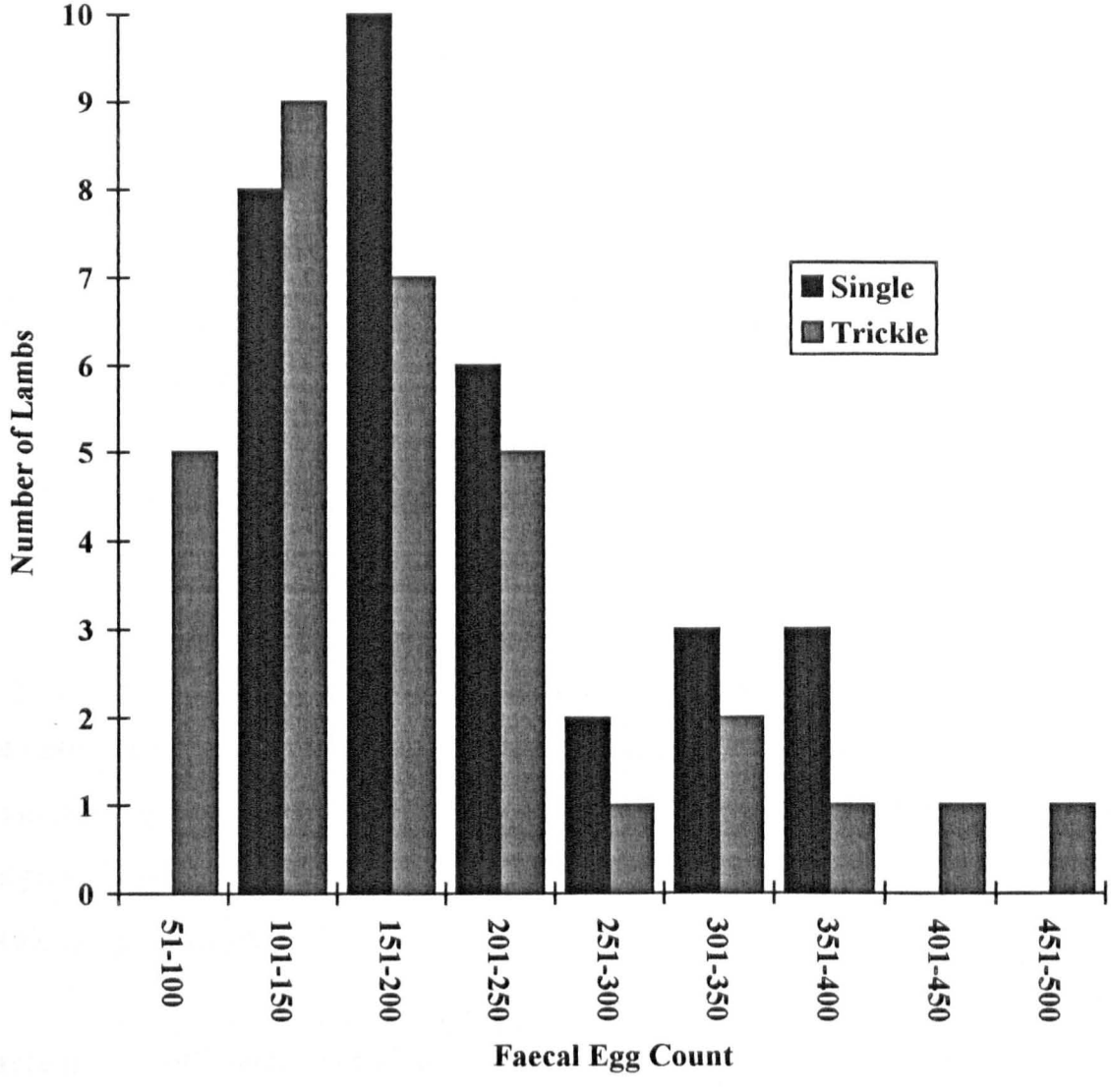


Fig 4.3 : Frequency distribution of mean FEC for lambs experimentally infected with single or trickle infections of *O. circumcincta* (Data not transformed to log form)



Correlation coefficients between individual samples and the overall mean were all significant ($p < 0.01$) except 27, 30, and 33 days post-infection for single infections. Only the penultimate faecal sample taken, i.e. 42 days post-infection was non significant for trickle infections (Table 4.3).

Table 4.3 : Correlations between samples taken at various stages post infection and overall mean FEC

	Days Post-infection								
	19	21	23	27	30	33	38	42	49
Single	0.77	0.83	0.74	0.23	0.001	0.21	0.58	0.38	0.42
Trickle	0.43	0.61	0.62	0.80	0.77	0.53	0.70	0.23	0.60

level of significance $p < 0.05$ $r = 0.31$

$p < 0.01$ $r = 0.43$

The highest correlations between a single faecal sample and the overall mean of samples taken from 19 to 49 days post-infection were similar in both groups but occurred at 19 to 23 days in the single infection group (S) and at 21 to 27 days post- infection in trickle infection group (T), corresponding in each case to peak egg production .

Correlation coefficients between consecutive faecal samples were all significant apart from 23&27 days post-infection in the S group. Correlations between samples were generally lower in the S group than in the T group. (Table 4.4).

Table 4.4 : Correlation coefficients between consecutive faecal samples

	Days Post-infection						
	19/21	21/23	23/27	27/30	30/33	33/38	38/42
Single	0.54	0.78	0.30	0.31	0.69	0.36	0.56
Trickle	0.70	0.76	0.72	0.81	0.80	0.58	0.74

level of significance $p < 0.05$ $r = 0.31$

$p < 0.01$ $r = 0.43$

Correlation coefficients between consecutive faecal samples and the overall mean were non significant between 27&30, 30&33 and 33&38 days post-infection for single infections, however, all correlation coefficients were significant and generally higher for trickle infections ($p < 0.01$) (Table 4.5).

Table 4.5 : Correlation coefficients between two consecutive faecal samples and the overall mean

	Days Post-infection							
	19/21	21/23	23/27	27/30	30/33	33/38	38/42	42/49
Single	0.58	0.57	0.56	0.08	-0.07	0.19	0.38	0.40
Trickle	0.60	0.62	0.53	0.60	0.74	0.82	0.53	0.43

level of significance $p < 0.05$ $r = 0.31$

$p < 0.01$ $r = 0.43$

The five highest correlations between 2 sample means and the overall mean are shown in Table 4.6. Correlation coefficients were only slightly higher for the 2 sample means than 1 sample figure with a maximum of 0.87 and 0.90 for single and trickle infections compared with 0.83 and 0.80 respectively.

Table 4.6 : The five highest correlations between 2 sample means and overall mean FEC

	1	2	3	4	5
Single	19 & 23 0.87	21 & 38 0.87	23 & 38 0.84	21 & 42 0.83	23 & 30 0.82
Trickle	21 & 27 0.90	23 & 30 0.89	30 & 38 0.87	27 & 33 0.86	19 &27 0.85

level of significance $p < 0.05$ $r = 0.31$

$p < 0.01$ $r = 0.43$

The correlations between mean egg counts for the first part of the evaluation from 19 - 30 and the second part from 33 - 49 days post-infection were $r = 0.42$ ($p < 0.001$) for single infections and $r = 0.55$ ($p < 0.001$) for trickle infections.

Effect of sex of lamb

There was no effect of lamb sex on faecal egg production of lambs infected with single or trickle doses of *O. circumcincta*. This was also true for the same group of animals during earlier natural infections (Table 4.7).

Table 4.7 : Effect of sex of lamb on faecal egg production

		Infection Type			
Lamb Sex		Single	Trickle	Mean	Natural
Male	mean	183	144	164	398
	% S.D.	1.36	1.66	1.53	3.34
	n	16	16	32	32
	conf limits	121-278	109-189	140-191	258-615
Female	mean	176	147	161	380
	% S.D.	1.43	1.41	1.43	2.81
	n	16	16	32	32
	conf limits	145-214	122-177	141-183	262-552
	% S.D.	1.033	1.52	1.48	3.03
Mean	mean	180	146	125	390
	conf limits	160-203	125-169	147-179	296-514

Effect of sire

There was no effect of sire group on faecal egg production however, this was true for the same group of animals during natural infections too. There was also no interaction between infective dose and sire effects and within group variation was similar (Table 4.8).

Table 4.8 : Effect of sire on faecal egg production

		Infection Type			
Sire		Single	Trickle	Mean	Natural
20	mean	194	126	157	468
	% S.D.	1.36	1.53	1.52	2.03
	n	8	8	16	16
	conf limits	149-253	87-182	125-196	320-684
22	mean	184	150	165	447
	% S.D.	1.43	1.71	1.58	2.99
	n	8	8	16	16
	conf limits	136-250	94-238	129-211	249-804
32	mean	182	139	160	316
	% S.D.	1.53	1.51	1.53	3.41
	n	8	8	16	16
	conf limits	126-262	98-198	128-201	164-611
147	mean	160	173	166	339
	% S.D.	1.28	1.27	1.27	3.94
	n	8	8	16	16
	conf limits	130-198	141-212	146-188	163-707
Mean	mean	180	146	125	390
	% S.D.	1.39	1.52	1.48	3.03
	conf limits	160-203	125-169	147-179	296-514

Effect of Previous Infection Level

The high and low susceptibility groups had significantly different FEC in field conditions ($p < 0.05$) but in experimental infection conditions differences were not significant for lambs infected with either single or trickle doses (Tables 4.9).

Correlations between mean previous natural infections and the mean of samples taken at 19 & 23 days for single infections and at 21 & 27 days for

trickle infections (these dates were the highest correlations with the overall mean) were 0.16 and 0.27 respectively and were non significant.

Correlations between samples taken at various stages post experimental infection and the natural infection FEC were almost all non significant (Table 4.10).

Correlations of the overall mean faecal sample, the mean of 19 - 30 days post-infection and the mean of 33 - 49 days post-infection with the natural infection FEC were all non significant (Table 4.11).

Table 4.9 : Effect of previous infection level on faecal egg production

		mean	n	% S.D	conf limits
Experimental	Single F L	177	16	1.33	155-205
Infection	Single F H	182	16	1.48	150-220
	Single Mean	180	32	1.39	155-213
Experimental	Trickle F L	135	16	1.62	106-171
Infection	Trickle F H	160	16	1.39	135-186
	Trickle Mean	146	32	1.52	118-177
Natural	Single F L	258	16	1.92	187-352
Infection	Single F H	977	16	1.66	762-1253
	Single Mean	468	32	2.43	301-726
Natural	Trickle F L	120	16	2.29	84-189
Infection	Trickle F H	995	16	1.74	763-1311
	Trickle Mean	324	32	3.61	172-608

F H Field High FEC F L Field Low FEC

Table 4.10 Correlations between samples taken at various stages post infection and the previous natural infection FEC

	Days Post-infection								
	19	21	23	27	30	33	38	42	49
Single	0.18	-0.06	0.12	-0.17	-0.01	0.57	0.15	-0.15	0.08
Trickle	0.04	-0.03	0.23	0.03	0.02	-0.06	-0.08	-0.11	-0.15

level of significance $p < 0.05$ $r = 0.31$

$p < 0.01$ $r = 0.43$

Table 4.11 Correlations of the overall mean faecal sample, the mean of 19 - 30 and the mean of 33 - 49 days post-infection with the natural infection FEC

	Overall mean	19 - 30 days post-infection	33 - 49 days post-infection
Single	0.05	0.18	- 0.06
Trickle	0.12	0.04	- 0.03

Plasma pepsinogen concentrations

There was a non-significant correlation between plasma pepsinogen concentrations and faecal egg production, probably due to the low infective dose causing negligible gut damage, producing extremely low plasma pepsinogen levels. Plasma pepsinogen concentrations reached a peak at 20 days post-infection for both trickle and single infected lambs. However, those lambs infected with a single dose of larvae displayed significantly higher concentrations than those lambs infected with the trickle dose at this time ($p < 0.05$) (Figure 4.4) but subsequent mean plasma pepsinogen concentrations for single and trickle infections were similar. Correlation coefficients were non significant between consecutive samples for trickle infected lambs, but correlations between mean samples and the overall mean were all significant (Table 4.12). Correlation coefficients of consecutive samples for lambs infected with a single dose of larvae were all significant (Table 4.13). There was no effect of sire group or lamb sex on plasma pepsinogen concentration of lambs (Table 4.14).

Fig. 4.4 : The mean plasma pepsinogen concentrations of lambs experimentally infected with either a single or trickle dose of *Ostertagia circumcincta*

Within figure numbers = Coefficient of Variation

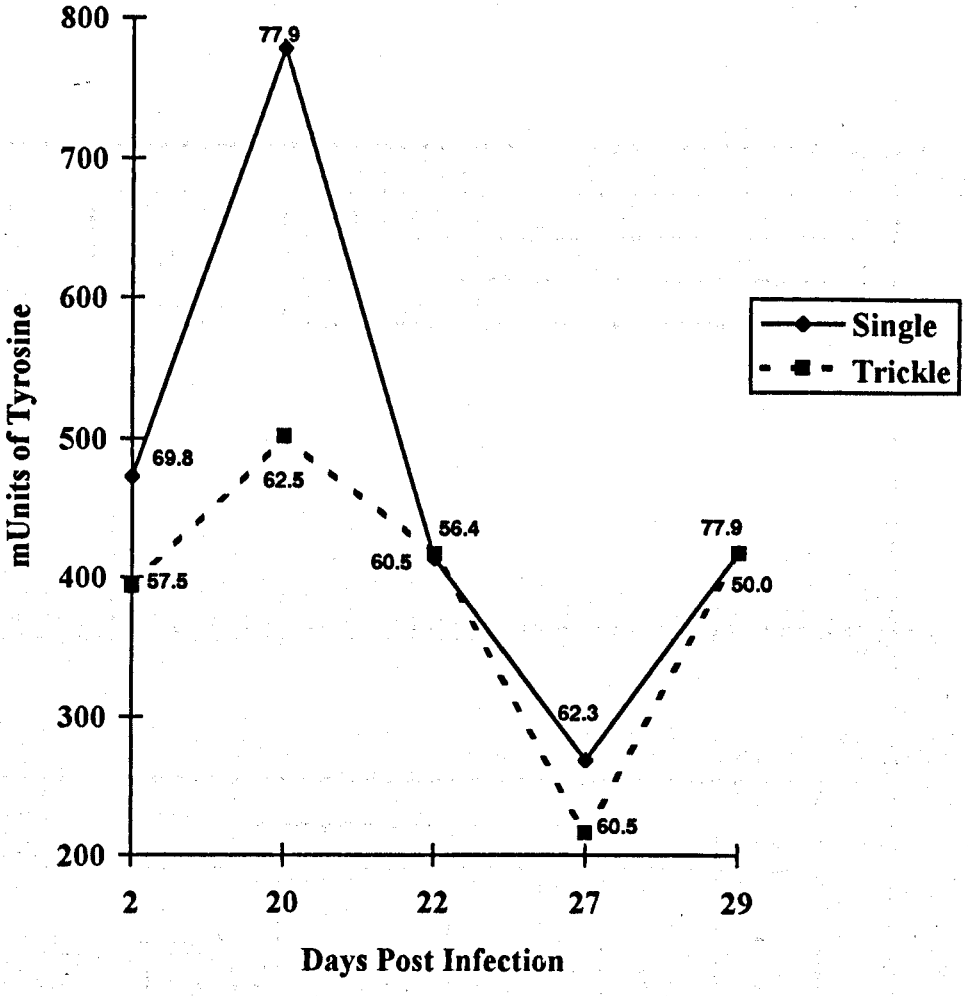


Table 4.12 : Correlations between plasma pepsinogen samples taken at various stages post-infection and overall mean sample

	Days Post-infection				
	2	20	22	27	29
Single	0.71	0.92	0.65	0.78	0.83
Trickle	0.66	0.47	0.46	0.37	0.38

Table 4.13 : Correlation coefficients between 2 consecutive plasma pepsinogen samples

	Days Post-infection			
	2 & 20	20 & 22	22 & 27	27 & 29
Single	0.39	0.39	0.43	0.49
Trickle	-0.06	-0.05	0.02	0.05

level of significance $p < 0.05$ $r = 0.31$

$p < 0.01$ $r = 0.43$

Table 4.14 : Effect of sire, sex and infective dose on plasma pepsinogen concentration

Infective Dose	Sex	Sire				Mean	s.e.
		20	22	32	47		
Single	M	502	484	343	350	419	39.8
	F	371	642	423	552	498	87.6
Trickle	M	346	517	498	355	418	25.4
	F	275	437	453	356	384	38.1
Mean		374	520	429	403		
s.e.		49.8	62.1	37.5	49.4		

Liveweight Gain

The mean weekly liveweight changes for infected and control lambs are shown in Figure 4.5. Weight increased over the experimental period for both groups of lambs, by 3.7 kg (males 4.2 kg, females 3.3 kg) for the infected lambs and 2.5 kg (males 3.1 kg, females 2.9 kg) for the control lambs but the difference between them was non significant

No effect of the administration method of the infective dose, was seen on liveweight change. The rate of growth decreased from 12 - 27 days post-infection for the lambs infected with a single dose and from 12 - 20 days post-infection for the lambs infected with trickle doses (Figure 4.6).

A significant effect of sire was observed on liveweight gain ($p < 0.0005$) progeny from rams 32 and 47 grew faster than the offspring of rams 20 and 22 (Figure 4.7). Males lambs also grew faster than females ($p < 0.05$) (Table 4.15).

Lambs selected as high egg producers from previous FEC in field conditions at the start of the trial had a mean liveweight of 28.0 kg compared with 31.2 kg for those selected as low faecal egg producers. On average the latter lambs gained slightly more weight (4.0 kg) over the experimental period than the H lambs (3.4 kg) but the difference was not significant (Figure 4.8). The difference between the liveweight gain of the H and L lambs during the 12-27 day post-infection plateau was significant ($p < 0.05$).

Figure. 4.5 : The growth pattern of infected and control lambs

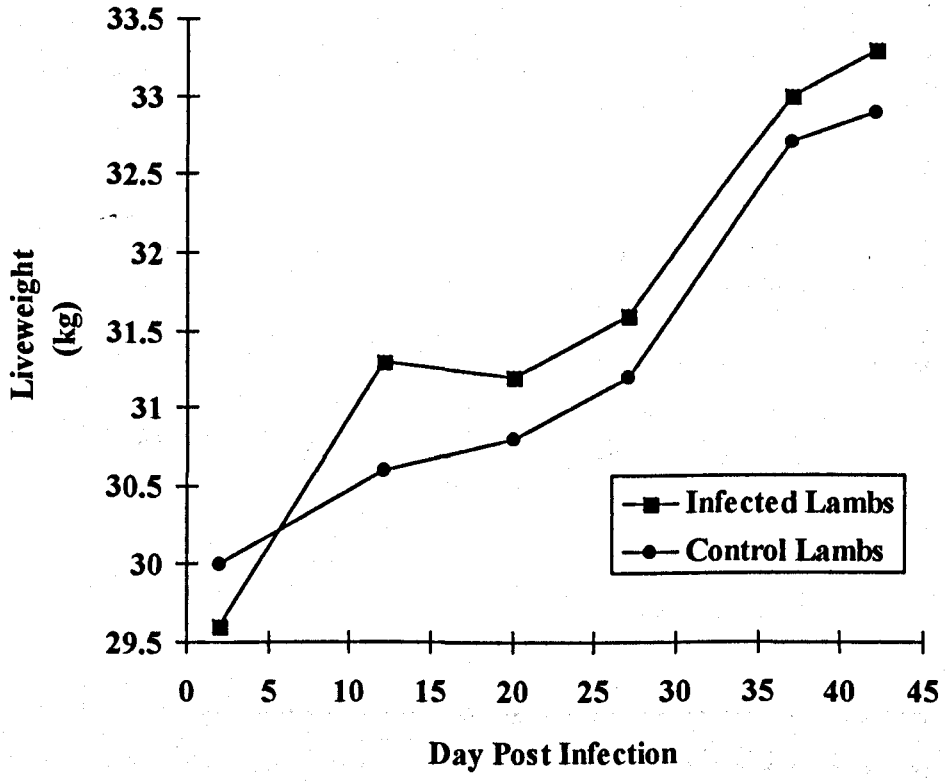


Figure. 4.6 : The growth pattern of lambs administered single and trickle infective doses

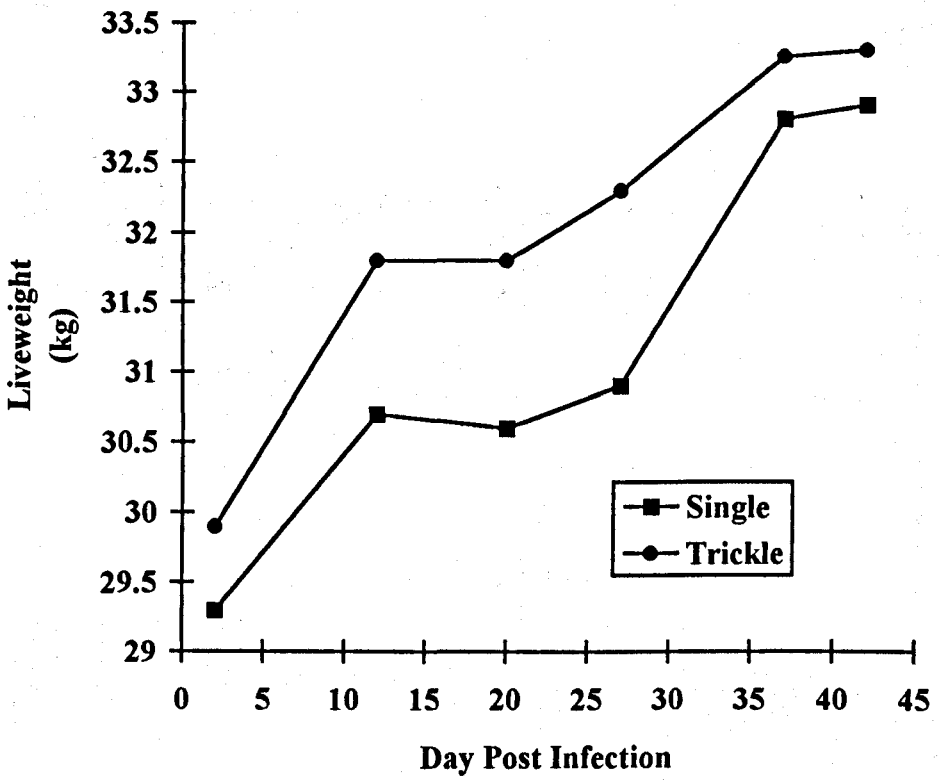


Figure. 4.7 : The growth pattern of lambs from different sire groups

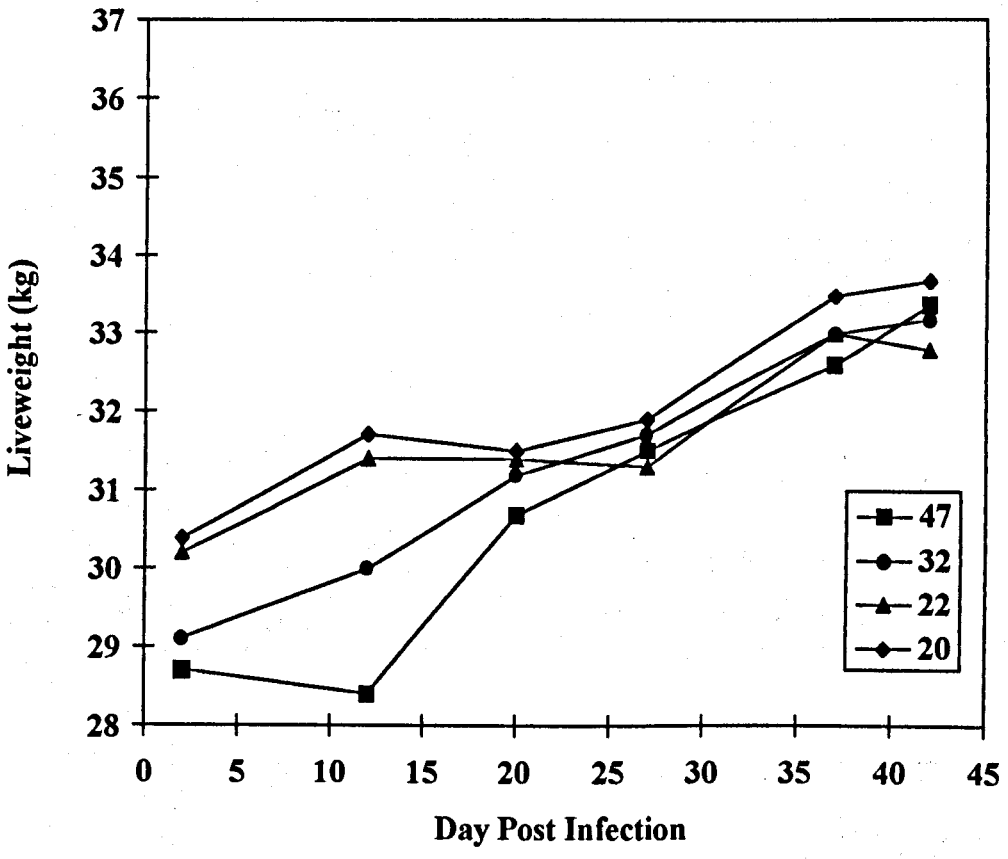


Figure. 4.8 : The growth pattern of lambs selected as low and high faecal egg producers in field conditions

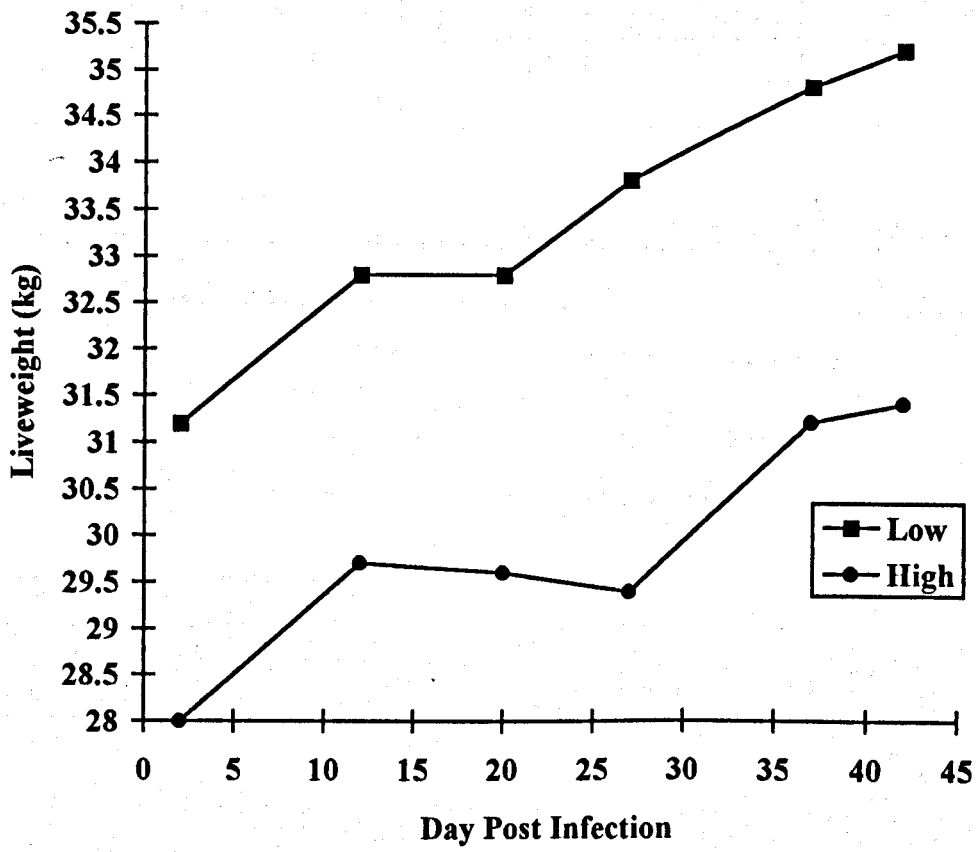


Table 4.15 : Effect of sire, sex and infective dose on live weight gain (kg) from 2 -42 days post-infection

Infective Dose	Sex	Sire				Mean	s.e.
		20	22	32	47		
Single	M	4.1	3.3	5.5	6.3	4.8	0.46
	F	2.5	2.1	2.8	3.8	2.7	0.40
Trickle	M	3.1	1.7	5.1	3.8	3.5	0.57
	F	3.6	2.5	4.5	5.4	3.9	0.39
Mean		3.3	2.5	4.4	5.0		
s.e.		0.38	0.41	0.49	0.43		

No correlation was observed between FEC, plasma pepsinogen concentration or live weight change (Table 4.16).

Table 4.16 : Correlation coefficients between FEC, plasma pepsinogen (PP) concentration and live weight gain (LWG)

	FEC	PP
LWG	r = -0.14	r = -0.10
PP	r = 0.05	.

level of significance $p < 0.05$ $r = 0.25$

$p < 0.01$ $r = 0.33$

4.3 Discussion

Experimental Infection rates produced relatively low FEC compared with field conditions. Nevertheless in this study the pattern of faecal egg output observed for both single and trickle infected lambs demonstrated the accepted 18-21 day prepatent period of the lifecycle of *O. circumcincta.* Lambs infected with a single larval dose displayed maximum mean FEC at 19 days post-infection the first day of sampling and lambs infected with a trickle dose over a 10 day period had mean FEC peaking at 27 days post- first infection, or 17 days post-the final infective dose. The pattern of trickle infection followed that of the single after the last infective dose was administered. The sharp decline in FEC following peak infection and the slower decline thereafter of both groups has been previously described by Michel (1963); Anderson and Michel (1977); Coadwell and Ward (1981), and Barger (1985).

The extent to which individual lambs displayed varying patterns of egg production is illustrated in Figures 4.9 and 4.10. Some lambs had high or low FEC throughout the trial whilst others an initial peak in FEC followed by a rapid decline.

Figure 4.9 : Widely different patterns of FEC of two lambs infected with a 10,000 single dose of *O. circumcincta*

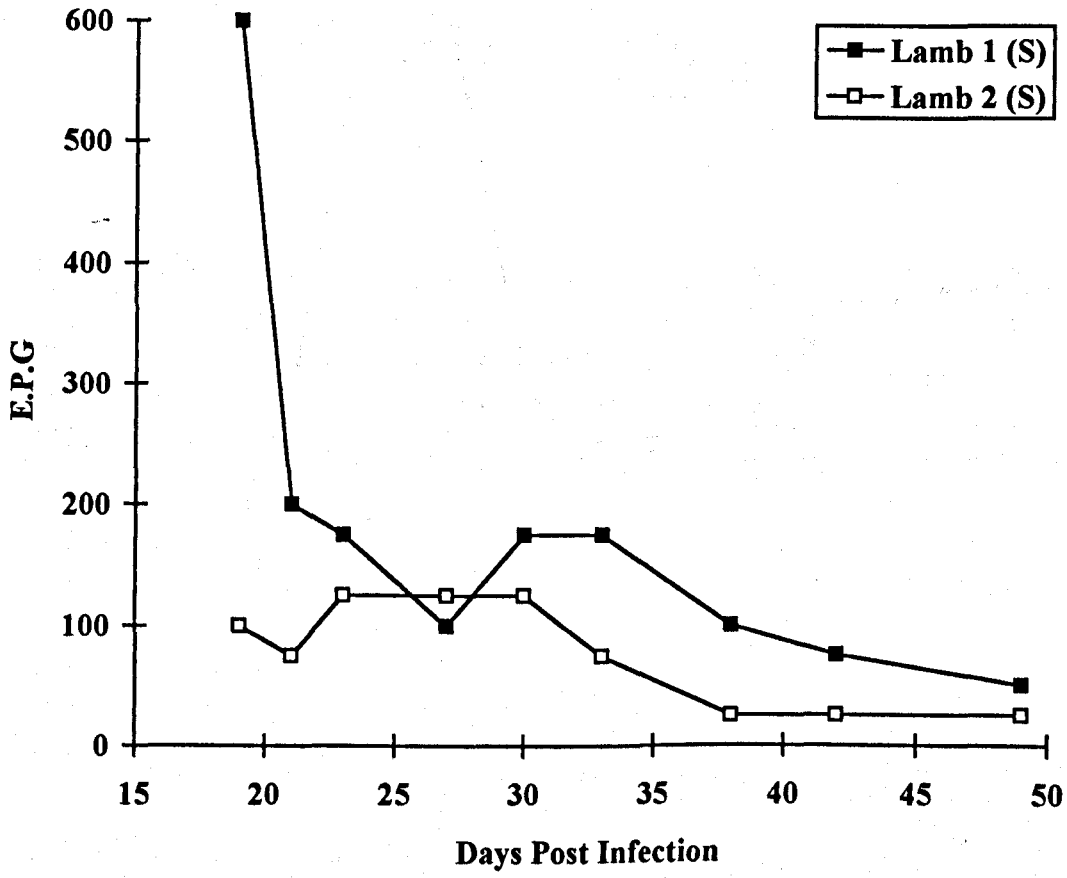
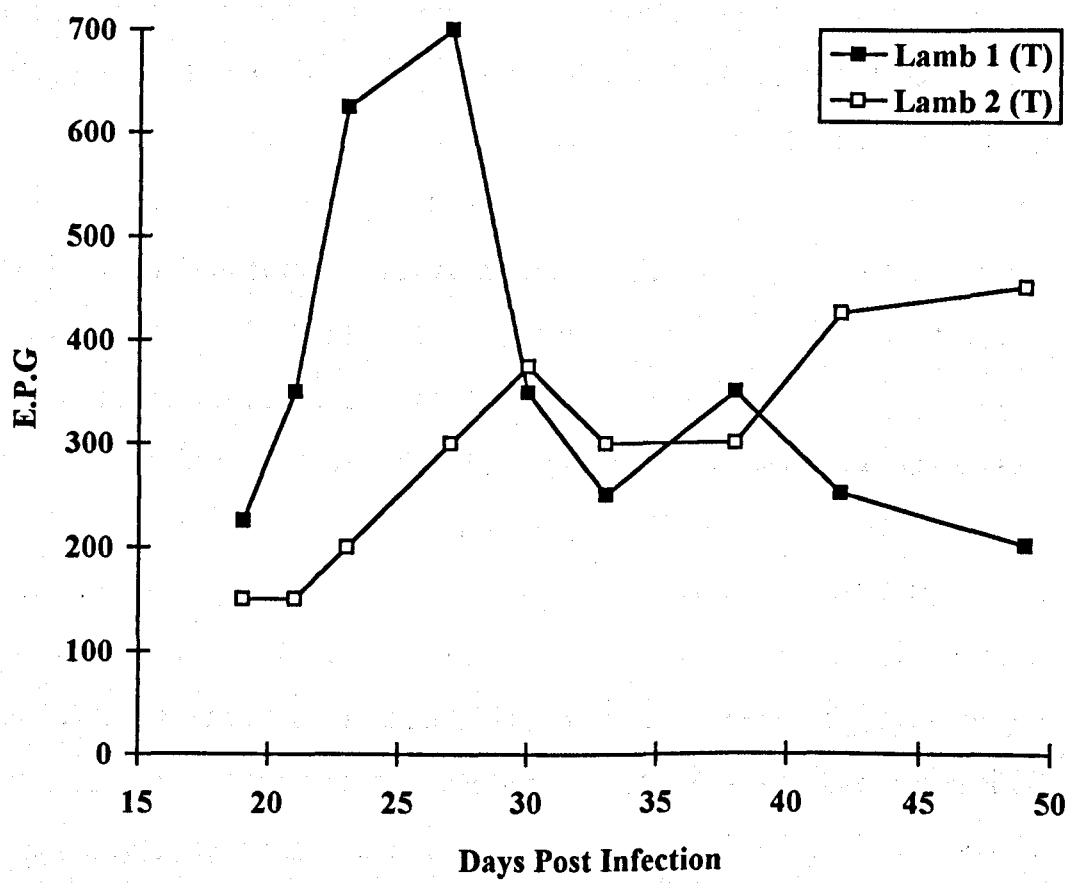


Figure 4.10 : Widely different patterns of FEC of two lambs infected with 5x2,000 trickle doses of *O. circumcincta*



In selection procedures, it would be easier to select individuals with high or low susceptibility when FEC of these would be at a maximum. In the case of the examples, this was in the pre- 30 day period. However, in the trickle infection examples, the two lambs could be definitely ranked depending on time of sampling. It has also been suggested by Hong, Michel and Lancaster (1987) that those animals which appear to be resistant by exhibiting very high initial worm burdens followed by a sudden decline exhibit a simultaneous loss of worm numbers and a decrease in worm fecundity. In the former case, lambs which had low counts throughout may be considered resistant. Low counts may be due to faecal output or fewer mature females and mature females being less prolific. Michel (1963) has stated that when worms develop in a resistant host they are less prolific and/or a reduced number of mature females develop. In conclusion it has to be argued that animals could be classified high or low responders depending on a number of genetically controlled mechanisms. Michel (1969) suggested that the varying manifestations of host resistance might be the effect of different underlying mechanisms. Wakelin (1982) suggested that speed of worm expulsion is related to speed of T lymphocyte cell response and is due to the genetic control of phenotype being exercised more strongly at the level of the bone marrow precursors of the inflammatory cells needed for the intestinal responses that remove worms from the host.

The correlation coefficients between consecutive samples were generally significant with maximum values of 0.78 (S) 21 and 23 days post-infection and 0.81 (T) 27 and 30 days post-infection. The lambs subjected to the trickle infection generally had more uniform relative FEC scores than those given the single dose. The need to take more than one sample to measure FEC would appear to be least valuable at the time of peak production. However, r^2 would have values of 0.61 (S) and 0.66 (T) indicating that a considerable amount of

variation in one sample appears to be independent of the influence of the other.

Correlations between single samples and the overall mean were highest at 21 (S) and 27 (T) days post-infection and were all significant for trickle infections and nearly all for single infections apart from 27, 30 and 33 days post-infection. The latter correlations between these individual samples and the overall mean may have been very low because the lambs which demonstrated peak egg production at the beginning of the trial (19 days post-infection) displayed a decline in FEC at 27 days presumably due to nematode age resulting in a decrease in egg production and adult worm expulsion. Barger (1988) suggested that adult female *O circumcincta* produce less eggs as they start to die from 20 days post-infection. Those lambs which displayed high FEC at 27-33 days probably were infected with inhibited larvae earlier in the infection and therefore demonstrated delayed egg production or the worms were older when egg production began to occur (Barger, 1988). At 38 days post-infection when correlation coefficients seemed to increase slightly, FEC were declining for all lambs.

There were no benefits of taking two consecutive samples as indicated by the slight reduction in correlation coefficients between the mean of 2 consecutive samples and the overall mean which were highest at 33/38 (r =0.80) and 21/23 (r =0.78) for trickle and single doses respectively. However, higher correlations with the overall mean were obtained when samples were taken a few days apart 21 & 27 (r =0.90) and 19 & 23 (r =0.87) for trickle and single infections respectively. In both trickle and single infections the increase in correlation coefficients was small but nevertheless 2 samples assessment taken a few days apart may be useful in the particular circumstances of animal

breeding where the requirement is to identify individuals which are high or low responders as accurately as possible.

The significant correlation coefficients between the mean FEC of samples taken between 19 - 30 days and those taken 33 - 49 days in both infection methods indicate that generally some long-term common response occurred over the whole period of investigation. Nevertheless, the r^2 estimates of 0.18 and 0.30 for single and trickle groups indicate that much of the within-group variation post 30 days was not dependent on the individual previous estimates and a sample taken pre- and post-30 days would give increased accuracy. Previous reports have shown somewhat more significant relationships between egg counts taken at different points in time. Woolaston, Windon and Gray (1991) described repeatability estimates of FEC between 28 and 35 days of an experimental infection with *Haemonchus contortus* to be 0.60± 0.01 and Dineen and Windon (1980) showed the repeatability of fortnightly egg counts between weeks 3 and 11 of an experimental infection of *Trichostrongylus colubriformis* to be 0.67± 0.01.

There was no effect of sex of lamb on FEC as both male and female lambs had similar FEC. However, this observation was also true for the same group of lambs in field conditions when many lambs were still at the prepubescent stage. In accordance with this observation many authors, (Woolaston, Barger and Piper 1990; Knight and Vegors, 1972; Albers, Gray, Piper, Barker, Le Jambre and Barger, 1987) have noticed no difference in mean FEC due to lamb sex in the pre-puberty period. Many other authors, (Dobson, 1964; Yazwinski, Goode, Moncul, Morgan and Linnerud, 1981; and Courtney, Parker and Herd, 1985a) have however, observed higher FEC in males as lambs become sexually mature.

The absence of an effect of sire on FEC is in agreement with natural infection figures for the 4 Cambridge sires and indicates that, in this case, although there was an overall reduction of phenotypic variability by reducing the effects of some of the common environmental factors, this did not result in progeny groups becoming significantly different from each other.

The correlations between FEC occurring in natural field conditions and those of the experimental infections were non significant for both single and trickle doses, except for the one instance at 33 days post-infection for lambs infected with a single larval dose. The absence of a significant correlation could be due to a number of factors; Behnke, (1987) stated that adult *Ostertagia* worms have a short lifespan under field conditions, surviving only up to 26 days, however, in experimental infections, especially single infections they can survive in excess of 100 days post-infection. The faecal egg output patterns and hence individual FEC taken on a limited number of occasions could be different in field and experimental conditions. Another possibility is that the lambs which displayed high FEC in field conditions could have had their immune systems sufficiently stimulated to limit subsequent infections (Thomas and Boag, 1972). A third alternative is that because in experimentally infected lambs the infection levels and FEC were low, it was not possible to distinguish between low and medium egg-producing animals (Barger, 1985; Wakelin and Blackwell, 1988). Probably the most plausible reason for the FEC of natural and experimental infections not correlating significantly is nutritional. The lambs when experimentally infected were housed indoors and fed 300g of a high protein concentrate (18% crude protein) diet plus hay whereas when they were naturally infected, pastures were of poor/moderate nutritional quality and concentrates were not fed. In Chapter 5, lambs fed a basal concentrate diet and described as high egg producers in field conditions

had significantly higher FEC than those lambs described as low faecal egg producers in natural conditions. Such a difference was not seen in lambs fed a supplemented diet. Several workers including van Houtert, Barger & Steel, (1992), Kambara, McFarlane, Abell, McAnulty and Sykes, (1993) Blackburn, Rocha, Figueiredo, Vieira, Cavalcante and Rosa (1991) and Wallace, Duncan, Fishwick, Gill, Holmes, McKellar, Parkins, Murray, and Stear (1993) have shown that feeding a high level of concentrates in the diet and thereby increasing intakes of both energy and protein decreases the production of nematode eggs in the faeces. Thus when using both natural and experimental infections in selection programmes, a number of factors need to be considered.

In the experimental infections, variation in FEC was reduced compared with natural infections but it was still high. The 95% confidence intervals were ±22 (% S.D. =1.39) and ±22 (% S.D. =1.52) for single and trickle infection groups compared with ±153 (% S.D. =2.43) and ±159 (% S.D. =3.61) respectively for experimental and natural infections. This reduction in variation could be due to the controlled time and frequency of infection, the low infective dose and the equal larval intake between low, medium and high egg-producing animals. In experimental infections, maximum mean individual lamb FEC were 350 and 378 for single and trickle infections respectively compared with means of 2138 and 3550 for high susceptibility lambs in the field. Eady and Woolaston (1992) stated that measurements carried out in controlled experimental infection may underestimate what occurs under natural field conditions where there will be further incoming larvae. Gamble and Zajac (1992) observed less variation in FEC of both St. Croix and Dorset lambs in experimental compared with natural infections.

Further work is required to determine the optimum nutritional environment and infection rates which would be necessary to identify resistant and non-resistant animals.

The plasma pepsinogen concentrations reached only 700 which were much lower than the 1000-3000 Units stated by Urquhart, Armour and Duncan (1987) to be usual in animals infected with *Ostertagia circumcincta* and suggests minimal gut damage. Also Soulsby (1986) suggested that young animals which have not experienced infection have concentrations less than 1000 Units. The lack of a significant correlation between FEC and plasma pepsinogen concentration may also have been due to the low infective dose administered resulting in minimal gut damage. Plasma pepsinogen concentrations could not be considered as an alternative to taking faecal samples of lambs to select high and low faecal egg-producing animals.

Plasma pepsinogen concentrations reached a peak at 20 days post-infection for both trickle and single infected lambs but a peak in concentrations may have occurred earlier, if samples were taken between 2 - 20 days post-infection. This pattern of plasma pepsinogen concentration was similar to that described by Smith, Jackson, Graham, Jackson, and Williams (1987) who observed an increase in lymph plasma pepsinogen concentrations of 10 - 14 month old Suffolk lambs 20 days after infection with a trickle dose of 2000 *O. circumcincta* larvae per day. Yadav (1987) however, witnessed a peak plasma pepsinogen concentration at 14 days post-infection with a single infection of 20,000 larvae.

The somewhat lower mean plasma pepsinogen concentrations of lambs infected with the trickle dose compared with those infected with a single dose, was non significant. However, a difference was seen at 20 days post-infection when the highest figures were recorded. The plasma pepsinogen concentrations were greater for single than trickle as may be expected because of the delayed build up of larvae in the trickle infection.

Correlations between consecutive plasma pepsinogen samples were all significant for single infections ($p < 0.05$). Correlations between consecutive trickle infections were however, all non-significant, probably due to the delayed build up of larvae in the trickle infection.

The absence of an effect of sire or sex of lamb on plasma pepsinogen concentration is in accordance with the observations recorded for FEC.

The mean liveweight gain was not related to FEC or plasma pepsinogen concentrations, probably due to the fact that a relatively small infective dose was given. There was no significant difference between liveweight gains for both control and infected lambs in the present study.

A temporary plateau in the growth curve of infected lambs was observed at 12-27 days post-infection for lambs infected with a single dose of larvae. This period post-infection coincides with the two phases of pathological changes occurring in animals infected with *O. circumcincta.* In the first stage, up to 17 days after infection, lesions are produced by the developing larvae in the gastric glands and functional cells are replaced by undifferentiated cells. Major morphological and functional changes are seen in the second phase, 17 - 35 days post-infection, and are associated with the emergence of adult parasites

from the gastric glands. These morphological changes in the abomasum result in a reduction in appetite and a temporary decrease in the efficiency of the abomasum at these times (Soulsby, 1986). The temporary plateau in weight gain was observed from 12 to only 20 days post-infection for lambs infected with trickle doses probably due to the lower FEC witnessed.

CHAPTER 5

INFLUENCE OF DIETARY CONCENTRATE LEVEL ON NEMATODE INFECTION RATES IN LAMBS INFECTED WITH A SINGLE DOSE OF 25,000 *OSTERTAGIA CIRCUMCINCTA*

5.1 Introduction

Several workers including Blackburn, Rocha, Figueiredo, Vieira, Cavalcante and Rosa (1991) have shown that feeding a high level of concentrates in the diet and thereby increasing intakes of both energy and protein decreased the production of nematode eggs in the faeces. Wallace, Duncan, Fishwick, Gill, Holmes, McKellar, Parkins, Murray, and Stear, (1993) found that when lambs were artificially infected with 10,000 *Ostertagia circumcincta* larvae three times a week for 13 weeks, those given a low protein diet containing 89g crude protein per kilo (DM) had lower plasma albumin concentrations and higher FEC than lambs fed a supplemented diet having a concentration of 163g crude protein per kilo. In a further experiment, lambs infected with 200 *Haemonchus contortus* larvae three times a week for 10 weeks on a basal diet showed lower PCV's, total plasma proteins, plasma albumins and plasma fructosamimes, but however, not higher FEC or worm burdens than those on a supplemented diet. Wallace *et al* (1993) suggested that the lambs on the supplemented diet infected with *H. contortus* displayed increased resilience but not increased resistance as measured by the high worm burdens and FEC. Kambara, McFarlane, Abell, McAnulty and Sykes (1993) noticed that young lambs aged 8-26 weeks were more susceptible to parasite challenge, i.e. they displayed higher FEC (FEC) when fed a lower protein diet. Lambs supplemented with fishmeal showed improved resistance to *Trichostrongylus colubriformis* (van Houtert, Barger & Steel, 1992), and lambs supplemented with meat and bone meal and soyabean meal also showed improved resistance to *Trichostrongylus colubriformis* (Kambara, McFarlane, Abell, McAnulty and Sykes, 1993).

This trial was conducted in order to investigate the relationship between level of nutrition and nematode infection rate. Effect on lamb mean infection rate and variation and pattern of infection were investigated observing factors such as FEC, plasma pepsinogens, eosinophil counts and liveweight gain.

5.2 Materials and Methods

Experimental Design

Twenty-three 10 month old lambs, (it would have been interesting to use younger animals however, these lambs were the only animals available at the time) 14 with low counts and 9 with high counts were selected from the previous artificially infected population of 64 lambs on the basis of FEC. Lambs were dosed with Ivermectin and 2 weeks later placed on either a high or low level of concentrate feeding and artificially infected with a single dose of 20,000 *O. circumcincta* larvae. The lambs were divided into groups of 2 to 4 animals (1 group consisted of only one animal because the other member of the group died at the start of the experiment) according to sex, male (M) or female (F), previous response to infection, high (H) or low (L) resistance, level of concentrate feeding high (H) or low (L) in a 2x2x2 randomised block experimental design. Each lamb in the H group was fed 900g/day and in the L group 450g/day of a concentrate lamb pellet diet, containing 160g/kg crude protein (DM) (18% C.P, M.E= 10.8 MJ/kg) (BOCM Pauls). Lambs were also each fed 300g/day sugar beet pulp (C.P =10.0%, M.E= 12.0 MJ/kg). Hay was available ad libitum (C.P =4.8%, M.E= 8.8 MJ/kg) and hay residues were recorded daily. The concentrates were fed twice daily at 09.00h and 16.30h.

The lambs were faecal sampled twice weekly from 14 to 47 days post-infection and on three occasions (22, 30 and 37 days post-infection) blood samples were taken and plasma pepsinogen concentrations and eosinophil counts determined on day 22. Lambs were weighed weekly from 1 to 43 days post-infection. At 33 and 43 days post-infection 6 (3 high diet, 3 low diet, 5 males and one female) and 8 (4 high diet, 4 low diet, 4 males and 4 females)

lambs respectively were slaughtered and their abomasums retained for necroscopy. At necroscopy the pH of abomasal contents was measured using a glass electrode (Corning 107). Lambs were slaughtered at 07.00h.

Parasitological Techniques

FEC were recorded using the Modified McMaster techniques as described previously.

Adult and immature nematodes were recovered from the abomasal contents and the abomasum digested in order to recover immature nematodes. The number of worms and the number of eggs in each female worm were counted and any morphological differences noted. The method used was adapted from 'Manual of Veterinary Parasitological Laboratory Techniques (1986) and is outlined below.

Technique for counting nematodes of the alimentary tract

1. The abomasum was ligatured and removed from animal and placed in a bowl.
2. The abomasum was then cut open over the bowl in which all the contents were caught. The stomach wall was washed thoroughly under a stream of water from a tap and the mucous membrane was rubbed carefully in order to remove any worms adhering to it.

3. The contents of the bowl were then poured through a wire mesh screen with an aperture of 0.038 mm and washed with a stream of water from a rubber tube attached to the tap until no more coloured matter passed through.
4. The screen was the inverted and placed over a bowl and by using a stream of water the worms were washed into it.
5. The wall of the abomasum was digested in pepsin and HCl to extract immature worms from it Eight grams of pepsin, 20 ml of HCl and 8.5g NaCl were added to 1000 ml of water in order to digest 500 g of tissue.
6. The abomasum was placed in a large glass bowl containing the protein digesting fluid and incubated at 37° C overnight.
7. The digested material was then poured through 2 mesh screens of apertures of 0.075 mm (upper screen) and 0.038 mm, and washed with very hot water and the recovered material placed in the bowl with the nematodes recovered from the gut contents (step 4) .
8. The contents of the bowl were made up to 6 litres by the addition of water and stirred vigorously and two 30 ml samples were removed for examination.
9. The total number of worms counted in the two samples was multiplied by 100 to give the number of worms present in the abomasum.

Haematological Techniques

Plasma pepsinogen concentrations and eosinophil counts were performed as described previously in Chapter 4.

Statistical Analysis

Statistics were undertaken by the use of the computer package Statistical Analysis System (SAS). Data of FEC because of its skewed distribution was transformed into log form (log 10 x+1). The means presented for FEC are geometric means unless otherwise stated. The variability of each mean figure is described by its percentage standard deviation obtained by taking the antilog of the log form standard deviation, and by 95% confidence limits of the mean.

5.3 Results

Faecal Egg Counts (FEC)

The mean FEC of the lambs on the two different diets at each sampling date post-infection are shown in Figure. 5.1. Both groups of lambs showed FEC to be present at 16 days. Lambs on the low concentrate diet (L) had significantly higher FEC, ($p < 0.01$) especially up to 30 days post-infection.

When comparisons were made using the mean FEC of all samples taken for each lamb there was a significant effect of diet ($p < 0.05$). Egg counts in faecal samples 3 (21 days post-infection) and 5 (28 days post-infection) differed significantly due to diet ($p < 0.05$) (Table 5.2 and Figure 5.1.)

Although males had higher counts than females especially at certain times the difference between sexes was not significant and there were too few animals to compare. Egg counts in faecal samples 4 (24 days post-infection $p < 0.01$) and 9 (24 days post-infection $p < 0.05$) differed significantly due to sex of lamb (Table 5.3 and Figure 5.2.).

Although individual and overall figures for the groups which had been identified in field conditions as having high (H) or (L) FEC were not significantly different a pattern of production clearly shows a trend of H producing higher FEC.(Table 5.4 and Figure 5.3.). There was no interaction effect of diet with sex or diet with previous infection response.

Table 5.1 The effect of previous field challenge, sex and diet concentrate quantity on FEC

	Field FEC	Low Conc. Diet (750g/day)			High Conc. Diet (1200g/day)			All Lambs		
		n	Mean	%SD	n	Mean	%SD	Mean	%SD	conf limits
Males	High	2	598	1.19	2	128	2.02	275	2.67	33-2279
	Low	4	203	1.22	4	233	1.38	218	1.30	174-273
Females	High	4	340	1.18	1	150	.	288	1.48	165-506
	Low	2	130	2.92	4	141	2.07	138	2.09	61-313
Mean			268	1.86		166	1.74			
conf limits		181-399			114-242					

Table 5.2 : Significance of differences of means of samples between lambs on the two different diets

Days post-infection	16	18	21	24	28	31	33	37	40
Sig.	n.s.	n.s.	0.05.	n.s.	0.05.	n.s.	n.s.	n.s.	n.s.
Means L diet	366	120	240	295	363	145	195	148	20
Means H diet	166	35	20	148	58	56	144	170	33

Table 5.3 : Significance of differences of means of samples between both sexes of lambs

Days post-infection	16	18	21	24	28	31	33	37	40
Sig.	n.s.	n.s.	n.s.	0.05	n.s.	n.s.	n.s.	n.s.	0.05
Males	305	56	79	351	134	83	135	339	119
Females	198	79	67	122	172	103	65	74	6

Table 5.4 : Significance of differences of means of samples between high (H) or low (L) FEC made previously in field conditions

Days post-infection	16	18	21	24	28	31	33	37	40
Sig.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
H	409	126	117	354	224	123	282	251	49
L	182	44	54	152	117	76	47	126	19

Figure 5.1 : Mean FEC for the lambs on the two different diets

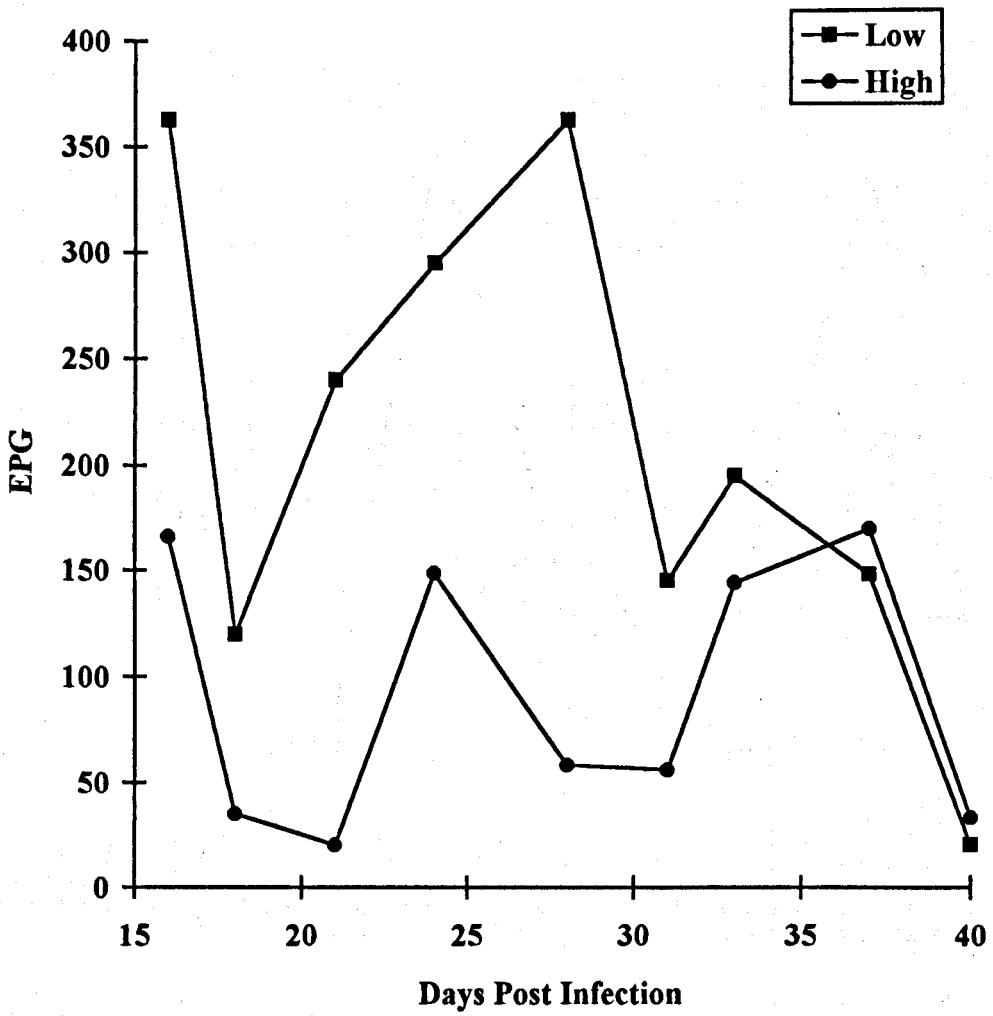


Figure 5.2 : Mean FEC for both sexes of lambs

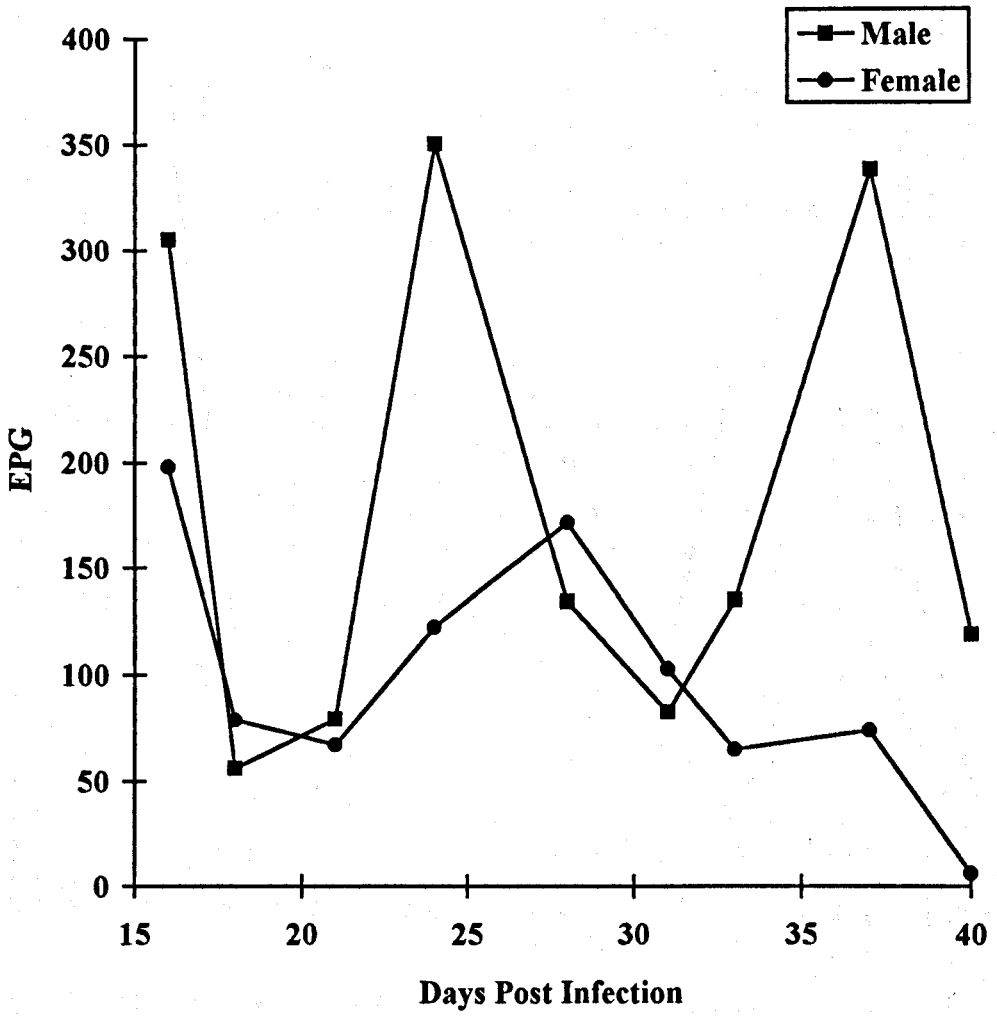
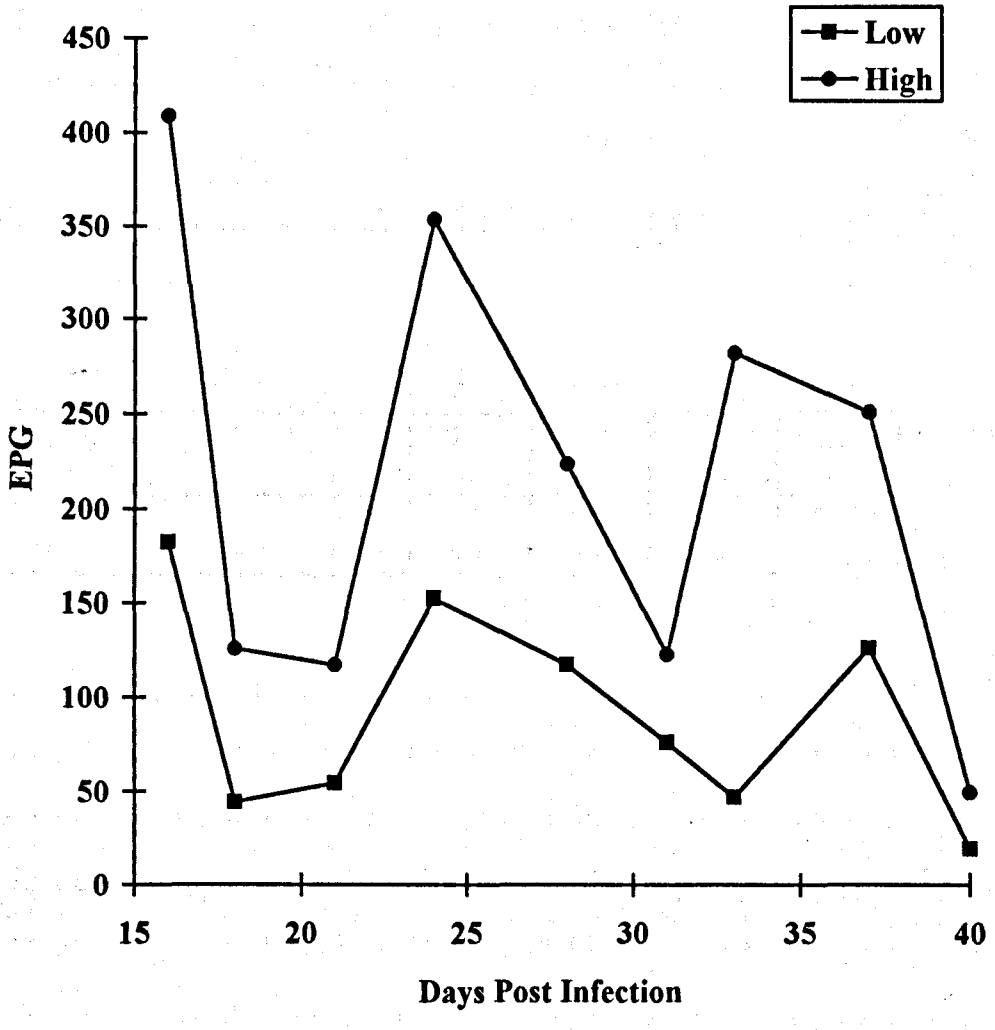


Figure 5.3 : Mean FEC for the lambs labelled high (H) or low (L) depending on FEC made previously in field conditions



Correlation coefficients for consecutive samples for the 2 groups of lambs are shown in Table 5.5. Lambs on a low diet had significant correlations between 1 & 2 and 4 & 5 weeks post-infection ($p < 0.01$) but correlations were non significant for those lambs on a high concentrate diet.

Table 5.5 : Correlation coefficients for consecutive faecal samples for lambs on the 2 different diets

Days	16/18	18/21	21/24	24/28	28/31	31/33	33/37
Low Diet	0.89 p<0.01	0.26 n.s.	0.56 n.s.	0.81 p<0.01	0.40 n.s.	0.66 n.s.	0.27 n.s.
High Diet	0.46 n.s.	0.23 n.s.	-0.01 n.s.	0.16 n.s.	0.52 n.s.	0.14 n.s.	0.62 n.s.

Correlation coefficients for samples and the overall mean are shown in Table 5.6. Coefficients were significant at 28 days post-infection ($p < 0.01$) and 21 33 and 37 days post-infection ($p < 0.05$) for those lambs on a high diet. Lambs on a low concentrate diet had significant coefficients at 16, 18, 31 and 40 days post-infection ($p < 0.01$) and 24, 15, 28 and 38 days ($p < 0.05$).

Table 5.6 : Correlation coefficients between faecal samples and the overall mean

Days	16	18	21	24	28	31	33	37	40
Low Diet	0.88 p<0.01	0.84 p<0.01	0.65 n.s.	0.76 p<0.05	0.71 p<0.05	0.84 p<0.01	0.71 p<0.05	0.75 p<0.05	0.82 p<0.01
High Diet	0.44 n.s.	0.09 n.s.	0.73 p<0.05	0.54 n.s.	0.81 p<0.01	0.57 n.s.	0.68 p<0.05	0.68 p<0.05	0.53 n.s.

Plasma pepsinogen concentrations

Mean plasma pepsinogen concentrations for lambs on the different diets were mostly low (less than 1000 Units of tyrosine) and are shown in Figure 5.4. The differences in plasma pepsinogen concentration between the means of the high and low diet lambs at each of the 3 sampling times were not statistically significant. The effect of, sex of lamb and high or low previous FEC in field conditions on mean plasma pepsinogen concentrations were also all non significant (Table 5.7, 5.8, 5.9 & 5.10).

Table 5.7 : The effect of previous field challenge, sex and diet concentrate quantity on mean plasma pepsinogen concentration

	Field FEC	Low Conc. Diet (750g/day)			High Conc. Diet (1200g/day)			All Lambs	
		n	Mean	s.e.	n	Mean	s.e.	Mean	s.e.
Males	High	1	1575	-	1	976	-	1276	302.6
	Low	4	578	250.6	4	690	97.5	634	125.2
Females	High	4	632	41.2	1	611	-	626	29.5
	Low	1	747	-	4	909	160.7	869	120.7
Mean			744	146.1		798	79.1		

Table 5.8 : The effect of previous field challenge, sex and diet concentrate quantity on the plasma pepsinogen concentration at 22 days post infection

	Field FEC	Low Conc. Diet (750 g/day)			High Conc. Diet (1200 g/day)			All Lambs	
		n	Mean	s.e.	n	Mean	s.e.	Mean	s.e.
Males	High	2	1112	-	2	952	48.7	1032	238.0
	Low	4	568	116.2	4	820	125.9	712	135.5
Females	High	4	862	176.5	1	56	569.9	700	211.4
	Low	2	744	145.9	4	848	280.5	813	85.4
Mean			806	132.0		785	93.7		

Table 5.9 :. The effect of previous field challenge, sex of lamb and diet concentrate quantity on the plasma pepsinogen concentration at 30 days post-infection

	Field FEC	Low Conc. Diet (750 g/day)			High Conc. Diet (1200 g/day)			All Lambs	
		n	Mean	s.e.	n	Mean	s.e.	Mean	s.e.
Males	High	2	1522	150.4	2	782	173.7	1152	233.3
	Low	4	619	155.2	4	773	242.9	696	136.6
Females	High	4	740	163.6	1	1012	-	795	222.5
	Low	2	964	276.6	4	802	296.4	856	203.5
Mean			807	130.6		867	121.9		

Figure 5.4 : Mean plasma pepsinogen concentrations for the lambs on the two different diets

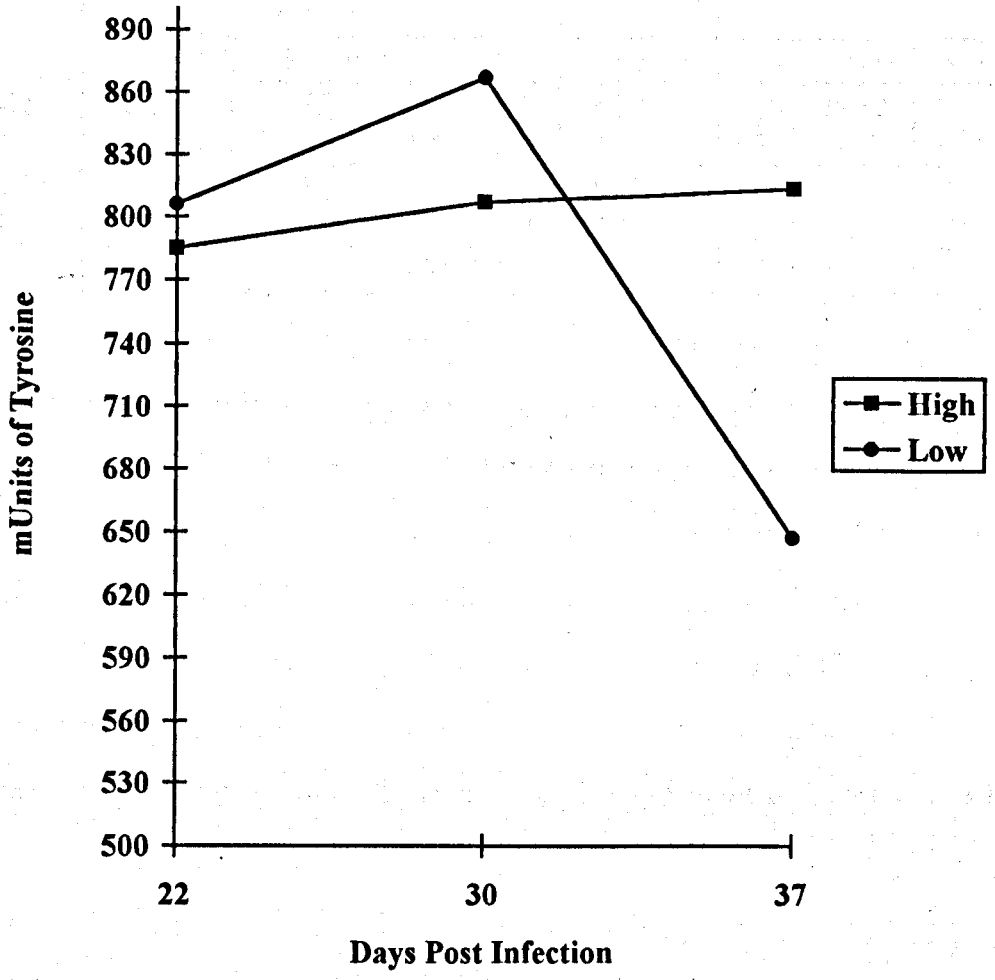


Table 5.10 : The effect of previous field challenge, sex of lamb and diet concentrate quantity on the plasma pepsinogen concentration at 37 days post-infection

	Field FEC	Low Conc. Diet (750 g/day)			High Conc. Diet (1200 g/day)			All Lambs	
		n	Mean	s.e.	n	Mean	s.e.	Mean	s.e.
Males	High	1	767	-	1	972	-	1172	199.8
	Low	4	879	96.9	4	709	37.3	622	147.4
Females	High	4	425	245.6	1	1372	-	511	105.9
	Low	1	956	-	4	556	268.3	898	71.2
Mean			647	154.2		813	49.6		

Correlation coefficients between consecutive plasma pepsinogen concentrations for lambs on a high concentrate diet were non significant, however, coefficients for those lambs on a low concentrate diet were all significant, as seen in Table 5.11. Correlation coefficients between samples and the overall mean were significant for only sample 2 (30 days post-infection) for those lambs on the high concentrate diet. Correlation coefficients between samples and the overall mean were all significant for lambs on a low concentrate diet, as shown in Table 5.12.

Table 5.11 : Correlation coefficients for consecutive plasma pepsinogen samples for lambs on the 2 different diets

Days	22 & 30	30 & 37
Low Diet	0.55 n.s.	0.78 $p<0.02$
High Diet	0.24 n.s.	0.21 n.s.

Table 5.12 : Correlation coefficients between plasma pepsinogen samples and the overall mean

Days	22	30	37
Low Diet	0.86 p<0.01	0.96 p<0.001	0.89 p<0.005
High Diet	0.66 n.s.	0.82 p<0.05	0.56 n.s.

Eosinophil counts

Eosinophil counts performed 22 days post-infection ranged from 0 - 20 thousands/ml. The association between eosinophil counts and FEC was significant ($p < 0.01$) with the two parameters correlating negatively ($r = -0.74$). However, eosinophil counts were not correlated with plasma pepsinogen concentrations. Eosinophil counts were also not affected by diet type (Table 5.13).

Table 5.13 : The effect of previous field challenge, sex and diet concentrate quantity on eosinophil count

	Field FEC	Low Conc. Diet (750g/day)			High Conc. Diet (1200g/day)			All Lambs	
		n	Mean	s.e.	n	Mean	s.e.	Mean	s.e.
Males	High	2	7.0	.	2	7.0	4.04	3.5	2.60
	Low	4	10.3	5.07	4	4.5	1.66	5.5	1.02
Females	High	4	1.0	0.58	1	0.0	0.0	2.2	1.28
	Low	2	7.0	4.04	4	6.5	1.19	9.2	3.44
Mean			3.7	1.16		7.3	1.92		

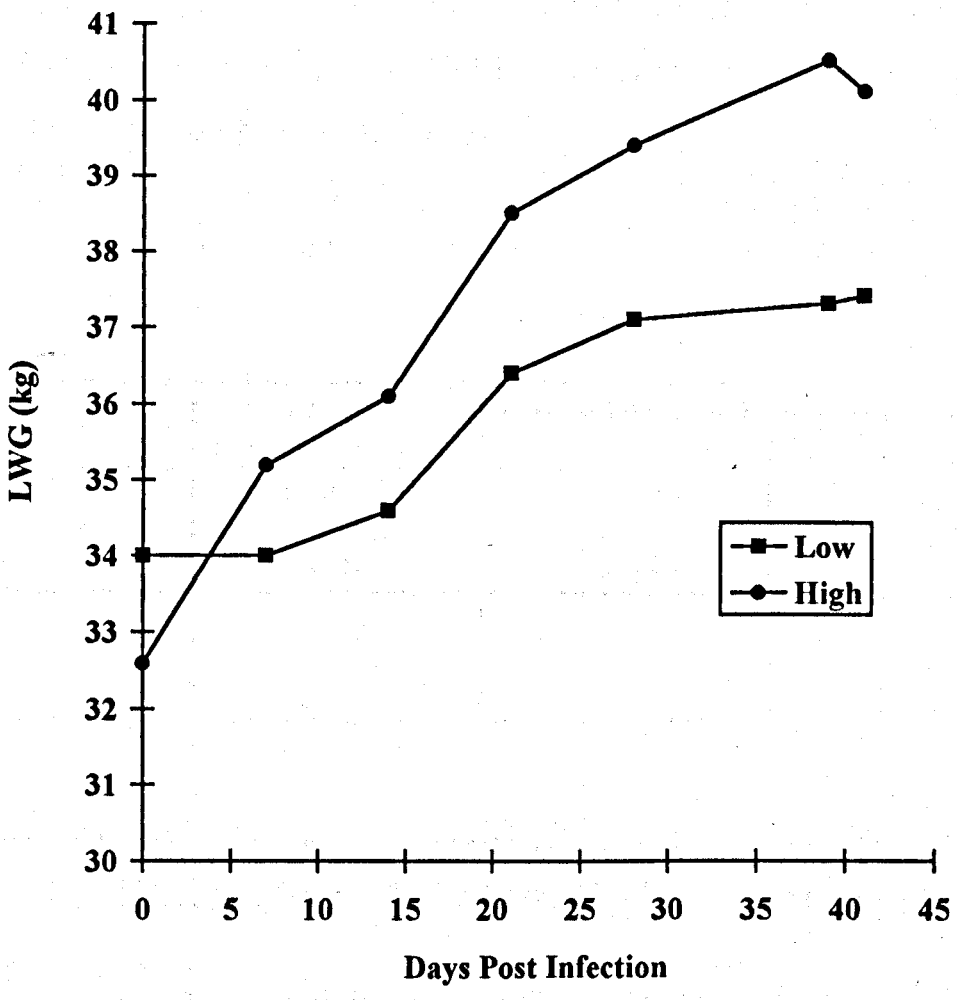
Liveweight gain

The mean liveweight gains for both groups of lambs are shown in Figure 5.5. The lambs fed on a high concentrate diet gained significantly more during the 41 day period than those on a low concentrate diet ($p < 0.001$). Sex of lamb and previous FEC in the field had no effect on live weight gain and the interaction between high and low field FEC and diet was also non significant. (Table 5.14).

Table 5.14 : The effect of previous field challenge, sex and diet concentrate quantity on live weight gain

	Field FEC	Low Conc. Diet (750 g/day)			High Conc. Diet (1200 g/day)			All Lambs	
		n	Mean	s.e.	n	Mean	s.e.	Mean	s.e.
Males	High	2	2.3	0.79	2	9.2	1.85	5.8	0.80
	Low	4	4.7	0.68	4	7.1	1.25	5.9	2.12
Females	High	4	2.8	1.80	1	7.0	.	5.0	0.89
	Low	2	3.9	0.86	4	7.2	0.80	4.9	1.63
Mean			3.5	0.58		7.5	0.61		

Figure 5.5 : Mean liveweight gains for the lambs on the two different diets



A significant correlation (r = 0.76) was seen between mean FEC and mean plasma pepsinogen concentrations ($p < 0.05$) for lambs in the group fed a low concentrate diet however, no correlation was observed for lambs on a high concentrate diet. Live weight gains and plasma pepsinogen concentrations did not correlate significantly for lambs fed both types of diet nor did liveweight gain and FEC (Tables 5.15, 5.16 & 5.17).

Table 5.15 : The correlation coefficients between mean FEC, plasma pepsinogen concentration and live weight gain

	Plasma Pepsinogen Conc.	Live Weight Gain
FEC	r = 0.57 p <0.05 n=16	r = -0.41 n.s. n=23
Live Weight Gain	r = 0.05 n.s. n=16	.

Table 5.16 : The relationship between FEC, plasma pepsinogen concentration and live weight gain for lambs on a low concentrate diet

	Plasma Pepsinogen Conc.	Live Weight Gain
FEC	r = 0.76 p <0.05 n=8	r = -0.35 n.s n=12
Live Weight Gain	r = -0.33 n.s. n=8	-

Table 5.17 : The relationship between FEC, plasma pepsinogen concentration and live weight gain for lambs on a high concentrate diet

	Plasma Pepsinogen Conc.	Live Weight Gain
FEC	r = -0.09 n.s. n=8	r = 0.30 n.s. n=11
Live Weight Gain	r = 0.16 n.s. n=8	.

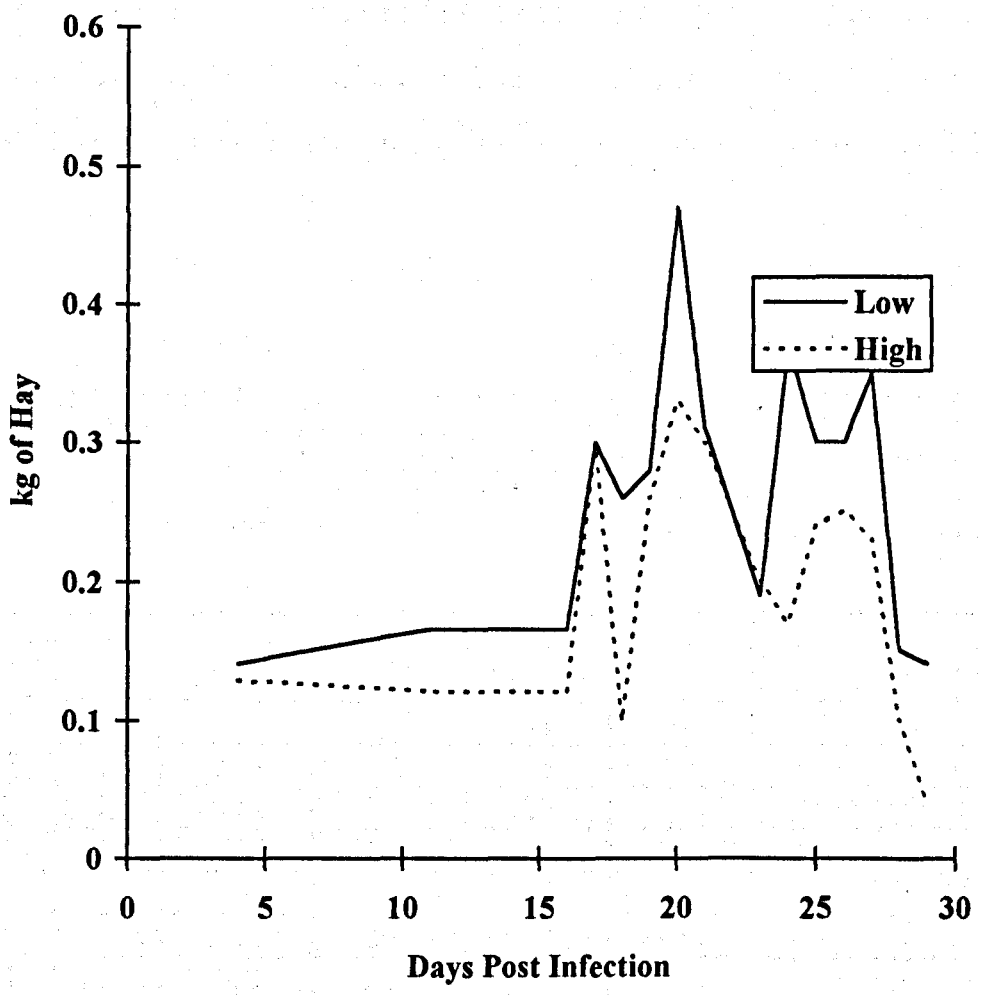
Hay intake

Daily hay intakes from 4 days post-infection are shown in Figure 5.6. Hay intakes reached a peak at 20 days post-infection for the lambs on both the low and high concentrate diets. Lambs on the low concentrate diet had significantly higher ($p < 0.01$) hay intakes than those on the high concentrate diet. Male lambs consumed significantly more hay than female lambs ($p < 0.001$). No effect of previous field challenge was seen on daily hay consumption (Table 5.18).

Table 5.18 The effect of previous field challenge, sex of lamb and diet concentrate quantity on mean hay intake (kg/d)

	Field FEC	Low Conc. Diet (750 g/day)		High Conc. Diet (1200 g/day)		All Lambs	
		Mean	s.e.	Mean	s.e.	Mean	s.e.
Males	High	0.48	0.045	0.36	0.005	0.42	0.038
	Low	0.45	0.004	0.35	0.007	0.40	0.018
Females	High	0.34	0.035	0.24	-	0.30	0.023
	Low	0.32	0.020	0.30	0.033	0.31	0.024
Mean		0.39	0.022	0.33	0.017		

Figure 5.6 : Mean daily hay intake for the lambs on the two different concentrate diets



Post mortem worm burdens

The numbers of *O. circumcincta* recovered at necropsy are shown in Table 5.19. The numbers of adult *O. circumcincta* recovered at necropsy were significantly related to the mean FEC of each lamb (r = 0.64) but negatively correlated with the number of arrested larvae found (r = -0.51). The number of arrested larvae found correlated significantly with the number of larvae found in the gut lumen (r = 0.47).

Diet of lamb had no significant effect on post mortem worm burdens.

Table 5.19 : Adult *O. circumcincta* worms (including mean eggs per female worm) and larvae recovered at necropsy from 14 lambs sent to slaughter

Lamb	Diet	Adult Worms	Mean FEC	Eggs/ Female	Larvae	Arrested Larvae	Male : Female
254	L	800	225	31	0	0	1 : 1
304	L	200	61	21	200	200	0 : 2
32	H	800	339	27	0	200	1 : 3
174	L	1600	167	3	0	0	2 : 1
81	H	200	211	42	0	800	0 : 2
82	H	1200	261	22	100	0	1 : 5
274	L	600	267	24	400	800	1 : 1
16	H	200	150	33	0	200	0 : 2
125	L	1600	278	33	100	250	1 : 2
262	L	2400	428	35	100	0	1 : 1
328	H	400	50	9	0	100	0 : 4
341	H	1200	157	7	0	400	1 : 1
345	H	1600	78	21	0	0	1 : 3
350	L	2400	528	15	0	0	1 : 5

Abomasal pH

The abomasal pHs of the lambs slaughtered at 33 and 43 days post-infection are presented in Table 5.20. Abomasal pHs were all high and not significantly different and did not change from subgroup to subgroup and were unaffected by diet. A significant correlation ($p < 0.05$) was observed between FEC and pH, however, a non significant correlation was observed between pH and worm numbers.

Table 5.20 : Abomasal pH of lambs post-mortem

Lamb	Diet	pH	FEC	Days
262	L	5.0	428	33
125	L	4.8	278	33
345	H	4.8	78	33
32	H	4.9	339	40
16	H	4.6	150	40
304	L	4.5	61	40
82	H	4.8	261	40

5.4 Discussion

The results of the present study show that lambs fed the low concentrate diet had significantly higher FEC than those fed the high concentrate diet i.e. mean = 268, compared with mean = 166. There was some evidence that this was particularly noticeable in the individuals identified as having low resistance and high FEC in field conditions. In the present study it was not possible to distinguish between differences in energy and protein intakes. M.E. values of the concentrate diet of 10.8 MJ/kg and crude protein content of 18% support the findings of Bown, Poppi and Sykes (1986) who showed that consumption of extra protein reduced FEC. Blackburn *et al* (1991) suggested that the extra protein suppressed egg-laying in mature female worms. Abbott and Holmes (1990) however, suggested that high FEC in lambs on a low concentrate diet is due to a reduced faecal output due to a reduced feed intake. In the present trial, total dry matter intake, hay and concentrates were greater for the lambs on the high diet so that faecal output may have been greater but extra concentrates would have made the diet more digestible and so reduced faecal output.

There was no effect of sex of lamb on FEC as both male and female lambs had similar FEC. This observation was also true for the same group of lambs in field conditions and in the previous artificial infection as mentioned in the preceding chapter. This observation contrasts with findings of many authors, (Dobson, 1964; Yazwinski, Goode, Moncul, Morgan and Linnerud, 1981; and Courtney, Parker and Herd, 1985a) who observed higher FEC in males as lambs become sexually mature. In the present trial, lambs were 6 and 3 months older than the lambs in the preceding chapters and at 8-9 months old were sexually mature so differences in FEC due to sex of lamb should have been

observed. The males consumed more hay than the females in the present trial which may have resulted in higher faecal output and a reduction of the proportion of eggs in the faeces. Also in natural infections on pasture the increased appetite of male animals leads to an increase in intake of larvae, thus contributing to increased infection rates (Herd, Queen and Majewski, 1992). In addition, only small sample sizes were used in the present trial.

Most plasma pepsinogen concentrations were less than the 1000-3000 Units stated by Urquhart, Armour and Duncan (1987) to be usual in animals infected with *Ostertagia circumcincta* and suggest minimal gut damage. However, a significant correlation was observed between FEC and plasma pepsinogen concentration. However, no effect of diet was seen on mean plasma pepsinogen concentration and in fact at 37 days post-infection, lambs on the low concentrate diet had significantly lower plasma pepsinogen concentrations than those on the high diet. The absence of a significant effect of diet on plasma pepsinogen concentration is in agreement with the points mentioned above by Blackburn *et al* (1991) and Abbott *et al* (1990) who suggested that decreased FEC in lambs fed supplemented diets is not due to factors affecting the larval stage of the parasite or the resilience of the lambs.

The absence of an effect of sex of lamb on plasma pepsinogen concentration is in accordance with the observations recorded for FEC.

The significant negative correlation which was observed between eosinophil counts and mean FEC ($p < 0.01$) is in agreement with other authors (Blackburn *et al* 1992; Buddle, Jowett, Green, Dough and Risdon, 1992; and Windon Gray and Woolaston, 1993). Circulating eosinophils are recognised as indicators of resistance (Kimambo, MacRae and Dewey, 1988b; MacRae,

1993: Windon, *et al,* 1993). Buddle *et al* (1992) observed that the relationship between elevated eosinophil numbers and resistance was greater for some sire groups compared with others. T helper cells are said to be responsible for eosinophilia via IgE activation of serosal mast cells and the effector responses observed in genetically resistance lambs (Gill, Gray and Watson, 1991; Windon, 1991b). However, Rothwell, Windon, Horsburgh and Anderson (1993) observed no differences in eosinophil numbers in lambs bred for resistance or susceptibility, although following vaccination and challenge, resistant lambs had increased numbers of circulating eosinophils. The absence of the effect of diet on eosinophil counts during ostertagiasis is in agreement with Wallace *et al* (1993) and may be due to the reduction in concentrate quantity not having a detrimental effect on T helper cell activity.

The liveweight of lambs from both diet groups increased at a slow, but steady, pace. The lambs fed the high concentrate diet had significantly greater liveweight gains than those fed the low concentrate diet but this was more the result of increased dietary intakes of energy and protein rather than a sign of parasitism.

Mean daily hay intakes reached a peak at 20 days post-infection for lambs fed a low concentrate diet and a high concentrate diet. This observation is in agreement with findings by Wallace *et al* (1993), who noticed an increase in refusals of diet from 6-12 days post-infection followed by a decrease in refusals at 20 days post-infection when 10 month old Scottish Blackface lambs were infected with a trickle infection of 10,000 *O. circumcincta* larvae three times a week. Many authors (Soulsby, 1986; Barker, 1973; Sykes, 1995) have described anorexia as a clinical sign of ostertagiasis. The higher hay intakes of

the lambs fed the low concentrate diet indicates their ability to compensate for the low concentrate allowance by consuming more hay. Even so, ME and CP intakes were higher in the lambs fed the higher concentrate diet. This observation is in disagreement with Wallace *et al* (1993), who noticed more refusals of diet in infected lambs fed a basal diet than those on the supplemented diet. The authors concluded that this difference was due to the lambs on the supplementary diet being able to withstand infection better thus their appetites were less severely affected by the challenge.

Post-mortem adult worm burdens were significantly correlated to terminal FEC ($r = 0.64$) in agreement with Roberts and Swan (1981) who observed a high correlation coefficient of 0.83 between adult *Haemonchus contortus* and terminal FEC. Thus the fluctuating faecal egg output observed may be due to fluctuating egg production by the female nematodes, a phenomenon which is known to occur in laboratory model animals (Croll and Matthews, 1977). The small number of abomasal pH values measured showed similar values of 4.5-5.0. However, these values were considerably higher than the quoted figures of pH 2-3 (McKellar, Duncan, Armour, Lindsay and McWilliam, 1986). Despite the small number, a significant correlation was observed between pH and FEC ($p < 0.05$) and there was some suggestion of a correlation with abomasal adult worm burdens. Many authors have associated elevated pH with ostertagiasis (Yadav, 1987, Soulsby, 1986) due to the damage to hydrochloric acid-producing parietal cells by adult worms emerging out of the gastric mucosa.

CHAPTER 6

COMPARISON OF CONSECUTIVE INFECTIONS

1. The effect of sensitising infection on FEC following a subsequent infection.

2. Comparison of experimental infection levels at 12-13 months of age compared with natural field infections at 4-5 months of age.

6.1 Introduction

Genetic differences in FEC of lambs have been well documented. There is increasing evidence that differences in FEC are very much more obvious after the immune system has been triggered by an initial large nematode infection. (Eady and Woolaston, 1992), i.e. adaptive immunity rather than innate resistance. Eady and Woolaston (1992) observed no genetic differences in terms of FEC in 6-8 month old lambs which had not previously been exposed to parasitic infection but after an initial exposure in which a dose of infective larvae was administered, and the infection allowed to progress for 4 weeks before removal by drenching, differences in FEC were observed. However, the response has been found to differ from breed to breed. Altaif and Dargie (1978) observed that both four month old Scottish Blackface and Finn Dorset lambs when infected with a primary dose of *Haemonchus contortus* had FEC although those of the Finn Dorset lambs were higher when the animals were given a secondary infection whereas the Scottish Blackface were almost totally immune, the Finn Dorset lambs continued to have high FEC.

The aims of the first trial were to investigate if variation in response to a primary infection of *Ostertagia circumcincta* was apparent and if these differences correlated with those seen after a secondary challenge infection.

It is important to determine the long-term consequences of selection and whether response of young lambs is correlated with that in older lambs and adults (Windon, Gray and Woolaston, 1993). Selection for resistant lines of sheep should therefore demonstrate this trait throughout the animal's life.

The aims of the second trial were to investigate how FEC of 12 - 13 month old ram lambs following an experimental infection of *Ostertagia circumcincta* compared with egg counts taken during their first grazing season when they were 16-20 weeks old.

6.2 Materials and Methods

Trial 1

Experimental design

Seventeen parasite naive, experimentally reared lambs were experimentally infected with a single dose of 10,000 *Ostertagia circumcincta* larvae when 9 weeks old and with a single dose of 20,000 larvae when 13½ weeks of age. The lambs were weighed weekly from day one of the trial.

The lambs had been kept indoors from birth, experimentally reared on a milk substitute (Milkivit Lamb, BP nutrition) and weaned at 5 weeks of age. Post-weaning, the lambs were fed ad libitum a concentrate diet of lamb pellets (BOCM Pauls) and hay.

Parasitological techniques

The lambs were faecal sampled twice every 8-10 days throughout the experimental period from 18 - 43 days post first infection and 14-68 days post second infection. FEC were performed by the modified McMaster technique.

Statistical Analysis

Statistics were performed using the computer package SAS as mentioned previously.

All FEC data was transformed to log form (log10 x+1) (unless otherwise stated) and the means are presented as geometric means.

6.3 Results

FEC

The mean FEC of the lambs over the experimental period from 18- 65 days post-infection at 9 weeks are shown in Figure 6.1. The overall mean FEC for the samples taken throughout the first post-infection was 140. The wide 95% confidence limits of this mean 65-304 reflects the wide range of values recorded from 0-700 EPG. In the period following the second infection The mean was 93 with again wide 95% confidence limits from 3-267 reflecting an even wider range of actual figures from 0-925 EPG. FEC were highest at 18 days post-infection, a steady decline in output was then observed. Following the secondary infection a slight increase was seen 10 days post-infection and then FEC production was somewhat erratic with highest output increased at 27 days post second infection or 57 days post primary infection.

Correlation coefficients of consecutive faecal samples for both the primary and secondary infections are shown in Tables 6.1 and 6.2. Correlation coefficients were significant only between FEC 1&2 and 3&4 during the primary infection. Correlation coefficients were significant between FEC 5&6, 6&7 and 7& for the secondary infection ($p < 0.001$). The correlation coefficient between the mean of the primary and the mean of the secondary infection was significant $r = 0.77$ ($p < 0.001$).

Table 6.1 : Correlation coefficients of consecutive samples for the primary infection

1 & 2	2 & 3	3 & 4
0.70 $p < 0.005$	0.57 n.s.	0.93 $p < 0.001$

Table 6.2 : Correlation coefficients of consecutive samples for the secondary infection

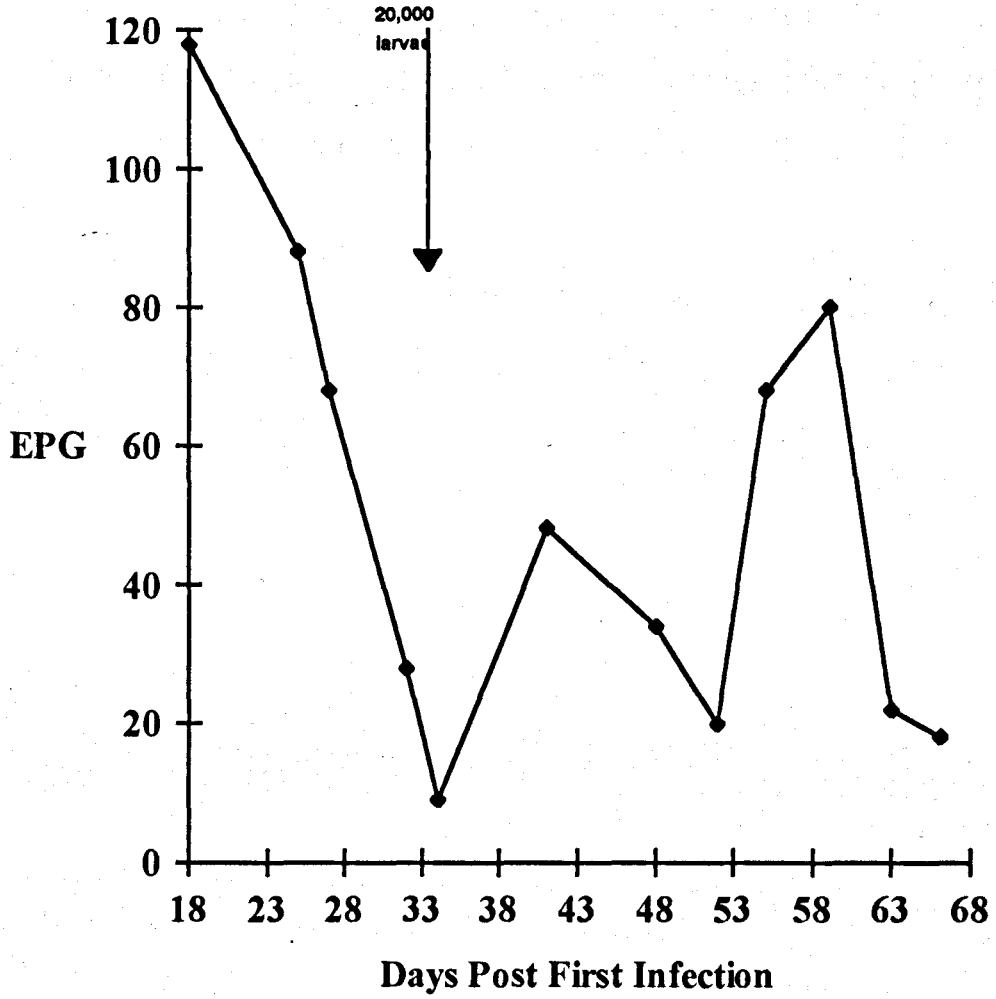
1 & 2	2 & 3	3 & 4	4 & 5	5 & 6	6 & 7	7 & 8
0.30 n.s.	0.57 n.s.	-0.07 n.s.	-0.18 n.s.	0.83 $p<0.001$	0.71 $p<0.001$	0.81 $p<0.001$

The correlation coefficients between individual FEC were mainly non significant and inconsistent as can be seen from Table 6.3.

Table 6.3 : Correlation coefficients between primary and secondary FEC

		Primary Infections							
		FEC1	FEC2	FEC3	FEC4	FEC5	FEC6	FEC7	FEC8
Secondary Infection	FEC1	0.07 n.s.	0.20 n.s.	0.62 p<0.01	0.66 p<0.01	0.40 n.s.	0.19 n.s.	0.55 n.s.	-0.18 n.s.
Secondary Infection	FEC2	0.38 n.s.	0.37 n.s.	0.53 p<0.05	0.55 p<0.05	0.56 p<0.05	0.38 n.s.	0.70 p<0.01	-0.24 n.s
Secondary Infection	FEC3	0.36 n.s.	0.35 n.s.	0.30 n.s.	0.41 n.s.	0.49 p<0.05	0.35 n.s.	0.80 p<0.01	-0.13 n.s.
Secondary Infection	FEC4	0.28 n.s.	0.50 p<0.05	0.50 p<0.05	0.52 p<0.05	0.65 p<0.01	0.17 n.s.	0.65 p<0.01	-0.03 n.s.

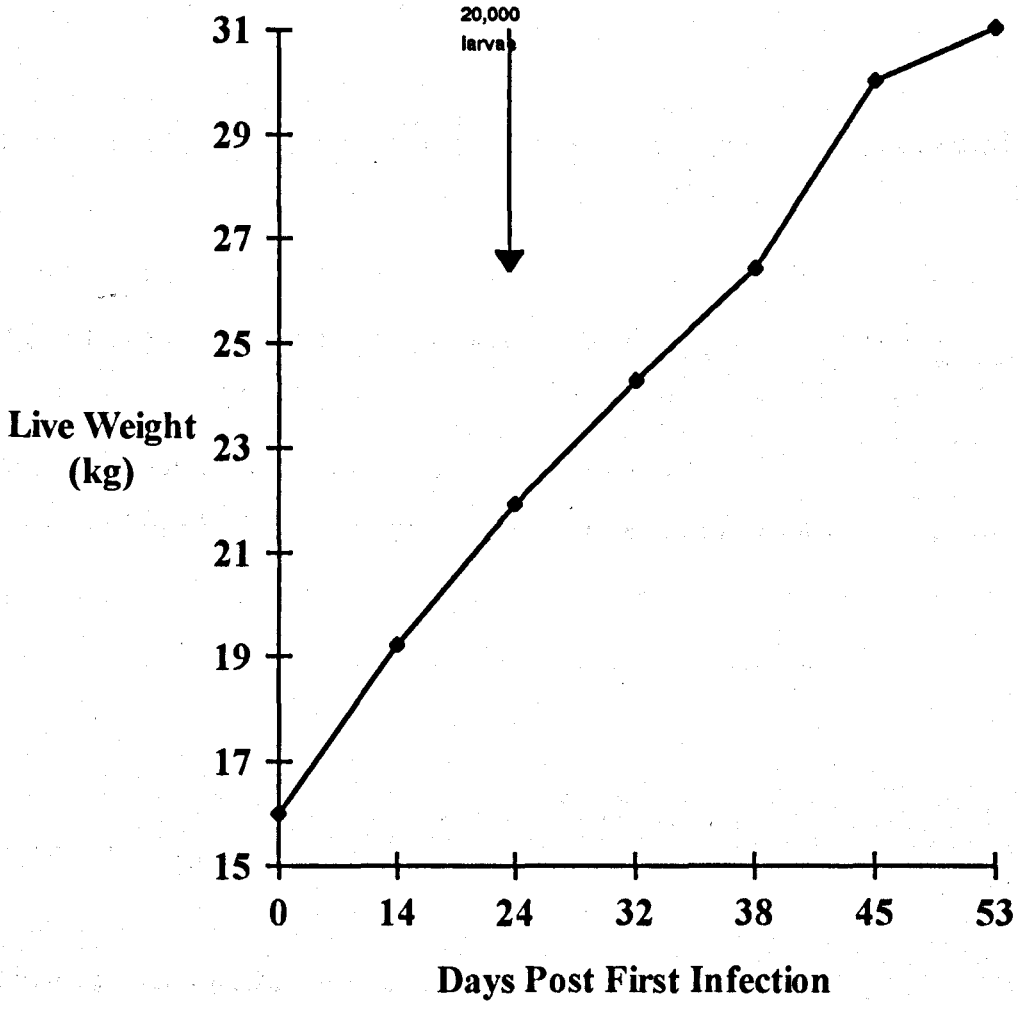
Figure. 6.1 : Mean FEC from 18- 65 days post-infection of lambs infected with consecutive doses of 10,000 and 20,000 *Ostertagia circumcincta* larvae



Liveweight

The mean liveweight change of the lambs over the 65 day experimental period is shown in Figure 6.2. Lamb weights increased steadily and the correlation coefficient between liveweight gain and FEC in both primary and secondary infections was non significant ($r = 0.1$ and 0.3 respectively). The relationship between the mean liveweight change during the primary infection period and that in the secondary infection period was non significant, ($r = 0.12$).

Figure. 6.2 : Mean liveweight of the lambs over the 65 day experimental period



Trial 2 - Rams

6.2a Materials and Methods

Experimental design

Thirty-seven, 12 - 13 month old ram lambs were experimentally infected with 18,000 *Ostertagia circumcincta* larvae in a single dose. Faecal samples were taken from each ram twice weekly from 19 - 28 days post-infection inclusive. Trichostrongyle eggs per gramme were estimated by the modified MacMaster technique, as before. All rams were dosed with a therapeutic dose of ivermectin (Oramec MSD Agvet) 2 weeks prior to infection in order to remove any gastrointestinal parasites present (dose rate 2.5 ml per 10 kg body weight). Rams were kept indoors and fed a concentrate diet with hay ad libitum and were weighed twice weekly from 13 - 28 days post-infection.

Statistical Analysis

Statistics were performed using the computer package SAS as mentioned previously.

All FEC data was transformed to log form (log10 x+1) (unless otherwise stated) and the means are presented as geometric means.

6.3a Results

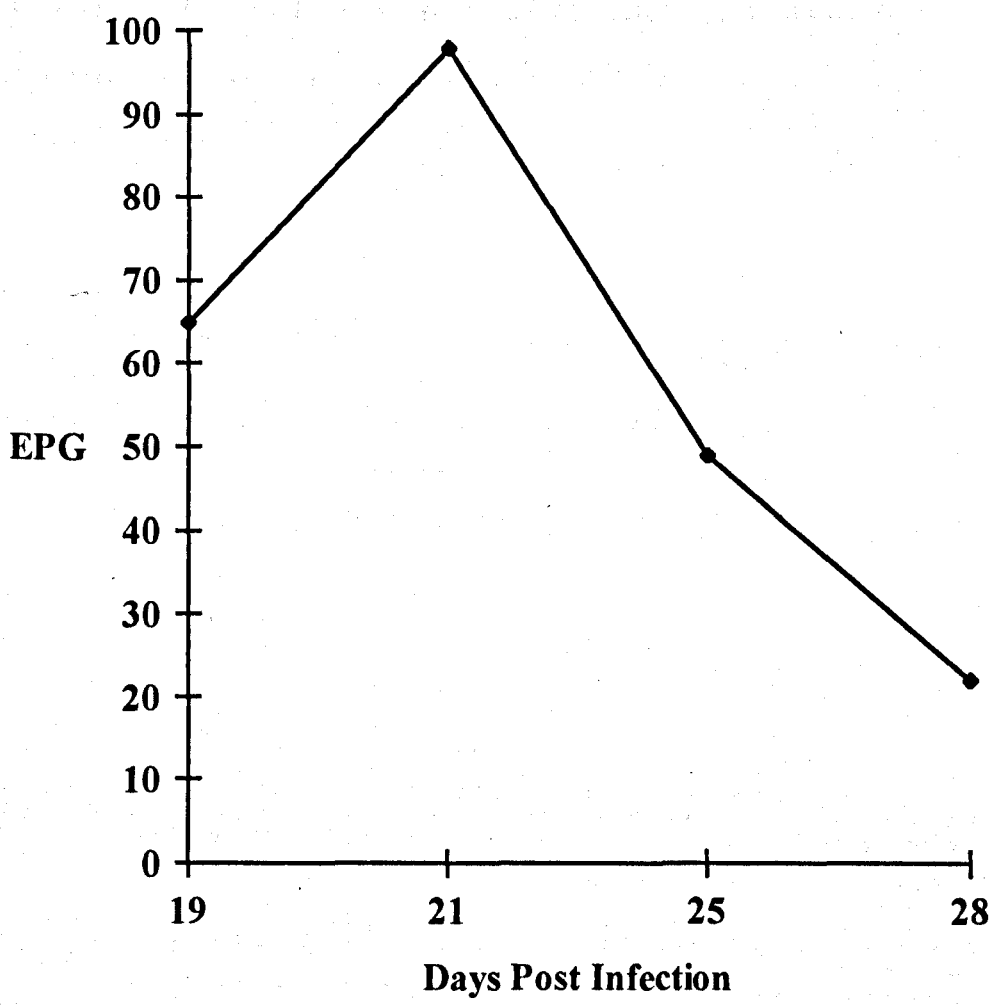
Trial 2 - Rams

FEC

The mean FEC for the experimentally infected rams are shown in Figure 6.3. FEC reached a peak at 21 days post-infection and after this point a steady decline in FEC was observed. Mean FEC varied from 3 - 500, with an overall mean of 106, a percentage standard deviation of 2.81 and 95% confidence limits of 74-152. When the rams were lambs mean FEC varied from 0 - 1901, with an overall mean of 248, a percentage standard deviation of 3.69 and 95% confidence limits of 152-400.

A significant correlation ($r = 0.49$, $p < 0.01$) was observed between FEC of the rams following the infection with a dose of 18,000 *Ostertagia circumcincta,* at 12 - 13 months of age and when they were growing lambs, aged 4 - 5 months.

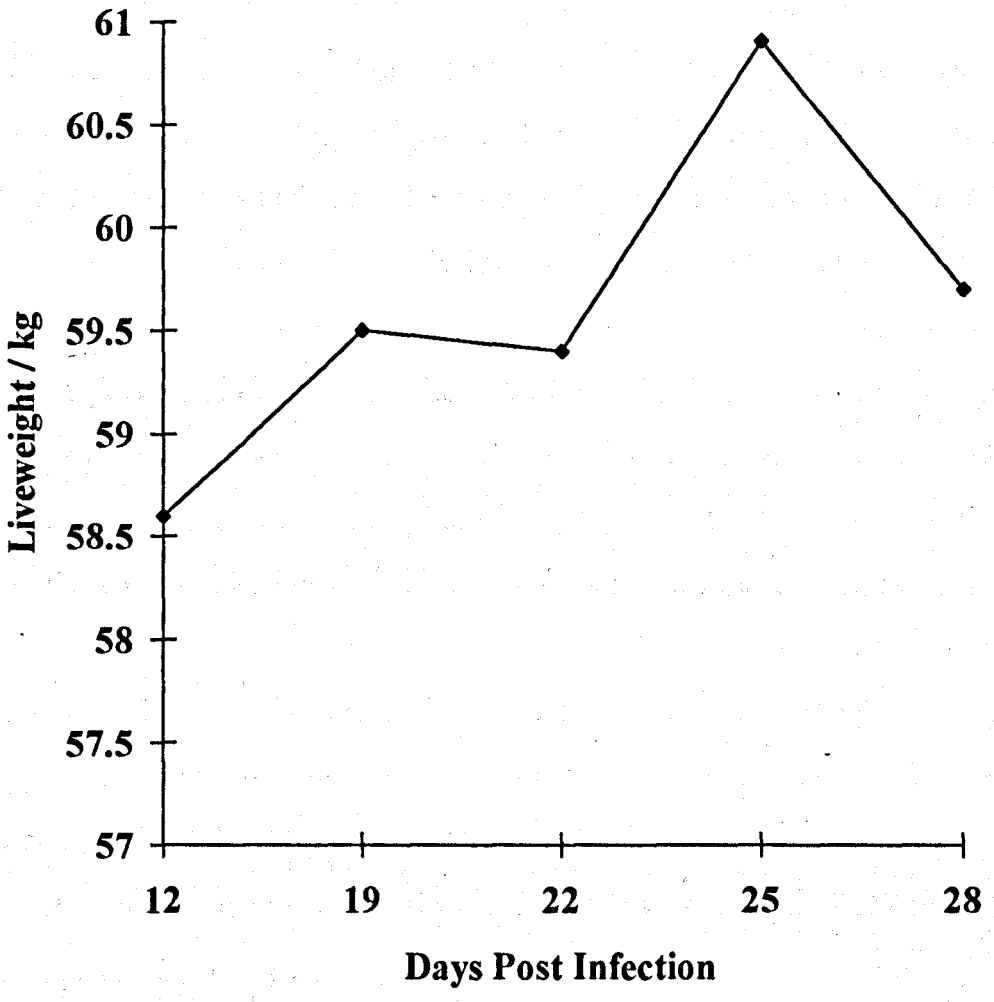
Figure 6.3 : Mean FEC for rams experimentally infected with 18,000 *Ostertagia circumcincta* larvae



Liveweight

Mean live weight of the ram lambs was 59 kg with a range from 49 - 70 kg and a coefficient of variation of 8.6. The lambs for 12 - 13 months of age were growing slowly (Figure 6.4). No significant relationship was observed between mean liveweight change and mean FEC (r = 0.044).

Figure 6.4 : Mean live weights for rams experimentally infected with 18,000 *Ostertagia circumcincta* larvae



6.4 Discussion

Trial 1

A wide variation in FEC was observed between the lambs following both first and second infections. All lambs were however, fed very well so that even greater differences which may be seen in field conditions could have been camouflaged by diet. The extremely low mean FEC of some lambs even when naive, with one lamb expressing a zero FEC throughout the trial had also been seen in a previous infection (Hughes, 1993 unpublished data) when four, five month old lambs were each experimentally infected with 25,000 *O. circumcincta* larvae. Two of the lambs displayed FEC of less than 100 until 29-30 days post-infection and thereafter maximum counts only reached 400 EPG. A second lamb displayed a maximum FEC of 1300 at 21 days post-infection, which quickly declined to 100 EPG until day 30 when the FEC started to increase, reaching a further peak of 1000 EPG at 43 days post-infection (the last day of the trial). The final lamb reached a maximum FEC of 1300 EPG at 29 days post-infection, a decrease was then seen followed by a further peak of 1050 EPG at 37 days post-infection. This wide variation in FEC with some animals displaying extremely low counts in naive animals contrasts with reports of other authors. Jenkins and Carrington (1981) investigating primary infections in mice observed no difference in faecal egg output of mice with the genetic capacity of low or high antibody production. However, a repeated infection demonstrated differences between the two groups of mice. Douch (1988) witnessed only high egg counts (range 1667-4400 EPG) when 8, 12 and 16 week old lambs were infected with 28,000, 35,000 and 42,000 *T. colubriformis* larvae and only during subsequent infections were worm burdens reduced.

In trial 1, the mean of the second infection was lower than that of the first (93 compared with 140 EPG) even though the infective dose rate was doubled to 20,000 larvae per lamb. This decrease in FEC thus suggests evidence of a 'self-cure' reaction. Bradley, Rhadakrishnan, Patil-Kulkarni and Loggins (1973) also noticed self-cure reactions in lambs when a second dose of larvae was administered. Stoll (1929) first described self-cure when 2 lambs subjected to natural infections of *H. contortus* displayed spontaneous termination of infections and Gordon (1948) and Stewart (1950) used the term to denote the termination of infections only when induced by reinfection. However, some other authors have reported contrasting results. Salman and Duncan (1984) observed that when lambs aged 6-8 weeks were given a primary infection of *Haemonchus contortus* followed 15 days later by a secondary infection, there were no signs of expulsion of the first infection. Dineen and Wagland (1966) observed that when a challenge infection was superimposed on the sensitising burdens, a large proportion of the challenge dose became established but when the sensitising burdens were removed by the administration of an anthelmintic 8 days before challenge, the animals were relatively resistant to the challenge dose.

In the second infection, the reduced egg count with the delayed peak 27 days post-infection compared with the 18 days post-infection peak seen in the first dose may have been due to a decline in the establishment of incoming larvae or a loss of adult worms or a reduction in egg production or a combination of 2 or more of these factors. A similar pattern of egg output was witnessed when Bradley *et al* (1973) infected Florida Native lambs (haemoglobin type Hb.B) with two doses of *H. contortus* larvae 28 days apart. They also reported that the adult worm population was lower after the second dose was given. Florida

Native lambs which were Hb.AB animals which had zero FEC during the first infection displayed elevated FEC up to one week after the administration of the second larval dose followed by a rapid decline. Bradley *et al* (1973) suggested the latter pattern of faecal egg output was due to the second dose of infective larvae either stimulating the rapid maturation of inhibited stages or a stimulation of egg production in females for a short period followed by self-cure and protection.

Lambs in trial 1 were growing rapidly and performance was not significantly affected by infection rates.

Trial 2

Ram lambs in trial 2 displayed a high correlation ($r = 0.49$, $p = 0.01$) between FEC of the two sampling periods. This observation is in agreement with that of several authors. Woolaston, Elwin and Barger (1992) observed significant differences ($p < 0.01$) in FEC in lambs selected for resistance and susceptibility to *Haemonchus contortus* and *Trichostrongylus colubriformis* at six months of age and then when adult ewes and rams. Gray, (1991) compared FEC of 318 lambs experimentally infected with *H. contortus* one month after weaning and the mean natural FEC of 93 of the lambs 18 months later and observed a correlation of $r = 0.44$ ($p < 0.05$).

CONCLUDING REMARKS

The work described in this thesis using a variety of techniques to identify infection levels/resistance, but principally FEC has underlined the large amount of variability which is likely to be observed in gastrointestinal nematode infections, even in low dose artificial infections.

Separate Chapters in this thesis describe various aspects of the work carried out over 3 years to advance the understanding of genetic resistance to nematodes.

In all 3 years, significant differences in FEC of progeny groups were seen and in year 3, FEC of progeny groups correlated significantly with their sires. This evidence suggests that some of the variation in resistance is genetically controlled and could be exploited by selecting lines of resistant animals.

Faecal sampling of ewes pre- and post-partum is unlikely to add to the identification of resistant individuals. However, from a management point of view the variability of infection rates of the ewes around partum highlights the fact that certain individuals can be susceptible as early as 8 weeks prior to lambing and if kept in field conditions could be contaminating the pasture. Also over the total periparturient rise 2 year old ewes were more susceptible to parasitic infection than older ewes, a fact which should be borne in mind when deciding on a suitable drenching programme for pregnant and lactating ewes.

To incorporate a selection programme to increase resistance to nematodes within a pedigree sheep breeder's flock a plan such as the one outlined below could be followed.

1. From the work in this study it was clear that taking FEC alone was the most reliable method to detect high and low responder lambs as the other methods add little accuracy. Factors such as live weight gain and incidence of scouring were also unrelated to FEC.
2. All lambs could be screened by placing them on contaminated pasture and faecal sampled once post-weaning at 16-20 weeks of age. However, lambs would graze contaminated pasture pre-weaning in order to ensure that they have become immunologically primed, but FEC taken pre-weaning would probably not be an accurate estimate of resistance because of the large effect of environmental factors such as availability of the ewe's milk and succession of nematode species, i.e. *Ostertagia spp.* followed by *Trichostrongylus spp.* In a pedigree flock it would be useful to screen both male and female lambs however, in the present study in many groups of lambs a higher percentage of female lambs had very low FEC (i.e. they were more resistant) compared with male lambs of which only a few had very low egg counts. A fact which needs to be considered when selecting lambs for resistance to nematode infection.
3. Following faecal sampling lambs would be drenched with an anthelmintic and those with a FEC less than 200 EPG should be brought indoors, fed a maintenance diet and artificially infected with a single dose of 25,000 *O. circumcincta* larvae in order to observe a variability in resistance. Feeding at maintenance may compromise live weight gain performance for a short time but lambs fed a high concentrate diet display a lower and less variable FEC than those on a low concentrate diet. Artificial infections reduce non-genetic variability due in part to unequal larval intake whilst grazing and they thus provide a better representation of genetic variation of resistance.

Single infections would appear to be the most suitable because they are easier to administer and they demonstrate variability within the flock.

4. Faecal samples would be taken at 18 and 21 days post infection (peak egg production in lambs infected with a single larval dose) and those lambs with mean FEC below 200 EPG should be used as the group of lambs which breeding stock is to be selected from the following year.

If time had permitted it would have been useful to leave a small group of lambs, consisting of some animals displaying high FEC and some low FEC, undosed in field conditions. These would have to be observed carefully for welfare reasons but it would enable self-cure to be witnessed in heavily infected animals and also if lambs displaying very low FEC demonstrated a build-up of infection levels over a period of time. Live weight gains and carcass conformation could also be observed in these lambs to establish if these traits correlate closely with FEC when studied in longer term trials..

Investigating further the interaction of infection rate and FEC with diet with lambs being fed varying quantities and qualities of diet, e.g. high/low fibre diets and looking at the rate of passage of food through the gut would help to clarify if when a high amount of faeces is produced egg counts are substantially reduced when a high fibre diet is provided.

By further looking at nematodes in the gut of artificially infected lambs the success of larval establishment, the percentage of larval inhibition, the fecundity of female worms and the rate of expulsion of third stage larvae and adult worms can be observed in individual lambs. The immune status of the host may affect the nematode at various stages of the life cycle.

Problems of exposing animals to infections may not be acceptable to some breeders, the use of genetic markers to identify resistant animals would however, be ideal. Such markers would include, investigation into the genes known to influence the immune response, i.e. the Major Histocompatibilty Complex (MHC). The MHC acts as restriction elements in antigen presentation. The region is extremely polymorphic making it suitable for investigating disease associations. Restriction Fragment Length Polymorphisms (RFLPs) which have been used to study the effect of genetic variation in the MHC class II region on resistance to *Trichostrongylus colubriformis* in sheep using human probes could also be investigated.

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APPENDICES

APPENDIX 1

EWE TABLES

Appendix Table 1 : FEC from 4 weeks pre- to 4 weeks post-partum for 1992, year 1 ewes

	Weeks pre- / post-partum								
Ewe no.	**4**	**3**	**2**	**1**	**P**	**1**	**2**	**3**	**4**
2084	50	400	900	600	600	400	0	150	0
2164	0	50	100	150	50	200	700	100	500
4321	0	250	350	250	300	300	250	.	200
5028	100	150	500	600	250	0	50	250	200
5114	0	450	500	200	300	0	650	100	0
5191	0	0	0	0	50	250	250	0	150
5244	0	400	1500	800	200	0	1150	300	0
5292	0	800	300	450	150	500	1150	0	250
6073	0	450	400	800	350	250	450	100	850
6135	0	50	100	100	0	0	0	50	0
6325	0	600	700	0	450	550	0	150	0
7126	150	0	800	400	300	950	50	.	150
7129	0	500	750	550	450	500	50	0	0
7141	100	0	150	300	350	200	0	0	200
7162	0	50	0	50	0	50	250	0	150
7204	0	350	650	200	400	0	0	300	0
7254	100	0	0	250	600	550	500	450	300
8067	0	150	700	650	650	450	500	300	900
8070	0	100	50	650	1000	1100	750	500	450
8129	650	1050	.	.	1050	850	800	950	250
8222	0	100	350	150	1400	1200	1000	950	450
8275	0	200	0	450	350	750	0	0	.
8317	0	50	100	50	0	100	50	150	0
8324	0	100	150	50	100	200	50	0	0
9072	50	450	650	1200	750	900	500	.	650
9153	0	150	300	350	300	0	50	50	0
9165	50	100	150	150	350	650	2050	1000	50
9200	0	550	0	250	450	50	100	50	0
9223	0	300	500	650	50	300	300	0	250
9251	0	150	300	0	300	1000	300	150	250
9256	0	0	400	350	600	250	450	350	50
9267	0	500	400	300	150	150	0	0	300
9279	0	450	650	450	600	0	100	0	0
0222	0	500	1200	350	1000	1200	500	300	700
0230	0	1100	2450	1050	1200	1300	1600	600	700
0276	100	450	850	650	950	600	500	0	.
9242	0	250	450	400	350	100	0	100	50
0304	100	1050	0	350	2650	1300	3050	1000	650

Table 1 continued

Ewe no.	Weeks pre- / post-partum								
	4	3	2	1	P	1	2	3	4
0305	100	700	700	1100	600	350	1300	400	0
0415	100	400	950	450	600	200	650	1000	400
0443	100	550	800	400	250	500	450	0	400
0451	0	300	500	350	300	350	300	0	450
0465	300	1350	0	0	250	0	0	0	0
0511	.	.	.	400	200	0	1150	350	250
2064	500	350	250	150	50	0	.	.	.
2185	350	100	0	450	.	350	200	700	150
3052	250	0	0	50	550	1400	500	300	200
4068	2200	350	750	550	600	650	400	0	850
5185	50	150	100	450	50	0	100	0	300
5190	300	500	550	200	0	0	0	0	0
5282	500	1200	0	850	100	0	850	100	350
5284	50	800	1000	0	1950	1000	800	0	0
6017	150	350	.	.	0	150	150	0	0
6163	1050	400	.	150	0	150	0	0	0
6231	550	50	0	.	350	400	50	50	150
6266	900	850	0	200	400	0	100	150	100
6285	300	600	0	250	0	0	0	0	150
7112	300	1200	800	0	0	0	0	0	600
7127	400	650	500	0	.	.	0	0	300
7160	50	250	50	0	100	0	0	100	0
7170	800	1150	250	0	.	350	300	0	50
7177	350	450	250	50	.	0	650	0	300
7233	600	1300	650	300	0	150	0	300	450
7316	800	0	450	150	100	300	200	0	50
8018	150	350	0	.	100	0	100	0	0
8049	100	250	300	600	1200	0	50	0	0
8058	450	400	200	0	0	0	0	.	0
8120	750	400	0	450	50	0	500	750	150
8223	200	1100	0	450	.	800	200	0	650
8319	250	0	100	0	.	850	350	0	0
9008	100	50	100	100	800	0	0	200	50
9051	0	700	500	50	300	1150	100	0	300
9052	0	150	250	200	0	850	450	0	0
9089	250	0	0	150	50	0	250	250	200
9135	200	400	500	0	0	200	100	0	50
9167	50	750	100	550	.	50	0	950	1200
9179	0	0	50	400	.	0	100	50	50
9199	950	100	50	250	550	300	0	200	50

Table 1 continued

Ewe no.	Weeks pre- / post-partum								
	4	3	2	1	P	1	2	3	4
9245	600	750	700	150	450	150	200	0	200
9255	0	600	0	150	400	200	750	250	350
9321	1200	1800	1700	200	0	.	1200	.	500
B10	100	300	50	0	0	0	0	0	100
0004	950	50	1000	950	650	600	250	100	250
0212	0	500	0	300	0	0	450	0	250
0238	300	0	50	100	0	0	0	250	250
0251	0	300	100	500	0	350	0	750	250
0252	100	1200	750	0	850	650	100	250	400
0272	300	450	400	50	1000	750	200	150	200
0310	400	1750	1150	1050	600	950	950	600	350
0318	250	350	250	50	150	0	150	350	500
0320	650	1250	600	1200	.	0	550	0	50
0326	300	0	50	850	0	600	550	50	700
0472	250	1350	200	300	250	0	650	850	1750

Appendix Table 2 : FEC at 8 weeks pre-partum of 1992, year 1 ewes

Ewe no.	year	eight	group	age	litter size	mean FEC
2084	1	0	1	3	3	359
2164	1	0	1	3	2	192
4321	1	0	1	3	4	237
5028	1	0	1	3	3	224
5114	1	100	1	3	2	264
5191	1	0	1	3	2	55
5244	1	0	1	3	3	527
5292	1	0	1	3	2	459
6073	1	500	1	3	3	336
6135	1	0	1	3	1	36
6325	1	0	1	3	1	277
7126	1	0	1	3	3	350
7129	1	0	1	3	2	406
7141	1	.	1	3	3	144
7162	1	0	1	3	1	57
7204	1	0	1	3	2	220
7254	1	0	1	3	2	284
8067	1	200	1	3	3	467
8070	1	0	1	3	4	480
8129	1	.	1	3	1	815
8222	1	500	1	3	3	575
8275	1	.	1	3	3	219
8317	1	.	1	3	3	55
8324	1	0	1	3	2	81
9072	1	0	1	3	3	644
9153	1	.	1	3	1	140
9165	1	0	1	3	2	466
9200	1	100	1	3	3	165
9223	1	0	1	3	1	271
9251	1	0	1	3	4	261
9256	1	0	1	3	4	264
9267	1	0	1	3	3	210
9279	1	0	1	3	3	264
0222	1	0	1	2	1	626
0230	1	0	1	2	5	1115
0276	1	0	1	2	1	512
9242	1	.	1	3	2	222
0304	1	0	1	2	1	1390

Table 2 continued

Ewe no.	year	eight	group	age	litter size	mean FEC
0305	1	200	1	2	2	590
0415	1	800	1	2	1	522
0443	1	600	1	2	4	431
0451	1	0	1	2	2	284
0465	1	0	1	2	2	231
0511	1	0	1	2	2	384
2064	1	0	2	3	3	198
2185	1	0	2	3	4	287.5
3052	1	0	2	3	3	332
4068	1	0	2	3	3	731
5185	1	.	2	3	2	139
5190	1	.	2	3	3	194
5282	1	0	2	3	2	459
5284	1	0	2	3	1	606
6017	1	0	2	3	4	163
6163	1	0	2	3	1	315
6231	1	0	2	3	3	200
6266	1	0	2	3	2	319
6285	1	0	2	3	1	159
7112	1	100	2	3	3	347
7127	1	0	2	3	4	244
7160	1	0	2	3	4	70
7170	1	0	2	3	3	362
7177	1	0	2	3	4	256.5
7233	1	0	2	3	3	446
7316	1	0	2	3	1	240
8018	1	0	2	3	3	107.5
8049	1	0	2	3	2	281
8058	1	0	2	3	4	131
8120	1	0	2	3	4	345
8223	1	0	2	3	2	425
8319	1	0	2	3	1	194
9008	1	0	2	3	4	149
9051	1	0	2	3	3	341
9052	1	0	2	3	2	205
9089	1	600	2	3	2	125
9135	1	0	2	3	2	181.5
9167	1	0	2	3	3	456
9179	1	0	2	3	2	812.5
9199	1	0	2	3	1	279

Table 2 continued

Ewe no.	year	eight	group	age	litter size	mean FEC
9245	1	0	2	3	2	375
9255	1	0	2	3	3	269
9321	1	0	2	3	5	311
B10	1	0	2	3	3	106
0004	1	50	2	2	1	494
0212	1	0	2	2	2	200
0238	1	350	2	2	3	112.5
0251	1	0	2	2	3	225
0252	1	0	2	2	2	512.5
0272	1	50	2	2	2	300
0310	1	50	2	2	1	1062.5
0318	1	100	2	2	4	225
0320	1	.	2	2	3	925
0326	1	0	2	2	3	300
0472	1	1250	2	2	2	525

Appendix Table 3 : FEC from 4 weeks pre- to 4 weeks post-partum for 1993, year 2 ewes

Ewe no.	Weeks pre- / post-partum							
	4	3	2	1	P	1	2	3
2164	950	1000	1100	550	200	500	200	500
4321	650	2100	950	700	500	850	600	800
5028	1200	2700	2200	1650	700	550	0	350
5284	200	100	800	1450	1200	1900	1600	400
6180	450	200	150	300	150	0	750	100
6135	200	100	100	100	200	700	800	2950
6285	500	850	100	1200	1000	500	400	450
6325	800	800	800	900	1150	1000	900	700
7112	600	150	1150	1800	650	550	700	.
7126	150	300	250	1000	400	150	350	650
7127	750	1400	200	400	600	500	350	150
7129	600	1850	800	1000	400	300	950	800
7170	200	350	200	450	600	750	250	1200
7177	500	350	450	850	350	600	400	500
7254	50	50	200	300	200	100	0	150
8049	950	750	50	50	350	550	500	600
8058	1050	1450	350	300	1100	900	750	50
8067	1000	1000	1200	1350	1100	350	800	900
8070	1100	1000	800	900	700	600	600	650
8223	750	500	50	500	100	200	350	350
8275	350	750	350	450	550	250	500	750
8317	.	50	150	600	250	250	200	100
8324	100	50	150	250	250	0	100	0
9008	100	100	150	450	700	150	0	0
9051	300	500	550	550	750	350	150	950
9052	100	50	0	50	700	550	150	350
9072	1650	900	1300	1150	500	750	300	50
9089	450	600	250	650	950	500	900	700
9135	200	150	200	600	500	50	100	50
9153	50	0	0	100	700	1400	650	0
9165	700	900	750	550	.	.	.	.
9167	300	250	150	0	0	0	150	400
9199	500	1200	.	.	.	.	.	.
9233	500	100	850	0	350	350	700	500
9255	150	200	100	250	100	750	1300	500
9321	350	550	1250	1900	750	300	300	300
B10	450	750	500	400	600	350	0	150
Susie	950	1950	900	1500	500	200	300	400

Table 3 continued

Ewe no.	Weeks pre- / post-partum							
	4	3	2	1	P	1	2	3
0238	300	350	300	600	900	500	400	200
0239	400	500	800	250	250	250	400	550
0251	350	100	450	350	200	450	800	200
0254	100	50	50	700	200	150	50	450
0260	150	100	200	450	400	450	300	500
0304	2750	2700	1000	2200	2550	.	.	.
0305	200	100	100	650	300	500	100	700
0310	150	550	200	250	400	600	600	750
0415	450	400	600	500	250	350	400	300
0451	150	200	150	250	400	400	250	250
0465	250	550	300	.	2300	1000	200	100
0472	350	200	750	600	500	400	.	.
1111	350	750	1150	750	200	500	350	300
8120	200	300	350	450	150	50	500	650
0276	300	350	350	400	450	.	.	.
0212	250	250	50	250	500	0	600	450
0222	500	550	350	550	750	450	400	650
0230	600	500	800	1000	650	850	700	2150
0232	500	600	500	500	600	650	400	350
0252	250	0	0	300	200	150	100	300
0272	800	400	600	700	900	600	850	1400
0318	50	0	50	450	400	350	300	400
0489	2800	1200	2300	1700	1400	1400	500	300
1016	50	0	150	150	50	450	450	400
1037	700	800	400	900	1700	1000	500	600
1048	0	0	100	450	400	500	500	450
1066	2150	800	1200	1200	1700	1000	1300	450
1076	900	1400	550	800	1400	950	450	750
1113	550	300	600	900	750	550	250	200
1119	1300	1000	800	800	1550	1700	1200	2300
1131	100	0	200	150	200	150	200	50
1142	150	200	150	100	0	100	.	.
1159	250	0	0	350	450	700	1400	500
1165	500	0	550	1000	600	750	450	1000
1173	50	50	450	350	500	650	400	450
1198	1100	1000	700	600	400	1300	1350	1350
1202	450	100	350	250	350	200	900	350
1203	200	250	400	450	400	550	850	.
1205	550	900	400	550	450	450	250	100
1210	50	350	400	300	150	300	500	0

Table 3 continued

Ewe no.	Weeks pre- / post-partum							
	4	3	2	1	P	1	2	3
1236	2500	1200	1200	1250	850	1000	5400	1700
NT	300	250	100	550	300	600	200	350
2064	0	0	0	0	0	100	0	0
2185	50	0	0	400	750	450	50	200
5185	200	0	100	150	700	0	200	50
5190	400	0	0	100	600	0	250	150
5191	900	200	250	300	250	150	400	400
5244	800	700	700	1100	700	750	850	1050
5282	900	550	400	150	50	150	300	300
5292	900	900	400	450	500	650	200	350
1175	300	100	250	50	.	.	.	.
6027	150	350	350	0	200	150	150	100
6073	500	550	850	1100	2100	850	700	750
6231	300	1100	1500	1450	1400	300	250	.
6266	1100	1000	1200	600	50	200	250	400
7162	0	0	200	400	500	400	100	200
7204	900	500	450	450	350	300	250	250
7233	400	100	200	600	950	850	550	400
7316	400	200	100	0	0	50	0	0
8018	650	0	100	200	0	0	300	750
8129	2300	2900	350	500	2900	3200	4000	3900
8222	1050	1000	600	450	550	950	700	450
8319	850	700	400	600	700	650	650	300
9063	150	450	550	300	250	0	50	150
9256	100	100	50	0	.	.	.	.
9279	550	0	650	1100	1100	700	1050	1050

Appendix Table 4 : FEC at 8 weeks pre-partum of 1993, year 2 ewes

Ewe no.	year	eight	group	age	litter size	mean FEC
2164	2	.	1	3	2	625
4321	2	600	1	3	5	357.5
5028	2	3850	1	3	3	1169
5284	2	1700	1	3	4	956
6180	2	150	1	3	4	262.5
6135	2	.	1	3	2	112.5
6285	2	300	1	3	4	625
6325	2	300	1	3	3	881
7112	2	1650	1	3	4	779
7126	2	350	1	3	3	405
7127	2	1250	1	3	3	544
7129	2	.	1	3	4	837.5
7170	2	50	1	3	3	500
7177	2	0	1	3	5	500
7254	2	50	1	3	3	131
8049	2	700	1	3	3	475
8058	2	300	1	3	5	744
8067	2	600	1	3	4	962.5
8070	2	2100	1	3	1	794
8223	2	500	1	3	2	812
8275	2	300	1	3	1	494
8317	2	100	1	3	5	250
8324	2	50	1	3	5	112.5
9008	2	200	1	3	2	206
9051	2	450	1	3	2	512.5
9052	2	.	1	3	1	244
9072	2	550	1	3	4	825
9089	2	50	1	3	4	650
9135	2	350	1	3	3	231
9153	2	450	1	3	3	362.5
9165	2	400	1	3	.	725
9167	2	150	1	3	1	142.5
9199	2	350	1	3	.	.
9233	2	1250	1	3	4	419
9255	2	650	1	3	2	419
9321	2	200	1	3	5	712.5
B10	2	350	1	3	4	400
Susie	2	.	1	3	5	837.5

Table 4 continued

Ewe no.	year	eight	group	age	litter size	mean FEC
0238	2	400	1	3	4	369
0239	2	450	1	3	4	425
0251	2	300	1	3	3	362.5
00254	2	300	1	3	2	219
0260	2	.	1	3	4	319
0304	2	.	1	3	3	2356
0305	2	.	1	3	4	331
0310	2	.	1	3	1	437.5
0415	2	.	1	3	4	406
0451	2	.	1	3	2	256
0465	2	.	1	3	2	633
0472	2	.	1	3	2	819
1111	2	.	1	2	1	544
8120	2	.	1	3	1	331
0276	2	600	2	3	3	422.5
0212	2	200	2	3	3	289
0222	2	300	2	3	2	530
0230	2	150	2	3	3	894
0232	2	500	2	3	2	512.5
0252	2	100	2	3	3	179
0272	2	300	2	3	2	856
0318	2	600	2	3	5	294
0489	2	3100	2	3	2	1450
1016	2	0	2	2	1	206
1037	2	200	2	2	4	825
1048	2	200	2	2	1	300
1066	2	800	2	2	3	1225
1076	2	100	2	2	2	900
1113	2	50	2	2	4	512.5
1119	2	2100	2	2	3	1140
1131	2	0	2	2	1	131
1142	2	50	2	2	4	117
1159	2	50	2	2	2	456
1165	2	150	2	2	4	606
1173	2	1400	2	2	1	362.5
1198	2	500	2	2	4	975
1202	2	300	2	2	4	369
1203	2	300	2	2	1	443
1205	2	150	2	2	3	456
1210	2	.	2	2	4	312.5

Table 4 continued

Ewe no.	year	eight	group	age	litter size	mean FEC
1236	2	400	2	2	2	1887.5
NT	2	200	2	2	3	331
2064	2	0	2	3	2	12.5
2185	2	100	2	3	3	322.5
5185	2	100	2	3	2	214
5190	2	800	2	3	3	215
5191	2	250	2	3	2	337.5
5244	2	350	2	3	3	819
5282	2	1200	2	3	3	306
5292	2	400	2	3	3	450
1175	2	.	2	2	4	.
6027	2	0	2	3	3	181
6073	2	300	2	3	3	866.5
6231	2	50	2	3	3	900
6266	2	150	2	3	4	572.5
7162	2	0	2	3	5	212
7204	2	800	2	3	3	440
7233	2	1000	2	3	3	548
7316	2	2800	2	3	4	124
8018	2	500	2	3	4	254
8129	2	2200	2	3	3	2745
8222	2	100	2	3	4	815
8319	2	300	2	3	2	577
9063	2	500	2	3	3	271
9256	2	250	2	3	4	.
9279	2	150	2	3	3	837.5

APPENDIX 2

LAMB TABLES

Appendix Table 5 : FEC and live weight gains for group 1, year 1 lambs

Lamb No.	Sex	LWG 1	LWG 2	FEC 1	FEC 2	FEC 3	LSR	Sire	Gp
1	M	3.0	1.5	450	350	950	3	C4	1
2	F	2.0	0.0	0	250	650	3	C4	1
3	F	3.5	-1.0	350	150	450	3	C4	1
5	M	3.5	3.0	250	400	250	2	C4	1
7	F	8.5	-1.0	50	250	450	1	S	1
8	M	0.0	-3.0	550	350	.	2	C3	1
9	F	2.5	-3.0	650	50	.	2	C3	1
10	M	3.0	0.0	600	200	900	3	C4	1
11	F	1.0	1.5	750	650	1300	3	C4	1
13	F	5.5	0.5	.	200	750	3	C4	1
14	F	3.5	0.0	300	400	0	1	S	1
15	M	4.5	-4.0	800	650	450	2	C3	1
16	F	2.0	0.5	1200	50	250	2	C3	1
17	M	2.0	1.5	400	450	.	2	C3	1
19	F	4.0	1.0	1200	350	100	2	C3	1
20	M	5.0	-1.0	500	900	1750	3	C4	1
21	M	3.0	1.0	650	0	1000	3	C4	1
22	M	5.5	-1.0	650	0	500	3	C4	1
23	M	5.0	6.5	550	150	.	3	C4	1
24	F	4.0	1.5	200	50	0	3	C4	1
25	F	3.0	1.5	100	0	.	3	C4	1
27	F	4.5	0.5	400	200	.	2	C4	1
28	F	2.5	-0.5	250	100	.	2	C4	1
29	M	3.5	-1.0	450	400	600	3	C4	1
30	F	0.0	2.5	900	200	.	3	C4	1
31	F	2.5	1.0	450	50	300	3	C4	1
32	M	2.0	1.5	1600	50	250	2	C2	1
33	F	3.5	-0.5	.	400	200	2	C2	1
37	F	1.0	.	700	550	250	3	C3	1
38	M	3.5	-3.0	250	0	.	3	Ch	1
39	M	4.4	3.0	200	150	.	3	Ch	1
35	M	2.5	2.5	1300	0	300	1	C3	1
41	F	4.0	0.0	400	200	450	2	C3	1
44	F	4.4	0.0	0	0	0	2	C2	1
45	F	2.5	0.5	.	200	50	1	C4	1
46	F	-3.5	-0.5	150	850	100	1	V	1
51	F	3.0	1.5	0	200	1650	1	S	1
52	M	3.5	0.0	250	300	.	2	S	1
53	F	3.5	-2.0	.	400	1100	2	S	1

Table 5 continued

Lamb No.	Sex	LWG 1	LWG 2	FEC 1	FEC 2	FEC 3	LSR	Sire	Gp
54	M	1.5	.	700	.	.	3	Ch	1
55	M	2.5	5.5	1000	50	.	3	Ch	1
56	F	2.5	1.5	850	700	.	3	Ch	1
62	M	6.5	-1.5	450	100	400	3	C3	1
63	F	4.0	-2.0	100	100	.	3	C3	1
64	F	2.0	0.0	250	0	350	3	C3	1
65	M	5.5	.	550	0	.	1	C2	1
66	F	0.5	1.5	550	250	250	2	C2	1
67	F	1.5	-1.5	650	50	2250	2	C2	1
68	M	4.5	-0.5	1150	650	850	2	C2	1
69	F	2.5	-0.5	250	0	50	3	C2	1
70	F	3.5	0.5	350	250	250	3	C2	1
71	F	-3.5	1.0	850	250	200	3	C2	1
72	F	2.0	1.0	550	0	.	2	Ch	1
73	F	4.0	1.0	352	50	.	2	Ch	1
74	M	8.0	.	850	.	.	3	Ch	1
75	M	4.0	-0.5	600	.	150	3	Ch	1
76	M	3.0	.	450	.	.	3	Ch	1
77	M	3.5	.	500	.	.	3	Ch	1
79	M	5.0	2.0	350	900	.	3	Ch	1
80	F	4.5	-1.5	800	.	300	3	Ch	1
81	F	3.5	1.0	400	200	.	2	C2	1
82	F	-1.5	0.0	900	100	250	2	C2	1
83	M	3.5	-0.5	650	0	450	3	C2	1
84	F	3.5	-0.5	750	450	1250	3	C2	1
85	F	3.5	-1.0	500	400	400	2	V	1
86	F	3.5	1.0	300	0	200	3	C2	1
89	F	3.0	-3.0	.	1850	550	2	C3	1
90	F	2.5	-1.0	150	50	.	2	C3	1
91	M	4.0	-1.0	350	100	.	3	Ch	1
92	M	2.5	-2.0	250	0	.	3	Ch	1
93	M	-0.5	2.0	500	350	.	3	Ch	1
94	F	3.5	2.0	850	0	550	2	S	1
96	F	0.5	-2.0	800	300	2250	2	C2	1
98	M	1.0	-1.0	400	300	.	1	C2	1
101	M	5.5	0.0	0	0	.	3	V	1
103	F	7.5	0.0	0	50	425	1	Ch	1
104	F	0.5	-4.5	0	50	.	1	V	1
106	F	6.5	0.0	550	250	350	2	C2	1
107	F	4.0	2.0	450	150	0	2	C2	1
120	F	8.5	2.5	900	1400	850	1	C4	1

Appendix Table 6 : FEC and live weights for group 2, year 1 lambs

Lamb No.	Sex	LWG 1	LWG 2	FEC 1	FEC 2	FEC 3	LSR	Sire	Gp
117	M	29.0	31.0	1350	450	200	.	C2	2
118	F	19.0	28.5	0	400	0	.	C2	2
122	M	27.5	.	850	350	.	.	Ch	2
123	M	24.0	21.5	500	.	0	.	Ch	2
124	F	21.0	28.0	100	0	.	.	Ch	2
125	M	24.5	29.0	350	500	0	.	Ch	2
126	F	21.5	27.0	.	100	0	.	Ch	2
127	F	28.0	31.0	550	0	0	.	Ch	2
129	F	31.0	36.0	100	1950	350	.	C3	2
130	F	34.5	38.0	100	100	450	.	C3	2
132	F	34.5	39.0	250	850	700	.	S	2
133	F	35.5	41.5	300	650	150	.	S	2
134	M	32.5	37.5	300	650	1250	.	C3	2
135	F	33.0	37.0	.	0	100	.	C3	2
136	M	31.0	34.5	50	0	0	.	Ch	2
137	F	25.5	30.0	450	200	200	.	Ch	2
138	M	24.5	29.5	300	850	.	.	C3	2
139	F	31.0	34.5	500	100	150	.	C3	2
140	M	29.0	34.5	1600	500	150	.	C1	2
141	F	28.0	32.0	0	150	0	.	C1	2
142	F	31.5	35.5	50	250	0	.	C1	2
144	M	33.5	38.5	800	700	.	.	S	2
145	F	32.5	38.0	0	0	50	.	Ch	2
146	F	28.5	33.0	50	800	400	.	C3	.
147	F	31.0	34.0	.	0	0	.	C3	2
148	F	26.5	29.5	150	.	0	.	C3	2
151	F	21.0	27.0	200	250	150	.	Ch	2
152	M	22.0	22.0	3150	1150	0	.	C3	2
153	M	17.0	.	450	150	0	.	C3	2
154	F	26.5	.	200	400	600	.	C3	2
155	M	26.0	36.0	450	150	50	.	Ch	2
156	M	33.5	38.0	500	450	300	.	Ch	2
157	F	32.5	33.5	250	.	0	.	Ch	2
160	.	29.0	.	200	.	600	.	Ch	2
162	M	16.5	40.5	650	.	0	.	S	2
163	M	36.5	39.5	150	750	0	.	S	2
165	F	33.5	.	100	450	100	.	S	2
169	M	29.0	32.5	250	300	450	.	C3	2
170	F	31.5	32.5	.	1200	300	.	C3	2

Table 6 continued

Lamb No.	Sex	LWG 1	LWG 2	FEC 1	FEC 2	FEC 3	LSR	Sire	Gp
171	F	34.5	38.0	600	1150	550	.	C3	2
173	F	23.5	26.0	1250	600	1200	.	V	2
174	M	32.5	38.0	50	1350	300	.	C4	2
175	F	25.0	27.5	0	50	400	.	C4	2
176	M	26.5	29.0	600	900	850	.	C4	2
177	M	33.0	36.0	150	1300	350	.	V	2
178	F	28.0	29.0	0	200	0	.	V	2
181	F	31.5	32.0	.	1050	550	.	S	2
184	F	38.0	40.5	1200	1050	250	.	S	2
182	F	33.0	36.0	250	50	100	.	S	2
183	F	31.0	33.0	0	0	0	.	S	2
186	M	25.0	27.0	.	100	0	.	C1	2
187	F	33.0	.	.	.	1050	.	C1	2
189	M	33.0	.	300	400	.	.	V	2
190	F	27.5	30.0	950	50	400	.	V	2
191	M	36.0	40.0	850	400	250	.	V	2
192	F	32.0	34.0	250	50	0	.	V	2
193	M	27.5	31.5	100	2700	250	.	C2	2
194	F	32.5	38.0	50	100	600	.	C2	2
195	M	29.0	32.0	350	1850	500	.	C2	2
196	M	36.0	39.5	800	0	.	.	S	2
197	M	37.5	40.0	110	1800	1050	.	C2	2
198	M	24.0	.	250	650	0	.	Ch	2
200	F	29.5	.	100	450	.	.	Ch	2
201	F	27.5	32.5	100	450	250	.	Ch	2
202	F	33.0	39.0	200	.	0	.	S	2
203	F	39.5	.	0	550	.	.	Ch	2
204	M	34.0	39.0	350	150	450	.	C1	2
205	F	27.5	31.0	550	350	0	.	C1	2
206	F	19.5	23.0	70	450	150	.	C1	2
207	F	30.5	36.0	150	0	0	.	C4	2
208	F	30.5	35.0	900	50	0	.	C4	2
209	F	26.0	33.0	100	.	300	.	C4	2
211	F	26.5	30.0	600	600	650	.	C2	2
212	F	37.0	38.5	300	0	250	.	C2	2
214	M	21.5	29.5	150	250	50	.	V	2
215	F	28.0	29.5	50	300	150	.	C4	2
217	F	28.5	33.0	0	50	0	.	C4	2
218	F	20.0	23.5	50	200	0	.	C4	2
219	M	36.0	.	1200	100	450	.	C2	1
221	M	25.0	33.0	900	600	550	.	S	2

Table 6 continued

Lamb No.	Sex	LWG 1	LWG 2	FEC 1	FEC 2	FEC 3	LSR	Sire	Gp
224	M	30.0	32.5	0	1050	350	.	S	2
226	M	27.5	.	950	.	100	.	V	2
227	M	26.5	30.5	4400	2750	2350	.	V	2
228	F	29.0	33.0	1050	2500	800	.	V	2
230	F	28.5	.	0	50	.	.	V	2
231	M	29.0	32.5	250	600	550	.	V	2
237	F	26.0	28.0	0	100	0	.	Ch	2
241	F	27.0	30.5	750	200	1300	.	C2	2
242	F	28.0	28.5	600	550	0	.	C2	2
243	F	35.5	.	0	250	.	.	Ch	2
244	M	30.0	33.0	1000	950	900	.	V	2
245	F	27.0	33.0	250	1100	100	.	V	2
246	M	29.5	33.5	2500	.	100	.	C2	2
247	M	37.5	41.0	350	.	350	.	C2	2
248	F	22.0	.	600	.	50	.	C2	2

Appendix Table 7 : FEC and live weight changes for group 1, year 2 lambs

Lamb No.	Sex	LWGTOT	LWG1	FEC	FEC1	LSR	Sire	Gp
13	F	1.5	-1	0	0	1	Ch	1
14	M	5.5	0.5	175	150	3	C7	1
15	M	3.5	-1	200	300	3	C7	1
16	F	2.5	-0.5	75	50	3	C7	1
17	M	1.5	-2	200	300	2	C2	1
18	M	3	-2.5	350	500	2	C2	1
19	M	2.5	-2	175	100	3	C1	1
21	F	4	-1.5	25	0	3	C1	1
23	M	2.5	-0.5	650	450	3	C1	1
24	M	4.5	-1	50	0	2	C3	1
25	F	1.5	-1.5	325	200	2	C3	1
26	M	.	0	250	200	3	C6	1
28	F	3.5	-1.5	575	550	3	C6	1
29	F	4	-2.5	400	450	3	C6	1
31	M	1.5	-2.5	1700	1200	3	C6	1
32	M	2	-0.5	325	200	3	C6	1
33	F	2.5	-1	475	700	3	C6	1
36	F	4	0	100	100	2	C1	1
37	F	2.5	-1	100	50	3	C1	1
38	F	4.5	-1	200	.	3	C1	1
39	F	3	-1	75	0	3	C1	1
40	M	1.5	2	125	50	2	C3	1
41	M	.	-1.5	225	50	2	C2	1
42	M	2.5	-2	525	50	3	C2	1
43	F	3	-0.5	50	100	3	C2	1
44	F	3	-0.5	100	100	3	C2	1
45	M	2	-2.5	275	50	1	C4	1
47	M	1	-3	175	250	2	C6	1
48	F	0	-3	100	.	2	C6	1
49	F	2.5	0	700	0	3	C2	1
50	F	3.5	0.5	300	.	3	C2	1
51	F	1.5	0	325	400	3	C2	1
53	M	.	-1	225	200	2	C4	1
54	F	4.5	-2.5	175	100	2	C4	1
57	M	1.5	-3	1875	1650	3	C7	1
58	M	0.5	-1.5	550	.	3	C7	1
60	M	-0.5	-3	1025	1250	2	C7	1
62	M	2.5	-1	175	150	3	C3	1
64	F	4	-0.5	0	.	3	C3	1
65	F	3	-2.5	756	0	3	C3	1

Table 7 continued

Lamb No.	Sex	LWGTOT	LWG1	FEC	FEC1	LSR	Sire	Gp
59	M	2	-1	1150	1150	3	C7	1
66	M	1.5	-2	25	50	3	C4	1
67	M	1	-2.5	300	.	3	C4	1
68	M	-1.5	-5.5	200	400	3	C4	1
70	M	5.5	-0.5	400	400	3	C7	1
71	F	4.5	0	400	400	3	C7	1
72	F	0.5	-3	150	.	3	C7	1
76	F	.	.	1350	1200	3	Ch	1
77	M	2.5	-0.5	400	500	3	C4	1
78	M	-1	-2.5	675	350	3	C4	1
79	M	4	0	350	550	3	C4	1
81	M	1.5	-1	1200	1200	3	C7	1
82	M	1.5	0	275	350	3	C7	1
83	F	6	-1	225	150	3	C7	1
84	M	1.5	-4	150	200	4	C2	1
88	F	.	-1.5	50	.	4	C2	1
90	M	4.5	-0.5	700	250	3	C3	1
91	M	2.5	-2	300	400	3	C3	1
92	F	2.5	-2.5	0	0	3	C3	1
97	F	1	-2.5	50	0	2	Ch	1
98	F	4.5	-1	250	200	2	Ch	1
103	F	3	0	300	300	2	C6	1
107	F	2	-2.5	50	50	2	T	1
108	F	2.5	-1.5	325	200	2	T	1
109	F	-2	0	100	200	2	C4	1
110	M	2	-2	50	0	3	C2	1
111	M	2	1	375	250	3	C2	1
114	F	6	1.5	50	0	.	C2	1
115	M	6	4	425	250	2	T	1
116	M	5.5	0	0	0	2	T	1
117	F	4.5	0.5	150	250	1	T	1
118	M	7	1.5	75	50	3	C2	1
119	M	7	1.5	50	50	3	C2	1
120	F	2	0	175	350	3	C2	1
126	M	4	0	150	200	2	C3	1
127	F	4	-0.5	100	100	2	C3	1
123	M	3	1	50	0	3	C7	1
124	F	5	0	125	150	3	C7	1
125	F	3.5	-2	275	150	3	C7	1
129	M	3	-1	625	800	3	C5	1
130	M	5	0.5	150	100	3	C5	1

Table 7 continued

Lamb	Sex	LWGTOT	LWG1	FEC	FEC1	LSR	Sire	Gp
131	F	3.5	-1.5	250	350	3	C5	1
132	M	.	.	425	550	3	Ch	1
134	F	1.5	-2	100	150	3	Ch	1
135	M	1.5	-2.5	475	550	3	Ch	1
139	F	3	0	75	0	2	Ch	1
140	F	4	-1	100	50	2	Ch	1
143	M	.	.	300	300	2	Ch	1
146	F	3	-0.5	200	250	2	T	1
147	F	2.5	0.5	225	300	2	T	1
148	M	0.5	-2.5	225	100	2	Ch	1
149	M	.	-3	0	0	5	C6	1
150	M	4	-1	650	400	5	C6	1
152	F	0.5	-2	200	.	5	C6	1
153	F	4	0	350	250	5	C6	1
154	F	2.5	-0.5	25	0	1	C6	1

Appendix Table 8 : FEC and live weights for group 2, year 2 lambs

Lamb No.	Sex	LWGTOT	LWG1	FEC	FEC1	LSR	Sire	Gp
160	M	.	.	100	.	2	C5	2
161	F	.	.	175	150	2	C5	2
165	M	.	.	100	150	1	C5	2
167	F	.	.	400	600	2	C5	2
168	M	.	.	100	100	2	T	2
169	M	.	.	150	200	2	T	2
170	M	.	.	175	150	3	C2	2
171	M	.	.	200	150	3	C3	2
172	F	.	.	1700	1550	3	C3	2
173	M	.	.	425	300	2	C5	2
174	M	.	.	375	300	2	C5	2
176	M	.	.	675	600	3	C4	2
177	F	.	.	1500	2100	3	C4	2
178	F	.	.	600	650	3	C4	2
179	M	.	.	100	50	3	C6	2
180	F	.	.	900	500	3	C6	2
181	F	.	.	250	200	3	C6	2
182	M	.	.	425	400	3	C1	2
183	M	.	.	250	250	3	C1	2
184	F	.	.	425	300	3	C1	2
185	M	.	.	250	250	2	Ch	2
186	F	.	.	175	100	2	Ch	2
187	M	.	.	225	200	2	Ch	2
188	F	.	.	375	350	2	Ch	2
190	M	.	.	625	200	2	Ch	2
191	F	.	.	100	100	2	Ch	2
197	M	.	.	500	600	3	C5	2
198	M	.	.	275	350	3	C5	2
199	F	.	.	125	150	3	C5	2
205	M	.	.	675	850	3	C6	2
206	F	.	.	550	200	3	C6	2
210	M	.	.	50	.	2	Ch	2
208	M	.	.	225	200	2	C10	2
209	F	.	.	675	750	2	C10	2
213	F	.	.	150	.	3	C4	2
214	F	.	.	1100	.	3	C4	2
215	F	.	.	400	.	3	C4	2
216	M	.	.	875	900	2	C8	2
217	F	.	.	0	0	2	C8	2

Table 8 continued

Lamb No.	Sex	LWGTOT	LWG1	FEC	FEC1	LSR	Sire	Gp
219	M	.	.	200	.	3	Ch	2
220	F	.	.	900	150	3	Ch	2
223	F	.	.	475	350	2	T	2
224	F	.	.	525	600	2	T	2
227	F	.	.	100	100	2	C1	2
228	F	.	.	200	.	2	C1	2
229	M	.	.	450	.	1	C1	2
230	M	.	.	900	.	3	C4	2
231	F	.	.	525	650	3	C4	2
232	F	.	.	250	250	3	C4	2
234	M	.	.	300	.	2	C4	2
236	M	.	.	375	250	2	C8	2
237	F	.	.	925	1000	2	C8	2
240	M	.	.	1250	100	2	T	2
241	M	.	.	875	700	2	T	2
242	F	.	.	50	0	2	T	2
246	M	.	.	550	.	2	C9	2
247	F	.	.	775	800	2	C9	2
249	M	.	.	425	300	3	C6	2
250	M	.	.	650	500	3	C6	2
251	M	.	.	975	1200	3	C6	2
252	M	.	.	50	50	1	C8	2
253	M	.	.	1300	.	3	C10	2
254	F	.	.	450	250	3	C10	2
255	F	.	.	2150	1200	3	C10	2
256	M	.	.	325	400	2	C10	2
257	M	.	.	675	450	2	C10	2
259	M	.	.	325	.	2	C10	2
260	F	.	.	50	0	2	C10	2
261	F	.	.	600	700	3	C4	2
262	F	.	.	1800	1450	3	C4	2
263	F	.	.	500	350	3	C4	2
264	M	.	.	200	200	3	T	2
265	F	.	.	325	200	3	T	2
266	F	.	.	400	500	3	T	2
270	M	.	.	100	.	2	Ch	2
272	M	.	.	475	400	2	C1	2
273	M	.	.	350	300	2	C1	2
274	M	.	.	50	.	3	C5	2
275	M	.	.	100	.	3	C5	2

Table 8 continued

Lamb	Sex	LWGTOT	LWG1	FEC	FEC1	LSR	Sire	Gp
277	M	.	.	100	100	3	T	2
278	F	.	.	0	.	3	T	2
279	F	.	.	250	.	3	T	2
280	M	.	.	0	.	2	T	2
282	M	.	.	350	.	2	T	2
285	M	.	.	1300	.	3	Ch	2
286	M	.	.	1050	850	3	Ch	2
287	M	.	.	150	.	3	Ch	2
284	F	.	.	1650	800	1	C5	2

Appendix Table 9 : Live weights for group 1, year 3 lambs

Lamb No.	Sex	Pre-weaning FEC	LWG1	LWG2	LWG3	LWG4	Gp
1	M	250	24	34.5	40	43	1
2	M	200	25	26	39	44	1
3	M	100	24.5	37	41	45.5	1
4	M	.	24.5	35	38.5	43	1
5	M	300	25.5	33.5	37	39	1
6	M	150	25	38	41.5	44	1
7	F	150	23.5	32.5	36.5	38.5	1
8	F	100	22	30.5	32.5	32	1
9	M	100	19.5	28.5	33	36.5	1
10	M	0	20.5	31	35.5	38.5	1
11	F	400	21.5	31.5	35	37	1
12	F	100	23	32.5	35	37	1
15	F	50	18.5	27.5	31	34	1
16	F	700	17.5	26.5	31	33	1
17	F	250	24	32.5	37	38	1
23	F	200	19	28.5	29.5	33	1
24	F	350	18.5	27.5	30	29	1
25	F	0	21.5	31	36	38	1
29	M	50	24.5	35	39	44	1
30	F	100	22.5	29.5	33	33.5	1
31	M	100	25	35	38	41.5	1
32	M	700	25	33	39	41.5	1
33	M	150	26.5	38.5	43	46	1
38	M	100	19.5	28	31	36	1
39	M	250	25.5	36.5	40.5	43	1
40	M	200	21.5	32.5	37	41	1
41	M	150	29.5	41.5	47	48.5	1
42	F	450	21.5	33.5	35.5	37.5	1
43	F	400	25	34.5	37	40.5	1
44	M	350	24.5	34.5	39	44	1
45	F	100	22.5	31.5	36.5	40	1
47	M	150	19	27.5	31	34.5	1
48	M	550	17	28	32	33	1
49	F	0	21.5	33	36	38	1
50	F	200	19.5	28.5	33	32.5	1
52	M	150	21.5	33	32	39	1
53	F	250	18	24.5	26.5	26.5	1
54	M	200	24.5	36.5	41.5	45	1
55	F	50	23.5	31.5	36	39	1
56	F	250	23	33	33	36.5	1

Table 9 continued

Lamb No.	Sex	Pre-weaning FEC	LWG1	LWG2	LWG3	LWG4	Gp
60	M	50	20	31	35	37	1
61	F	400	20	30.5	34	35.5	1
62	F	200	22	29.5	33	35	1
68	M	150	21.5	33.5	39	41	1
71	M	350	31.5	42.5	48	52	1
73	M	150	25.5	36.5	41	43	1
74	F	150	21	32	36	36.5	1
75	F	500	27	38.5	40.5	40	1
76	M	.	22.5	33.5	38	.	1
78	F	100	18.5	27.5	32.5	33.5	1
79	F	.	23.5	32	34.5	36.5	1
80	M	150	22.5	33.5	36.5	39.5	1
81	M	200	23.5	33	36	38	1
82	M	300	21	33	36.5	40	1
83	M	.	21.5	31.5	35.5	40	1
84	F	.	18.5	26.5	30	32.5	1
86	F	250	17.5	27.5	30.5	30.5	1
87	F	150	16.5	25.5	29	31	1
91	M	0	24	38.5	40.5	43	1
92	M	0	24.5	36	41.5	44	1
93	M	400	23.5	34.5	40	42	1
94	F	100	19.5	29.5	33	34.5	1
95	M	200	15.5	25.5	31.5	30.5	1
97	M	150	20	33	38	40	1
77	M	150	31	32	35	38.5	1
98	M	350	20.5	31.5	35	39.5	1
99	F	150	20.5	30.5	34	34	1
100	M	50	21.5	31.5	34.5	36.5	1
101	F	400	19	28	32.5	34.5	1
102	F	450	19	26.5	30.5	33	1
103	F	50	22.5	32.5	37	39	1
104	M	750	21.5	32.5	37	39.5	1
105	M	.	23	32.5	37	40	1
106	F	50	20.5	30.5	34.5	36	1
107	M	50	29	41	45	48.5	1
108	M	100	19	32.5	36	39.5	1
109	M	100	18.5	26.5	29	30	1
110	F	200	19	28.5	32.5	32.5	1
114	M	0	20.5	30	32	35	1
115	M	150	17.5	27.5	32	36	1

Table 9 continued

Lamb No.	Sex	Pre-weaning FEC	LWG1	LWG2	LWG3	LWG4	Gp
116	F	50	18.5	30	34	39.5	1
117	M	250	27	39.5	44	47.5	1
118	M	.	24.5	35.5	40.5	44	1
119	M	.	20.5	30	34	39.5	1
120	F	350	21.5	36.5	41.5	45.5	1
124	F	0	20	31.5	36	37	1
125	F	200	18	30.5	32.5	34.5	1
126	F	100	15	24.5	28	31	1
128	M	50	27	34.5	37.5	40.5	1
69	F	0	22.5	31.5	36	39	1
130	F	200	.	29.5	33.5	36.5	1
129	M	200	.	.	.	37	1

Appendix Table 10 : Live weights for group 2, year 3 lambs

Lamb No.	Sex	Pre-weaning FEC	LWG1	LWG2	LWG3	LWG4	Gp
138	F	0	.	38	.	36.5	2
139	F	0	.	32	.	33	2
140	F	0	.	27.5	.	30	2
141	F	100	.	35	.	35.5	2
143	M	100	.	35.5	.	37	2
145	F	50	.	28.5	.	33	2
150	M	.	.	.	.	31.5	2
111	M	200	.	29	.	31	2
112	M	.	.	28	.	30	2
151	M	100	.	33.2	.	36	2
152	F	0	.	.	.	24.5	2
154	M	300	.	33.5	.	36.5	2
155	M	.	.	38.5	.	40.5	2
156	F	200	.	33	.	37	2
157	M	.	.	29.5	.	32.5	2
158	F	300	.	30.5	.	34	2
159	M	100	.	33.5	.	35.5	2
160	M	.	.	25.5	.	30	2
161	F	0	.	25.5	.	26	2
162	F	0	.	26	.	28.5	2
164	F	0	.	35.5	.	37	2
165	F	0	.	26.5	.	29.5	2
166	F	100	.	26.5	.	28.5	2
167	F	0	.	29	.	30	2
171	F	400	.	34	.	35.5	2
172	F	50	.	32	.	35.5	2
173	M	350	.	34	.	36.5	2
176	F	200	.	26	.	29.5	2
177	F	.	.	.	.	24	2
179	M	750	.	22	.	27	2
181	M	1400	.	21	.	24	2
184	M	0	.	29	.	33.5	2
190	M	100	.	30	.	.	2
191	M	200	.	23.5	.	27	2
200	M	0	.	38	.	41.5	2
201	M	1300	.	31.5	.	33.5	2
202	M	600	.	27	.	.	2
208	M	400	.	39.5	.	41.5	2
219	M	.	.	.	.	.	2
226	M	150	.	32	.	38	2

Table 10 continued

Lamb No.	Sex	Pre-weaning FEC	LWG1	LWG2	LWG3	LWG4	Gp
243	F	300	.	20.5	.	22.5	2
244	F	950	.	20.5	.	24.5	2
245	F	450	.	23	.	0	2
260	M	300	.	25.5	.	30.5	2
261	F	0	.	30	.	34.5	2
268	F	50	.	27	.	27.5	2
269	F	50	.	27	.	29.5	2
259	.	1200	.	23.5	.	27	2

Appendix Table 11 : Live weights for group 3, year 3 lambs

Lamb No.	Sex	Pre-weaning FEC	LWG1	LWG2	LWG3	LWG4	Gp
287	M	450	.	25	30	30.5	3
288	M	.	.	18	25	25.5	3
289	F	650	.	29	29	29.5	3
290	M	800	.	21	24	24.5	3
292	M	350	.	24	29	29.5	3
293	M	300	.	25	28.5	28.5	3
297	F	.	.	20	23.5	23.5	3
298	F	5400	.	18	19.5	20.5	3
301	M	700	.	31	33.5	33.5	3
302	F	0	.	22	25	25	3
319	M	0	.	16	19	19	3
320	F	0	.	15	17	17.5	3
321	M	50	.	21.5	23.5	24	3
324	F	0	.	16	19.5	19.5	3
333	F	450	.	22.5	25	25	3
335	M	500	.	18	23.5	23.5	3
336	F	850	.	18	18	19.5	3
206	F	200	.	36	40	40	3
207	F	100	.	19	24	22.5	3
216	M	500	.	22.5	28	28.5	3
218	F	250	.	21.5	23.5	24	3
224	M	700	.	23.5	26	26	3
225	F	350	.	21	24	25	3
228	M	350	.	26	30.5	30.5	3
229	F	1000	.	17	20.5	21	3
232	F	600	.	22.5	24.5	23.5	3
234	F	.	.	17	19.5	19.5	3
235	F	2400	.	22	24	24	3
237	M	150	.	24	31.5	31.5	3
239	M	650	.	30.5	34	34.5	3
241	F	200	.	25	27.5	27.5	3
242	F	650	.	25	29	29	3
246	M	500	.	20	25.5	25.5	3
247	M	550	.	21.5	24.5	24.5	3
253	F	500	.	20	25.5	25.5	3
254	F	1300	.	22	26	25.5	3
255	F	200	.	28	31.5	31.5	3
256	F	300	.	26.5	31	30.5	3
257	M	550	.	25	31.5	32.5	3
258	M	550	.	30	33	33.5	3

Table 11 continued

Lamb No.	Sex	Pre-weaning	LWG1	LWG2	LWG3	LWG4	Gp
263	M	1150	.	18.5	19.5	19.5	3
264	M	1400	.	18	24	18.5	3
279	M	500	.	29	34	32.5	3
280	M	450	.	26	30.5	30	3
221	M	950	.	28	31	31.5	3
259	F	1200	.	23.5	.	27	3

Appendix Table 12 : FEC for group 1, year 3 lambs

Lamb No.	FEC Post1	Faecal Score1	FEC Post2	Faecal Score2	LSR	SIRE	Gp
1	300	3	250	3	2	C5	1
2	150	4	150	2	2	C5	1
3	100	4	50	3.5	1	C3	1
4	50	5	250	4	2	C10	1
5	250	5	300	4	2	C10	1
6	250	5	200	.	3	C8	1
7	0	5	50	4	3	C8	1
8	0	2	0	1.5	3	C8	1
9	150	3.5	0	3.5	1	C3	1
10	0	.	50	4	1	C3	1
11	0	5	300	4	2	C11	1
12	50	5	50	5	2	C11	1
15	150	3	150	4	1	C8	1
16	450	3	400	3	2	C8	1
17	250	.	100	4	2	C8	1
23	350	4.5	350	4	3	C5	1
24	550	5	650	.	3	C5	1
25	100	5	200	4	3	C5	1
29	300	.	250	5	2	C6	1
30	0	5	100	5	2	C6	1
31	0	5	0	4	3	C8	1
32	50	5	100	3.5	3	C8	1
33	300	5	250	2.5	3	C8	1
38	450	4	200	3	3	C3	1
39	100	5	50	4	3	C3	1
40	250	5	300	5	3	C3	1
41	400	5	300	.	2	C6	1
42	150	4	150	3.5	2	C6	1
43	0	4.5	100	3.5	1	C4	1
44	600	4	450	3.5	2	C10	1
45	300	5	350	4	2	C10	1
47	250	4	300	2	3	C4	1
48	400	4.5	350	4	3	C9	1
49	100	5	200	4.5	3	C9	1
50	600	5	300	5	3	C9	1
52	400	.	350	1.5	3	C4	1
53	200	4.5	200	3.5	3	C4	1
54	200	5	350	3.5	3	C11	1
55	250	2.5	200	3.5	3	C11	1
56	400	5	400	5	3	C11	1

Table 12 continued

Lamb No.	FEC Post1	Faecal Score1	FEC Post2	Faecal Score2	LSR	SIRE	Gp
60	100	5	50	5	3	C14	1
61	400	5	400	5	3	C14	1
62	250	5	300	4.5	3	C14	1
68	400	2	350	2.5	2	C9	1
71	200	5	150	4	2	C8	1
73	100	5	50	4	3	C6	1
74	200	5	150	5	3	C6	1
75	100	5	100	5	3	C6	1
76	150	4	100	5	3	C6	1
78	150	5	350	5	3	C6	1
79	250	5	250	4.5	3	C6	1
80	1000	1	600	3	2	C9	1
81	0	5	100	5	2	C9	1
82	350	4	450	4	2	C10	1
83	500	5	500	3	2	C10	1
84	300	.	350	3.5	1	C10	1
86	250	4	200	2	3	C6	1
87	100	5	150	5	3	C6	1
91	950	5	600	5	2	C11	1
92	750	3.5	350	1.5	2	C11	1
93	150	4	250	3.5	2	C4	1
94	0	5	50	3.5	2	C4	1
95	100	2.5	100	1	1	C3	1
97	250	4.5	250	5	3	C6	1
77	300	4	250	3	.	C6	1
98	300	4	200	3	3	C6	1
99	1000	5	900	5	3	C6	1
100	50	4	150	2	3	C6	1
101	200	5	250	4.5	3	C6	1
102	250	5	200	3.5	3	C6	1
103	50	5	50	3	1	C1	1
104	450	5	300	4.5	4	C9	1
105	150	5	150	4	4	C9	1
106	200	5	300	4	4	C9	1
107	250	5	300	5	4	C9	1
108	250	5	100	4.5	3	C9	1
109	100	5	250	5	3	C9	1
110	250	4.5	300	3.5	3	C9	1
114	50	3.5	200	3.5	3	C1	1
115	400	5	350	3.5	3	C1	1

Table 12 continued

Lamb No.	FEC Post1	Faecal Score1	FEC Post2	Faecal Score2	LSR	SIRE	Gp
116	150	5	200	3.5	3	C1	1
117	300	4	400	4.5	3	C8	1
118	100	5	100	4	3	C8	1
119	650	5	450	3	3	C8	1
120	300	4.5	200	4	1	C5	1
124	0	4	50	5	3	C4	1
125	100	4	250	4	3	C4	1
126	50	3.5	100	3	3	C4	1
128	600	5	250	4	1	C4	1
69	0	3.5	0	2.5	.	C9	1
130	100	.	150	4	1	C3	1
129	50	5	100	5	1	C8	1

Appendix Table 13 : FEC for group 2, year 3 lambs

Lamb No.	FEC Post1	Faecal Score1	FEC Post2	Faecal Score2	LSR	SIRE	Gp
138	0	1.5	0	.	4	C1	2
139	50	1	0	1	4	C1	2
140	0	.	0	.	4	C1	2
141	0	3	0	3	4	C1	2
143	50	4	50	5	3	C1	2
145	0	1.5	0	1.5	3	C1	2
150	0	.	0	.	1	C1	2
111	200	.	100	5	2	C1	2
112	0	.	50	3	2	C1	2
151	300	4	100	5	2	C1	2
152	0	.	0	5	2	C1	2
154	0	5	0	4	3	C3	2
155	0	2	0	2	3	C3	2
156	400	5	250	4	3	C3	2
157	0	3	150	1	2	C7	2
158	200	4	100	4.5	2	C7	2
159	50	3	0	3	1	C13	2
160	150	5	100	5	3	C2	2
161	0	3	50	4	3	C2	2
162	0	5	0	5	3	C2	2
164	0	4.5	0	2	1	C13	2
165	0	5	0	2	3	C2	2
166	0	2	0	3	3	C2	2
167	0	1	0	1	3	C2	2
171	100	5	250	5	1	C13	2
172	50	4	50	2	1	C12	2
173	50	5	50	5	1	C12	2
176	50	5	50	3	4	C10	2
177	0	.	50	4	4	C10	2
179	700	5	1350	4	3	C14	2
181	900	3	1100	5	3	C14	2
184	500	5	900	4	1	C13	2
190	0	5	50	5	3	C5	2
191	0	4.5	0	4.5	3	C5	2
200	0	3	0	3.5	1	C1	2
201	1000	2.5	1100	3	2	C14	2
202	300	5	100	.	2	C14	2
208	150	5	450	5	1	C11	2
219	100	.	150	.	1	C5	2
226	100	4.5	100	4	1	C5	2

Table 13 continued

Lamb No.	FEC Post1	Faecal Score1	FEC Post2	Faecal Score2	LSR	SIRE	Gp
243	0	2	0	2	2	C5	2
244	0	5	0	5	2	C5	2
245	500	.	0	4.5	1	C5	2
260	300	5	100	4	3	C5	2
261	0	3	0	3	3	C5	2
268	0	5	0	5	2	C13	2
269	50	5	300	5	1	C13	2
259	700	5	900	4	1	C10	2

Appendix Table 14 : FEC for group 3, year 3 lambs

Lamb No.	FEC Post1	Faecal Score1	FEC Post2	Faecal Score2	LSR	SIRE	Gp
287	800	2.5	900	4	2	C10	3
288	100	2.5	600	4	2	C10	3
289	1150	3	850	4	1	C14	3
290	400	1.5	600	1	2	C5	3
292	50	1	50	3.5	2	C5	3
293	250	1	400	1	1	C5	3
297	2400	5	1950	5	2	C14	3
298	2750	5	2500	3.5	2	C14	3
301	900	4	1050	3.5	1	C13	3
302	450	1	450	1	1	C13	3
319	1850	2.5	1050	1	3	C13	3
320	1300	1.5	3000	1.5	3	C13	3
321	100	1.5	350	5	3	C13	3
324	100	1	300	4	3	C13	3
333	400	2	400	2	1	C13	3
335	650	2	950	2	2	.	3
336	650	1.5	1000	3.5	2	.	3
206	150	4.5	150	5	4	C10	3
207	50	2	50	3	4	C10	3
216	50	2	100	3.5	2	C2	3
218	50	5	150	5	2	C2	3
224	400	4	500	4	1	C5	3
225	750	3	700	3	1	C5	3
228	350	5	350	5	2	C10	3
229	500	2	.	.	2	C10	3
232	100	4	400	4.5	2	C12	3
234	1000	3	1100	1	3	C14	3
235	2600	1.5	650	2	3	C14	3
237	.	.	250	2	1	C12	3
239	450	5	900	4	2	C2	3
241	100	5	400	5	2	C12	3
242	700	4	100	4	2	C12	3
246	550	3	400	3	2	C13	3
247	100	3.5	200	3	2	C13	3
253	550	.	1200	1.5	2	C12	3
254	250	3	400	3	2	C12	3
255	500	3.5	950	4	2	C10	3
256	150	2.5	200	4.5	2	C10	3
257	0	3	0	4	1	C2	3
258	50	3.5	50	5	2	C10	3

Table 14 continued

Lamb No.	FEC Post1	Faecal Score1	FEC Post2	Faecal Score2	LSR	SIRE	Gp
263	700	5	500	5	2	C14	3
264	750	2.5	1000	5	2	C14	3
279	400	1.5	100	1.5	2	C10	3
280	200	3	150	5	2	C10	3
221	300	.	400	.	2	C2	3
259	700	5	900	4	2	C10	3

Appendix Table 15 : FEC for experimentally infected lambs .

Lamb No.	FEC1	FEC2	FEC3	FEC4	FEC5	FEC6	FEC7	FEC8
16	100	100	75	100	75	100	75	50
26	125	125	75	100	150	150	125	100
28	475	375	375	225	200	250	225	175
29	325	225	100	200	375	300	150	125
31	450	225	125	200	325	225	75	75
32	100	75	50	75	25	50	100	75
57	600	200	175	100	175	175	100	75
58	125	200	300	400	250	175	150	50
66	100	200	350	325	325	200	175	225
78	75	175	200	175	125	100	275	275
81	375	500	425	250	150	100	25	175
82	500	500	325	200	150	175	225	175
83	75	125	200	325	250	150	125	50
123	300	325	425	600	650	525	275	100
124	75	225	250	125	50	100	175	75
125	0	0	100	175	100	125	125	25
130	225	225	150	175	175	150	150	125
131	100	50	50	175	225	150	125	75
149	50	75	125	150	100	50	75	100
150	200	300	275	275	150	25	75	125
151	100	250	300	125	150	175	75	100
173	100	125	175	150	125	100	50	50
174	100	75	125	125	125	75	25	25
176	225	350	625	700	350	250	350	250
178	100	100	100	150	200	150	125	100
179	200	150	75	125	125	100	150	200
180	525	675	425	175	125	125	150	200
181	75	200	225	125	100	25	125	250
198	150	150	200	225	150	125	75	75
199	125	75	100	300	425	225	125	100
209	200	125	125	225	175	50	75	100
215	150	150	125	125	175	125	75	100
230	100	100	175	175	150	75	100	75
250	150	125	125	125	150	150	175	125
253	150	150	200	300	375	300	300	425
254	225	300	325	200	25	M	50	100
255	75	25	75	200	225	100	M	50
256	125	125	125	375	475	225	100	100
262	150	125	25	200	225	125	175	75
263	275	225	200	100	50	25	125	150

Table 15 continued

Lamb No.	FEC1	FEC2	FEC3	FEC4	FEC5	FEC6	FEC7	FEC8
274	0	25	25	50	150	125	125	125
275	100	175	275	300	225	200	200	125
292	50	125	150	75	200	175	50	75
302	0	0	175	300	275	300	275	225
304	300	250	300	275	175	100	75	200
310	125	225	275	125	100	125	150	175
313	50	75	175	250	175	125	225	175
314	200	375	350	200	200	175	125	175
315	275	325	175	375	375	175	150	125
322	175	225	225	150	125	250	250	225
324	150	300	275	125	125	125	50	50
325	50	50	125	175	150	125	150	175
328	225	225	125	25	100	150	100	50
341	175	100	175	225	175	100	75	100
344	200	125	100	75	50	25	75	50
345	150	275	175	200	275	175	75	100
347	100	25	75	275	300	100	25	75
350	300	175	175	225	175	100	125	125
358	175	325	300	325	125	75	275	275
359	325	75	250	325	275	225	75	0
362	125	350	200	25	175	200	75	75
365	300	275	225	175	125	200	250	250
338	700	300	300	0	200	325	200	75
363	150	200	200	125	75	100	200	3050

Appendix Table 16 : FEC for the experimentally infected lambs

Lamb No.	Sex	Mean FEC	Field FEC	Single/ Trickle	Plasma pep	Field H/L	Sire
16	F	83	75	T	284.95	l	23
26	M	117	250	T	260.63	l	20
28	F	300	575	S	215.45	h	20
29	F	222	400	S	32.43	l	20
31	M	228	1700	T	271.05	h	20
32	M	66.7	325	T	371.83	l	20
57	M	172.2	1875	S	635.93	h	23
58	M	188.9	550	S	281.48	h	23
66	M	211	25	T	396.15	l	47
78	M	161	675	T	309.15	h	47
81	M	255.6	1200	S	477.2	h	23
82	M	277.8	275	S	523.6	l	23
83	F	150	225	T	634.77	l	23
123	M	377.8	50	T	597.7	l	23
124	F	122.2	125	T	344.03	l	23
125	F	72.2	275	T	628.98	l	23
130	M	166.7	150	S	500.5	l	32
131	F	116.7	250	T	458.7	l	32
149	M	88	1	T	481.9	l	20
150	M	166.7	650	S	375.3	h	20
151	F	155.6	200	S	653.3	l	20
173	M	105.6	425	S	135.5	l	32
174	M	344.4	375	S	518.9	l	32
176	M	144.4	675	T	378.8	h	47
178	F	133.3	600	S	505.03	h	47
179	M	194.4	100	S	769.13	l	20
180	F	300	900	S	583.8	h	20
181	F	144.4	250	T	472.6	l	20
198	M	144.4	275	S	521.3	l	32
199	F	177.8	125	T	521.25	l	32
209	F	138.9	675	T	291.9	h	23
215	F	138.9	400	S	423.95	l	47
230	M	127.8	900	T	337.1	h	47
250	M	138.9	650	S	364.8	h	20
253	M	277.8	1300	T	491.13	h	23
254	F	238.9	450	S	1303.6	h	23
255	F	94.4	2150	S	681.1	h	23
256	F	194.4	325	T	486.5	l	23
262	F	127.8	1800	S	1035.55	h	47
263	F	150	500	S	114.68	h	47

Table 16 continued

Lamb No.	Sex	Mean FEC	Field FEC	Single/ Trickle	Plasma pep	Field H/L	Sire
274	M	72.2	50	T	469.1	l	32
275	M	188.9	100	T	531.68	l	32
292	F	105.6	650	T	156.38	h	20
302	F	183.3	3550	T	559.7	h	32
304	F	216.7	350	S	427.4	l	32
310	F	155.6	300	S	444.8	l	32
313	F	144.4	50	T	340.6	l	47
314	F	222.2	700	T	312.8	h	47
315	F	227.8	50	T	285	l	47
322	F	222.2	550	T	114.68	h	20
324	F	138.9	700	S	139	h	23
325	F	122.2	550	T	358	h	20
328	F	127.8	50	S	396.2	l	32
341	M	144.4	950	S	500.2	h	20
344	M	94.4	1550	T	493.4	h	32
345	M	155.6	350	S	239.8	l	47
347	M	122.2	1500	T	462.2	h	23
350	F	183.3	400	S	444.75	l	23
358	M	277.8	1950	S	430.9	h	47
359	M	161.2	.	S	392.7	l	47
362	M	177.8	100	S	261.4	l	47
365	F	205.6	1350	T	271.1	h	32
338	F	316.7	800	S	194.6	h	32
363	M	155.56	282.6	T	.	h	47

Appendix Table 17 : Plasma pepsinogen estimates for the experimentally infected lambs

Lamb No.	PP1	PP2	PP3	PP4	PP5	Single/ Trickle
16	132	69.5	361	125	584	T
26	35	403	167	28	445	T
28	257	417	139	0	306	S
29	90	0	0	.	97	S
31	382	487	83	83	431	T
32	354	320	667	195	306	T
57	785	862	681	348	653	S
58	438	459	278	195	195	S
66	281	626	334	14	612	T
78	785	695	264	97	181	T
81	354	737	417	278	.	S
82	910	1056	278	236	.	S
83	271	792	306	.	806	T
123	507	834	695	83	778	T
124	281	139	236	639	361	T
125	243	917	945	111	542	T
130	841	.	598	375	528	S
131	855	737	14	348	737	T
149	980	.	542	278	626	T
150	521	348	403	292	.	S
151	354	1348	459	389	417	S
173	966	125	97	56	261	S
174	660	890	473	.	195	S
176	382	83	500	320	612	T
178	271	339	723	.	403	S
179	341	890	375	.	1043	S
180	354	959	431	403	542	S
181	549	292	653	306	639	T
198	174	1668	528	278	1265	S
199	35	639	222	514	709	T
209	1911	0	487	195	487	T
215	438	959	83	97	556	S
230	90	375	389	278	306	T
250	0	598	403	209	250	S
253	295	931	181	.	361	T
254	1188	2641	917	737	931	S
255	813	1029	639	334	723	S
256	63	528	528	500	389	T
262	841	2363	264	681	834	S
263	116	42	209	209	0	S

Table 17 continued

Lamb No.	PP1	PP2	PP3	PP4	PP5	Single/ Trickle
274	549	1043	264	361	209	T
275	.	1168	320	278	361	T
292	118	278	83	28	236	T
302	227	403	1195	.	81	T
304	493	459	431	209	612	S
310	1242	445	681	.	209	S
313	229	278	584	222	278	T
314	0	612	306	42	292	T
315	0	0	500	0	639	T
322	521	139	181	28	250	T
324	0	1112	320	97	28	S
325	202	361	584	153	334	T
328	561	.	681	.	111	S
341	771	681	598	361	334	T
344	146	487	181	236	56	S
345	160	848	529	292	181	T
347	427	598	542	56	584	S
350	549	612	473	209	.	S
358	262	751	348	209	264	S
359	275	500	195	278	83	S
362	130	375	292	167	250	T
365	867	500	348	.	0	T
338	382	583	904	222	292	S
363	90	70	111	361	236	S

Appendix Table 18 : FEC of the experimentally infected lambs on the two different concentrate diets

Lamb No.	FEC								
	1	2	3	4	5	6	7	8	9
16	100	100	0	100	100	300	200	400	150
28	200	100	600	600	900	500	600	200	0
32	0	0	400	300	500	500	650	400	300
81	100	0	0	500	200	350	200	300	250
82	300	300	200	600	200	300	100	200	150
125	100	100	200	200	400	350	600	.	.
173	300	50	300	250	300	200	200	350	100
149	200	400	300	100	200	150	200	400	0
180	300	400	550	700	500	250	150	300	200
253	700	1000	400	700	700	1050	500	700	700
254	50	0	0	500	200	300	100	400	300
255	200	200	400	200	200	700	500	100	400
274	100	100	400	400	600	300	100	300	100
292	100	200	200	100	300	250	100	200	0
304	0	0	0	250	150	150	0	0	0
315	100	200	200	200	400	750	0	300	0
328	300	50	0	0	0	0	0	0	0
341	200	100	400	200	0	200	0	.	.
345	0	100	0	200	0	150	100	.	.
350	400	500	600	500	800	450	450	.	.
262	200	100	700	300	500	600	600	.	.
174	200	100	200	100	100	400	200	200	0
123	200	150	200	200	500	100	400	500	100

Appendix Table 19 : Sex, diet quantity and previous challenge response of the experimentally infected lambs on the two different concentrate diets

Lamb No.	Sex	Diet	Field FEC
16	F	H	L
28	F	L	H
32	M	H	L
81	M	H	H
82	M	H	L
125	F	L	L
173	F	L	L
149	F	L	L
180	M	L	H
253	M	L	H
254	M	H	L
255	F	L	H
274	M	L	L
292	F	H	H
304	F	L	L
315	F	H	L
328	F	H	L
341	M	H	L
345	M	H	H
350	M	L	H
262	F	L	H
174	M	L	L
123	M	H	L

Appendix Table 20 : FEC for the lambs infected with a primary experimental dose of *Ostertagia circumcincta*

Lamb	EPG Prim 1	EPG Prim 2	EPG Prim 3	EPG Prim 4
21	700	200	100	0
22	100	100	0	0
26	1100	400	100	0
46	400	600	1400	2000
134	1200	600	400	100
146	0	0	0	0
148	400	0	100	100
204	200	200	200	600
182	400	200	400	200
183	200	100	300	300
240	700	400	500	600
0	100	0	600	1000
306	100	200	100	0
262	0	100	0	0
272	0	150	0	100
308	350	300	250	100
311	300	300	150	0

Appendix Table 21 : FEC for the lambs infected with primary and secondary experimental doses of *Ostertagia circumcincta*

Lamb	EPG Sec 1	EPG Sec 1	EPG Sec 1	EPG Sec 1	EPG Sec 1	EPG Sec 1	EPG Sec 1	EPG Sec 1
21	0	100	0	300	300	200	300	100
22	0	0	0	400	200	0	100	100
26	0	100	200	0	0	400	700	200
46	800	0	300	100	800	600	500	600
134	400	0	100	0	700	1000	400	400
146	0	0	0	0	0	0	0	0
148	100	1000	0	0	100	100	0	0
204	500	1000	1000	100	700	1000	1200	800
182	0	100	150	100	300	300	200	400
183	0	100	200	300	0	200	0	100
240	700	700	300	200	100	700	200	100
0	0	500	500	0	1200	1200	400	0
306	0	200	0	100	300	200	0	0
262	0	100	200	200	100	0	0	0
272	0	0	0	0	0	0	0	0
308	50	200	100	0	100	50	0	0
311	50	300	200	100	50	100	0	0

APPENDIX 3

EXAMPLES OF STATISTICAL ANALYSIS

An Example of a General Linear Models Procedure

Dependent Variable: POSTMEANL

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SEX	1	1.26871781	1.26871781	4.50	0.0417
SIRE	7	4.58623044	0.65517578	2.32	0.0490
SEX*SIRE	4	0.25345099	0.06336275	0.22	0.9225

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SEX	1	0.28626350	0.28626350	1.02	0.3211
SIRE	7	3.53690779	0.50527254	1.79	0.1231
SEX*SIRE	4	0.25345099	0.06336275	0.22	0.9225

An Example of a TTest Analysis

LH	N	Mean	Std Dev	Std Error	Minimum	Maximum
1	12	2.55591858	0.26394908	0.07619554	2.17609126	3.02118930
2	11	2.22041130	0.77322870	0.23313723	0.00000000	2.87506126

Variances	T	DF	Prob>|T|
Unequal	1.3679	12.1	0.1962
Equal	1.4182	21.0	0.1708

For H0: Variances are equal, F' = 8.58 DF = (10,11) Prob>F' = 0.0014

An Example of a Correlation Analysis

Pearson Correlation Coefficients / Prob > |R| under Ho: Rho=0 / N = 63

	ALL	LWG	PLASMA
ALL	1.00000 0.0	-0.14069 0.2714	0.04983 0.6981
LWG	-0.14069 0.2714	1.00000 0.0	-0.09746 0.4473
PLASMA	0.04983 0.6981	-0.09746 0.4473	1.00000 0.0

APPENDIX 4

PLASMA PEPSINOGEN ESTIMATION

(obtained from Glasgow University)

Reagents

2% Bovine Serum Albumin (BSA) Sigma Chemical Co. Ltd. No. A-4503

2N Hydrochloric Acid (HCl)

4% Trichloroacetic Acid (TCA)

0.25N Sodium Hydroxide (NaOH)

Folin-Ciocalteu's Reagent (Diluted 1+2 v/v with distilled water immediately before use) BDH Chemicals Ltd. No. 19058.

Tyrosine BDH Chemicals Ltd. Not less than 98.5%.

Stock Standard Tyrosine 1.812g Tyrosine in 1000 ml 0.1N HCl (10μ mols/ml)

Working Tyrosine Standards:-

(1) 10 ml Stock Standard Diluted to 1000 ml (2.0 ml Contains 0.2μ mols)

(2) 20 ml Stock Standard Diluted to 1000 ml (2.0 ml Contains 0.4μ mols)

Preparation of 2% Albumin

Weigh the required amount in a clean dry conical flask and then pour carefully down the side the required amount of distilled water so that the powered albumin floats on top of the water. Tap the flask gently, to spread the power evenly over the waters surface and leave, undisturbed, until the albumin has dissolved. this solution is reasonably stable but if not used within 2 - 3 days is best kept at -20°c.

Preparation of Acid/Albumin

Adjust the pH of 40 ml of 2% BSA to pH 1.5 with 2N HCl, and add distilled water to make the total volume up to 60 ml. This should be prepared fresh on the day of setting up the test. This is sufficient for 9 test sera plus appropriate controls any excess is discarded.

Procedure

(In 30 ml universal bottles)

Plasma tests

Two universals for each test, 1 labelled 'control' and the other 'test'. 2.5 ml acidified BSA and 0.5 ml of plasma in both universals. 'test' universal incubated for 24 hrs at 37°C, the 'control' immediately after the addition of plasma, is precipitated by the addition of 5.0 ml of 4% TCA.

BSA blanks (in duplicate, i.e. 4 universals)

2.5 ml acidified BSA and 0.5 ml distilled water in each universal. 2 universals (incubated-blanks) are incubated for 24 hrs at 37°C. The other 2 universals (unincubated-blanks) are immediately precipitated with 5.0 ml of 4% TCA.

Allow precipitated 'controls' and unincubated-blanks to stand for 10 minutes after mixing, to ensure efficient flocculation of the precipitate, and then filter through a Whatman No. 44 filter paper.

After the 24 hrs incubation period is completed, 5.0 ml of 4% TCA is added to each universal and undigested BSA is precipitated and filtered through a Whatman No. 44 filter paper. N.B. filtrates are stable and can be stored at 4°C at this stage until the incubated samples are also available.

Treatment of filtrates

Pipette 2.0 ml of each filtrate into suitably labelled flasks (50 ml conicals) containing 20 ml of 0.25N NaOH.

Set up flasks (in duplicate) containing 2.0 ml of each working tyrosine standard (i.e. 0.2μ mols and 0.4μ mols).

Set up reagent blank containing 2.0 ml distilled water with 20 ml 0.25N NaOH.

To all flasks add 3.0 ml diluted Folin-Ciocalteu's reagent. Allow to stand for 30 minutes, and then read the blue colour in a spectrophotometer at 725mμ. (colour is fairly stable but it is good practice to keep a standard time interval between addition of Folin-Ciocalteu's reagent and reading the resultant colour).

Calculation of results

1. Subtract 'reagent blank' from all spectrophotometer reading.

2. From 'tyrosine standards' calculate a factor for the conversion of all spectrophotometer readings to μ mols tyrosine and convert all readings into an equivalent 'μ mols tyrosine'.

3. If incubated BSA and plasma ('test') .....A

Non-incubated BSA and plasma ('controls') .....B

A - B = total release of tyrosine on incubation.

4. Incubated BSA alone ('incubated BSA-blank') C

Non-incubated BSA alone ('non-incubated BSA-blank').....D

C - D = tyrosine released from BSA substrate due to incubation alone (no pepsinogen).

5. (A - B) - (C - D) = tyrosine in μ mols released on incubation due to action of activated pepsinogen in 0.125 ml plasma in 24 hrs.

6. Calculate tyrosine in μ mols released by 1000 ml plasma per minute and multiply by 5.56.