PARASITISM AND PRODUCTION IN FLEECEWEIGHT-SELECTED AND CONTROL SHEEP

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Abstract

Internal parasitism in sheep selected for increased wool production for 37 years (FW) and in unselected controls (C) was studied. FW sheep were shown to consistently develop higher FECs than C sheep when grazing naturally infected pasture. Resistance to establishment of infective larvae of *Haemonchus contortus* and *Ostertagia circumcincta*, but not *Trichostrongylus colubriformis*, was lower in artificially infected adult FW sheep.

Several parasitological and immunological parameters were compared between lines. Antiparasite antibody levels in grazing lambs and artificially infected adult sheep did not differ between lines. There was a typical strong inverse relationship between numbers of mucosal mast cells and numbers of parasites in FW sheep, whereas no relationship was evident in C sheep. Packed cell volume was lower in FW sheep than C sheep and thymus weights were heavier in FW sheep. Blood gastrin levels tended to rise more in C sheep than FW sheep when infected.

Production loss associated with infection in each line was examined. Albendazole controlled release capsules (CRC) were used to prevent infection in some sheep from each line while the remainder were allowed to become subclinically infected. Despite FW sheep developing a larger burden than C sheep there was little evidence that this resulted in greater production loss in FW sheep. Production loss was not found to be associated with decreased feed intake.

Decreased resistance to internal parasites (of some species) following selection for increased wool production has been clearly demonstrated. This suggests that resistance traits and wool production are unfavourably genetically correlated, which will slow selection responses when all traits need to be simultaneously improved.

Despite the effectiveness of CRC treatment in preventing establishment of an adult parasite burden, production in treated sheep, particularly rams, was lower than

in subclinically infected sheep at some stages of the trial. The cause of this effect is unknown.

The effect of CRC treatment of young sheep (aged 6 months) was examined 10 months later when sheep were artificially infected. Resistance to establishment of *T. colubriformis* larvae was lower in previously treated sheep, as were thymus weights. It appears that CRC treatment of sheep may have a detrimental long term effect on resistance to parasites.

In an unrelated study the effect of kiwifruit vinegar (8.2g/100ml) on parasitological and production parameters in lambs and fertility in two-tooth ewes was investigated. There was a tendency (not significant) for FECs in vinegar treated lambs to be lower than in untreated lambs. Treatment had no effect on liveweight gain, wool growth, or wool colour, but caused a small reduction in wool yield. Pre-mating liveweight of two-tooth ewes and reproductive status 38 days after removal of the ram was not affected by vinegar treatment.

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Chapter One: Introduction

Internal parasites have been causing production loss, mortality, and reduced returns to New Zealand sheep farmers for over one hundred and fifty years. For the last thirty years broad-spectrum anthelmintics have provided a highly cost-effective means of controlling much of this production loss and mortality. However continued reliance on anthelmintics is no longer possible or desirable as a result of several recent developments. First and foremost is the increasing prevalence of anthelmintic resistant parasite populations. Failure of one or more of the currently available drench families is becoming a reality for a growing number of farmers.

World wide consumer pressure is mounting to reduce chemical inputs in agricultural production, for environmental and health reasons. There is the potential for overseas markets to exploit the real or perceived dangers of anthelmintic residues to justify creating trade barriers. Lucrative European 'organic' markets could also be more fully exploited if effective non-chemical control methods were readily available.

An alternative method of parasite control in sheep which is currently the subject of considerable research effort is selection for improved natural resistance to infection. Several selection studies in New Zealand (Baker *et al.* 1991) and Australia (Gray, 1991) have confirmed that selecting sheep on the basis of low faecal egg count results in progeny with increased resistance to infection. This has also been demonstrated in a commercial farming environment (Bisset *et al.* 1992).

However the issue of whether low faecal egg count is the ideal selection criterion when increased returns to the farmer is the ultimate aim of selection, has yet to be resolved. Despite clear differences in worm burden between sheep selected for low faecal egg count, and unselected sheep, superior production in a parasitised environment has proven more difficult to demonstrate. Based on studies to date it is unclear whether the underlying genetic relationship between resistance to internal parasites and production traits is favourable, neutral or unfavourable.

Two lines of sheep at Massey University, one selected for high wool production for 36 years (FW), the other randomly selected controls (C), were studied to further examine the genetic relationship between resistance and production. It was hypothesised that if this relationship is unfavourable, resistance to infection in the FW line would have decreased relative to the C line. Such a decrease in resistance has previously been observed in lines of sheep selected for high production (McEwan *et al.* 1992b). It was also of interest to compare the production response to removal of parasites in each line.

In a two year study, faecal egg counts (as an indicator of resistance status) of FW and C sheep grazing naturally infected pasture were monitored. Liveweight gain and wool growth were measured in sheep which were either subclinically infected, or in which parasitism was prevented by the use of albendazole controlled release capsules. A second trial was conducted to determine whether differences in FEC between lines accurately reflected differences in worm burden. Various indicators of immune responsiveness were also examined in each line. Preliminary results of the second trial have been published previously (Williamson *et al.* 1994). Final results of the first and second trial have been submitted to the New Zealand Journal of Agricultural Research for publication (see Chpts 3 and 4). Additional data and discussion which was not included for publication is presented in Chapter 5.

An additional trial was also conducted in the area of alternative control methods for internal parasites. This trial examined the effect of kiwifruit vinegar, an 'organic remedy', on worm burden, production and fertility of grazing outbred sheep. Results have been published previously (Williamson *et al.* 1993, see Appendix 1).

Chapter Two: Literature Review

Any study in the field of selection for improved genetic resistance to parasites brings together three branches of science: parasitology, immunology and animal breeding. While some may claim expertise in one or even two of these areas, few would be able to claim more than passing knowledge of all three. Therefore this review is divided into two sections. The first is intended to familiarise the reader with basic concepts in parasitology and immunology, the second then reviews current issues in selection for improved genetic resistance to internal parasites of sheep.

Section 1: Parasitology and Immunology

1.1 Parasite biology

Gastrointestinal parasites of sheep arrived in New Zealand with the first importations of sheep about 1814. Twenty-three species have been identified from sheep, and are found throughout the country. The most important of these are *Haemonchus contortus*, *Ostertagia* species, *Trichostrongylus axei* occurring in the abomasum, and *Trichostrongylus* species, *Nematodirus* species and *Cooperia* species in the small intestine (Vlassoff and McKenna, 1993).

With the introduction of thiabendazole in 1962, control of a wide spectrum of nematodes was finally possible. Modern anthelmintics now provide a highly effective and relatively low cost means of control. Anthelmintics were seen for many years as the ultimate answer to parasitism, but this is no longer the case for two main reasons. First, it is now recognised that even with regular drenching significant production losses still occur as a result of continuing larval challenge. Second, parasite resistance to anthelmintics is becoming an increasing problem. In order to maximise the benefits of drenching, in conjunction with other control measures (such as selection for genetic resistance), it is essential that the biology and epidemiology of parasites is well understood.

1.1.1 Life cycle

The biology of all the important nematodes is superficially similar. Female worms in the gut lay eggs which pass out in the sheep's faeces, each egg then hatches and passes through three larval stages on pasture. Third stage larvae are infective, and if eaten by susceptible sheep, may develop into adult worms. For most nematode species the time between ingestion of infective larvae and the appearance of eggs in the faeces is about three weeks. The way disease actually develops in a flock of sheep is determined by a complex interaction between individual hosts, parasites and the environment.

1.1.2 Epidemiology

Development of parasites is greatest in lambs, before immunity has developed, and in ewes during late pregnancy and lactation when immunity is depressed (resulting in the well-known 'peri-parturient rise' in egg output). Adult sheep pass few eggs during most of the year. Enormous numbers of eggs are passed by susceptible sheep but only a small proportion survive and develop to the infective stage to reinfect sheep. The main determinants of larval development on pasture are temperature and moisture, with warm, wet conditions being optimal.

The number of susceptible animals and environmental conditions change throughout the year resulting in a characteristic pattern of numbers of infective larvae on pasture (Fig. 1). Charleston (1986) provides an excellent review of the causes and implications of this seasonal pattern. Briefly, lambs are born onto pasture with few larvae, and their intake of grass is low, resulting in little larval intake initially. By November the number of infective larvae on pasture is increasing because of depressed immunity in lactating ewes. With increasing feed intake, larval intake by lambs also increases. As summer approaches, conditions for larval development deteriorate as moisture levels fall. Immunity of ewes has also been restored after weaning, resulting in a decline in numbers of infective larvae on pasture.

However during summer, egg output from the lambs themselves has been increasing. In Autumn conditions become wet enough for a sudden increase in development of larvae to the infective stage. Feed intake is also increasing so serious disease is most likely to occur at this time. By early winter, temperatures are declining, and lamb numbers have also fallen. The number of larvae falls and remains low until a new generation of susceptible lambs is born to begin the cycle again.

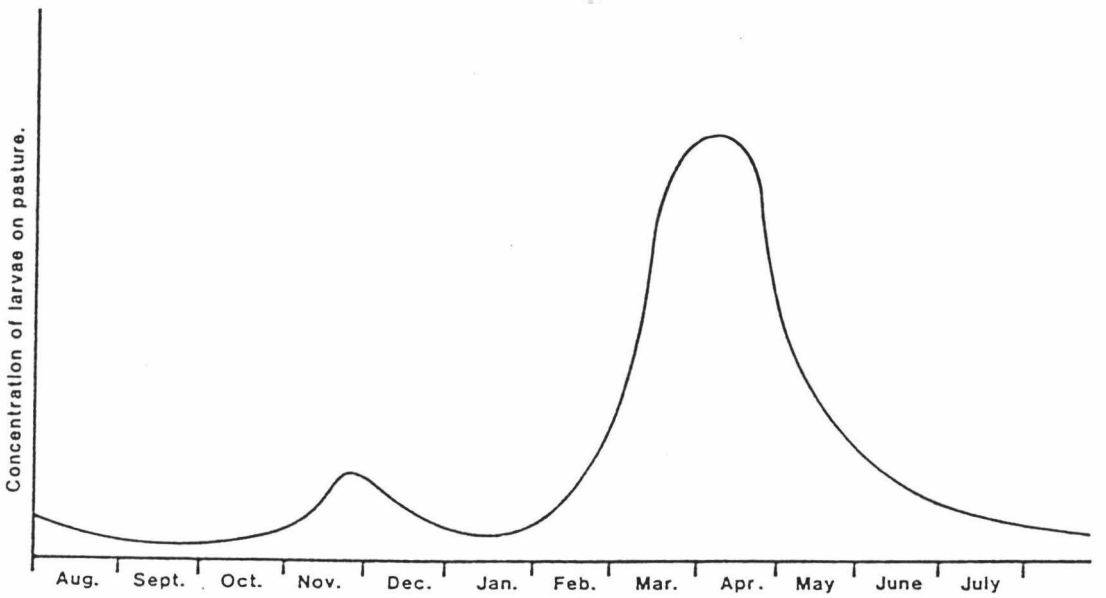

Figure 1 The general seasonal pattern of populations of infective larvae on sheep pastures (diagrammatic). From Charleston (1986).

1.2 Control Strategies

Modern drenching strategies for nematode control focus on preventing the build-up of infective larvae on pasture (Anderson,1990). Ideally lambs are drenched and moved to 'safe' pasture, ie. pasture with low levels of larvae. In practice it is difficult to provide safe pasture since it takes many months without grazing before larvae have declined to 'safe' levels. However the principle of preventing infection, rather than just treating it, is used in 'integrated control' strategies. Grazing with other species (usually cattle) and the use of new pasture or crops can also help

provide safe pasture. Commercial use of genetically resistant sheep is likely to be in conjunction with conventional control strategies rather than in isolation.

1.3 Effects of Parasites

1.3.1 Pathology

Parasites cause production loss as a result of complex host-parasite interactions, rather than simply competing with the host for nutrients. At least some of the pathology associated with infection is attributable to the host's response to parasites rather than the effects of the parasites *per se.* In some host-parasite systems it has even been shown that artificially suppressing the immune response can result in reduced pathology. *H. contortus* is one of the few common gastrointestinal parasites which affects the host directly, by sucking blood which may result in anaemia.

Reduced appetite and feed intake (anorexia) is a common feature of infection, and in severe cases intake may cease altogether. Infection also causes loss of blood and tissue protein into the gut. Some of this protein is re-utilised, but the rest is lost to the animal, contributing to decreased efficiency of feed utilisation. Production depression associated with this protein loss can be reduced by feeding animals a high protein diet (Poppi *et al.* 1990), and can also enhance immune responsiveness. Damage to the gut lining and subsequent loss of water and dissolved materials may cause diarrhoea. However diarrhoea can occur for reasons other than parasitism, and is not a reliable indicator of the level of infection. Anaemia may also occur, particularly when *H. contortus* is present.

1.3.2 Production depression

Production losses due to parasitism are well documented and accepted (Barger, 1982), costing New Zealand millions of dollars every year, much of it as a result of subclinical infection suffered under accepted drenching regimes. For

example Albers *et al.* (1989) found that lambs infected with a single dose of 11 000 *H. contortus* larvae grew 38% slower than control lambs, and produced up to 16% less wool. This wool growth depression occurred three to six weeks after infection and persisted for at least 14 weeks after termination of infection.

Similar results have been obtained with other parasites of economic importance. It is also known that adult sheep can suffer significant production loss when challenged, despite high levels of resistance.

Single dose, monospecific studies such as those of Albers *et al.* (1989) above are useful, but hardly reflect the typical grazing situation with sheep exposed to continuous challenge by a number of species. It is known that complex inter-species interactions occur (eg. Adams *et al.* 1990) which are likely to influence the pathology of a mixed infection.

1.4 Anthelmintic Resistance

Considering the inevitability of the evolutionary process and the general mis-use of anthelmintics over the years, it is hardly surprising that resistance to one or more anthelmintic families is a fact of life for a growing number of farmers in New Zealand and Australia (McKenna, 1991; Overend *et al.* 1994). There are unlikely to be any new anthelmintics developed in the near future because of the enormous financial cost involved. Thus, anthelmintic resistance will increasingly become one of the main driving forces behind breeding for improved genetic resistance in sheep.

Briefly, anthelmintic resistance develops in the following manner. Resistance genes arise by mutation and initially exist only in the heterozygous state, at a low frequency. Recommended dose rates rarely remove all parasites, leaving those that are resistant to contribute to the next generation with consequent increase in resistance alleles. The recommended practice of drench and move to clean pasture can exacerbate the problem. Clean pasture will become contaminated predominantly with larvae derived from resistant adults which survived drenching.

Continued anthelmintic use will almost inevitably lead to resistant parasites, although some steps can be taken to slow the process. Farmers need to ensure that they are not under-dosing sheep, and anything which reduces the frequency of drenching (improved genetic resistance?) is likely to be beneficial. It is usually recommended that anthelmintic families be rotated between years. However in the long run this may be no better than exhausting one family and moving on to the next.

Once anthelmintic resistance occurs on a property there is little that can be done to reverse the situation. Having switched to a different action family, loss of resistance alleles is slow, and re-accumulation is rapid when returning to the original action family (Anderson, 1990). There is some hope that it may be possible to 're-seed' a farm with susceptible parasite genotypes, swamping the resistant population. But it is likely that the only long-term solution, short of changing farming enterprises, will be to use genetically resistant sheep. Even then farmers could be faced with the spectre of parasites becoming resistant to these improved sheep.

1.5 Host Responses to Infection

Ingestion of infective larvae and recognition of parasite antigen by a resistant, eg. adult, host initiates a cascade of events which culminate in expulsion of (most) adult and incoming larvae. Even immunoparasitologists admit that these events are extremely complex and remain poorly understood, particularly in domestic species. Nevertheless it is important that anyone working in the field of breeding for resistance to parasites has some understanding of immune responses to parasites.

1.5.1 Basic immunology

A host's response to infection can be classified as either innate or acquired. The first time an animal experiences a particular pathogen (primary infection) various non-specific 'innate' defences are available to try to prevent infection. The next time

the animal encounters the same pathogen (secondary infection) it will also be able to mount a much more effective 'acquired' response.

1.5.1.1 Innate immunity

Innate responses are non-specific and arose early in evolution, long before the more complex acquired immunity developed. There are a number of different mechanisms involved. The first line of defence is the external physical and chemical barriers, including skin, mucous membranes, the cough reflex, and pH. Once foreign material gets past the external mechanisms there are a variety of internal protective mechanisms, including fever, interferon, and serum proteins (lysozyme etc.)

If the foreign material has still not been dealt with, the phagocytic cells, including granulocytes and macrophages, come into play.

1.5.1.2 Acquired immunity

Acquired immunity developed comparatively late in evolutionary terms and is only present in vertebrates. It supplements the protection of innate immunity. In simple terms, contact with antigen (ie. foreign matter) leads to activation of lymphocytes and synthesis of antibodies specific for that antigen.

Three major cell types are involved in acquired immunity, interacting together. The first two, T cells and B cells (lymphocytes) exhibit 'antigenic specificity'. Antigenic specificity refers to the fact that these cells will only recognise, and react with, the particular antigen for which they were developed. Various functional types of lymphocytes (phenotypes) can be distinguished by cell surface markers. A nomenclature system is used for these markers in which the term CD (cluster determinant) is followed by a number indicating the order of discovery (by 1991 this list had reached at least CD78). Two of the more important and well-defined T cell phenotypes are $CD4^+$ and $CD8^+$. $CD4^+$ 'helper' T cells recognise

foreign antigen bound to MHC class II molecules, while $CD8^+$ 'cytotoxic' T cells recognise antigen bound to MHC class I molecules.

The third important cell type is the macrophage, which is known as an 'accessory' cell. Macrophages ingest antigen, process it and present it to T cells, to initiate an immune response. In contrast to T and B cells, macrophages are non-specific.

In addition to these three cell types there are also the cells involved in the final 'effector phase' of the immune response, including neutrophils, eosinophils and mast cells. These cells are also non-specific.

The effector mechanisms of the acquired response can be divided into two arms, humoral and cellular.

a) Humoral immunity

Humoral immunity involves antibodies (also known as immunoglobulins), secreted by B-cells. Structurally, antibodies consist of two heavy chains and two light chains, making a symmetrical molecule. The part of an antigen that an antibody molecule binds to is called the antigenic determinant or epitope.

On the basis of differences in heavy chains, there are 5 classes of antibody: G, M, A, E, and D. Different classes of antibody can have the same specificity against an antigen while having different functions.

The humoral arm also involves the complement system, which is activated by the reaction between antibody and antigen. It consists of a group of serum enzymes which have a number of functions including lysis of foreign cells, enhancement of phagocytosis, and attraction of polymorphonuclear cells of the innate system (which are highly phagocytic), to the site of infection.

b) Cellular (cell mediated) immunity

Cell mediated immunity involves T lymphocytes (T cells). T cells carry many identical antibody-specific receptors on their surface, and move to where the antigen is (as opposed to B cells which release antibodies to go to the antigen). There are various types of T cells, as discussed above, of the same specificity but with different functions. These include helper and suppressor T cells which regulate the immune response, killer cells which attack foreign cells directly, and T cells which induce migration and activation of cells involved in the inflammatory response.

Overall the immune system works by a complex interaction between humoral and cellular branches of the acquired response, along with innate responses.

1.5.2 Resistance mechanisms

As mentioned above, the way in which sheep prevent establishment of larvae, and expel adult parasites remains largely unknown. Various substances have been shown to be elevated in resistant sheep facing challenge, but demonstrating a direct effect on parasite survival is more difficult. Several mechanisms appear to play a part in resistance.

1.5.2.1 Antibodies

Levels of circulating antibodies are commonly found to rise during infection. Various antibody isotypes are secreted into the gut lumen, where they affect worms in some way, possibly by interfering with nutrient absorption. They may also play a part in mediating hypersensitivity reactions.

1.5.2.2 Hypersensitivity reactions

Hypersensitivity reactions are what most people know as allergic reactions. These reactions usually occur when the immune system 'over-reacts' to an antigen (eg. pollen). Generally this is undesirable, even fatal, but one of the few times when it seems to be useful is in expulsion of parasites from the gastro-intestinal tract.

The most important type of hypersensitivity reaction with regard to parasite resistance seems to be the Type I or anaphylactic reaction. This involves IgE antibodies which bind to mast cells, the reaction occurring within minutes of exposure to parasite antigen.

IgE (one of the 5 immunoglobulin isotypes) has the shortest half life and is found in the lowest concentrations in the body. It has a strong affinity for granulocytes (particularly mast cells), and is mainly found bound to these cells. It is thought that when an antigen (eg. from a parasite) binds to IgE, the mast cell is stimulated to release its stored granules. These granules consist of substances such as histamine, leading to a hypersensitivity reaction, and expulsion of the parasite. However Kennedy (1990) states "The protective role of IgE against nematode infection in vivo remains controversial..."

1.5.2.3 Mucus

A number of studies by P.G.C Douch and coworkers have suggested that antiparasite factors in gut mucus may be important in resistance (Douch *et al.* 1983). For example, they have shown that mucus from resistant sheep reduces larval migration, relative to susceptible sheep.

1.6 Genetic Control of Host Immune Responses

Genetic regulation in expression of immunological function is well documented (Wakelin, 1985). A large number of studies have demonstrated that

genes within the major histocompatibility complex (MHC) are associated with resistance or susceptibility to disease. However non-MHC genes are also involved.

All higher life forms possess an MHC (known as the ovine lymphocyte antigen complex, or OLA, in sheep), that codes for three classes of protein molecules (antigens). Class I genes code for antigens involved in T cell recognition and are necessary to initiate an immune response. Class II genes ('immune response genes') control the interaction of T cells, B cells and macrophages. Class III genes are involved in the complement cascade. Although the MHC has been linked to resistance or susceptibility to disease (including helminths) in many species, it is still not clear how it exerts its effects (Kennedy, 1990). Numerous studies are currently looking at the MHC as a potential source of genetic markers for resistance, although Kennedy (1990) states "...the net effect of the MHC on disease resistance and pathology might be of minor importance in outbred populations".

1.7 Analysis of Parasite Data

Parasites are not randomly distributed amongst their hosts but tend to be aggregated, with a variance which is much greater than the mean. This has important implications when analysing parasite or FEC data, because assumptions of normality of residual effects and constant variance are commonly violated. A number of transformations have been used by various researchers to try to overcome these problems, particularly squareroot and logarithm. If transformation does not achieve normality and homoscedasticity, a non-parametric analysis technique should preferably be used instead of analysis of variance. The negative binomial distribution has been widely used to describe aggregated distributions, including that of common nematodes in grazing lambs (Barger, 1985).

Section 2: Selection for Increased Resistance to Parasites

2.1 Genetic Variation in Resistance

2.1.1 Between breeds

Between-breed genetic variation in resistance to internal parasites was first documented by Stewart *et al.* (1937), and has since been confirmed in numerous studies. Gray (1991) gives a comprehensive review of breed comparisons although few are relevant to New Zealand. Watson *et al.* (1992b) found that Perendale sheep had lower FECs than Romneys. However, they point out that this difference could have arisen as a result of differences in grazing behaviour and feed intake rather than genetic resistance. Earlier comparisons of these two breeds were inconclusive (reported in Watson *et al.* 1992b). Texel cross lambs have also been shown to be more resistant than pure bred Romneys (McEwan *et al.* 1992a).

Although between-breed variation has been clearly demonstrated, little is known about resistance genes involved, and mechanisms through which they are expressed. It is unclear whether these resistance mechanisms differ between breeds. If they do, there could potentially be large benefits from combining mechanisms from different breeds. Unfortunately resistant 'native' breeds tend to be of lower productive potential. Combining them in a breeding programme solely to increase resistance is unlikely to be a practical option.

Alternatively, resistance mechanisms may be similar in each breed, with susceptible breeds merely having a low frequency of resistance alleles. Given that the magnitude of between-sire differences in resistance is of the same order as the largest between-breed differences (Gray *et al.* 1987), selection within breed is a more likely option.

2.1.2 Within flocks

A number of studies in New Zealand (Watson *et al.* 1986; Bisset *et al.* 1992; Douch *et al.* 1994b; Watson *et al.* 1992a), and Australia (reviewed by Woolaston, 1990) have conclusively demonstrated genetic variability in resistance (as measured by FEC), in sheep.

Albers and Gray (1987) state "...there is no real justification for averaging heritabilities estimated in different host-parasite systems, under different conditions, on different resistance parameters and with different precision...". Nevertheless heritability estimates have generally been around 0.3 to 0.4, indicating that moderate genetic variation is available for selection.

The variance of parasite populations (and FECs) is typically found to be proportional to the mean. Consequently selection for low FEC is likely to lead to decreased variance in this trait (Albers and Gray, 1987). Assuming the relative importance of genetic contributions to resistance is not altered by average level of FEC, a higher mean FEC indicates more genetic variation exists. The observation that it is easier to select for increased FEC than for decreased FEC (eg. Baker *et al.* 1990), supports this idea. Predicting response to selection therefore requires variance to be re-estimated for each generation of selection.

Heritability of resilience (usually measured as production when facing parasite challenge) is rather less well quantified, compared to heritability of resistance. One of the first major attempts to quantify genetic variation in this trait (Albers *et al.* 1987), estimated heritability of liveweight gain and wool growth when infected to be low, not differing significantly from zero. Studies with New Zealand research flocks (Watson *et al.* 1986; Douch *et al.* 1994b), and on commercial farms (Bisset *et al.* 1992, 1994) have produced heritability estimates which are variable but tend to also be low. It appears that direct selection for improved production when parasitised is likely to result in only slow progress in this trait.

2.1.3 Major Genes

Albers *et al.* (1987) postulated the existence of a rare major gene controlling resistance to *H. contortus*. Selection for resistance would be considerably faster if controlled by one, or a few major genes, compared to polygenic control. However there has been little further evidence of major gene involvement (eg. Woolaston *et al.* 1990). Gray and Gill (1993) state that resistance is "the consequence of the action of many genes rather than one or a few major genes".

2.2 Selection Criteria

In order to improve genetic resistance, the breeder must be able to accurately identify superior animals. There are a number of criteria which have been used, or are being assessed for potential use.

2.2.1 Faecal egg count (FEC)

FEC is currently the most widely used selection criteria in experimental studies in New Zealand and Australia, and the only criteria being used commercially. Although only an indirect measure of resistance, it has been shown to be well correlated with parasite burden (McKenna, 1981). In addition low FEC is a useful trait *per se* since reduced egg output is likely to result in lower pasture contamination. However McEwan (1993) points out that FECs are inaccurate, expensive, and require infection with associated production depression. Nevertheless in the words of Gray (1991) "...faecal egg counts are the standard against which all potential techniques should be measured and their extensive use will continue, at least while new techniques are being evaluated". These new techniques are considered in the following sections.

2.2.2 Ovine lymphocyte antigens

Particular genes within the ovine lymphocyte antigen (OLA) complex (the sheep major histocompatibility complex) may be associated with resistance or susceptibility to parasitic infection, but the evidence is equivocal. Differences in the frequency of two OLA haplotypes were found in lines of Merino sheep selected for high or low responsiveness to infection with *T. colubriformis* (Outteridge *et al.* 1985). This association was subsequently confirmed in outbred Merinos mated on the basis of OLA type (Outteridge *et al.* 1988), and has also been reported in Romneys (Douch and Outteridge, 1989). However other workers have found no such association (Cooper *et al.* 1989; Riffkin and Yong, 1984; Luffau *et al.* 1986). Bisset *et al.* (1991) concluded that OLA type is not a useful predictor of responsiveness in genetically resistant sheep at Wallaceville.

Genetic variation in the MHC can also be detected using restriction fragment length polymorphisms (RFLPs). Application of this technique in sheep is in its infancy and is considered at present to have no useful predictive value (Gray and Gill, 1993). Work is also under way to assess the value of random amplified polymorphic DNA markers (RAPDs), which have advantages over RFLPs, as genetic markers for resistance (Pulford *et al.* 1994).

2.2.3 Immunological function

It is now well established that a wide range of immune functions are enhanced in genetically resistant sheep relative to unselected sheep (see section 2.9) and a number of these functions have been considered as selection criteria. One of the more promising examined so far is anti-parasite antibody level following infection (Douch *et al.* 1994b). Work is currently under way in New Zealand to evaluate selection of sheep for high circulating antibody level following infection. Preliminary results show large direct responses to selection on antibody level, and favourable correlated changes in liveweight (McEwan *et al.* 1994c). However there has been little response in FEC.

2.2.4 Haemoglobin genotype

For many years there has been interest in the relationship between haemoglobin genotype and resistance. Several studies have demonstrated higher resistance in sheep of haemoglobin genotype AA (HbAA) relative to HbAB or HbBB (reviewed by Windon, 1991). However other studies have found no association, and at present it would have to be concluded that Hb genotype has no practical value as a selection criterion.

2.2.5 Gastrin level

Each of the selection criteria discussed above are indirect predictors of resistance to infection, rather than predictors of superior production when challenged (resilience). Gastrin level is a potential predictor of resistance to the pathological effects of infection, and therefore indirectly measures resilience.

Blood gastrin levels are commonly found to rise in sheep following infection with nematode parasites in the abomasum and small intestine. Elevated gastrin level may cause feed intake depression (Fox *et al.* 1989), and depressed feed intake is one of the most important causes of production loss in infected sheep (Sykes and Poppi, 1982). The extent of gastrin elevation above 'normal' levels has been used to indicate the severity of gastric dysfunction in infected sheep (Fox *et al.* 1988). The ability to maintain a 'normal' gastrin level when challenged is potentially an indirect predictor of ability to maintain an undepressed production level when infected.

2.3 Parasite Adaptation

If parasites can adapt genetically to changes in host resistance, selection for improved resistance will be an on-going process. Selection could conceivably become an extension of the 'evolutionary arms race' (Behnke and Barnard, 1990) between host and parasite, making rate of progress difficult to predict. The much shorter generation interval of parasites relative to sheep gives parasites a considerable

advantage in this arms race. The fact that parasites can become resistant to anthelmintics indicates that they certainly have the capacity to rapidly adapt to changes in their environment. However the ability to adapt to the single mode of action of an anthelmintic does not necessarily imply that parasites will be able to adapt to polygenically controlled host resistance.

Studies with serial passage of *H. contortus* through genetically resistant sheep (Albers and Burgess, 1988; Adams, 1988; Woolaston *et al.* 1992) have found no evidence for parasite adaptation. However a similar study with *T. colubriformis* found that infectivity of parasites did increase after passage through resistant sheep (reported in Windon, 1991). If parasite adaptation is found to be a problem, resilience as a selection objective becomes more attractive.

2.4 Specificity of Resistance

An important point to consider when selecting for improved resistance is whether selection for resistance to one species of parasite confers resistance to other species. This is particularly important in Australia where selection programmes have focused on single-species infection. Selection for resistance to *H. contortus* or *T. colubriformis* has resulted in increased resistance to other economically important parasites, although the level of resistance tends to be lower than for the target species (Woolaston, 1990).

In New Zealand, selection has primarily been based on resistance to natural field challenge, so this issue is of less importance. However the prevalence of economically important parasite species does vary between regions within New Zealand. A possible sire by location interaction was found when resistant and susceptible rams were exchanged between Ruakura and Wallaceville (Baker *et al.* 1990). More recently McEwan *et al.* (1994a) found little evidence of such an interaction, and concluded that genotype by environment effects would not be a problem in commercial selection programmes.

The effect of selection for resistance to parasites, on resistance to other non-parasite diseases, is also of major importance. Little work has been done in this area with regards to sheep diseases. Gray (1990) concluded that most associations between disease traits in sheep are positive or neutral, although there is some evidence that resistance to *H. contortus* may be associated with susceptibility to fleece rot and clostridial diseases. A negative association between resistance to internal parasites and resistance to ticks has been found in cattle (McKinnon, 1990). More work has been done in mice and chickens where it has been shown that selection for high antibody level can increase resistance to some diseases but reduce resistance to others (reported in Gray, 1991). This author concluded that "...it should not necessarily be expected that sheep selected for resistance to one parasite will be resistant to other diseases and ailments...". A better understanding of resistance at the genetic and mechanistic level may enable effects of selection on other diseases to be predicted to some extent.

2.5 Stability of Resistance

Selection programmes in New Zealand and Australia have focused on improving resistance in young animals, since they are normally the most susceptible to infection. However it is important that resistance is also manifest later in life. Adult sheep are usually considered to be highly resistant to infection, but immunity is typically depressed around parturition, resulting in increased FEC (the 'peri-parturient rise'). In this regard Woolaston (1992) found that differences in FEC between genetically resistant ewes and susceptible ewes were maintained during the peri-parturient rise.

Adult sheep are also vulnerable when nutritionally stressed, and when suffering from other diseases. It would be hoped that superior genetic resistance is also maintained at these times. It is encouraging to note that genetic resistance has been found to remain effective up to 5 years of age, with differences between resistant and susceptible lines only disappearing by 7 years of age (Gray, 1991).

2.6 Relationship Between Resistance and Production

2.6.1 Liveweight gain and wool growth

Gray (1991) states "There is general agreement that, in sheep, genetic correlations between faecal egg output and production traits such as wool quality and growth and liveweight gain are zero when animals are uninfected and moderately favourable when animals are infected". This implies that selection for improved resistance will lead to increased production when sheep are grazing infected pasture, and will not compromise production when sheep are uninfected. However the few available estimates of the genetic correlation between FEC and production in sheep are equivocal at best, and provide little support for the view of Gray (1991).

Watson *et al.* (1986) estimated the genetic correlation between FEC and liveweight gain in several strains of naturally infected Romney lambs. Estimates were not consistent, being favourable for the first sampling period (-0.43), but unfavourable for the second period (0.49). Interestingly, strain means for FEC and liveweight gain were unfavourably correlated. In particular it was found that the Ruakura High Fertility strain had the highest FEC, and above average liveweight gain.

The first comprehensive study (Albers *et al.* 1987), looked at the relationship between FEC and production (liveweight gain and wool growth) in Merino lambs when artificially infected with *H. contortus* and when uninfected. When infected, genetic correlations were favourable, and moderate to high (-0.39 ± 0.31 to -0.68 ± 0.34), although it should be noted that standard errors were also high. When uninfected, correlations were low and did not differ significantly from zero. These results are presumably the basis for the view expressed by Gray (1991), above.

Woolaston (1990) presents estimates of the genetic correlation between FEC and production in adult merino rams. FEC was measured following artificial infection with *H. contortus*, and production was measured in individual rams and in

uninfected relatives. Correlations were low, but were favourable between FEC and liveweight gain and unfavourable between FEC and wool growth. Standard errors were again high. Cummins *et al.* (1990) reported similar findings in naturally-infected Merinos, with correlations being low and favourable for liveweight gain and unfavourable for wool growth.

Bisset *et al.* (1992) examined the relationship between FEC and production in naturally-infected Romney lambs on a commercial New Zealand farm, in a three year study. FEC was measured during a two to four month untreated period. Liveweight gain was measured through to autumn (including the untreated period) and wool production measured at one year. In contrast to the previous two studies, the relationship between FEC and production was favourable for both liveweight gain (-0.48 ± 0.21) and woolgrowth (-0.31 ± 0.16).

Douch *et al.* (1994b) estimated genetic correlations in lambs from resistance selection lines at Ruakura and Wallaceville over a three year period. The genetic relationship between FEC and liveweight gain within flocks was found to be favourable (-0.30 ± 0.25), although it was noted that at Ruakura, sheep from the resistant line had lower liveweight gain than susceptible sheep. The relationship between FEC and woolgrowth was not mentioned.

McEwan *et al.* (1994b) undertook a large scale evaluation of genetic relationships between FEC and production traits (liveweight at 8 months and hogget fleece weight) in two commercial group breeding schemes. Correlation estimates were consistently low and unfavourable for both liveweight and fleece weight (mean 0.15).

Garrick *et al.* (1992) found a large, unfavourable genetic correlation between FEC and hogget fleeceweight in a commercial ram-breeding flock, exposed to natural challenge. There was no such relationship evident the following year, but the presence of facial eczema tended to interfere with resistance to internal parasites.

Two further studies suggest an unfavourable relationship between FEC and production in sheep. In lines of sheep selected for high production, FEC increased and genetic correlations were found to be large and unfavourable (McEwan *et al.* 1992b). FEC has also increased following selection for high fleeceweight (Howse *et al.* 1992), but genetic correlations have not been estimated.

The relationship between antibody level (as an indicator of resistance status) and production is also of interest (see section 2.2.3). Douch *et al.* (1994b) found that genetic correlations were generally low, but some were unfavourable, and it was suggested that this may be due to competition between pathways for antibody production and liveweight gain. In contrast McEwan *et al.* (1994b) found genetic correlations between antibody level and liveweight to be favourable (mean 0.27).

2.6.2 Fertility

The effect that selection for increased resistance might have on fertility is also an important consideration. As mentioned above, Watson *et al.* (1986) found that the Ruakura High Fertility strain had the highest FEC of five strains compared. In contrast, (Woolaston, 1990) reported that the fertility of sheep selected for resistance to *H. contortus* did not differ significantly from control sheep. Fertility had in fact decreased in sheep selected for susceptibility.

McMillan *et al.* (1992) compared reproductive performance in the Ruakura FEC selection lines and found that the low FEC line tended to have larger litter size. This was a result of better embryo survival and ovarian activity. However these authors concluded that this was an effect of foundation ewes selected rather than a correlated genetic response.

2.6.3 Dagginess

Another trait which has been examined in relation to resistance is breech soiling or 'dagginess'. There has traditionally been a belief that dagginess (and diarrhoea) is associated with large worm burdens. However Watson *et al.* (1986) found a strong negative (unfavourable) relationship between FEC and dag score, with the daggiest sheep having the lowest FEC. More recently Douch *et al.* (1994b) reported that genetically resistant sheep at Wallaceville had higher dag scores and more fluid faeces than susceptible sheep. It was suggested that this may be due to greater immunological function in resistant sheep resulting in increased gut permeability and leakage of serum into the gut lumen.

In contrast, Bisset *et al.* (1992) found a favourable genetic correlation between dag score and FEC in a commercial flock. It is important that this relationship be clarified, since increased incidence of dags would be a highly undesirable correlated response to selection for reduced FEC.

It is difficult to conclude from these studies whether the relationship between FEC and other economically important traits is favourable, unfavourable or neutral. In lines of sheep selected for improved resistance, there has so far been little evidence that production while infected has increased relative to unselected sheep. There have been no reports of attempts to assess production of genetically resistant sheep while uninfected.

A small number of studies have looked at the relationship between disease resistance and production in other species. An unfavourable association between resistance to parasites and liveweight gain and fertility has been demonstrated in cattle (McKinnon, 1990), although the relationship was favourable for ticks. Results from studies with pigs and poultry appear to be as variable as those with sheep (Warner *et al.* 1987). It seems that a review of this important aspect of selection for disease resistance is well over-due.

Attempts to estimate genetic correlations between FEC and production tend to be confounded by the effect of infection on production. It is impossible to distinguish between effects due to parasites and those due to genetic potential when sheep are infected. The key point is that the true underlying genetic relationship between resistance and production can only be 'unmasked' when parasitism is completely controlled. The genetic relationship between FEC and production should be estimated in uninfected, and preferably parasite naive, sheep. This is true even if the records of relatives are used to estimate production.

When infection is only for a short period, production may not be greatly affected, but it is likely that some bias is present in all the studies discussed above. Particularly when it is considered that even under 'accepted' drenching regimes, production losses still occur. In these studies it is often difficult to determine whether estimates are of the true underlying genetic correlation between resistance and production, or in fact the correlation between resistance and resilience (ie. production when infected).

In summary, it might be argued that as the incidence of anthelmintic resistance increases, the value of improved genetic resistance also increases, regardless of whether genetic correlations with production are favourable or not. Implicit in this argument is the assumption that resistant sheep are able to maintain a profitable level of production while receiving less (or no) drenching. This has yet to be demonstrated.

2.7 Resistance Versus Resilience

Albers *et al.* (1987) defined resistance as 'the ability to suppress establishment and/or subsequent development of infection' and resilience as 'the ability to maintain a relatively undepressed production level when infected'. There is little consensus as to which of these host responses is the most desirable selection objective. To date most work in New Zealand and Australia has focused on selection for improved

resistance rather than resilience. There appears to have been little consideration of the potential benefits of resistance as opposed to resilience, to the farmer.

The rationale behind resistance as a selection objective is that as resistance increases, so will production and therefore returns to the farmer. This idea was supported by the work of Albers *et al.* (1987) which indicated that resistance and resilience traits were favourably genetically correlated in sheep infected with *H. contortus*. Thus selection for improved resistance (based, for example, on low FEC) was expected to lead to increased production in a parasitised environment.

Unfortunately this has not been backed up by subsequent work. It is now well established that selection for resistance can result in sheep with substantially lower worm burdens, but there is little evidence to show that this is reflected in higher production (Bisset *et al.* 1991). Nevertheless resistance *per se* may be a desirable trait. Resistant sheep pass fewer eggs which could lead to lower pasture contamination as discussed previously, and perhaps indirectly to increased production.

Resilience can be a simple trait to measure, involving only the usual measures of production (liveweight gain, wool growth), which are directly related to farm returns. Bisset *et al.* (1994) used a rather more complex measure of resilience, based on an individual animal's requirement for anthelmintic treatment. Requirement for treatment was assessed as age at first drench, time of first drench, and total number of drenches administered.

Any direct measure of resilience requires a level of challenge which allows differentiation between resilient and susceptible sheep. Thus there is a cost in terms of lost production. Selection on the basis of FEC also requires infection (natural or artificial), but other indicators of resistance which do not require infection will almost certainly become available in the future. Resilience as a selection criterion also has the disadvantage of a lower heritability than resistance (see section 2.1.2).

What might be the consequences of selection for resilience? If resistance and resilience are favourably genetically correlated then resistance should also improve ie. when facing parasite challenge, resilient sheep will be more productive and also more resistant. However Garrick *et al.* (1992) suggested that the relationship between resistance and production (resilience) may be unfavourable at low levels of infection. If this is true then it is possible that selection for increased resilience in such an environment could lead to sheep with decreased resistance, and consequent increase in pasture contamination.

Resilience was defined above in terms of production level when infected. However the production level of an animal facing parasite challenge depends on both the number of parasites which establish (ie. resistance), and resistance to the pathological effects of the parasites (ie. resilience). Thus while resistance and resilience are often considered as two separate traits, in reality they cannot be separated. Any measure of resilience is to some extent also a measure of resistance.

Bisset *et al.* (1992) used the term 'tolerance' defined as 'the ability to maintain good growth rates despite untreated nematode infection'. This appears to be essentially the same as 'resilience'. However our work with FW selected and C sheep suggested that there may have been a tendency for FW ewes to lose relatively more production than C ewes when infected, while still maintaining a better growth rate than C ewes. Therefore FW ewes would be considered 'tolerant' but not 'resilient', highlighting the need for clear definitions when discussing this issue.

2.8 Vaccination

Vaccination of sheep to hasten development of normal resistance or to stimulate different mechanisms of resistance is another potential control option. While no commercial vaccines against gastrointestinal parasites of sheep are currently available, there have been some promising research results. High levels of protection have been achieved in 11 week old lambs challenged with *H. contortus*, using a vaccine developed from 'cryptic antigens' from the intestinal brush-border of adult

parasites (Tavernor *et al.* 1992). If these results are confirmed, it seems likely that a vaccine against *H. contortus* will appear on the market before long.

Development of effective vaccines might appear to render advances in selection for genetic resistance obsolete. However it should be noted that vaccines work by stimulating the animals own immune system. Therefore genetically resistant sheep can be expected to have an improved response to vaccination. If highly effective vaccines do become available, it could be envisaged that sheep might one day be selected directly for improved response to vaccination.

Vaccines are not without drawbacks. Tavernor *et al.* (1992) found that despite substantial protection against establishment of parasites, packed cell volumes still declined. There is also likely to be a production cost associated with vaccination. Sheep vaccinated with irradiated larvae for 10 weeks grew more slowly than unvaccinated controls (Emery, 1991). The possible problem of parasite adaptation to improved immunological responses is also of concern for vaccines. As Kennedy (1990) asks "If nematodes can develop resistance to the most powerful anthelmintics available, then how long is a simple recombinant vaccine likely to be effective?"

2.9 Immune Function in Genetically Resistant Sheep

It is well known that the ability of sheep to resist infection varies between breeds, sexes, ages, and individuals. What is less well known is the differences in immunological function that contribute to this variation. By looking at the way immunological responses have changed in flocks selected for increased or decreased resistance, it is possible to gain useful insight into mechanisms involved. This may lead to new selection criteria for identifying genetically resistant sheep as discussed above.

In sheep selected for resistance to *H. contortus*, thymus weights and number of globule leucocytes in abomasal tissue were found to be greater than in unselected

sheep (Presson *et al.* 1988). When treated with dexamethasone (an immunosuppressant) these differences disappeared. Gill (1991) compared responses in these lines to primary and secondary infection with *H. contortus*. Interestingly, genetically resistant lambs had higher worm burdens following primary infection, but lower burdens following a second challenge. There were no immunological differences following primary infection, but after secondary infection, resistant lambs had higher levels of antibodies, mucosal mast cells and eosinophils. Further work (Gill *et al.* 1993a) showed that elevated antibody levels in resistant sheep following infection were of isotype IgG1 and IgA (but not IgG2 or IgM).

Gill (1993) suggested that enhanced T lymphocyte function may contribute to greater resistance to *H. contortus* in these genetically resistant lambs. Gill *et al.* (1993b) were able to show that $CD4^+$ T cells (and not $CD8^+$) were vital in expressing resistance, by selectively depleting $CD4^+$ or $CD8^+$ T cell subsets using monoclonal antibody. $CD4^+$ depletion suppressed a number of responses including levels of mast cells, eosinophils, and antibodies, and abolished differences between resistant and susceptible lines.

Selection for increased response to *T. colubriformis* challenge has also resulted in changes in a number of immunological functions. These include higher anti-parasite antibody levels, greater in-vitro phagocytosis in peripheral blood leucocytes, greater levels of histamine in intestinal tissue, higher concentrations of leukotrienes C4 and B4 in duodenal mucous, and more globule leucocytes and circulating eosinophils (reviewed by Windon, 1991).

Chapter Three

'Parasitism and production in fleeceweight-selected and control sheep'

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Abstract

In two successive years young sheep (<6 mnths old) from a line selected for high wool production for 37 years and an unselected line at Massey University were either treated with an albendazole controlled release capsule (CRC), or were allowed to become subclinically infected while facing natural parasite challenge.

Subclinically infected FW sheep developed higher faecal egg counts (FEC) than C sheep, suggesting that wool production and resistance to infection may be unfavourably genetically correlated in an environment of regular anthelmintic treatment. Despite higher FECs in FW sheep, there was little evidence that infected FW sheep suffered greater production depression than C sheep. There was no between-line difference in antiparasite antibody levels indicating that lower resistance in FW sheep is not associated with a decreased antibody response, nor was there a difference in antibody levels between CRC and non-CRC treated animals.

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Serum gastrin levels were higher in C sheep than in FW sheep and were higher in infected sheep than in CRC-treated sheep. There was some evidence that infected FW sheep may have suffered less gastric dysfunction than C sheep.

Although CRC treatment was effective in preventing establishment of an adult worm burden, the production response was variable. During some periods liveweight gain and wool growth in CRC-treated sheep (particularly rams) were lower than in subclinically infected sheep.

Key words: sheep; selection; internal parasites; faecal egg count; resistance; albendazole controlled release capsule; production; genetic correlation; antibodies; gastrin

Introduction

Pastoral sheep industries in Australasia rely heavily on anthelmintic treatment to control internal parasites. Increasingly, reports of anthelmintic-resistant parasites, and consumer demands for products free from chemical residues have made it imperative that alternative control methods be found. Studies in New Zealand (Baker *et al.* 1991) and Australia (Gray, 1991) have demonstrated that selection of sheep for low faecal egg count (FEC) results in progeny with reduced parasite burdens. However the genetic relationship between resistance and production is not clear and long-term effects of selection for low FEC on sheep productivity are yet to be determined.

This paper reports the results of a two-year study examining production and resistance in two lines of sheep at Massey University. One line has been selected for high greasy fleece weight for 37 years (FW), the other has been randomly selected during the same period (C) (Blair *et al.* 1985). FW sheep are more productive than C sheep when clinical parasitism is controlled by anthelmintic treatment (Blair *et al.* 1985), and preliminary investigations suggest that FW sheep may also develop higher FECs than C sheep between drenches. This suggests that there may be an

unfavourable genetic relationship between fleece weight and FEC. A similar increase in FEC has been observed in lines of sheep selected for high production at Woodlands Research Station, Southland (McEwan *et al.* 1992b) and in a line selected for high fertility at Ruakura (Watson *et al.* 1986).

FECs were monitored to confirm earlier observations that FW sheep develop higher FECs than C sheep under natural field challenge. Production was measured in FW and C sheep when subclinically infected and when a controlled release anthelmintic device was used to prevent infection, allowing production depression due to infection to be compared between lines. Feed intake, serum gastrin level and anti-parasite antibody levels were also measured. Preliminary findings from one year have been reported previously (Howse *et al.* 1992).

Materials and methods

Experimental design

Eighty 1990-born lambs were used in the first trial aged between 5 and 6 months at the start, and 117 1991-born lambs aged between 2 and 3 months were used in the second trial (Table 1). The 1991 trial began (day 0) on 25th February (ewes) or 6th March (rams) and in 1992 on 21st Nov for both sexes (weaning). All animals received anthelmintic treatment ('Ivomec' 0.08% w.v. ivermectin, dose rate 1ml/4kg, in 1991; 'Leviben' 20g/l ricobendazole and 37.5g/l levamisole hydrochloride, dose rate 2ml/5kg, in 1992) at day 0 to remove existing internal parasites.

Within sex and line, animals were randomly allocated to either a treatment group or control group. The treatment group (CRC) received an albendazole controlled release capsule (Nufarm), preventing establishment of an adult parasite burden for approximately 100 days with a constant anthelmintic release rate of about 0.5 mgAlb/kgLW/day (manufacturer's specifications). The control group (non-CRC) received anthelmintic treatment at day 42 in 1991 ('Ivomec') and at days 25 and 60

in 1992 ('Leviben') to prevent the occurrence of clinical but not sub-clinical parasitism. This group was not a true control since sheep were not treated with an inactive CRC. Both lines were grazed together on naturally infected pasture at all times, although in 1991 ewes and rams were grazed separately.

Liveweight Gain and Wool Growth

Liveweight gain and wool growth were measured at intervals up to day 76 (ewes) or 90 (rams) in 1991 and up to day 116 in 1992. Wool growth was estimated from samples taken from the left midside area of each animal (Bigham, 1974).

Wool growth was measured up to day 116 despite the fact that albendazole controlled release capsules (CRC) were expected to expire at around day 100, because of possible time lag between parasitic infection and wool growth depression (Albers *et al.* 1989).

Faecal Egg Counts

Faecal egg counts (FEC) were measured at intervals in all sheep to monitor the build up of internal parasites. A sample at day 12 served as a check to ensure that all sheep had been effectively drenched. Strongyle eggs (excluding *Nematodirus*) were counted using a modified McMaster technique where each egg counted represented 50 eggs per gram of faeces.

Feed Intake

Feed intake was estimated by intraruminal chromium controlled release capsule (Nufarm). Chromium content of faecal samples was determined by the method of Costigan and Ellis (1987). Pasture dry matter digestibility was estimated by *in vitro* analysis of a hand-plucked representative sample of pasture (Khadem *et al.* 1993).

In statistical analyses, feed intake was expressed on a metabolic liveweight basis ie. dry matter intake per kg liveweight raised to the power 0.75. This corrects for differences in feed intake between lines resulting from the greater maintenance requirement of FW sheep (by virtue of their larger body size), (Blair *et al.* 1985).

Serum gastrin level

Serum gastrin levels were estimated in 1991 at day 28 in both ewes and rams and at day 76 in ewes only, using a radioimmunoassay based on that of Hansky and Cain (1969) and modified by Simpson *et al.* (1993).

Antibody level

In 1991, levels of polyclonal antibody (Ab) to infective larvae of *Haemonchus contortus* and *Trichostrongylus colubriformis* were estimated at day 28 in all animals and at day 76 (ewes) or day 90 (rams), using an enzyme linked immunosorbent assay (ELISA) technique (Douch *et al.* 1994a). In 1992, levels of polyclonal antibody and specific immunoglobulin isotype G1 (IgG1) to infective larvae of *H. contortus* were estimated at day 56 in all animals.

Statistical analysis

Faecal egg counts were squareroot transformed to normalise error distributions and reduce the relationship between mean and variance. The significance of differences between groups was assessed using analysis of variance. Relationships between variables were assessed using linear regression. Low number of animals within groups limited the power of statistical tests.

Results

Liveweight gain and wool growth

Mean liveweight gain and wool growth of each group is presented in Table 1. While CRC treatment was effective in preventing establishment of an adult worm burden as indicated by FEC (see below), the effect on production was variable.

In 1991, CRC treatment increased liveweight gain in both ewes and rams. Over the total trial period there was no significant line by treatment interaction, but initially (up to day 42) the response to CRC treatment tended to be greater in FW rams (+87%) than in C rams (+30%) ($p=0.09$).

In 1992, CRC-treated sheep initially (up to day 25) grew more slowly than untreated sheep ($p<0.05$, pooled across all groups). CRC-treated ewes (but not rams) then grew faster than untreated ewes. There was no significant line by treatment interaction.

In 1991, a significant line by treatment interaction was evident with the wool growth response to CRC treatment over the trial period being greater in FW ewes (+29%) than in C ewes (+6%). In rams, the effect of CRC treatment was variable, tending to increase wool growth in the C line but to decrease growth in the FW line (interaction approaching significance, $p=0.08$).

In 1992, CRC treatment had no significant effect on wool growth in ewes, but CRC-treated rams had lower wool growth than untreated rams in both lines.

There was no consistent relationship between production and FEC or feed intake at any time in either year.

Faecal Egg Counts

Faecal egg counts of subclinically infected and CRC-treated sheep are presented in Table 2. FECs were consistently higher in infected FW sheep than C sheep in both years, although not always significantly so. CRC treatment was not completely effective in suppressing egg output, with occasional low FECs detected in CRC-treated sheep.

Larval cultures from faecal samples in both years showed that non-CRC sheep were infected with three genera of nematode parasite, *Haemonchus*, *Ostertagia* and *Trichostrongylus*.

Feed Intake estimated from faecal chromium

There were no consistent differences in feed intake between lines or treatment groups and no relationship between feed intake and other variables in either year.

Serum gastrin level

Gastrin levels were higher in C sheep than FW sheep and were higher in subclinically infected sheep than CRC-treated sheep of both sexes in the 1991 trial (pooled across sampling dates for ewes) (Table 1). Elevation of gastrin in response to infection tended to be greater in the C line (+43%, both sexes) than in the FW line (+23%, ewes; +11%, rams) but this interaction only approached significance in rams ($p=0.08$) and was not significant in ewes.

Antibody levels

Levels of anti-parasite antibodies did not differ significantly between lines or treatment groups in either year. The phenotypic relationship between antibody levels and FEC in non-CRC groups was generally weak.

Table 1. Production and serum gastrin levels in Fleeceweight-selected (FW) and Control (C) sheep when subclinically infected (-) and with infection controlled by CRC treatment (+).

			ewes								rams							
			C		FW		PSE[1]	significance[2]			C		FW		PSE	significance[2]		
			-	+	-	+		flk	trt	int	-	+	-	+		flk	trt	int
n	1991		11	11	6	9					10	10	13	10				
	1992		20	12	26	14					9	4	22	10				
Liveweight gain (g/day)																		
	1991		59	77	57	87	9	ns	**	ns	75	115	87	149	9	**	***	ns
	1992[3]	a.	119	100	130	107	9	ns	*	ns	140	114	128	108	18	ns	ns	ns
		b.	53	89	80	127	17	***	***	ns	92	76	101	113	12	+	ns	ns
Wool growth (μg/mm²/day)																		
	1991		17.1	17.9	18.1	23.1	0.9	**	**	*	14.7	16.3	18.9	17.9	0.7	***	ns	+
	1992		17.0	17.9	21.5	22.6	0.7	***	ns	ns	17.2	16.7	21.3	19.0	0.8	***	*	ns
Serum gastrin (pmol/l)			80	56	58	47	7	**	***	ns	43	30	30	27	3	**	**	+

ns=not significant +=p<0.10 *=p<0.05 **=p<0.01 ***=p<0.001

1. Pooled standard error
2. Significance of flock (flk), treatment (trt) and interaction (int) effects
3. a=day 0 to day 25
   b=day 26 to day 66

Table 2. Faecal egg counts (untransformed means ± s.e.m[1]) in subclinically infected Fleeceweight-selected (FW) and Control (C) sheep and in CRC-treated (+CRC) sheep (epg).

	ewes			rams			+CRC[2]
	C	FW	sign[3]	C	FW	sign[3]	
1991							
Day 0	550 ± 205	631 ± 177	ns	56 ± 37	1144 ± 545	*	-
Day 28	885 ± 427	2467 ± 1037	+	289 ± 135	604 ± 219	ns	7
Day 42	1486 ± 413	2050 ± 936	ns	1233 ± 381	2207 ± 582	ns	16
Day 76/90	63 ± 28	42 ± 20	ns	908 ± 297	2409 ± 568	*	35
1992							
day 0	359 ± 50	667 ± 64	***	415 ± 72	737 ± 86	*	-
day 25	163 ± 38	296 ± 46	*	328 ± 108	393 ± 65	ns	9
day 60	837 ± 200	1426 ± 144	**	941 ± 325	1548 ± 152	*	13

ns=not significant +=p<0.10 *=p<0.05 **=p<0.01 ***=p<0.001

1. Standard error of mean
2. Pooled across lines and sexes
3. Significance level based on squareroot transformed data

Discussion

For at least 17 years parasitism in both FW and C sheep has largely been controlled by regular anthelmintic treatment. Garrick *et al.* (1992) suggested that in such an environment sheep responding to parasite challenge in a way involving a high physiological (and therefore production) cost would be selected against ie. there would be a production advantage to having a low response to infection. Under such an hypothesis resistance is expected to decline following long term selection for high wool production. This trial confirms that resistance to infection (as indicated by FEC) is lower in FW sheep than C sheep.

It appears that in an environment of regular drenching, wool production and resistance may be unfavourably genetically correlated. This has important implications for sheep breeders intensively selecting for high wool production, and perhaps also for those considering selection for low FEC. This result is in contrast to studies which have indicated that selection for increased resistance (low FEC) in sheep is unlikely to adversely affect production (Woolaston, 1990; Bisset *et al.* 1992), although McEwan *et al.* (1994b) also concluded that resistance and production are unfavourably genetically correlated.

Production

There was little evidence to suggest that FW sheep suffer more production depression than C sheep despite developing higher FECs when subclinically infected. However there was a tendency toward greater production depression in FW ewes. Interpretation of results was hampered by low numbers of animals within groups, and the variable production response to CRC treatment (particularly in rams).

Faecal Egg Counts

Faecal egg counts tended to be higher in infected FW sheep than C sheep at each sampling time. Parasite burdens in infected sheep were kept low (ie subclinical)

for ethical reasons. It is likely that between-line differences in FEC would have become larger had infection continued for longer periods before anthelmintic treatment. These results support those of Williamson *et al.* (1994) who found that FECs were higher in 16-month FW rams compared to C rams when artificially infected with a mixed parasite burden.

FEC has been shown to be a useful indicator of the size, and potential pathogenicity, of a mixed parasite burden in outbred lambs grazing pasture (McKenna, 1981). Williamson *et al.* (1994) showed that higher FECs in FW sheep do indicate greater susceptibility to establishment of parasites (of some species), but may overestimate the true between-line difference in parasite burden. Care should be taken in extrapolating these findings based on artificially infected adult sheep to naturally infected lambs grazing pasture. Nevertheless it is likely that in the present study the actual difference in parasite burden between lines was less than that suggested by differences in FEC. There was some evidence that a low level of infection may have occurred in a small number of CRC-treated sheep, but this is unlikely to have had a major effect on production results.

Feed Intake

There was no evidence that feed intake was depressed in subclinically infected sheep in either line. This may partly be due to limitations in the method used for estimating feed intake which assumes that dry matter digestibility and chromate release rates are constant across lines and treatment groups.

Serum gastrin level

The gastrointestinal hormone gastrin produced in the abomasal mucosa of ruminants has many actions, one of the most important being stimulation of gastric acid secretion. Secretion of gastrin is regulated by a negative feed-back system involving abomasal pH: when abomasal pH rises gastrin secretion rises, resulting in increased gastric acid production and a fall in pH. The abomasal pH of sheep can

rise when infected with *H. contortus* (Nicholls *et al.* 1988), and *O. circumcincta* (Anderson *et al.* 1976), and possibly also when infected with the small intestine parasite *T. colubriformis* (Barker and Titchen, 1982), resulting in elevated gastrin levels. The extent of gastrin elevation above 'normal' levels in parasitised outbred sheep has been used to indicate the severity of gastric dysfunction (Fox *et al.* 1988).

Lower gastrin level in CRC-treated sheep indicates that prevention of a developing and adult parasite burden resulted in less gastric dysfunction than in sheep which were subclinically infected, although gastrin levels were not particularly high in any group. Nevertheless gastrin levels tended to be elevated to a greater extent in infected C sheep than FW sheep, suggesting infected FW sheep may have suffered less gastric dysfunction than C sheep.

Antibody levels

Levels of circulating antibodies have been shown to be elevated, and to be positively correlated with resistance status, in outbred sheep facing parasite challenge (McClure *et al.* 1992; Douch *et al.* 1994a). Lines of genetically-resistant sheep have also been shown to have higher antibody responses following challenge than random-bred sheep (Windon and Dineen, 1981; Gill, 1991; Gill *et al.* 1993a; Douch *et al.* 1994b).

At no time were parasite-specific antibody levels found to differ between FW and C lines despite clear differences in resistance between lines in the present trial and in additional work (Williamson *et al.* 1995 (in preparation), and unpublished observations). Therefore it appears that lower resistance in the FW line is not due to a lower antibody response, in contrast to the above studies which suggest that a difference might be expected.

No difference in antibody level was observed between infected (non-CRC) and uninfected (CRC) groups either. This appears to indicate that antibody levels reflected the level of larval challenge, rather than the burden acquired. Nevertheless

a between-line difference in the ability to mount an antibody response should still have been evident if it exists. The extent to which antibody levels were elevated above base levels is unknown, since levels in this trial cannot be directly compared with other published values.

There was little evidence of a relationship between antibody levels and FEC in either year but this may be due to the low numbers of animals within groups. In addition, in 1992 antibody levels were measured when sheep were between four and five months old at which time they were unlikely to have had a well-developed immune response.

Use of CRCs to prevent parasitism

The use of CRCs to compare the response to prevention of parasitism, between lines, was complicated by two problems. First, CRCs may not have been preventing all production depression, because of continuing larval challenge. Antibody responses suggest that ingested larvae may still have been immunogenic. The physiological (and production) cost of this response is unknown. Results of this study pertain to differences between lines in the response to elimination of a developing and adult burden, but not to differences which might arise had larval challenge also been prevented.

The second problem with the use of CRCs in this study was the highly variable nature of the production response to treatment. Liveweight gain and wool growth were significantly lower in CRC-treated sheep, compared to subclinically infected sheep during some sampling periods in both years. The cause of this effect, which was particularly evident in rams, is unknown.

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(For references see chapter seven)

Chapter Four

'Parasitological characteristics of fleeceweight-selected and control sheep'

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Abstract

Male sheep (aged 15 mnths) from a Massey University flock selected for increased fleece weight for 37 years (FW, n=13) and unselected controls (C, n=13), were infected with larvae of *Haemonchus contortus* (n=4000), *Ostertagia circumcincta* (n=22750), and *Trichostrongylus colubriformis* (n=25000). Some FW sheep (n=7) and C sheep (n=5), had previously been treated with an albendazole controlled release capsule (CRC), at 6 mnths of age.

Faecal egg counts (FEC) were higher in FW than C sheep (4204 *vs* 300 epg, $p<0.0001$), as were numbers of adult *H. contortus* (1151 *vs* 249, $p<0.01$) and *O. circumcincta* (2268 *vs* 600, $p<0.05$), when slaughtered at day 28. Numbers of *T. colubriformis* (5838 *vs* 5266) and total worm burden (9257 *vs* 6115) did not differ significantly between lines. Numbers of *T. colubriformis* were higher in previously CRC-treated sheep than in untreated sheep (7585 *vs* 3810, $p<0.05$) as was total worm burden (9918 *vs* 5773, $p<0.05$). The regression coefficient relating FEC to total worm burden was larger in FW than C sheep (0.35 *vs* 0.05 eggs per g faeces/worm, $p<0.01$).

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Packed cell volume (PCV) was lower in FW sheep than C sheep at day 27 ($p<0.05$). Thymus weight (corrected for liveweight) was higher in FW sheep than C sheep ($p<0.01$) and was lower in CRC-treated sheep than in untreated sheep ($p<0.01$). A line by treatment interaction was evident for number of mucosal mast cells in the small intestine ($p<0.005$). In the CRC-treated group C sheep had higher cell counts than FW sheep ($p<0.05$) while in the untreated group C sheep had lower cell counts than FW sheep ($p<0.05$). There was no effect of line or CRC treatment on number of mucosal mast cells in the abomasum. Numbers of mast cells were inversely related to number of parasites in FW sheep, but not C sheep. There was no significant effect of infection, line or CRC treatment on levels of circulating antibodies to *H. contortus*.

Keywords: sheep, selection, internal parasites, faecal egg count, mucosal mast cells, packed cell volume, thymus weight, antibodies, albendazole controlled release capsule.

Introduction

A line of sheep which has undergone 37 years of single trait selection for high fleece weight (FW) at Massey University (Blair *et al.* 1985), has been shown to have higher faecal egg counts than unselected controls (C) (Howse *et al.* 1992). A similar increase in faecal egg count has been reported in sheep selected for high production at Woodlands Research Station in Southland (McEwan *et al.* 1992b).

McKenna (1981) showed that faecal egg count was closely correlated with worm burden in young outbred sheep (up to 12 months of age), with a weaker correlation in sheep greater than 12 months of age. However faecal egg count was a good indicator of potential pathogenicity of the burden in both age groups. It can be argued that the pathogenicity of a mixed infection, as determined by the relative proportion of species present, is potentially of more significance than the number of worms alone.

The main objective of this trial was to confirm that higher faecal egg counts in fleeceweight-selected sheep actually reflect higher worm burdens, or greater potential pathogenicity of the burden, in the gastro-intestinal tract.

In addition the response of mast cells in the gastro-intestinal mucosa, and levels of circulating antibodies to *Haemonchus contortus* larvae were examined following infection. Thymus weights were measured as part of an unrelated trial (McCutcheon *et al.* 1993). The thymus has an important role in the immune system, particularly in its early development. Little is known about possible immunological differences between FW and C sheep which might account for differences in resistance to parasite establishment. Packed cell volume, which is a measure of anaemia, and rectal temperature were also compared between lines.

Adult sheep used in this trial were involved in a previous trial (Howse *et al.* 1992) in which some were treated with an albendazole controlled release capsule at 6 months of age, while the remainder were allowed to become subclinically infected. The effect of this treatment on variables measured in the present trial was examined. This trial was not designed to examine the effect of previous treatment and as a result numbers of animals within line and treatment group were low.

Materials and Methods

Sheep

The sheep used in this trial were drawn from the Massey University fleece weight selection lines. One line is a randomly bred control (C), the other (FW) has undergone single trait selection for greasy fleece weight for thirty-seven years. The origins and management of these Romney-based lines has been described by Blair *et al.* (1985). Both flocks are grazed together at all times except during mating. Thirteen rams from each line were available after selection of breeding stock, aged between fourteen and fifteen months at the start of the trial and reared on naturally infected pasture. Seven FW sheep and 5 C sheep were treated with albendazole

controlled release capsules (CRC, Nufarm) during their first Autumn (aged 6 months). Ten sheep drawn from both lines were included in the trial but left uninfected (and were not slaughtered) to serve as a control group for the analysis of antibody, packed cell volume and rectal temperature data.

All sheep were drenched with an anthelmintic eight days prior to infection to remove any existing parasite burden ('Leviben', 20g/l ricobendazole and 37.5g/l levamisole hydrochloride, dose rate 2ml/5kg liveweight).

Housing and feeding

Sheep were housed in raised pens, with two sheep per pen. Allocation to pens was random across lines and infection groups. A maintenance diet was fed consisting of lucerne chaff and formulated pellets, each supplying 50% of daily energy requirement. Water was available *ad libitum*. A period of 8 days elapsed before infection (day 0) to allow adjustment to the diet.

Parasites

Each sheep was infected with a mix of three species of infective (third stage) nematode larvae: *Haemonchus contortus* (n=4000), *Ostertagia circumcincta* (n=22750), and *Trichostrongylus colubriformis* (n=25000). These larvae were sheep-derived strains originating from Massey University (*H. contortus*), AgResearch Palmerston North (*O. circumcincta*) and Wallaceville (*O. circumcincta* and *T. colubriformis*). All sheep were known to have had previous exposure to these three parasite genera (Howse *et al.* 1992). The number of larvae in the infective dose was expected to achieve a low to moderate level of parasitism consistent with ethical considerations. Larvae were administered directly into the rumen by tube, in a water medium.

Sampling techniques

Faecal egg counts (FEC) were assessed at days 21 and 27 using a modified McMaster technique where each egg counted represented 50 eggs per gram of wet faeces. FEC was also assessed on day 0 to determine the efficacy of the anthelmintic drench (no eggs were found).

All infected animals were slaughtered on day 28 using a captive bolt pistol. The abomasum and small intestine were removed, ligated separately, and frozen for later parasite counts. Mucosal mast cells (including globule leucocytes) were counted as described by Douch *et al.* (1986). Sections for cell counts were taken from the fundic region of the abomasum, and one metre below the pylorus. Numbers of parasites were estimated by flushing the abomasal and small intestine contents and washing the mucosa of each into 2 litres of water. Aliquots were randomly drawn to make up 100 mls in which the total number of male and female parasites of each species were counted including 4th stage larvae.

The 'potential pathogenicity' of the total adult parasite burden was estimated using a 'points system' described by McKenna (1981) in which 500 *Haemonchus*, 4000 *Ostertagia* and 6000 *Trichostrongylus* are each equal to one point. This enabled a 'Total Pathogenic Index' to be calculated for each sheep. Actual pathogenicity of a parasite burden can only be measured in terms of effects on the host, which may differ between host genotypes. However a 'Total Pathogenic Index' is still a more useful measure than total burden when comparing the potential seriousness of a mixed infection between lines.

Packed cell volumes at days 5 and 27 were estimated following centrifugation at 3000 rpm for 15 minutes. Levels of polyclonal antibody (Ab) and specific immunoglobulin isotype G1 (IgG1) to infective larvae of *Haemonchus contortus* were estimated on day 27, using an enzyme linked immunosorbent assay (ELISA) technique (Douch *et al.* 1994a).

Rectal temperature was measured 8 days before and 8 days after infection. Organ weights of the 26 infected animals (including the thymus) were measured in a separate study (McCutcheon *et al.* 1993), although the effect of CRC treatment was not examined.

Statistical analysis

Differences between flock means for the variables measured were examined using analysis of variance techniques. The model included the fixed effects of selection line and previous CRC treatment. Between-line differences in the relationship between numbers of parasites and faecal egg count were examined using a general linear regression homogeneity of slopes model (H_o: $ß_{FW}=ß_C$).

The error distribution of parasite counts and FECs were negatively skewed, particularly in the C line, and variances tended to be larger in the FW line. Squareroot (sqrt) transformation gave the best improvement in normality and reduced the relationship between mean and variance. Where values were below 10 the transformation sqrt(x+0.5) was applied (Steel and Torrie, 1980).

Results

Preliminary results have been reported previously (Williamson *et al.* 1994) but did not include packed cell volumes, antibody levels, thymus weights and rectal temperatures, or the effect of CRC treatment on these and other parasitological parameters.

During the infection period there were no clinical effects of parasitism such as inappetance, diarrhoea, or listlessness.

Numbers of *Haemonchus* and *Ostertagia* at slaughter were greater in FW sheep than C sheep, but there was no significant difference in numbers of *Trichostrongylus* or in total worm count (TWC), between lines. However numbers

of *Trichostrongylus* and TWC were greater in sheep which had received previous CRC treatment than in untreated sheep. There was no significant effect of CRC treatment on numbers of *Haemonchus* or *Ostertagia* (Table 1).

The female to male ratio of the three parasite species did not differ between groups. Seven sheep from the FW line had early 4th stage larvae present at post-mortem, whereas larvae were present in only one C sheep. However the numbers of these larvae were very low and unlikely to be of biological significance.

The calculated 'Total Pathogenic Index' (TPI), was higher in FW sheep than C sheep (3.8 *vs* 1.5; $p<0.005$) with no significant effect of CRC treatment.

Faecal egg counts (sqrt transformed) were higher in FW sheep compared to C sheep at day 21 and day 27 and tended to be higher in CRC-treated sheep at day 21 ($p=0.09$) (Table 1).

The regression coefficient relating FEC at day 27 to total parasite burden was significantly larger ($p<0.01$) in FW sheep than C sheep (0.35 ± 0.13, $p<0.05$ *vs* 0.05 ± 0.01, $p<0.005$ eggs per gram faeces/worm) (Fig.2).

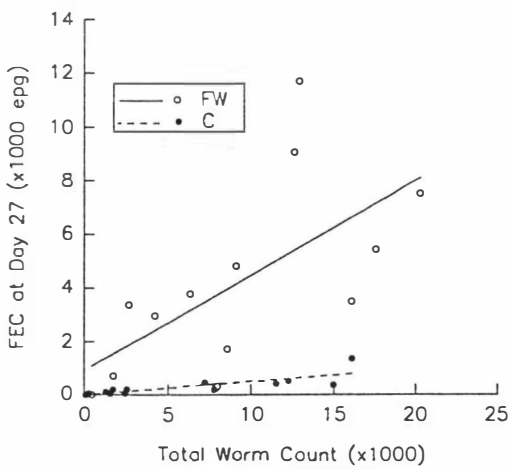

Figure 2 The relationship between faecal egg count at day 27 and total worm count in FW and C sheep artificially infected with nematode larvae

The regression coefficient relating FEC at day 27 to TPI was significantly larger ($p<0.001$) in FW sheep than in C sheep (1410±200, $p<0.0001$ *vs* 202±71, $p<0.05$, eggs per gram faeces/index unit) (Fig.3).

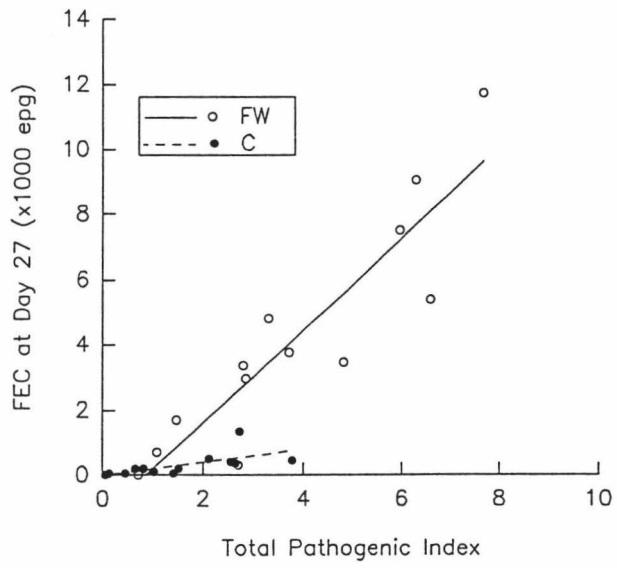

Figure 3 The relationship between faecal egg count at day 27 and Total Pathogenic Index in FW and C sheep artificially infected with nematode larvae

Mucosal mast cell counts in the small intestine appeared to be affected by previous CRC treatment with this effect being opposite between lines (interaction $p<0.005$). In the CRC-treated group C sheep had higher cell counts than FW sheep ($p<0.05$) while in the untreated group FW sheep had higher counts than C sheep ($p<0.05$). There was no effect of line or previous CRC treatment on numbers of mucosal mast cells in the abomasum (Table 1).

In FW sheep numbers of mucosal mast cells in the abomasum were strongly correlated with numbers of *Haemonchus* ($r=-0.79$, $p<0.001$) and with numbers of *Ostertagia* ($r=-0.66$, $p<0.01$) and small intestine mucosal mast cells were correlated with numbers of *Trichostrongylus* ($r=-0.67$, $p<0.01$). There was no evidence of a relationship between numbers of cells and parasites in C sheep (Fig.4).

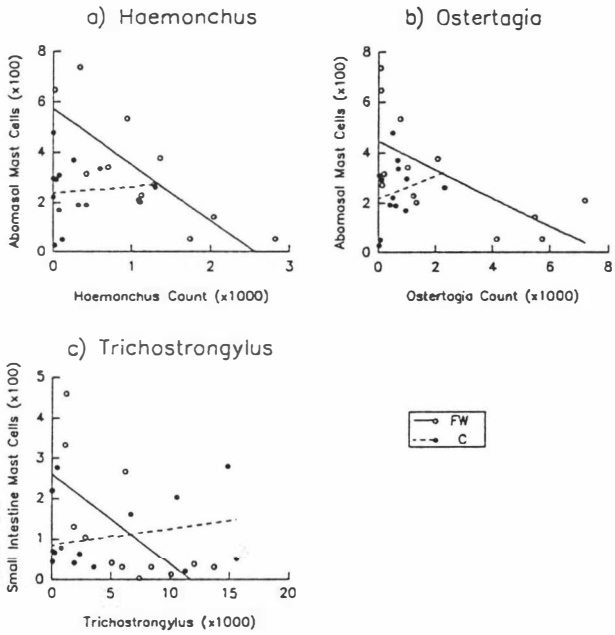

Figure 4 The relationship between mucosal mast cell count and worm count in FW and C sheep artificially infected with nematode larvae

Packed cell volume (PCV) was higher in C sheep than FW sheep at day 27, and this difference approached significance at day 5 ($p=0.07$). PCV declined between days 5 and 27 but this decline did not differ significantly between lines or between infected and uninfected sheep. There was no significant effect of previous CRC treatment on PCV level or decline (Table 2).

There was no significant effect of line, infection, or previous CRC treatment on levels of antibodies to *H. contortus* larvae (Table 2).

An interaction between the effects of line and previous CRC treatment on rectal temperature at day 8 was evident ($p<0.05$). In the CRC-treated group C sheep had higher rectal temperatures than FW sheep ($p<0.05$), while in the untreated group FW sheep had higher rectal temperatures than C sheep ($p<0.05$). There was no significant effect of infection on temperature at this time, and there was no significant effect of line or CRC treatment on temperature before infection (Table 2).

Results of organ weight analysis have been reported previously (McCutcheon *et al.* 1993). Thymus weights (corrected for liveweight) were greater in FW sheep than C sheep and were lower in sheep which had received previous CRC treatment than in those which had not (Table 1). CRC treatment did not appear to have affected other organ weights.

Table 3. Parameters measured in Fleeceweight-selected (FW) and Control (C) sheep at 16 months of age which received previous CRC treatment (+) or were untreated (-) at 6 months of age, when artificially infected with larvae (untransformed means)

	C		FW		PSE[1]	significance[2]	
	-	+	-	+		flk	trt
Number of worms							
H. contortus	293 (7%)[3]	180 (5%)	1157 (29%)	1146 (29%)	241	***	ns
O. circumcincta	668 (3%)	492 (2%)	2143 (9%)	2374 (10%)	741	*	ns
T. colubriformis	4245 (17%)	6900 (28%)	3230 (13%)	8074 (32%)	1945	ns	*
Total worm count	5205 (10%)	7572 (15%)	6530 (13%)	11594 (22%)	2371	ns	*
Faecal Egg Count (eggs/g faeces)							
Day 21	275	240	742	1779	388	*	+
Day 27	350	220	3575	4743	1003	***	ns
Mucosal Mast Cell Count							
Abomasum	233	268	292	338	71	ns	ns
Small Intestine	53	192	199	71	41	§	§
Thymus Weight (grams)[4]							
	25.8	17.7	53.1	29.9	7.4	**	**

ns=not significant +=p<0.10 *=p<0.05 **=p<0.01 ***=p<0.001

§=significance level not relevant due to presence of flock by treatment interaction, p<0.01

1. Pooled standard error, untransformed data
2. Significance of flock (flk) and treatment (trt) effects based on transformed data where appropriate (see text)
3. Establishment rate (adult worms recovered as a percentage of larvae in the infective dose)
4. Least squares means, corrected for liveweight

Table 4. Parameters measured in Fleeceweight-selected (FW) and Control (C) sheep at 16 months of age which received previous CRC treatment (+) or were untreated (-) at 6 months of age, when artificially infected with larvae, and in uninfected sheep (untransformed means)

	C		FW		Uninf[1]	PSE	significance[2]		
	-	+	-	+			flk	trt	inf
Packed Cell Volume									
Day 5	31.9	34.4	30.6	30.9	32.1	1.0	+	ns	ns
Day 27	30.3	32.2	28.4	28.7	29.7	0.9	*	ns	ns
Antibody Level									
Ab	1.02	1.01	0.94	1.11	1.03	0.12	ns	ns	ns
IgG1	0.86	0.74	0.76	0.97	0.84	0.17	ns	ns	ns
Rectal Temperature									
Day -8	38.75	38.80	38.87	38.93	38.86	0.07	ns	ns	ns
Day 8	39.33	39.50	39.48	39.28	39.50	0.08	§	§	ns

ns=not significant +=p<0.10 *=p<0.05 **=p<0.01 ***=p<0.001

§=significance level not relevant due to presence of flock by treatment interaction, p<0.05

1. Uninfected group (n=10)

2. Significance of flock (flk), treatment (trt) and infection (inf)

Discussion

The rams from the FW selection line used in this trial were those which remained after selection of breeding sires and therefore did not constitute a random sample of the total flock. If there is an unfavourable genetic correlation between FEC and wool weight as suggested by Garrick *et al.* (1992), the rams used in this trial would be expected to have lower FECs than the flock average. This difference would be small and would tend to underestimate any between-line differences in the parasitological parameters measured. The C rams used were those which remained after random selection of breeding sires and can therefore be considered a random sample themselves.

Parasite establishment

The generally low level of establishment of parasites in both lines indicates that most sheep had acquired a degree of immunocompetence, making them largely refractory to infection. However there were clear differences between sheep selection lines in the level of establishment and/or development of *H. contortus* and *O. circumcincta*, with numbers of adult parasites at slaughter being higher in the FW line than the C line. Total parasite burden did not differ significantly between lines although this was primarily due to the fact that establishment of *T. colubriformis* (which made up the largest, and most variable, proportion of parasites in the burden) did not differ between lines.

Sheep which received CRC treatment at 6 months of age appeared to have a reduced ability to prevent establishment and/or development of *T. colubriformis* in the small intestine at 15 months of age. This difference was also reflected in higher total parasite burden.

Jacobs *et al.* (1989) reported that a controlled release anthelmintic device can interfere with normal development of resistance to internal parasites. Calves treated with an oxfendazole pulse release bolus during their first summer had higher parasite

burdens than untreated controls in their second summer. In that trial it was suggested that the low level of pasture contamination achieved during the first summer may have been below threshold levels of antigenic information (Windon *et al.* 1984) required to stimulate immunity. However this is unlikely to have been the case in the present study. CRC treatment prevented parasite establishment for up to 100 days, but did not prevent exposure to infective larvae during this period or adult parasites before and after CRC treatment.

An alternative explanation is that albendazole had a direct effect on development of the immune system. It has recently been shown that benzimidazole drenches can affect a range of immunological functions in parasite-free sheep (Stankiewicz *et al.* 1994; Cabaj *et al.* 1994, in press).

The worm burden in FW sheep was made up of a higher proportion of species normally considered to be highly pathogenic, in particular *H. contortus*, and therefore the mean 'Total Pathogenic Index' (TPI) of the FW line was greater than that of the C line. Although the total parasite burden was not significantly larger in the FW line, on a theoretical basis the potential pathogenicity of the burden in FW sheep is considerably greater. However there is little evidence to suggest that the 'realised pathogenicity' of the burden acquired by FW sheep grazing pasture is greater than that of C sheep, with FW and C sheep tending to suffer similar production depression when challenged (Williamson *et al.* 1995, in preparation).

Faecal egg count

Higher mean faecal egg count in the FW line is consistent with observations by Howse *et al.* (1992) under field conditions. FEC increased rapidly between days 21 and 27 in the FW line, but increased only slightly in the C line. Little is known about possible differences in the kinetics of parasitic infection between these two lines, but the more rapidly increasing FEC in FW sheep may reflect greater parasite fecundity in this line. In utero egg counts of female worms were not performed in

this trial so it was not possible to determine whether parasite fecundity differed between lines.

The tendency for higher FEC at day 21 in CRC-treated sheep is likely to be, at least in part, a reflection of the greater number of *T. colubriformis* present. However the possibility of an effect of CRC treatment on parasite fecundity cannot be excluded.

Relationship between FEC and parasite burden

The relationship between FEC and parasite burden appears to differ between lines as a consequence of selection for fleece weight, as suggested by preliminary results (Williamson *et al.* 1994). Even though both lines carried a similar total burden, the FW line had a larger FEC at day 21 and a considerably larger FEC at day 27. This difference reflects, in part, the greater fecundity of *H. contortus* compared to the other species (McKenna, 1981). FW sheep, having a greater proportion of this species, had higher FECs even though the total burden was not significantly different. There was no evidence that a higher female to male parasite ratio was a contributing factor in the higher egg output of FW sheep.

The relationship between FEC and total parasite burden was compared between FW and C sheep at day 27 since at day 21 only 6 out of 13 C sheep had positive FECs. However in FW sheep the correlation between FEC and worm burden was stronger at day 21 ($r=0.80$, $p<0.001$) than at day 27 ($r=0.64$, $p<0.05$). This may be due to differences in the kinetics of infection between parasite species and/or lines. Between-line differences in the relationship between FEC and worm burden appear to be dependent on stage of infection.

Mucosal mast cells

A large number of trials using a range of host-parasite systems have shown a close association between inflammatory cells of the gut mucosa (including mast

cells and globule leucocytes) and resistance to internal parasites (reviewed by Rothwell, 1989).

Numbers of mast cells in the abomasal mucosa did not differ between lines despite clear differences in establishment of *H. contortus* and *O. circumcincta* in this part of the alimentary tract. Interpretation of between-line differences in levels of small intestine mucosal mast cells was complicated by an effect of CRC treatment. It is unclear why CRC treatment tended to decrease the inflammatory response in the small intestine of FW sheep, but to increase it in C sheep. This effect of CRC treatment may be related to between-treatment differences in numbers of *T. colubriformis* found in the small intestine.

The expected inverse relationship between mucosal mast cell numbers and parasite numbers was observed only in the FW line. It is unclear why there was no evidence of such a relationship in the C line.

Packed cell volume

Lower PCV in FW sheep has not been reported previously. It is unclear whether this difference is due to infection or independent of it. The magnitude of the difference in PCV between lines was similar at days 5 and 27 suggesting that the difference may be independent of infection. Infection did not appear to have any significant effect on PCV, with a general decline across both infected and uninfected groups.

It has been shown that there is a higher frequency of the haemoglobin type A allele in the C line (Pijls *et al.* 1988). A number of trials have shown that PCV is higher in sheep with this type of haemoglobin (eg. Luffau *et al.* 1990), which could account for the observed difference between lines. It has been suggested that this is a compensatory mechanism for the relatively inefficient oxygen carrying capacity of haemoglobin B (Agar *et al.* 1972). The lower oxygen and methionine availability in sheep with haemoglobin type B may also explain why a number of

trials have shown an association between this haemoglobin type and resistance to parasites (Agar *et al.* 1972).

Antibody level

There was no significant elevation in circulating levels of antibodies to *H. contortus* larvae (Ab and IgG1) in either line (relative to uninfected sheep). This may be because antibody levels resulting from natural infection in the pre-trial period remained elevated by day 27. A between-line difference in antibody level should still have been evident if it existed.

Thymus weight

Presson *et al.* (1988) reported that thymus weights were greater in sheep selectively bred for improved resistance to *H. contortus*, compared to sheep of normal resistance (aged 12 months). This contrasts with the present finding that susceptible (FW) sheep had greater thymus weights than sheep of normal resistance (C sheep).

CRC treatment appeared to interfere with normal development (or atrophy) of the thymus, leading to lower (residual) thymus weights in sheep at 16 months of age. This effect may be associated with reduced resistance to *T. colubriformis* observed in CRC-treated sheep.

Rectal temperature

There was some evidence that previous CRC treatment may have affected rectal temperature following infection, but the effect was small and may not be of biological significance. However mean rectal temperature within line and CRC treatment group exhibited the same pattern as mean mucosal mast cell counts in the small intestine, suggesting that rectal temperature may reflect the inflammatory response to parasite infection.

Conclusions

This trial confirms that resistance to establishment of internal parasite larvae (of some species) has decreased as a result of long term selection for high fleece weight, but that FEC tends to overestimate this difference. The relationship between numbers of mucosal mast cells and numbers of parasites, and thymus weights differed between lines suggesting an immunological basis for decreased resistance.

Interpretation of results was complicated by an effect of previous treatment with albendazole controlled release capsules on some parameters measured in this trial. Further work is required to determine the mechanism(s) involved.

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(for references see chapter seven)

Chapter Five: Additional Material

Statistical analysis

Data previously analysed by Howse *et al.* (1992) were reanalysed with some changes. A number of animals were excluded because they were missing at day 0 and consequently were not drenched. FEC data was transformed before analysis since variance at most sampling times was significantly larger in the FW line (variance was proportional to the mean). Distributions also tended to be significantly different from normal.

Feed intake was not used as a covariate for liveweight gain or wool growth (in contrast to Howse *et al.* 1992) because the relationship between feed intake and production tended to be weak (within groups) and variable (between groups). Using covariance analysis in these circumstances is not appropriate.

As a consequence of these changes, results presented in this thesis differ to some extent from those reported by Howse *et al.* (1992).

Development of immunity

In 1992 six additional FEC measurements were taken from non-CRC ewe lambs of both lines from day 146 until day 224 (mid-July) when lambs were aged between 10 and 11 months (Fig 5). FECs were measured every ten days during this period and lambs were drenched when mean FEC reached a trigger level of approximately 1500 eggs per gram (day 157 and day 224). These FECs were used to determine whether there were differences between lines in the pattern of development of immunity.

FECs were higher in FW ewes than in C ewes at each sampling time ($p<0.05$), except at day 188 when the difference approached significance ($p<0.10$). Outbred lambs grazing pasture generally begin to develop resistance around 6 months

of age and expel most of their parasite burden ('self-cure') by 9-10 months of age (Brunsdon, 1970; Waller and Thomas, 1981). Douch *et al.* 1984 found that when lambs were divided into low FEC and high FEC groups, low FEC lambs showed evidence of resistance to establishment by approximately 6 months of age whereas high FEC lambs remained susceptible until 9 months of age.

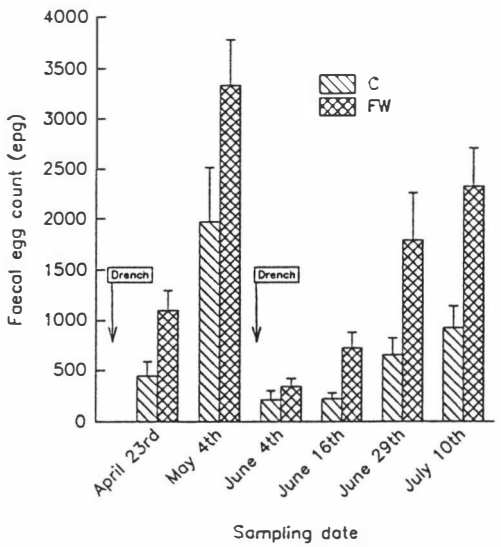

Figure 5 Faecal egg counts of non-CRC FW and C ewes in 1992 (untransformed means and std errors).

Mean FEC in the 1992 trial continued to increase in both lines up until day 224 when sampling ended (due to management constraints). This continuing increase is in contrast to typical patterns of infection where FEC declines from an Autumn peak as a result of lower larval development and increasing host resistance. However examination of the FECs of individual animals revealed that the FECs of a number of C lambs (8/20) remained low (<350 epg) during the last 5 weeks of the trial, whereas in the FW line FECs continued to increase in all but one lamb (1/20). This may indicate that C lambs were developing immunity (ie. self-curing) earlier than FW lambs. Results from the 1991 trial were inconclusive.

FEC was also assessed in previously CRC-treated ewes and untreated ewes at day 146 to determine whether CRC treatment affected development of immunity. FEC did not differ significantly between groups suggesting that previous exposure to CRCs had not affected immunity at this time.

Faecal consistency

Faecal samples taken from day 146 to day 224 were scored for faecal consistency (on a scale of 1=fluid to 5=pellets) to determine whether between-line differences existed. It has been reported that sheep bred for increased resistance to parasites have softer faecal consistency (and more dags) than random bred sheep (Douch *et al.* 1994b). It was suggested that this could be due to greater proteinase release from mucosal mast cells in resistant sheep, causing increased gut mucosal permeability and leakage of serum into the gut lumen and consequently softer faeces. If resistance is lower in FW sheep than C sheep as a result of lower immune responsiveness then it might be expected that FW sheep would have firmer faecal consistency. At day 213 faecal consistency actually tended to be slightly softer in FW lambs ($p=0.09$), but overall there was little evidence for a between-line difference. It should be noted that faecal consistency is likely to be softer in susceptible sheep when the level of infection is high due to the effects of diarrhoea.

Relationship between resistance and production

The fact that selection for high fleece weight has led to decreased resistance implies that FW sheep with a strong response to parasites tend to be genetically below average for wool production. The biological basis for this unfavourable genetic relationship is unclear. It may be that there is competition for limiting resources as suggested by Douch *et al.* (1994b). Alternatively it may be due to pathological effects of the immune response on the host resulting from inappropriate hypersensitivity reactions directed at small numbers of parasites.

Genes within the major histocompatibility complex (MHC) are known to have a strong influence on resistance to a range of diseases including nematode parasitism in sheep (Outteridge *et al.* 1988). The MHC has also been shown to be associated with production traits in several species (Warner *et al.* 1987). It is possible that selection for high wool production in an environment of low challenge has led to an increase in the frequency of MHC haplotypes associated with susceptibility to parasitic infection.

Evidence that a strong immune response is not necessarily beneficial is provided by Meeker *et al.* (1987), who found that high immune responsiveness in pigs was associated with low weaning weight and slower growth rate up to 100 kg liveweight. In addition Edfors-Lilja *et al.* (1986) showed that pigs with the intestinal receptor K88 (which confers susceptibility to *E. coli* infection) had lower growth rate initially, possibly due to diarrhoea, but grew faster than resistant pigs up to 100 kg liveweight.

Effect of CRC treatment on production

The most likely explanation for lower production in CRC-treated sheep compared to untreated sheep is an unfavourable effect of albendazole. An alternative explanation is that continuous larval challenge without establishment may have caused more production loss than when a burden was allowed to develop between anthelmintic treatments. It is known that incoming larvae play an important role in intra-host regulation of nematode populations (of some species) in sheep and CRC treatment may have increased the immunological cost, or pathological effects of larval challenge.

Gastrin

The difference between lines in gastrin level tended to be small in uninfected sheep and to become larger when infected although this line by CRC effect was not significant at the $p<0.05$ level. It is possible that when sheep are uninfected gastrin

level is similar in both lines and that a difference only becomes evident when infected, with gastrin being elevated to a greater extent in C sheep than FW sheep.

One probable physiological role of gastrin in ruminants is that it reduces gastrointestinal motility (Carr *et al.* 1970) which would slow the passage of ingesta, leading to reduced feed intake. Fox *et al.* (1989) showed that feed intake was depressed by up to 40% when gastrin levels were artificially elevated in parasite-free calves, and concluded that this was due in part to the effect of gastrin on motility. It is suggested that a superior ability of FW sheep to maintain a 'normal' gastrin level when parasitised would allow feed intake to remain relatively undepressed while carrying a larger parasite burden. This is supported by production results which show that FW sheep did not lose significantly more production than C ewes when infected, despite developing a larger burden.

This argument implies that maintaining a lower gastrin level in the face of parasite challenge is beneficial to the host. However Nicholls *et al.* (1988) suggested that hypergastrinaemia may play a role in the host's defence against parasites through stimulation of increased gastrointestinal motility and histamine release. Thus C sheep having a high gastrin response to infection would be more resistant but would lose more production due to decreased feed intake, when the level of challenge is low. FW sheep on the other hand, with a lower gastrin response, would be less resistant but able to maintain feed intake. Unfortunately it seems to be more accepted that gastrin reduces motility in ruminants rather than increases it (Carr *et al.* 1970; Fox *et al.* 1989) and while histamine is associated with level of infection, Douch *et al.* (1984) found no evidence of a direct role for histamine in parasite expulsion.

Relationship between mucosal mast cells and parasites

An inverse relationship between numbers of globule leucocytes, and numbers of parasites has been demonstrated in sheep in several trials (eg. O'Sullivan and Donald, 1973; Dineen *et al.* 1978; Dineen and Windon, 1980; Douch *et al.* 1986). There has been some controversy over the relationship between mast cells and

globule leucocytes. However it is now generally accepted that globule leucocytes are partially degranulated mast cells and that these two cell types can be included in one count as was done in this trial.

The inverse relationship between cell numbers and parasite numbers in FW but not C sheep is unexpected. This appears to suggest that the inflammatory response is linked to worm expulsion in FW sheep but not C sheep.

However Rothwell (1989) points out three reasons why this result should be interpreted with caution. First, immature mast cells are not easily recognised even though they may be functionally active. C sheep may have had more of these immature cells which were not observed. Second, the number of cells present gives no indication of the level of cellular function. During function mast cells may degranulate and therefore lose the granules by which they are identified. C sheep may have had a higher level of cellular activity resulting in superior worm expulsion. Third, other cell types, such as basophils, may perform the same role as mast cells and globule leucocytes. These other cell types were not counted in this trial. The time between infection and slaughter may also be important. For example if the inflammatory response is more rapid in C sheep, mucosal cell numbers may have already declined by day 28 following effective expulsion. Thus numbers of cells might bear no relation to numbers of worms at post-mortem.

For these reasons the lack of association between numbers of cells and parasites in C sheep is not necessarily indicative of a poorer inflammatory response. The fact remains that the 'typical' relationship between numbers of cells and parasites was observed in the FW line rather than the C line. This result is at least clear evidence of a physiological difference between lines in the response to parasitism.

Chapter Six: Summary and Conclusions

FECs of subclinically infected FW sheep were shown to be consistently higher than those of C sheep when grazing naturally infected pasture. It was subsequently demonstrated that higher FECs in FW sheep reflect greater establishment of *H. contortus* and *O. circumcincta* in the abomasum, but not *T. colubriformis* in the small intestine. Decreased resistance to internal parasites (of some species) in response to long term selection for increased wool production has been clearly demonstrated.

Possible reasons why selection for increased wool production might have lead to decreased resistance in FW sheep were considered. For at least 17 years selection for superior wool production has been in an environment of regular anthelmintic treatment. In such an environment there is no advantage to having a strong immune response to parasites, rather there may be a small physiological (and therefore production) cost involved. Thus sheep which respond strongly to a low level of challenge would tend to be selected against, which may have led to the observed decrease in resistance in the FW line.

Several indicators of immune responsiveness were examined in each line to determine whether FW sheep have a reduced response to parasite challenge, as the hypothesis above would suggest. There was no evidence of a between-line difference in antiparasite antibody levels in grazing lambs or in artificially infected adult sheep. Numbers of mucosal mast cells in infected adult sheep also did not appear to differ between lines. However there was a clear between-line difference in the relationship between numbers of mucosal mast cells and numbers of adult parasites recovered at slaughter. A typical inverse relationship was observed in FW sheep, while no relationship was evident in C sheep. In addition, thymus weights of adult FW sheep were greater than those of C sheep. It appears that some aspects of the immune response against parasites may have changed following selection for wool production.

Selection for increased wool production has also resulted in lower packed cell volume in FW sheep than C sheep, which may be related to known differences in frequency of haemoglobin genotypes between lines. Gastrin levels were also lower in FW sheep on pasture than in C sheep, but it is unclear whether this is independent of infection, or a result of a lower gastrin response to infection in FW sheep.

At subclinical levels of parasitism, FW sheep are more productive than C sheep despite carrying a larger parasite burden. Comparing production in sheep which were subclinically infected or which had infection controlled by albendazole CRCs suggests that FW ewes do not suffer significantly more production depression when infected than C ewes. Thus FW sheep can be considered more 'resilient' than C sheep, at least at the level of parasitism experienced in this study.

Unexpectedly, production in CRC-treated sheep was lower than in subclinically infected sheep at some stages of the trial, despite effectively preventing establishment of an adult parasite burden. The cause of this effect is unknown.

The effect of treatment of young sheep (aged about 6 months) with albendazole CRCs was examined 10 months later when sheep were mature. Resistance to establishment of *T. colubriformis*, but not *H. contortus* or *O. circumcincta*, was found to be lower in previously treated sheep than in untreated sheep. Thymus weight of treated sheep was also lower, and there appeared to be an effect on numbers of mucosal mast cells in the small intestine. It is unclear why this long term effect of treatment occurred, but it may be related to the known (short term) effect of albendazole on immune function in sheep.

Decreased resistance to internal parasites following long term selection for increased wool production suggests that resistance traits and wool production are unfavourably genetically correlated. This finding adds to a growing body of evidence indicating that slower selection responses can be expected when all traits (including resistance) need to be simultaneously improved.

Selection for improved genetic resistance (or resilience) to internal parasites in sheep is in its infancy. As a result of this study, several areas were identified which require further research before improved resistance can confidently be included as an industry objective. The sign, and magnitude, of the genetic correlations between resistance and other economically important traits (production, dagginess, other diseases etc.) need to be confirmed. With appropriate economic weights selection can then be on the basis of an index, rather than the less efficient method of independent culling levels. Whether resistance, as opposed to resilience, is actually the ideal selection objective when increased returns to the farmer is the ultimate aim of selection, also needs to be addressed. It is important to note that at present there is little evidence that selection for increased resistance results in increased returns to the farmer.

Surprisingly little is known about what makes one sheep more resistant to internal parasites than another. As our understanding of resistance at the mechanistic and genetic level improves, new selection criteria are likely to become available which will speed selection progress.

Despite the gaps in our understanding of parasitism and production in sheep, it appears that selection for improved genetic resistance will be one of the best long term solutions to parasite problems facing the sheep industries of New Zealand and Australia.

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Appendix One *Proceedings of the New Zealand Society of Animal Production 53*:413-415

'The evaluation of kiwifruit vinegar as a stock feed'

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ABSTRACT

Two trials were conducted to assess the effect of kiwifruit vinegar on a number of production parameters in sheep. Trial A evaluated the effect of kiwifruit vinegar (8.2g/100ml, as acetic acid) on lamb liveweight gain, fleece production (midside growth rate, yield and colour), and internal parasite burdens. Trial B evaluated the effect of kiwifruit vinegar on two-tooth liveweight change prior to mating, and subsequent fertility.

In trial A 138 ewe lambs were randomly assigned to one of four treatment groups: i) "Extender 100" Albendazole drench capsule + 10 ml vinegar; ii) "Extender 100" Albendazole drench capsule only; iii) normal drenching (trigger level 1500 epg) + 10 ml vinegar; iv) normal drenching. The only trait for which there was strong evidence of an effect was wool yield which decreased in response to kiwifruit vinegar. Vinegar treated lambs tended to have reduced faecal egg counts during the period of the trial.

In trial B 140 two-tooth ewes were randomly assigned to one of two treatment groups: i) 15 ml vinegar administered at 12, 8, 4 and 1 weeks prior to mating; ii) control. Reproductive status was assessed by ultrasound 38 days after removal of the ram. No significant differences were found in liveweight or in reproductive status between the two groups. Evidence from these two trials would suggest that any effect of kiwifruit vinegar on performance traits in sheep is small and not necessarily beneficial.

Keywords kiwifruit vinegar, internal parasites, albendazole capsule, wool growth, wool yield wool colour, liveweight gain, fertility.

INTRODUCTION

Kiwifruit vinegar is sold in New Zealand as a stock feed, and there are some suggestions among farmers that the product can improve stock health and performance. However to date there have been no published data to support this belief. Other types of vinegar have traditionally been the subject of claims regarding beneficial properties, particularly cider vinegar. Rice vinegar has been used in Japan for thousands of years in the belief that it promotes good health and cures a wide range of ailments in man (Kuroiwa 1977).

Kiwifruit vinegar is produced by fermentation of kiwifruit wine, in the same way that apple wine has traditionally been used to produce cider vinegar. The main component is acetic acid (8.2 g/100 ml). Chemical analysis of kiwifruit vinegar indicates it containes a range of micronutrients such as magnesium (52 mg/l), calcium (92 mg/l), phosphorus (143 mg/l) and some trace elements (eg copper, 0.28 mg/l; zinc, 0.56 mg/l). Total solids amount to 2.6 g/100 ml.

The aim of this experiment was to investigate the effect of kiwifruit vinegar on liveweight gain and wool production and quality in lambs, and the fertility of two-tooth ewes. It is possible that kiwifruit vinegar may affect production in lambs due to anthelmintic properties (Rutherford 1987). The trial design therefore allowed a comparison of the effects of vinegar in both a parasitised and an unparasitised environment.

MATERIALS AND METHODS

Trial A

One hundred and thirty eight ewe lambs were randomly allocated to one of four treatment groups in January 1992. These were: extender 100 Albendazole capsules (CAP); extender 100 Albendazole capsules plus 10ml vinegar (CAP+VIN); Normal drenching only (NONCAP) and normal drenching plus 10ml vinegar

(NONCAP+VIN). The extender 100 Albendazole capsules contain 2.1 g Albendazole released at a rate of 0.5 mg Albendazole/kg LWT/day for a 40 kg animal.

Of the 138 ewe lambs, 101 were born at the usual time (Aug/Sept) and 37 were early-born (May). Both age groups were evenly distributed amongst the four treatment groups. Prior to allocation to treatment groups all lambs were drenched at a rate of 1 ml/4 kg of levamisole (Nilverm, Coopers-Pitman-Moore, New Zealand Limited) to eliminate any existing internal parasite worm burden. Midside wool growth was measured from samples taken on the right midside of lambs while they lay on a flat surface (Bigham 1974). An initial patch measuring 20 cm x 20 cm was cleared of wool on day 1. Subsequently an area of approximately 100 cm^2 was clipped and measured using callipars. Kiwifruit Vinegar (Prestons Kiwifruit Winery, Tauranga, New Zealand) was administered orally on days 1, 28, 56 and 86 of the trial (10ml per animal of an 8% kiwifruit vinegar solution diluted with equal parts of water) according to the manufacturer's recommendations. All animals were weighed on days 1, 28, 56, and 86, and the midside wool patch was clipped on day 116. Greasy wool samples were scoured to determine clean growth rate, yield and colour (Bigham *et al.* 1984) under standard conditions (Parker *et al.* 1991).

"Extender 100" albendazole slow release drench capsules (TM FERNS Corporation Ltd) were inserted into CAP and CAP+VIN lambs (day 1) to suppress adult worm burden. The capsules provide protection from internal parasites for 100 days. Four capsule treated animals were sampled for faecal egg count on day 86 of the trial and were found to have zero eggs per gram of faeces.

NONCAP and NONCAP + VIN lambs were allowed to develop subclinical parasitism, until a trigger level of 1500 eggs per gram was reached, at which time all lambs in these two groups were faecal sampled and drenched (days 55, 91 and 119). The rise in internal parasite burden was monitored using a random sample of 20 animals, measured 28 days after each drench, and then every 10 days until the trigger level was reached. This trigger level has been used by other workers, to indicate when drenching is required (Watson *et al.*, 1986). FEC was assessed using

the Modified McMaster Technique where each egg counted represents 50 eggs per gram (Watson *et al.* 1986).

Trial B

One hundred and forty two-tooth ewes were randomly allocated to either a control or kiwifruit vinegar treatment group in December 1991. All animals were drenched with an anthelmintic on day 1. Kiwifruit vinegar was administered on days 1, 31, 60 and 87 (15ml of 8% vinegar solution, according to manufacturer's recommendations). All animals were weighed at these times. Mating took place between days 84 and 132 and pregnancy status was assessed by ultra-sound on day 170, 38 days after removal of the ram.

In Trial A liveweight gain and wool traits were analysed using standard univariate analysis of variance techniques. The model used took account of the effects of vinegar, capsule and age. The distribution of faecal egg counts at each sampling time was found to be positively skewed. Transformation by squareroot gave the best improvement in normality so all FEC data has been analysed using this transformation. Repeat measures analysis of variance was considered for the analysis of FEC but was found to be unsuitable due to the number of missing values. All results are presented as least squares means and standard errors, or for faecal egg counts as re-transformed least squares means. In trial B reproductive status was analysed using maximum likelihood estimation of parameters.

RESULTS

Trial A

Age of lamb was found to have no effect in all traits except wool colour. It was therefore decided to pool results across ages for all other traits to give increased statistical power for the detection of differences between vinegar-treated and control groups.

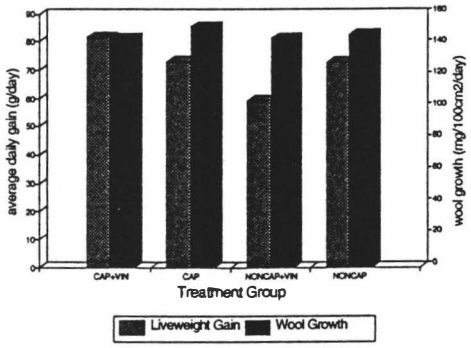

Figure 6 The effect of kiwifruit vinegar (+VIN) and anthelmintic capsules (CAP) on liveweight gain and clean midside wool growth in ewe lambs

Fig. 6 shows the effect of vinegar on liveweight gain and clean wool growth. In non-capsule animals the vinegar treatment caused a reduction in liveweight gain whereas in the capsule-treated animals, kiwifruit vinegar caused a slight increase (significant vinegar x capsule interaction, $P<0.05$). Clean wool growth was not affected by vinegar treatment in either the capsule-treated or non-capsule groups. However wool yield (%) was lower in the vinegar-treated lambs compared to the untreated controls (81 ± 0.35 *vs* 82 ± 0.36; $P<0.05$) with no effect of capsule treatment.

Early-born lambs which received vinegar had poorer wool colour compared to controls (Y minus Z values of 1.42 ± 0.15 *vs* 0.98 ± 0.15; $P<0.05$). In the normal aged lambs this trend was reversed with vinegar treated lambs having slightly better colour compared to the non-vinegar lambs (Y minus Z values of 2.15 ± 0.09 *vs* 2.26 ± 0.10; significant vinegar x age interaction, $P<0.05$). Capsule treatment had no effect on wool colour.

Faecal egg counts are given in Fig. 7 and indicate that at all faecal sampling times except for day 28 the vinegar-treated lambs had lower faecal egg counts. However this difference could only be shown to approach significance at day 38 ($P=0.092$) and day 55 ($P=0.087$). Only on days 55, 91 and 119 were samples from all non-capsule animals included, the other four sample times were monitoring counts of a sub-sample of 20 animals.

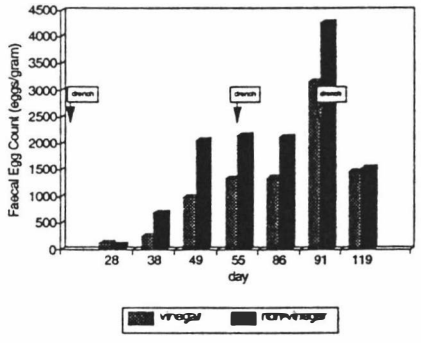

Figure 7 The effect of kiwifruit vinegar on faecal egg count in non-capsule treated ewe lambs

Trial B

Ewe liveweight prior to mating, and at mating, was not significantly different between vinegar-treated and control groups (Table 1). Reproductive status (dry, single or twin) also did not differ between treatment groups, 38 days after removal of the ram. The respective proportions of dry, single and twin bearing ewes were 5.9%, 42.6% and 51.5% in vinegar treated ewes and 7.4%, 38.9% and 53.7% in non-vinegar treated ewes.

TABLE 1 The effect (±SEM) of kiwifruit vinegar on liveweight (kg) in two-tooth ewes during the mating period.

Day of Trial	Vinegar	Non-vinegar
Day 1[1]	54.70±0.68	55.31±0.70
Day 31	58.07±0.70	58.47±0.72
Day 60	56.54±0.65	57.46±0.66
Day 87	56.74±0.62	56.88±0.64

[1]day 1 = 18th January

DISCUSSION

Trial A addressed two main issues. Firstly, whether kiwifruit vinegar had an effect on liveweight gain and wool traits. Secondly whether any differences which did arise could be attributed to an anthelmintic effect. It should be noted that although the capsules prevented the establishment of an adult worm burden, the lambs were still exposed to larval challenge. There are no published data available on what effect, if any, this exposure might have on production (e.g. as a result of an immune response), when capsules are being used. Thus it would be unwise to assume that the capsule-treated animals are strictly in an "unparasitised environment", when comparing capsule and non-capsule groups.

The vinegar-treatment did not appear to have any effect on wool growth overall. In the case of liveweight gain there was the suggestion of an interaction between vinegar and capsule treatments, but in the opposite direction to that expected if vinegar has an anthelmintic effect.

The decrease in wool yield due to vinegar treatment implies an increase in the yolk content of the fleece. Yolk is made up of two fractions, wool wax produced by the sebaceous glands, and suint which is mainly potassium salts (Henderson 1967). An increase in sebaceous gland activity would tend to support the claim by users of vinegar that it improves the shine on an animal's coat. This trial did not determine which part of the yolk increased, or whether it was a combination of both parts. Although the amount of greasy wool grown was not significantly different between groups there is no evidence to suggest that the increase in yolk comes at the expense of clean wool grown. This lower yield is in contrast to unpublished evidence that suggests cider vinegar reduces wool grease in adult ewes (New Zealand Farmer 1991). The detrimental effect of kiwifruit vinegar on wool colour in lambs is unlikely to be economically important. The interaction between vinegar and age group is likely to be the result of a combination of age differences and the small management differences which occurred.

The faecal egg count data suggests that the rate of re-infection by internal parasites following anthelmintic drenching may be lower in the vinegar-treated lambs. However this result should be interpreted with caution since the relationship between FEC and worm burden may have been disrupted by vinegar treatment. Fecundity of the worms may have been reduced rather than the actual number of worms. If this is the case we would expect the relationship between FEC and production to be different for the vinegar and non-vinegar groups. There was however, no significant relationship between FEC and production at any sampling time. A lower FEC in itself can still be considered a desirable effect because of the possibility of reduced pasture contamination. It should also be noted that running vinegar and non-vinegar animals together would have tended to underestimate any differences in FEC which may exist, because of cross-infection.

Kiwifruit vinegar had no effect on pre-mating liveweight change or fertility (dry, single or twin) in two-tooth ewes. This is to be expected considering the infrequent dosing intervals and low dose rates administered throughout the trial.

Evidence from these two trials suggest that any effect of kiwifruit vinegar on performance traits in sheep is small and not necessarily beneficial. Further work will be needed to confirm these findings and will require varying the treatment frequency and dose rates.

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