CHAPTER 1

GENERAL INTRODUCTION

Gastrointestinal (GI) worms represent the major health cost to the Australian sheep industry, which is estimated at $369 million annually (Sackett *et al.* 2006). The escalating cost of worms is due to the overwhelming prevalence of anthelmintic resistance on most Australian sheep farms. Besier and Love (2003) collated data which indicated resistance to benzimidazoles on 90-95% of sheep properties and resistance to levamisole on 80-90% of properties in NSW, VIC and WA. Resistance to the macrocyclic lactone drench group has also increased significantly in the last decade (Bailey & Neilsen 2005). Reliance on chemical treatment and the declining effectiveness of these treatments leads to increased mortality and production loss. Adoption of alternative worm control practices that constitute integrated parasite management programs is essential to ensure production losses do not reach an even higher cost.

Breeding for resistance to GI worms is a long-term practice that has been adopted by some leading sheep breeders. The benefits of selection for resistance are lower worm burdens, reduced frequency of anthelmintic use and reduced pasture contamination by infective larvae (Gray 1997). There are a number of experimental flocks and commercial properties within Australia that have shown the value of genetic selection for nematode resistance. Genetic variation in resistance to *Haemonchus contortus* has been demonstrated in the CSIRO *Haemonchus* selection flock. The flock comprises three divergent lines selected for increased resistance (IRH) and decreased resistance to *H. contortus* (DRH), compared with an unselected control line (C) (Woolaston *et al.* 1990). Selection is based on worm egg count (WEC) following artificial challenge with infective *H. contortus* larvae at approximately six months of age. The heritability estimates of WEC of experimental flocks range from 0.21 to 0.44 (Albers *et al.* 1987, Woolaston *et al.* 1991). Genetic correlations calculated in a range of Merino resource flocks across Australia show correlations between resistance and production traits were close to zero. Pooled estimates (s.e not available) for greasy fleece weight, clean fleece

weight, fibre diameter and bodyweight were 0.15, 0.10, -0.06 and -0.21 respectively (Eady *et al.* 1998).

Comparison of alternative worm control measures revealed genetic selection for resistance to be the most effective treatment for reducing WEC, with a 70% reduction between selected and random bred animals. In contrast, the use of protein supplementation (31% CP at 100g/hd/d twice weekly) and strategic drenching (Wormkill) produced a 35 and 12% reduction in WEC, respectively (Woolaston *et al.* 1997). However, the genetic comparison occurred between lines of sheep selected for approximately 20 years, whereas the other treatments could be used immediately.

It appears genetic selection is the most effective long-term treatment for reducing WEC, however correlated responses with production traits, such as growth rate and wool production, indicate that such selection has undesirable consequences. Applying WEC selection in industry flocks requires a selection index to account for unfavourable correlations with resistance (Morris *et al.* 2000). The production trait parameters measured from the study reported by Woolaston *et al.* (1997) indicated that despite a 70% reduction in WEC, genetic selection had no effect on bodyweight gain and resistant animals had 9% less clean wool growth than unselected animals. The protein supplemented group had a 44% greater bodyweight gain and grew 17% more clean wool than unsupplemented counterparts (Eady *et al.* 2003). Eady & Smith (2001) showed following an *H. contortus* trickle challenge in sheep selected for worm resistance and unselected controls, greasy clean wool growth and bodyweight were similar between groups. The main productive advantage shown by resistant animals was nil mortality, compared to a 25% mortality rate (based on packed cell volume (PCV) < 14%) in unselected animals (Eady & Smith 2001). Kahn *et al.* (2003) observed that random bred lambing ewes were no different for bodyweight gain and actually had greater annual clean fleece weight, clean wool growth rate and mean fibre diameter than resistant ewes, whilst ewes had a five-fold difference in WEC. In the absence of nematode infection, Doyle (1999) showed resistant ewe hoggets displayed a reduced productivity compared to random bred counterparts. Random bred animals gained an extra 1.1 kg bodyweight and 0.6 g/d greater clean wool growth rate, compared to resistant animals.

The expectation is that resistant sheep should suffer less production loss due to a reduced parasite infection. Yet, productivity is comparable to or lower than that of animals carrying a large parasite burden (susceptible genotype) or even in the absence of infection, but presence of incoming infective larvae. It appears that while resistant animals are exposed to nematode parasites an immune response is triggered which assists to reduce establishment and development of larvae. Even in the absence of infection, resistant animals may not be able to effectively 'down regulate' their immune response resulting in excessive activity. Consequently, protein supply may be redirected away from production traits into immune function (Symons & Jones 1975, Jones & Symons 1982, MacRae 1993, Coop & Sykes 2002). In contrast, susceptible animals show similar bodyweight gains and wool growth rates as resistant animals, while carrying an infection significantly greater. Clearly these susceptible animals are displaying stronger resilience to parasite challenge, which is demonstrated in selection flocks selected for superior production traits (Howse *et al.* 1992, Williamson *et al.* 1994, 1995).

It is important to understand the apparently separate mechanisms that arise from divergent selection on the same trait. Resistant animals effectively prevent establishment and/or development of nematode infection, yet seldom have greater productivity. In contrast, susceptible animals carry large parasite burdens and have equal or higher productivity than resistant animals. Understanding these mechanisms will clarify the biological basis for genetic correlations between parasite resistance and production traits and allow resistant genotypes to be exploited to their greatest potential and used more effectively in an integrated GI parasite control program.

Host nutrition has long been considered to be an important factor influencing the host-parasite relationship and the impact of GI nematode infections (van Houtert & Sykes 1996). The main aspects of the interaction between host nutritional status and GI parasitism have been extensively reviewed (Steel 1974, 1978, Sykes 1987, Preston & Leng 1987, Parkins & Holmes 1989, Poppi *et al.* 1990, MacRae 1993, Holmes 1993, Coop & Holmes 1996, van Houtert & Sykes, 1996, Coop & Kyriazakis, 1999, Coop & Kyriazakis 2001, Walkden-Brown & Kahn 2002, Coop & Sykes 2002). The effect of parasitism on the host metabolism can be summarised in three areas: nutrient intake, nutrient digestion and absorption and protein and amino acid utilisation. Quantifying the

differences in nutrient intake, digestion and partitioning among divergent selection lines may help to identify the mechanisms that influence resistance and resilience.

In this thesis the aim was to determine if divergent selection for resistance to *H. contortus* has produced correlated changes in voluntary feed intake and diet selection, ruminal digestion, nutrient partitioning and immunological responses to gastrointestinal nematode infection.

CHAPTER 2
PHYSIOLOGICAL RESPONSES TO GASTROINTESTINAL NEMATODE INFECTION IN SHEEP SELECTED FOR GENETIC DIFFERENCE IN RESISTANCE TO *HAEMONCHUS CONTORTUS*: A REVIEW

2.1 Introduction

In this review the influence of nutrition on the host-parasite relationship and the impact on productivity of gastrointestinal nematode infection in sheep is examined. The main aspects believed to be involved in a host's response to nematode infection are discussed, with emphasis on *Haemonchus contortus* (*H. contortus)* infection where literature exist: 1) nutrient intake and diet selection, 2) rumen function and nutrient supply, 3) partitioning of nutrients among organs and tissues and 4) immunological responses to infection. The focus in this review is the correlated response of the host to infection following years of divergent selection for resistance to *H. contortus*.

2.2 Distribution and lifecycle of *Haemonchus contortus*

H. contortus or Barber's pole worm is a prolific roundworm parasite of sheep commonly found in summer rainfall areas of Australia. Climatic conditions determine where *H. contortus* occurs and when they are most prevalent during the year. *H. contortus* is endemic throughout the New England area and northern New South Wales, south east Queensland and along the south coast of Western Australia. Studies have shown *H. contortus* eggs to develop best at a temperature range between 20-30°C, with low recovery of 3rd stage larvae below 10°C and above 30°C (Coyne & Smith 1992b). Optimal development of 3rd stage larvae requires higher faecal moisture content, approximately 70%, than for other dominant nematode parasites (Rossanigo & Gruner 1995).

The life cycle of *H. contortus* is typical of the major gastrointestinal parasites of sheep (Figure 2-1). Adult worms live in the abomasum of the sheep and lay eggs that pass out in the faeces. *H. contortus* is distinguished from other sheep roundworm parasites by

being the most prolific egg layer. Female worms are capable of laying between 5,000-10,000 eggs/d (Gordon 1948, Le Jambre 1995). The eggs hatch on pasture into 1st stage larvae within 4-6 d; 2nd stage larvae emerge from the cuticle and then develop into 3rd stage larvae. Pre-infective larval stages feed on bacteria in the faeces. The 3rd stage infective larvae are ingested by the grazing sheep and moult into 4th stage larvae before establishing in the abomasum. The 4th stage larvae penetrate the lining of the abomasum and begin to feed on blood and develop into adults. It takes around 21-28 d from ingestion of larvae to the appearance of worm eggs in faeces (Dineen *et al.* 1965, Dineen & Wagland 1966).

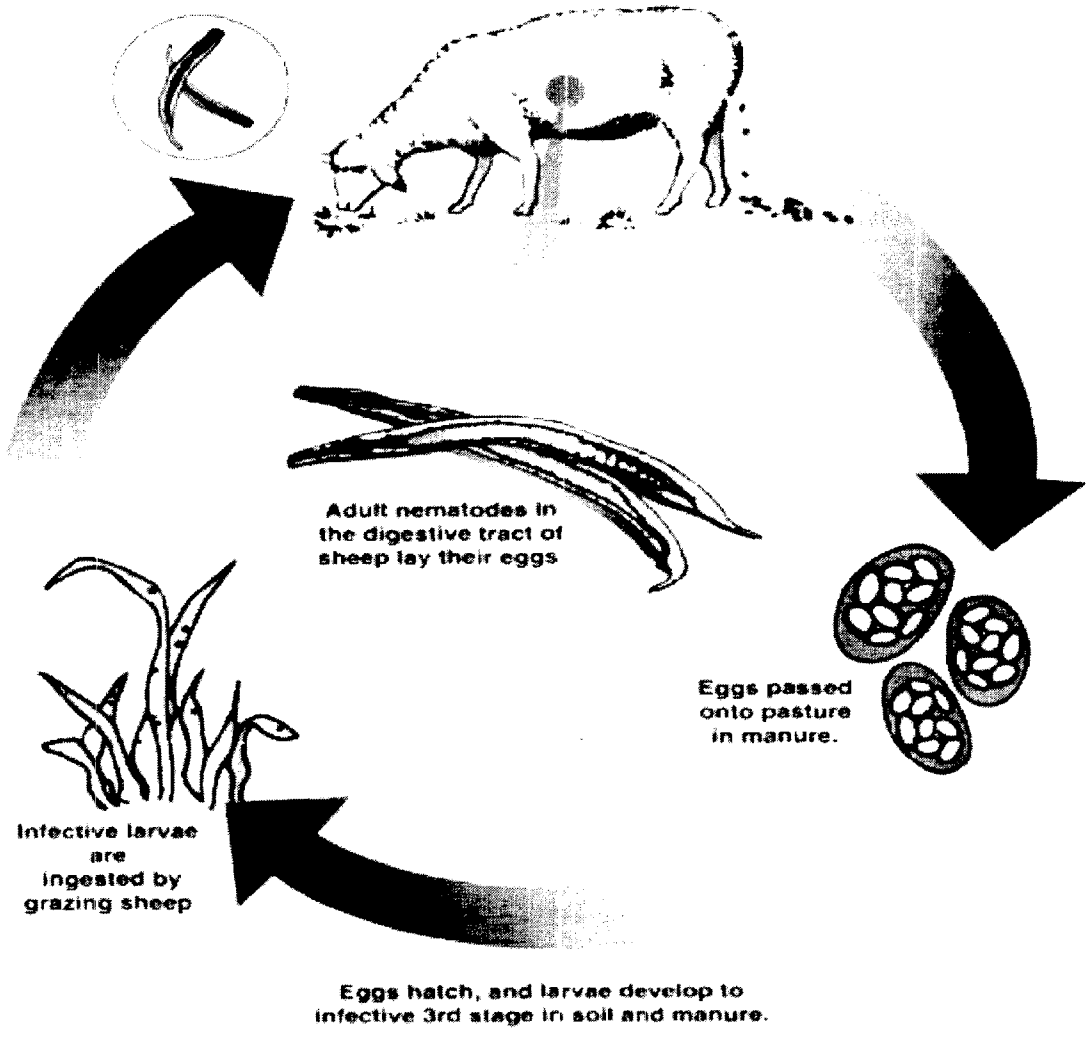


Figure 2-1 Generalised lifecycle of nematode parasites (Whittier *et al.* 2003).

2.3 Effects of a gastrointestinal nematode infection on host

2.3.1 Blood loss

Blood loss is chiefly confined to infections with *H. contortus*. The 4th stage larvae and adults suck blood and cause haemorrhage in the abomasum, consequently the main symptom of haemonchosis is anaemia. Estimates of average blood loss per worm per day range from 0.003 ml (Dargie & Allonby 1975) to 0.005 ml (Clark *et al.* 1962). Radio-tracer studies using ^{51}Cr-labelled erythrocytes show sheep carrying burdens of 3,000-4,000 worms could incur daily blood loss of 150-200 ml (Dargie 1975; Altaif & Dargie 1978; Abbott *et al.* 1986). Progressive anaemia stimulates an increase in erythrocyte production, which in turn may cause an iron deficiency in the parasitised animal (Albers *et al.* 1990). Daily catabolism of haemoglobin in sheep infected with *H. contortus* is increased five-fold compared to normal losses attributed to red cell breakdown (Steel 1978). In addition Rowe *et al.* (1988) reported that 9 month old Merino wethers carrying a mean *H. contortus* burden of 5,008 adults had a blood loss into the gastrointestinal tract of 253 ml/d, which equated to a loss of 2.6 g blood nitrogen /d.

The end result of chronic blood loss is often seen as declining haematocrit. Haematocrit values, as a measure of anaemia, have been shown to fall from normal levels of 0.27-0.45 *l/l* to 0.17 *l/l* within 4 weeks post-infection in animals fed a low protein diet (Abbott *et al.* 1986a).

2.3.2 Plasma loss

Plasma leakage resulting from the damage caused by the blood-sucking activities of the *Haemonchus* parasite, contributes to an even greater loss of plasma into the gut, than that ingested by the parasite (Steel 1978). Tissue damage is also caused by the emergence of adult worms into the abomasal lumen in abomasal infections (Parkins & Holmes 1989). *H. contortus* infection causes as much as 10% (per d) of circulating plasma volume to leak into the gastrointestinal (GI) tract (Parkins & Holmes 1989). Dargie (1975) reported plasma leakage in sheep infected with *H. contortus* to be 273 ml/d, compared with 38 ml/d in control animals.

The development of hypoalbuminaemia is associated with an increase in turnover rate of the plasma albumin pool, shown to increase by 40% in *H. contortus* infected sheep (Dargie 1975). The irreversible loss of albumin can be up to 50% higher in infected animals than in uninfected counterparts (Steel 1978). Severe cases of hypoalbuminaemia can result in odema under the jaw (bottle jaw).

2.3.3 Feed intake

Reduction in voluntary food intake has been described as one of the main features of gastrointestinal nematode infections, which can range from a progressive decrease in intake to almost complete anorexia in acute cases (Coop 1981). Reduced feed intake is more equivocal in *H. contortus* infections, as some studies show no change in feed intake between infected and uninfected animals (Wallace *et al.* 1998). A consequence of depressed feed intake is a reduction in animal productivity. Bodyweight gain has been shown to be reduced by 30-50%, partly as a result of 10-20% reduction in voluntary food intake (Coop 1981). The reduction in voluntary feed intake that accompanies gastrointestinal infection accentuates the need for a higher dietary protein intake (Symons, 1985). Abbott *et al.* (1986b) showed that animals infected with *H. contortus* and given a low protein diet had lower daily feed intakes than animals fed a high protein diet.

Voluntary feed intake has been demonstrated to decline from about week 3-4 of infection, during a small intestinal infection with *Trichostrongylus colubriformis* (Kyriazakis *et al.* 1994), at which time a proportion of the ingested larvae have developed into mature adults. Feed intake was shown to recover to a similar level to that of uninfected counterparts and remained unaffected within a few days after anthelmintic administration or once animals developed a strong immunity (Kyriazakis *et al.* 1996a).

Investigation into the mechanisms which induce a reduction in feed intake have suggested that central satiety signals might be associated with inappetence in sheep infected with *T. colubriformis* (Dynes *et al.* 1990). The central brain cholecystokinin (CCK) receptors may also play a role in regulation of feed intake (Dynes *et al.* 1998). The gastrointestinal hormone gastrin has also been shown to be elevated in parasitised animals. The actions of gastrin in parasitised animals have been summarised by Parkins

& Holmes (1989) as the ability to inhibit food intake in hungry sheep, reduce the frequency of reticular contractions, inhibit abomasal emptying and affect the normal myoelectric complex of the small intestine.

2.3.4 Nutrient supply

2.3.4.1 Digestibility

Impairment of feed digestion in the parasitised animal is one factor contributing to reduced performance (Parkins & Holmes, 1989). Barger (1973) described a reduction of up to 4% in apparent digestibility of organic matter, dry matter and nitrogen, which was associated with a 17% reduction in wool growth of sheep infected with *T. colubriformis*, despite no change in feed intake. The greatest influence of infection was on apparent N digestibility. Barger (1973) concluded that sheep infected with *T. colubriformis* are less efficient at converting feed to wool than uninfected animals.

Roseby (1973) described the dietary intake and digestibility in sheep infected with *T. colubriformis* and the results conflicted with those of Barger (1973). Infection with a single dose of 30,000L_3 did not affect the apparent digestibility of organic matter, dry matter or nitrogen. Interestingly, feed intake was reduced by up to 32% during weeks 4 to 7 of infection. Animals were fed about half the dry matter ration offered to sheep in the Barger study and were also fed 24 equal portions of the ration at hourly intervals, as opposed to once per day. The lower and more frequent feed intake would have reduced the chance of detecting effects of infection on digestibility, as the level of feed intake can affect the efficiency of food digestion (Roseby 1973). Reductions in performance of infected animals were similar to those reported by Barger, with a 12% reduction in wool growth and a reduced bodyweight gain of 13 g/d compared to uninfected animals (Roseby 1973).

A study conducted by Rowe *et al.* (1988) with Merino weaners infected with a bolus dose of 300 *H. contortus* L_3/kg bodyweight revealed a significant decrease in apparent whole tract organic matter digestion. Infected animals had a reduced organic matter digestibility of 61% compared to 65% in uninfected sheep. Nitrogen digestibility was not significantly different between treatments. Steel (1978) explains that rather than a reduced digestion and absorption of nitrogen, there is an increased loss of endogenous

nitrogen at the site of infection. Therefore in abomasal infections, such as *H. contortus*, nitrogen lost into the abomasum is largely reabsorbed during passage through the small intestine. In contrast, infection with *T. colubriformis* impedes reabsorption of endogenous nitrogen although there is evidence of compensation in more distal regions of the small intestine.

2.3.4.2 Volatile fatty acids

Volatile fatty acids (VFA) are the end products of fermentation. The principle VFA are acetic, propionic and butyric acids, which provide the major fuel for energy production in ruminants. Fermentation digestion is critical in supplying metabolisable energy and protein to ruminant animals, yet fermentation of feed can be impaired in parasitised animals. Infection with *T. colubriformis* reduced ruminal production of acetate by 30%, even after allowing for a reduced feed intake in parasitised animals (Steel 1972). Production of acetic acid during microbial fermentation of carbohydrate in the rumen is a major source of energy in ruminant tissues. Depression in bodyweight gain and wool growth as a consequence of nematode infections can, in part, be attributed to a decreased supply of energy (Steel 1972). In a later study, Roseby (1977a) reported that there was no change in rumen VFA concentration in weaners infected with *T. colubriformis* compared to uninfected controls. However, the rumen fluid volume was less in infected animals, which would result in a lower fermentation rate (Roseby 1977). There was no change in total VFA production measured in sheep challenged with *H. contortus*, however a change in fermentation pattern towards an increase in the proportion of propionate: acetate was observed (Rowe *et al.* 1988). There are few reports which document the effect of GI nematode infection on rates of production of VFA. The evidence that GI infection alters VFA production is equivocal, but a depression of acetate production appears likely.

2.3.4.3 Rumen turnover and flow rates

Rumen turnover and flow rates will have a major impact on the level of fermentation of digesta and nutrient supply to the animal. Rumen fluid volume have not been found to be affected by *Trichostrongylus* (Steel 1972) or *Teladorsagia* infections (Steel 1975). However, digesta flow rates from the abomasum and ileum in sheep infected with *Teladorsagia circumcincta* were higher than controls (Steel 1975). In contrast,

increased outflow rates in the rumen were found in *H. contortus* infected animals studied by Rowe *et al.* (1988). Rowe suggested that the faster outflow of liquid from the rumen may be caused by altered reticulo-rumen motility. Bueno *et al.* (1982) provide some support for this suggestion. These authors studied the effects of motor and transit disturbances associated with *H. contortus* infection and reported an increased rate of duodenal flow as a consequence of ionic permeability changes of the gastrointestinal mucosa and altered abomasal gastric acid secretions.

2.3.4.4 Microbial protein and ammonia

Dietary rumen degradable nitrogen is converted mainly to ammonia in the rumen, where it is used for microbial protein synthesis. An adequate rumen ammonia concentration is required for maximal flow of microbial amino acids to the small intestine (Datta *et al.* 1998). Ammonia concentration has been reported to be reduced in the rumen of sheep infected with *T. colubriformis* (Roseby 1977). In contrast, Rowe *et al.* (1988) showed *H. contortus* infection had no effect on rumen ammonia concentration or microbial protein synthesis. This was in spite of increased rumen outflow rate and a reduced ratio of acetate to propionate in the rumen VFA (discussed above).

2.3.4.5 Nitrogen metabolism

Reduced nitrogen retention is a characteristic feature of nematode infections and may markedly reduce deposition of carcass protein in growing animals even at low levels of infection (Symons & Steel 1978). Figure 2-2 displays the increased flow of nitrogen (N) from the liver and muscle to the small intestine when animals are infected with *T. colubriformis*. These changes are often accompanied by smaller amounts of ingested N and a greater loss of endogenous protein, creating a negative nitrogen balance. Elevated faecal N excretion has been associated with infections with *H. contortus* (Dargie 1973, Abbott *et al.* 1985b), *T. circumcincta* (Sykes and Coop 1977) and *T. colubriformis* (Steel 1974). Increased faecal N output is due to the loss of nitrogenous material, such as mucus, sloughed epithelia and plasma proteins as a consequence of physical damage and inflammatory responses at the site of infection (Steel *et al.* 1982). Increased urinary N has also been linked to the major nematode infections (Dargie 1973, Sykes and Coop 1977 and Poppi *et al.* 1981). However, Abbott *et al.* (1985b) found an increase in faecal N output of 1.3 g/d and 0.9 g/d rise in urinary N loss in Finn Dorset weaners infected

with *H. contortus,* resulting in daily N retention of -1.1 g/d. Many investigators (Steel 1975, Rowe *et al.* 1982, 1988, Bown *et al.* 1991b) have concluded that the loss in endogenous N in abomasal infections is reabsorbed in the small intestine. In support of this, Rowe *et al.* (1982, 1988) reported no increase in faecal or urinary nitrogen excretion as a result of *H. contortus* infection in Merino sheep. Steel (1975) showed that 80% of endogenous non-ammonium nitrogen (NAN) secreted in the abomasum of *Teladorsagia* infected animals was reabsorbed in the small intestine. Bown *et al.* (1991b) also found that the major site of digestion and absorption of protein occurred in the distal small intestine beyond the site of *Trichostrongylus* infection.

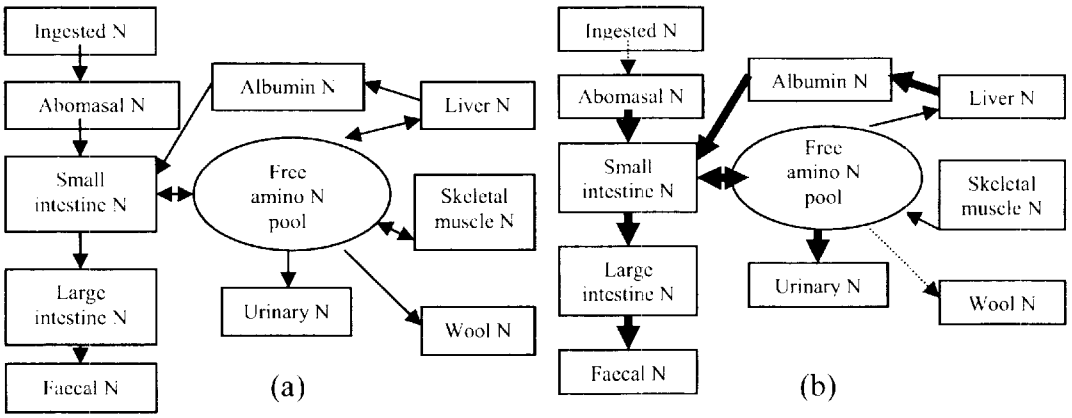


Figure 2-2 Schematic diagram of nitrogen flow in uninfected growing sheep with a positive nitrogen balance (a) and animals infected with *T. colubriformis* with poor growth and a negative nitrogen balance (b) → increased flow → reduced flow (from Symons *et al.* 1981a).

2.3.5 Partitioning of nutrients among organs and tissues

In the presence of a nematode infection, utilisation of nutrients for growth and wool production is usually impaired. The decrease in productivity in the absence of effects of food intake, is thought to be due to repartitioning of nutrients to the gastrointestinal tract for the immune response and tissue repair (Parkins & Holmes 1989), while body protein reserves mainly in the muscle are broken down (MacRae 1993). The schematic diagram displayed in Figure 2-3 shows that GI infection results in a reduced partitioning of amino acids to muscle, skeleton, wool and skin, while an increased flow of metabolisable protein is diverted to tissue repair, increased plasma protein supply and cell proliferation to mount an inflammatory response in the gut mucosa.

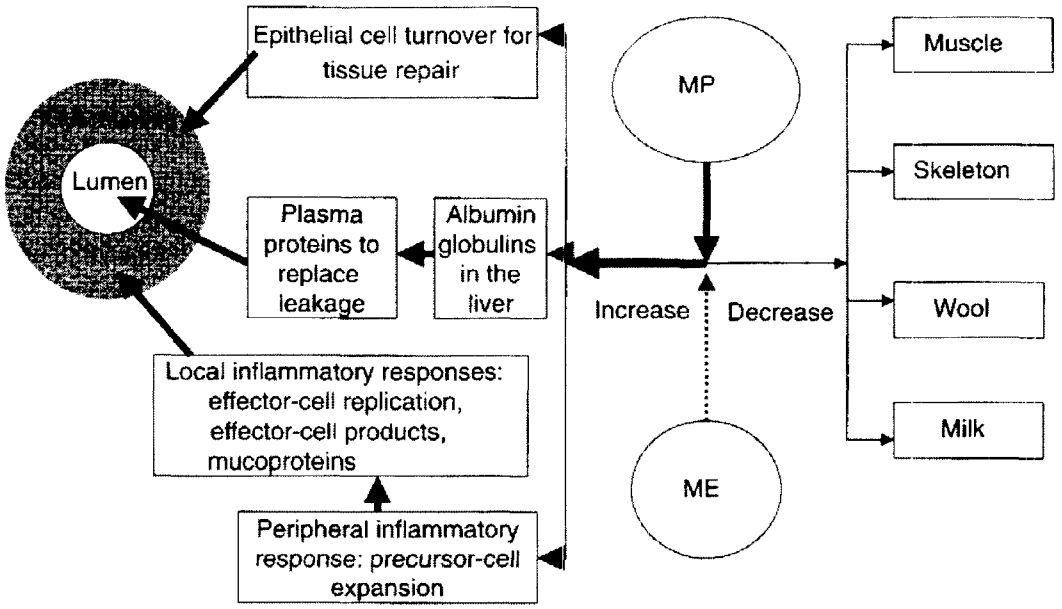


Figure 2-3 Schematic diagram of the effect of gastrointestinal nematode infection on protein metabolism in sheep. Amino acids from metabolisable protein (MP) are diverted from production (muscle, wool and milk) to mount an effective local immune response in the GI tract (from Coop & Sykes 2002)

A change in partitioning of amino acids when animals are infected with a GI nematode was shown in early studies using radioactive tracers. The incorporation of amino acid ^{14}C-$_{L}$-leucine decreased in skeletal muscle, while increasing in liver proteins of guinea pigs infected with *T. colubriformis*. This resulted in a loss of weight in infected animals, compared to a gain in weight in controls (Symons 1971). Further study confirmed that *T. colubriformis* infection results in an elevated hepatic protein synthesis. The increased rate of hepatic protein synthesis was associated with membrane-bound ribosomes that synthesise circulating plasma proteins (Symons *et al.* 1974). The increased rate of synthesis of proteins in the liver also diverts limited dietary nutrients from wool production. Symons & Jones (1975) reported that incorporation of labelled $_{L}$-leucine into sheep infected with *T. colubriformis* detected an increased protein synthesis in the liver, decreased protein synthesis in muscle and depressed incorporation of leucine in wool follicular homogenates, in both infected and pair-fed sheep.

Whole body metabolism was measured in guinea pigs infected with *T. colubriformis*. The percentage of radioactive label ^{14}C-$_{L}$-leucine recovered from each organ, faeces and urine were calculated at intervals up to 48 h after injection. There were no differences in

the total amount of label recovered between infected, uninfected or pair-fed animals. However, the percentage of injected ^{14}C-$_L$-leucine in liver, small intestine and large intestine was highest in infected animals, at the expense of carcass and skin. A reduced feed intake in infected animals did not affect the distribution of leucine, which suggests that change in protein distribution is independent of anorexia (Symons & Jones 1981c).

A constant infusion of ^{14}C-$_L$-tyrosine to measure tyrosine flux and fractional synthesis rate (FSR) was used to more precisely calculate protein synthesis in the whole body when lambs are infected with *T. colubriformis* (Jones & Symons 1982). The constant infusion method produced results comparable to earlier reports. The FSR of albumin and liver proteins increased two fold in infected animals, with a 38% increase in protein synthesis per day. The increase in FSR of albumin in animals infected with *T. colubriformis* is consistent with a faster rate of albumin turnover (Steel *et al.* 1980). Hypoalbuminaemia in infected lambs was attributed to enteric plasma loss, with a decrease in albumin concentration of 31%. The FSR and amount of protein synthesised per day was depressed in the kidney cortex and skeletal muscle, resulting in a 7.6 kg difference in bodyweight between infected and control animals (Jones & Symons 1982).

The previous report did not measure the protein synthesis in the small and large intestine, but further research using a flooding dose of $_L$-[4-^3H] phenylalanine showed increases in these organs in guinea pigs infected with *T. colubriformis* (Symons & Jones 1983). Daily protein synthesis in infected animals increased by 24% in the small intestine and over 70% in the large intestine. These increases were not due to anorexia since animals fed a reduced ration had lower daily protein synthesis than the infected group. The increase in protein synthesis could be explained by a faster rate of turnover of epithelial cells associated with villous atrophy and influenced by inflammation of the lamina propria (Symons & Jones 1983).

More recent reports investigating whole-body protein turnover in response to parasite infection with *T. colubriformis* reveal that infection had no affect on the amino acid irreversible loss rate (ILR), as an indicator of the availability of amino acids sourced from gastrointestinal absorption and breakdown of body protein (Yu *et al.* 2000,

Bermingham *et al.* 2000, Hoskin *et al.* 2002). Bermingham *et al.* (2000) concluded that the whole body ILR of amino acids may not change because an increase in protein synthesis in some tissues may be balanced by an increase in protein degradation in others. For example, parasite infection increased leucine sequestration (leucine removed from the circulation for either oxidation or protein synthesis) by 24% in the whole gastrointestinal tract and oxidation losses of leucine by a further 22 to 41%. As a consequence, availability of absorbed amino acids for peripheral tissue metabolism was reduced by 30%, during weeks 5 to 13 of *T. colubriformis* infection (Yu *et al.* 2000). This result confirms earlier studies of depressed rates of protein synthesis in skeletal muscle and wool in infected sheep (Symons & Jones 1975, Jones & Symons 1982). These findings have particular significance when consideration is made on the proportional distribution of protein synthesis in an unparasitised growing lamb. The GI tract accounts for only 5% of total body protein mass, but these tissues are responsible for 30-45% of whole body protein synthesis (MacRae 1993). A diagram of the protein turnover in a young growing lamb and the amounts of protein retained by the tissue is displayed in Figure 2-4.

Bermingham *et al.* (2002) found no difference in amino acid flux across the hind limb between control and parasite infected sheep However, the average daily weight gain was lower in infected weaners compared to control animals (-100 versus 200 g/d). Bermingham *et al.* (2002) suggested that an alteration in the way amino acids are utilised within the hind limb and changes in protein synthesis, degradation and /or amino acid oxidation, result in a similar net flux. Bermingham (2004) concluded that intestinal infection imposes little nutritional cost in the host 48 d post infection. The demand for available amino acids may be low, compared to times when the GI tract has higher priority for resources and consequently the supply of amino acids is limited to the hind limb. Effects of the parasite burden may occur during earlier stages of infection, shown by Symons & Jones (1975) at day 33. Voluntary feed intake was also depressed by almost 10% of intake pre-infection in the study by Symons and Jones, whereas feed intake was unaffected by *T .colubriformis* infection in animals investigated by Bermingham *et al.* (2002).

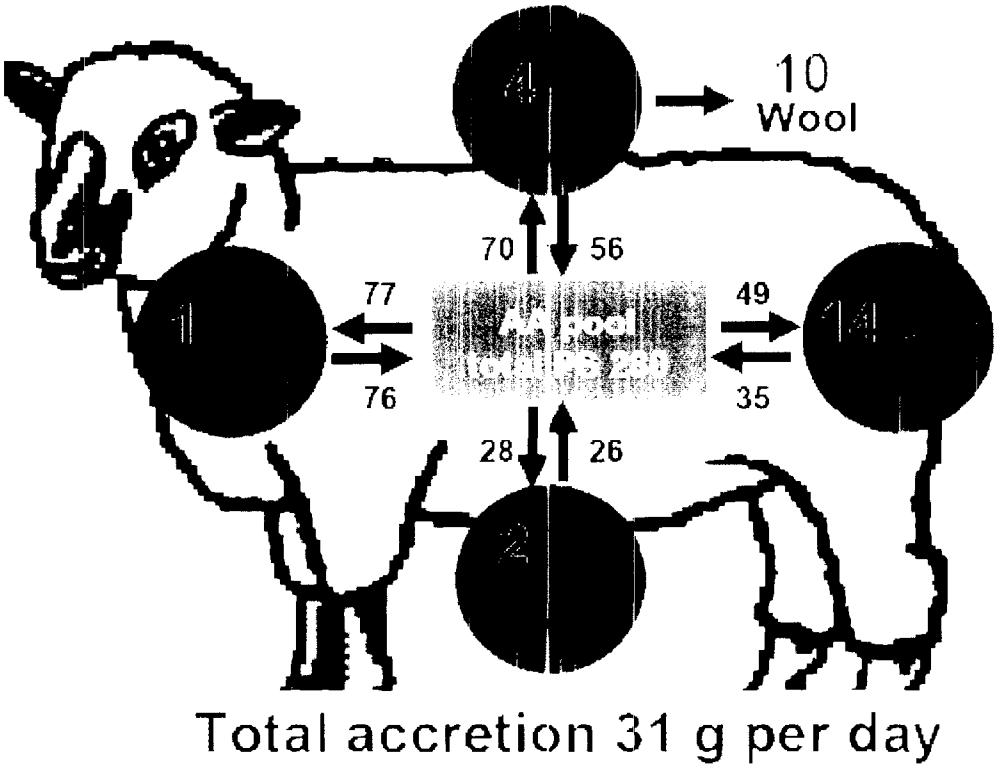


Figure 2-4 Protein turnover (g/d) in young growing lamb. Numbers in red represent the amount of protein retained by the tissue (g/d) (Bermingham 2004, adapted from MacRae 1993).

Although Symons & Jones (1981) suggested that a reduced feed intake did not affect change in protein distribution, a reduction in absorbed amino acids sourced from the gastrointestinal tract may affect the hosts response to infection depending on the stage of infection and population dynamics of the parasite. It should be noted that animals received almost double the number of infective larvae in the study by Symons & Jones (1975), which caused a reduction in voluntary feed intake. The extent of metabolic impairment has been identified to be influenced by the level of larval challenge and the number of worms established (van Houtert & Sykes 1996).

2.3.5.1 Nutrient partitioning to gut immune response

GI nematode infection stimulates an inflammatory response and activation of the immune system in the host. As a result, normal nutrient utilisation within the animal is redistributed away from protein production (growth, wool, lactation) towards tissues involved in inflammation and immune response (gut) (Colditz 2003). The net result of

repartitioning towards the gut immune response has been estimated to be up to 15% loss in productivity (Sykes 1994).

The modification of protein and amino acid metabolism during an immune response appears to be mediated by cytokines and hormones (Le Floc'h *et al.* 2004). Cytokines are produced mainly in immune cells, such as lymphocytes. There are three main cytokines reported to be involved in regulating protein metabolism during an immune response. Interleukin 1 (IL-1) is responsible for fever, anorexia and ACTH secretion, which will have an anabolic effect on the liver and catabolic effect on muscle. Interleukin 6 (IL-6) initiates the acute phase response and tumor necrosis factor (TNF) will inhibit muscle protein metabolism and stimulate muscle protein breakdown (Le Floc'h *et al.* 2004, Colditz 2002).

Acute phase proteins are synthesised in the liver and circulate in the blood. These proteins can override the normal control of nutrient utilisation, such that amino acid resorption from muscle is diverted toward the immune system (Colditz 2003). During inflammation, acute-phase protein plasma concentrations can increase up to 100-fold (Le Floc'h *et al.* 2004). Some acute phase proteins are also synthesised in gut mucosa (Wang *et al.* 1998), which may account for some of the increased protein sequestration in gut tissue (Yu *et al.* 2000) reported during parasitism.

As cited by Colditz (2002, 2003) cysteine is an amino acid required for synthesis of immunoglobulins, gastrointestinal mucins and the germinal layer of the wool follicle. During a GI nematode infection the partitioning of cysteine increases towards the host immune response and availability for wool synthesis is limited. During an infection with *T. colubriformis* wool growth was depressed by 18%, but with cysteine supplementation wool growth increased by 33% in both infected and uninfected sheep (Barger *et al.* 1973). Miller *et al.* (1998) supplemented animals from the high fleece weight selection line with cysteine and found they were less susceptible to *H. contortus* and *T. colubriformis* infection. Supplementation of selected animals with 2 g/d of cysteine via abomasal infusion, tended to have greater peripheral eosinophil concentration and higher globular leukocyte counts in the abomasum at slaughter (Miller *et al.* 2000).

The concentration of cysteine in muscle protein is low compared to levels in the liver and GI tract (MacRae *et al.* 1993). Therefore to meet the increased demand for cysteine by the liver and GI tract to mount an immune response, proportionally more muscle protein needs to be mobilised to partition to these organs (MacRae *et al.* 1993). This may, in part explain the decrease in weight gain observed during parasite infection.

2.3.5.2 Nutrient partitioning and production

Partitioning of absorbed amino acids can differ between productive tissues, such as skeletal muscle and skin. Specific nutrients, such as sulphur-containing amino acids, may act as a constraint to optimal protein gain as the demand for these amino acids is higher. For example cysteine and methionine are shown to be the first limiting amino acids for wool production in sheep (Liu *et al.* 2002), whereas for tissue gain there is a higher demand for lysine and histidine (Lobley 1986).

The FSR of amino acids in productive tissues can also vary depending on the breed and nutritional status of the animal. It has been demonstrated (Lobley *et al.* 1992; Liu *et al.* 1998) that the FSR of skin in sheep bred for superior wool production such as the Merino, may be double that of meat breeds such as Suffolk cross and Romney. Similarly, selection appears to have reduced the sensitivity of FSR of the target tissue (i.e. wool and muscle) to variations in feed intake. Lobley *et al.* (1992) and Liu *et al.* (1998) reported that in response to a change in feed intake, fed at maintenance or 0.6x maintenance, the FSR of skin in wool and meat breeds declined by 4 and 40% respectively. In contrast, the FSR of muscle declined by 40 and 20% for wool and meat breeds respectively.

Protein turnover within the hind limb muscle can vary between animals within the same breed through selection of phenotypic markers. Merino lambs that have been selected for over 50 years for high (W+) or low weaning weight (W-), differed by 42% in weight at weaning and maturity (Oddy *et al.* 1995). Oddy *et al.* (1995) reported no apparent differences in whole-body protein synthesis, but hind-limb muscle metabolism differed between lines. Protein degradation in W+ weaners decreased with increased feed intake, the rate of phenylalanine oxidation was lower, use of oxygen per kg hind limb muscle was less and the rate of blood flow to muscle was reduced, compared to W- animals.

Selection for high fleece growth rate has been shown to result in greater partitioning of available nutrients to wool and not increased efficiency of nutrient retention (Cronjé & Smuts 1994). Greasy fleece weights of Merino rams during *ad libitum* feeding were 8, 6 and 4 kg per annum for high, average and low producers respectively. The higher wool growth was achieved by partitioning a higher proportion of consumed N to wool (0.08, 0.06 and 0.05 in high, average and low producers respectively) and was not by increased energy intake or associated with whole-body N retention. When the same animals were fed a maintenance diet, clean fleece (g/d) was not significantly different between fleece growth rate categories. Thus the apparent priority for wool growth will depend on the relative nutrients required for other body functions (Cronjé & Smuts 1994). Interestingly, the low producers grew significantly faster than the average producers, emphasising the earlier discussion that animals will partition to tissue (muscle or skin) with the highest priority i.e. low wool producers partition more into skeletal muscle for higher weight gain.

2.3.6 Production

Clinical signs of parasitism such as scouring and ill thrift are accompanied by the occurrence of reduced weight gain and wool production. However, Barger (1982) demonstrated that *T. colubriformis* infection, even without evident clinical signs, can reduce bodyweight gain (BWG) in young sheep by 14-17% and wool growth by 9-30% in all classes of sheep.

Albers *et al.* (1989) investigated the effect of *H. contortus* on BWG and wool growth of young Merino sheep. Following a single infection with 11,000 *H. contortus* larvae, clean wool growth was reduced by 6.8% and fibre diameter was reduced by 0.57 μm. The BWG of infected weaners was also reduced by 38% when compared to uninfected controls.

Steel *et al.* (1980) studied similar effects of parasitism on BWG and wool growth in weaners infected with the intestinal nematode parasite *T. colubriformis*. The experiment used different larval dosing regimes, ranging from 300 infective larvae per week to 30,000 over a 24 week infection period. Productivity was reduced for BWG and wool

growth at dosing regimes from between 950 and 3,000 larvae per week. Reduced weight gain (49% gain of uninfected animals) and weight loss (in 30,000 infective larvae/week regime) occurred during the first 12 weeks of infection, at which time animals acquired resistance to the parasite challenge and WEC declined. Reduction in wool growth rate (44% growth of uninfected animals) peaked during weeks 8-12 and similarly bodyweight gain recovered from week 12 of infection.

Weaners infected with *T. circumcincta*, showed depressed BWG and wool growth, but only at high larval dosing regimes of 37,500 and 120,000 larvae per week. The reduction in productivity was accounted for mainly by a decrease in voluntary food intake, which was not evident in the lower infection regimes. Food consumption was reduced by 20% during the first 12 weeks of infection on the 120,000 larvae/ week infection rate (Symons *et al.* 1981).

Comparison of the two studies with *T. colubriformis* and *T. circumcincta* suggests that on a larval intake basis, *T. circumcincta* is considerably less pathogenic and detrimental to productivity than the intestinal parasite *T. colubriformis*. Moreover, the combined pathophysiological effects of both parasites are much greater than the effects of the monospecific infections (Steel *et al.* 1982).

2.4 Host response to infection

2.4.1 Establishment of *Haemonchus contortus* infection

H. contortus populations are regulated by the development of host resistance to infection. The onset of resistance decreases the number of worms establishing and increases the loss of established worms, which is proportional to the rate of larval intake. Barger *et al.* (1985) demonstrated that with a continuous infection of *H. contortus*, at infection rates of 600, 1,200, 2,400 or 4,800 larvae per week, worm numbers were proportional to the rate of infection for the first 6-9 weeks, at which time resistance became apparent, but began to decline or plateau thereafter. Establishment rate was not related to infection rate at any stage of the infection and declined from 45% at weeks 1 to 4 to insignificant numbers after weeks 10 and 13. In groups receiving

2,400 and 4,800 larvae per week, worm numbers declined rapidly from week 9 and were lower than groups given 600 or 1,200 larvae per week by week 15.

2.4.2 Rejection of *Haemonchus contortus* infection

Multiple larval infections are required for resistance to *H. contortus* to develop. Resistance has been observed after 9 weeks of thrice weekly dosing with 800-1,600 infective larvae, but young animals may require a longer exposure to mount an effective immune response (Barger *et al.* 1985). Immunity to *H. contortus* has been shown to develop in sheep as young as 4 months of age, but can be lost completely when animals are treated with an anthelmintic (Barger 1988). Barger (1988) concluded that protective immunity to *H. contortus* infection is achieved when moderate burdens are allowed to persist. Immunised animals, whose priming infections are removed, can be as susceptible as naive animals. The length of time in which immunity persists appears to be between 8 to 9 weeks following termination of an immunising infection. Wagland & Dineen (1967) demonstrated a strong immune response occurred when periods of 4 and 8 weeks elapsed between removal of infections and re-challenge. While, Coyne & Smith (1992a) showed no difference in total *H. contortus* worm burden between parasite-exposed or parasite-naïve lambs when re-challenged 9 weeks after a previous infection.

2.4.3 Immunological responses to infection

2.4.3.1 Lymphocytes

Lymphocytes are white blood cells that are the key operatives of the immune responses. There are two different classes of lymphocytes, T cells and B cells. T cells include the T helper cells which are responsible for the production of cytokines that regulate a cellular or humoral immune response. B cells differentiate upon antigen stimulation into plasma cells which are responsible for antibody production (Roitt *et al.* 1985).

T helper cells can be classified into two groups based on the type of immune response they promote. T helper cells 1 (Th1) produce cytokines that promote protection against bacterial pathogens and intracellular parasites (Roitt *et al.* 1985). A Th2 response (T helper cells 2) are involved in resistance against GI nematode parasites and are

characterised by an increase in immunoglobulins secreted by B cells (particularly IgG_1 and IgE) and the recruitment of eosinophils and mast cells (Schallig 2000).

Activation of the local lymph nodes and recruitment of lymphocytes to the abomasal mucosal tissue have been observed in sheep given a primary or challenge infection with *H. contortus* (Balic *et al.* 2002). However, increased concentrations of lymphocytes occured 5 days post infection during a primary infection, compared to 3 days in previously infected sheep. In addition, the percentage of lymphocyte populations varied between primary and secondary challenge (Balic *et al.* 2000, 2002).

2.4.3.2 Immunoglobulins

There are a number of immunoglobulin (Ig) isotypes involved in an immune response against GI nematode infection, including IgG_1, IgG_2, IgM, IgE and IgA (Schallig 2000). Serum antibody responses against GI parasites generally are greater and peak earlier after a secondary challenge than a primary infection (Balic *et al.* 2000). Serum Ig anti-parasite responses have been shown to be greater against larval antigens than against adult antigens in sheep infected with *H. contortus* (Schallig *et al.* 1995). Measurements of immunoglobulins in peripheral blood show an increased response after infection by nematode-specific IgG_1, IgG_2 and IgE antibodies. IgG_1 is the most dominant serum antibody isotype, against parasite antigens in sheep infected with GI infection (Schallig *et al.* 1995, Balic *et al.* 2000). IgA responses are more prominent in the local mucosal sites of infection, therefore are observed at lower concentration in serum (Balic *et al.* 2000, Schallig 2000). Serial biopsy of the abomasum during a *H. contortus* infection in sheep demonstrated increased numbers of immunoglobulin cells following infection, with peak values at 21 and 28 days after infection. IgA cells were the most frequently observed (Gill *et al.* 1992).

A significant association between reduced female worm length and increased IgA against third-stage larvae has been shown in sheep infected with *H. contortus* and *T. circumcincta* abomasal infections (Stear *et al.* 1999, Strain & Stear 2001). Worm length is positively associated with worm fecundity. Therefore, IgA may be the major mechanism controlling fecundity of abomasal parasites (Strain & Stear 2001).

A primary infection with *H. contortus* elevated IgE levels in serum which peaked 2-3 weeks after exposure to infective larvae (Kooyman *et al.* 1997). Measurement of IgE in lymph and serum of sheep infected with *T. circumcincta* showed a four-fold difference in IgE concentration in lymph than serum in both primary and secondary infections. This indicates production of IgE in the local lymph nodes (Huntley *et al.* 1998a).

2.4.3.3 Eosinophils

The general function of eosinophils is to fight evasion by promoting acute inflammation. They serve this function by migrating to the site of parasite invasion and releasing enzymes used to kill or severely damage the parasite (Tizard 1996). Following challenge with *H. contortus* larvae eosinophils were demonstrated to be concentrated around L3 larvae within 24 hours after dosing, suggesting that eosinophils may effectively destroy larvae at the site of larval penetration (Rainbird *et al.* 1998, Meeusen & Balic 2000).

Eosinophil numbers increased in response to infection in mucosal tissue and can be measured at increased levels in peripheral blood. The rise in number of eosinophils is greater in animals previously infected with GI parasites than during a primary infection (MacRae 1993, Balic *et al.* 2002).

2.4.3.4 Immune response of resistant genotypes

The immunological responses to parasite infection are most clearly displayed in lines divergently selected for resistance or susceptibility to infection. Windon (1996) reported that in both the *Trichostrongylus* selection flock and the Golden Ram flock, resistant animals have a greater cellular (mast cell, globule leukocytes, circulating eosinophils and mediator release) and antibody response compared to susceptible counterparts. Little information is available on the immunological basis of resistance in the *Haemonchus* selection flock (Woolaston *et al.* 1990). The three experimental flocks were used to investigate the presence of a number of T lymphocyte phenotypes (CD4+, CD8+, γδ+ and T19+), during infection with *T. colubriformis*. There were no differences in lymphocyte phenotypes in peripheral blood between lines within these flocks. However an increased proportion of CD8+ lymphocytes in susceptible animals

led the authors to speculate that selection for susceptibility to GI nematodes results in stimulation of CD8+ which may inhibit resistance during infection with *T. colubriformis* (Wong *et al.* 1997). The relative numbers of lymphocyte subpopulations were also compared between resistant and susceptible sheep from the Wallaceville selection flock. Pernthaner *et al.* (1995) reported a higher percentage of CD5+ and CD4+ cells observed in resistant compared to susceptible animals.

Resistant 12 month old wethers from the Golden Ram selection flock exhibit lower WEC, lower worm weights, heavier thymuses and more intense tissue infiltration by globule leucocytes compared to controls (Presson *et al.* 1988). When animals are immuno-suppressed with the glucocorticosteroid, dexamethasone, these characteristics are abolished in resistant animals. Presson *et al.* (1988) suggested there is an immunological basis to resistance to infection in resistant genotypes.

Immunological and parasitological parameters in rams from the low (LWEC) and high (HWEC) WEC lines in the Wallaceville selection flock were investigated by Bisset *et al.* (1996). Lines differed significantly for WEC (LWEC 16% of HWEC) and worm burden (LWEC 33% of HWEC). Total antibody titres in serum against *T. colubriformis* were higher in LWEC animals and IgG_1 concentration were 0.60 compared to 0.41 (optical density units) in HWEC animals. Mucosal mast cells, globule leucocytes and mucosal eosinophils were significantly higher in LWEC line. Circulating eosinophils did not differ between genotypes.

Circulating eosinophil numbers have also been measured in the *Haemonchus* and *Trichostrongylus* selection flocks. Woolaston *et al.* (1996) found eosinophils to be higher in the IRH line, but differences were not statistically significant. The numbers of eosinophils were significantly higher in the high responder lambs when compared to low responders after vaccination and challenge infection with *T. colubriformis* in the *Trichostrongylus* selection flock (Dawkins *et al.* 1989). It was concluded that peripheral eosinophil numbers were a measure of host responsiveness to infection (Dawkins *et al.* 1989, Woolaston *et al.* 1996).

The role of antibodies in accounting for the superior resistance of genetically resistant sheep was investigated in the Golden Ram flock (Gill *et al.* 1991). Following infection

with 20,000 *H. contortus* larvae resistant animals had higher antibody responses than susceptible counterparts. Isotype-specific antibody responses showed higher IgG_1 and IgA serum antibody levels in resistant animals. There was no difference in IgG_2 and IgM levels (Gill *et al.* 1991). Faecal antibody responses to *H. contortus* also showed higher IgG_1 and IgA levels in resistant sheep than controls. This suggests that IgA and IgG_1 antibodies produced at the site of infection may play an important role in genetically determined resistance of sheep to *H. contortus* (Gill *et al.* 1989). Antibody levels in guinea pigs selected for high and low responsiveness to *T. colubriformis* also showed higher anti-*T. colubriformis* IgG_1 antibody titres in high responders than low responders (Manjili *et al.* 1999). It was suggested by Manjili *et al.* (1999) that IgG_1 antibodies mediate the release of mast cells and basophil products at the site of infection which contribute to the more effective immune response in resistant animals.

2.5 Regulating host response by genetic selection

2.5.1 Definition of resistance and resilience

The need for alternative methods to control gastrointestinal parasite infection has encouraged research into the identification of host genotypes to improve resistance and resilience to parasite infection. Resistance is the ability of a host to reduce the establishment, survival and reproductive rates of the parasite. Resilience is the ability of the host to continue to maintain productivity, despite a parasite infection (Albers *et al.* 1987). Breeding for parasite resistance has been incorporated into commercial breeding programs across Australia, so that producers can take advantage of lower worm burdens, reduced pasture contamination and a decrease in anthelmintic use in their flocks. Selection for resilience is not as widely adopted in Australia, but management of host nutrition has demonstrated the benefit of resilience by improved animal performance.

2.5.2 Influence of breed on resistance

Resistance to parasite infection can be determined by breed. Comparison of six breeds of sheep for resistance to *H. contortus* in a field situation demonstrated clear differences in the susceptibility to *H. contortus* infection (Preston & Allonby 1979). Red Masai were by far the most resistant breed, with increasing susceptibility in Blackhead

Persian, Merino, Dorper, Corriedale and Hampshire Down. Resistance was determined by worm egg count and mortality during the period of exposure to *H. contortus* infection. All (n = 10) of the Hampshire Down animals died, while no deaths occurred among the Red Masai. Worm burden was determined in Merino and Red Masai breeds confirming that the worm egg count did reflect worm burdens and that establishment of worms was greater in Merinos than Red Masai. It was concluded that variations arose because of differences in an immune response against the parasite (*ibid*). Tests on abomasal mucosa found higher levels of anti-larval IgA in Red Masai compared to Merinos (Preston & Allonby 1979).

2.5.3 Selection method for increased resistance

The discovery in the early 1970s that worm egg count (WEC) was a heritable trait in Merino sheep (Le Jambre 1978) brought about the development of several selection lines with different levels of genetic resistance (selection flocks discussed below). The heritability of WEC has been estimated in a number of Merino flocks using artificial and natural infection, with the major economically damaging nematode parasites and ranged from 0.2 to 0.5 (Eady *et al.* 1996). The higher estimates have been detected in flocks where the WEC was measured from artificial infection (Woolaston *et al.* 1991) and repeated measures were made (Woolaston & Windon 2001).

The heritability of WEC and packed cell volume decline (PCVD) in lines of Merinos selected for divergent levels of resistance to *H. contortus* were 0.23 ± 0.03 and 0.21 ± 0.03 respectively (Woolaston & Piper 1996). The heritability estimates of WEC increased after cube root transformation to 0.29 ± 0.03. The genetic correlation between PCV and WEC was 0.87, therefore selection for reduced PCVD is likely to decrease WEC. However, the genetic relationship was not linear as the relationship appeared to be weaker in susceptible animals (genetic correlation estimate of 0.76) than in resistant counterparts (1.00). It appears that susceptible animals have a physiological limit, such that once severe anaemia exists an increase in worm burden is not indicated by PCV. WEC has also been shown to have the strongest association with worm burden compared to other measures, such as PCV and eosinophil count (Stear *et al.* 1995b). In summary, WEC has been generally accepted by industry to be the best indicator of resistance to parasite challenge.

The value of other phenotypic markers as an indicator of resistance has been considered. Woolaston *et al.* (1996) measured circulating eosinophils following *H. contortus* infection and found counts to be higher in animals bred for resistance to *H. contortus*, but they were not significantly different to susceptible animals. In a Merino flock bred for resistance to *Trichostrongylus* (Windon 1991) the heritability of circulating eosinophils was 0.19 ± 0.08 (Dawkins *et al.* 1989) following infection with *T. colubriformis*. But as the heritability was less than WEC and estimates inconsistent across selection lines, there is no advantage over WEC as a selection measure for resistance (Woolaston *et al.* 1996, Stear *et al.* 1995b).

Evaluation of antibody levels in serum as an indicator of resistance has generated heritability estimates for IgG_1 against *T. colubriformis* of 0.18 ± 0.05 in Romney sheep (Douch *et al.* 1995). However, the use of antibody as a selection parameter for resistance has been calculated to only give 67% of the genetic gain that would be achieved by selecting for WEC (Douch *et al.* 1996).

While variation in resistance to GI nematode infection differs among breeds of sheep, the major source of genetic variation for WEC within a breed is within bloodlines (22%), with progeny from individual sires showing measurable differences in resistance to nematode infection in Merinos (Eady *et al.* 1996). There is little genetic variation in resistance to nematodes between strains (1%) and bloodlines (3.5%).

The repeatability of WEC is found to be high (up to 0.97) when samples are measured at short intervals within an infection, but falls rapidly as interval time increases. For example, Stear *et al.* (1995a) reported that a repeatability of WEC between two infections of 0.57. Therefore selecting for resistance using the mean of two WEC would be equivalent to selecting a trait with a heritability of 0.39 (Morris *et al.* 2000).

2.5.4 Selection flocks

2.5.4.1 Haemonchus selection flock

The *Haemonchus* selection flock was established in 1977 at CSIRO, Chiswick, Armidale (latitude 30°31'S, longitude 151°39'E, elevation 1070 m) from fine-wool

Merinos allocated into increased resistance to *Haemonchus* (IRH) and decreased resistance to *Haemonchus* (DRH) lines, on the basis of PCVD following artificial challenge with *H. contortus* infective larvae. A random-bred line was selected at random from the same original flock as a control (C). From 1978 replacement animals were chosen within the IRH and DRH lines according to their maximum worm egg count (WEC) following *H. contortus* artificial challenge. Animals were born and reared in a common environment and challenged at 5-6 months of age with 10,000 *H. contortus* infective larvae and faecal samples taken at approximately 3, and 5 weeks post infection (*pi*). Weaners born in 1988 showed PCVD of 25.7, 20.3 and 22.0% and WEC at 2,730, 17,400 and 12,720 epg in IRH, DRH and C lines respectively. The differences in WEC among lines also persisted after natural challenge obtained from grazing infected pasture (Woolaston *et al.* 1990). The divergent genetic trend for the first 15 years of selection is displayed in Figure 2-5.

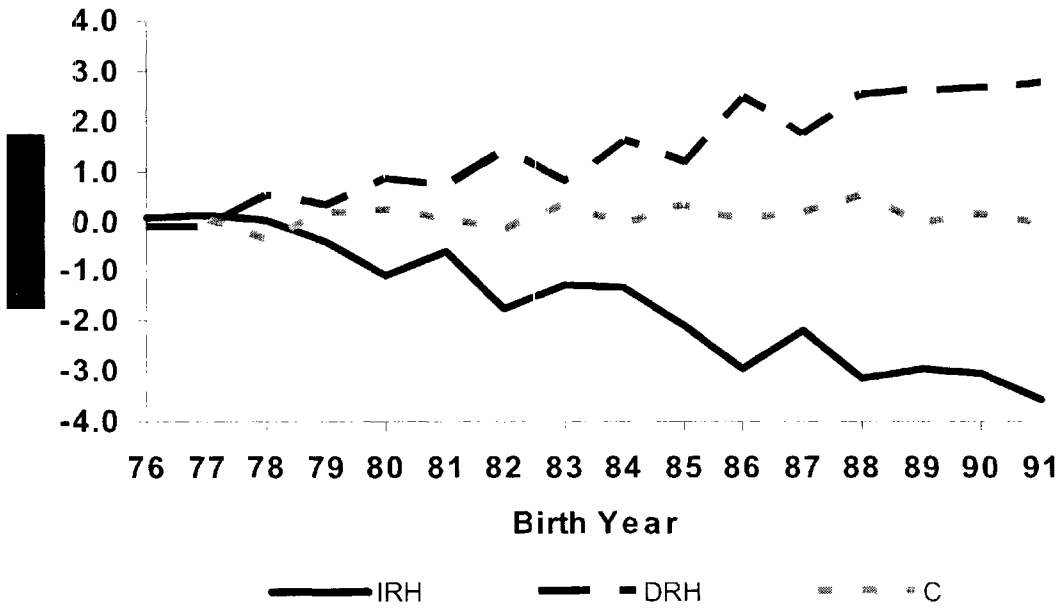


Figure 2-5 Estimated breeding values (EBV) for cube-root transformed worm egg count, classified by birth year and selection line for increased resistance (IRH), decreased resistance (DRH) and control (C) (from Woolaston & Eady 1995).

2.5.4.2 Golden Ram flock

The Golden Ram flock was established at the University of New England, Armidale in the early 1980's, resulting from progeny testing of fine and medium wool Merino rams over three years from three separate breeding flocks. Animals were challenged with

11,000 *H contortus* larvae after weaning at 3-4 months of age. WEC at 4 and 5 weeks *pi* were used to measure resistance to infection. The frequency distribution of the 60 sire group means showed that one sire group had an extremely high level of resistance. WEC and haematocrit of the 60 sire groups (including resistant group) at week 5 *pi* were 12,760 epg and 23.3%. The resistant sire group WEC and haematocrit at the same time point were 2,642 epg and 29.9% (Albers *et al.* 1987). The sire was named the 'Golden Ram' and thought to carry a major gene for parasite resistance. Backcross matings between relatives of this ram were made to establish a line of animals with increased resistance to *H. contortus* in an attempt to elucidate the mode of inheritance of the resistance gene (Woolaston & Eady 1995).

A major gene (i.e. large effects of one or a small number of genes) for resistance has been demonstrated to account for one third of the total genetic variation in the Golden Ram flock (Meszaros *et al.* 1999). By contrast, resistance in the *Haemonchus* selection flock and *Trichostrongylus* selection flock appears to be conferred as a polygenic trait (i.e. small effects of a large number of genes).

2.5.4.3 Trichostrongylus flock

The *Trichostrongylus* selection flock was established in 1975 from five resistant (high responders) and five susceptible (low responders) founder rams, from medium wool peppin Merinos (Windon 1991). The selection program was subsequently based on penned lambs raised helminthologically naive to standardise and define parasite load and measure parasitological and immunological responses. Lambs were vaccinated with 20,000 irradiated *T. colubriformis* larvae at 8 and 12 weeks of age, treated with anthelmintic at 16 weeks and challenged a week later with virulent *T. colubriformis*. Five WECs at fortnightly intervals were taken from week 3 *pi*, to determine the response to infection and identify resistance (Windon 1991).

2.5.4.4 Rylington flock

The Rylington flock was established in 1987 at Rylington Park in Western Australia. Rams used in the first two years were sourced from the IRH *Haemonchus* selection line, the Golden Ram flock, the high responder *Trichostrongylus* line and a closed flock at

Yalanbee, which had run without anthelmintic use for over 20 years (Karlsson *et al.* 1991). Selection was based on a combination of production traits and WEC after natural paddock challenge, however repeatability of WEC was low and heritability variable. Parasite resistance was then assessed after artificial challenge with *T. colubriformis* (Karlsson *et al.* 1991).

2.5.4.5 Wallaceville Romney breeding flock

Divergent breeding lines of Romney sheep were established at Wallaceville Animal Research Centre, NZ in 1979 (Bisset *et al.* 1996). The lines were selected on the basis of resistance to infection (resistant line) and resilience to infection (tolerant line). Animals were challenged with natural infection (predominately *Trichostrongylus vitrinus, T. colubriformis, T. circumcincta, H. contortus and Cooperia curticei*) and selected for low or high WEC and above average growth rate when left untreated with an anthelmintic. Since 1988 the lines have been selected for low and high WEC, based on a natural challenge at three periods, weaning (December), autumn and early winter (May/June). Each WEC is determined from a separate infection challenge, as each period is terminated by an anthelmintic treatment (Bisset *et al.* 1996).

2.5.5 Response to selection

Sheep under the age of 12 months are generally more susceptible to gastrointestinal parasite infection than when they are mature (Manton *et al.* 1962). Animals tested within the *Haemonchus* selection flock showed significantly lower WEC in IRH lambs at 65 d of age. The IRH were more resistant and expressed resistance earlier than the DRH and C lines (Ward *et al.* 1999).

Susceptibility to GI infection in endemic areas is mainly apparent in young animals and during the periparturient period (late pregnancy and lactation) in ewes. Woolaston (1992) illustrated in ewes from the IRH line that selecting for resistance reduces the periparturient rise in WEC. The rise in WEC 4 weeks prior to parturition followed a similar pattern in IRH, DRH and C lines, but increases were substantially smaller in IRH ewes. During lactation WEC increased further, but by week 4 of lactation WEC in IRH ewes showed signs of having peaked, while WEC continued to increase in DRH and C ewes. The mean WEC of each selection line before lambing, two weeks after the

beginning of lambing and at the end of lambing are shown in Figure 2-6. Woolaston (1992) noted that communal grazing of the selection lines may have led to an underestimate of the differences in WEC. If the lines had been grazed separately during this period the IRH ewes should have lower exposure to infective larvae and therefore an even smaller periparturient rise in WEC. DRH ewes would have had higher levels of pasture contamination which may have caused adverse effects on the performance of both ewes and lambs. Consequently, the reduction in pasture contamination produced by resistant animals (Kahn *et al.* 2003) before and during the periparturient period would be expected to have led to fewer mortalities and a reduced number of anthelmintic treatments in lambing ewes and weaners.

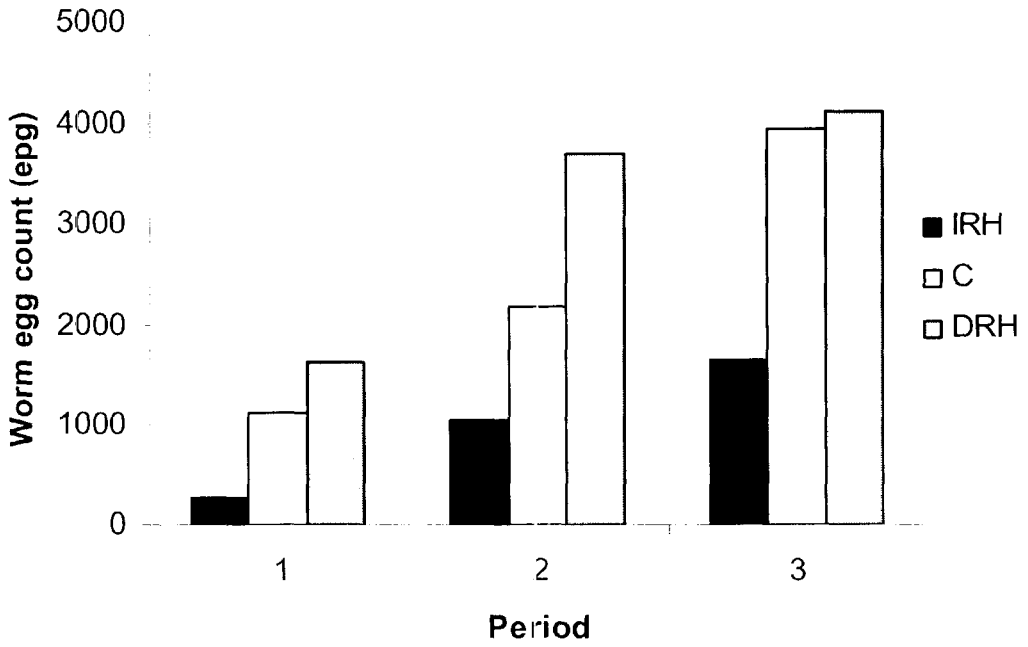


Figure 2-6 Mean worm egg count from ewes selected for either increased resistance to *Haemonchus* (IRH), decreased resistance to *Haemonchus* (DRH) or random-bred control (C) at different sample times during the periparturient period. 5 d prior to commencement of lambing (Period 1), two weeks after beginning of lambing (Period 2) and at the end of lambing (Period 3) (adapted from Woolaston 1992).

When divergent nematode resistant and susceptible lines from the Wallaceville Romney breeding flock (Bisset *et al.* 1996) were grazed separately in matching farmlets, the epidemiological benefit of resistance was apparent. Animals grazed separately from weaning to approximately 10-11 months old. In autumn (April) larval infestation levels on pasture were 5-6 fold greater on the farmlet grazed by susceptible weaners and

equated to a 56-fold difference in WEC between resistant and susceptible animals by winter. This was associated with significantly higher growth rates in resistant weaners, compared to susceptible counterparts (Bisset *et al.* 1997). A similar study was conducted using the *Haemonchus* selection flock, where a portion of the IRH ewes and weaners were grazed separately to the composite flock with the three genotypes; IRH, DRH and C lines. Over the two year experimental period IRH ewes were not drenched, while the composite group received 2 drenches (drenching was at regular intervals as WEC approached or exceeded approximately 700 epg). This was compared to a commercial flock managed similarly that received 6 drenches. The weaner groups received three drenches over this period, except for the IRH weaners, which only required drenching immediately after weaning and subsequently kept worm burdens low. The WEC of the composite group were higher than those of the IRH weaners. The corresponding commercial flock of weaners received 10 drenches during this time (Bell *et al.* 2003).

Most of the Merino selection lines have been selected for increased resistance under artificial challenge to a specific nematode species. However, cross resistance against other economically important nematode species, following priming with mixed species has also been displayed. Resistant lambing ewes and lambs from the *Haemonchus* selection flock have produced lower WEC with infections of predominantly *T. colubriformis* and *T. circumcincta* species (Woolaston 1992, Ward *et al.* 1999). Windon *et al.* (1987) demonstrated that selection for high response to *T. colubriformis* conferred some cross-protection against *H. contortus*.

The *Haemonchus* selection flock was used to demonstrate the relative effectiveness of genetic selection in reducing WEC of young Merino sheep grazing at pasture and infected with mainly *T. colubriformis* and *H. contortus*, compared to alternative worm control measures. Sheep selected for resistance to *H. contortus* reduced WEC by 70% compared to unselected controls. The use of protein supplementation (31% CP at 100g/hd/d twice weekly) and strategic drenching (Wormkill) produced a 35 and 12% reduction in WEC, respectively (Woolaston *et al.* 1997). Genetic selection was the most effective long-term worm control measure, whereas the other treatments, although not as effective, can be implemented immediately.

2.5.6 Genetic correlation with production traits

The genetic relationship between WEC and production traits was examined in a parasite-free environment and under artificial challenge (Albers *et al.* 1987). Albers *et al.* (1987) concluded that a favourable relationship between WEC, bodyweight gain and wool growth existed whilst animals are challenged, with little response in a worm-free environment. Genetic correlations calculated on a range of Merino resource flocks across Australia show correlations between WEC and production traits were close to zero (Eady *et al.* 1998). Pooled estimates (s.e. not available) for greasy fleece weight, clean fleece weight, fibre diameter and bodyweight were 0.15, 0.10, -0.06 and -0.21 respectively (Eady *et al.* 1998). Genetic correlations calculated from New Zealand studies in Romney sheep (Bisset *et al.* 1992) indicated negative correlations between WEC and weight gain and fleece weight of -0.48 ± 0.21 and -0.31 ± 0.16, respectively. Differences between genetic correlations presented in Australia and New Zealand may exist due to breed and environment, particularly the degree of parasite challenge.

2.5.7 Resilience

Breeding for resilience to GI nematode infection poses an attractive alternative to breeding for resistance as the benefit of unaffected productivity in spite of infection can be expressed in economic units. The potential to select for resilience to nematode infection was first demonstrated by Albers *et al.* (1987) who measured bodyweight gain and wool growth in the absence and presence of an artificial infection of 11,000 *H. contortus* infective larvae. The heritability estimate for the loss of production due to infection (resilience) was 0.09 ± 0.07. Such a low heritability indicates that selection for resilience would result in slower rate of progress than what is possible for host resistance (Albers *et al.* 1987). Following the methodology used by Albers *et al.* (1987) in a commercial situation would prove difficult as comparison of wool growth and body weight gain would be confounded by time and infection.

Resilience has also been demonstrated in some flocks selected for superior production traits. In studies using Merino weaners selected for increased fleece-weight, WEC was between 32 and 92% higher (Howse *et al.* 1992, Williamson *et al.* 1995) and total worm burdens were 75% greater than in unselected counterparts (Williamson *et al.* 1994; 1995). In addition, McEwan *et al.* (1992) demonstrated that WEC was positively

correlated with bodyweight gain (0.95) and wool growth (0.41) in lines selected for greater reproduction, bodyweight and fleece weight. It appears that selection for superior production may have indirectly selected for mechanisms which initiate resilience.

The benefit of resilient animals may appear to be an attractive selection trait in terms of maintaining productivity despite nematode infection. Nonetheless, the pasture contamination produced by resilient animals remains an issue for classes of animals, such as periparturient ewes and weaners, which are more susceptible to infection. Breeding for resistance reduces pasture contamination and reinfection of susceptible classes of sheep.

2.5.8 Productivity of resistant genotypes

Resistant genotypes appear to develop immunity against parasite infection more rapidly than susceptible counterparts. Yet the ability to resist infection does not generally result in higher productivity. Barger & Southcott (1975) conducted studies into the relationship between WEC and production traits. They found that challenging resistant sheep with *T. colubriformis* reduced wool growth by 11% and appeared to depress bodyweight gain. More recently, Eady & Smith (2001) reported that *H. contortus* trickle challenge (1,000L_3/week) in both sheep selected for worm resistance and unselected controls resulted in similar levels of production between groups. Selection had no effect on greasy or clean wool growth or bodyweight. The main productive advantage shown by resistant animals was nil mortality, compared to an assumed 25% mortality rate (based on packed cell volume (PCV) < 14%) in unselected animals (Eady & Smith 2001). Eady (2002) examined the relationship between bodyweight gain and WEC at regular intervals in young sheep from weaning to 10 months and found similar bodyweight gains in weaners in both the *Haemonchus* selection flock and Rylington flock. Kahn *et al.* (2003) observed that random bred lambing ewes were no different for bodyweight gain and actually had greater annual clean fleece weight, clean wool growth rate and mean fibre diameter than resistant ewes, despite a five-fold difference in WEC. In the absence of nematode infection, Doyle (1999) showed resistant ewe hoggets displayed a reduced productivity compared to random bred counterparts. Random bred

animals gained an extra 1.1 kg bodyweight and 0.6 g/d greater clean wool growth rate, compared to resistant animals.

The production traits measured from the study reported by Woolaston *et al.* (1997) indicated that despite a 70% reduction in WEC, genetic selection had no effect on bodyweight and resistant animals had 9% less clean wool growth than unselected animals (Eady *et al.* 2003).

Divergent breeding of lines of Romney sheep has achieved an 11-fold difference in WEC between high and low WEC lines. However, correlated responses in production traits include significantly decreased post weaning weight gain, yearling fleece weight and increased dags in lambs. Morris *et al.* (2000) suggested in a commercial environment an index selection for a combination of increased productivity and decreased WEC and dags is required if resistant selected animals are to be beneficial to a sheep enterprise.

Improvement in productivity of resistant sheep was seen in resistant Romney weaners when grazed separately from susceptible counterparts, from weaning to 11 months of age. A 56-fold difference in WEC between resistant and susceptible weaners was associated with a 2.34 kg higher weight gain in resistant weaners. However, when the two genotypes were grazed together the advantage in growth rate of resistant weaners disappeared. Yearling fleece weights did not significantly vary between genotypes grazing separately or together (Bisset *et al.* 1997). Interestingly, equivalent productivity was shown in IRH weaners from the *Haemonchus* selection flock only when grazed separately from DRH and C lines. Weight gain from weaning to 12 months of age in IRH weaners was similar to DRH and C weaners when grazed in separate paddocks. However, the IRH animals grazing together with DRH and C lines had a 6 and 1 kg lower weight gain, in consecutive years, than the IRH animals grazing alone. Fleece traits were similar among genotypes managed alone or grazed together (Bell *et al.* 2003).

It appears that while resistant animals are exposed to nematode challenge when grazing with susceptible genotypes, animal production is hampered. However with lower pasture contamination and re-infection rates when grazed alone, productivity can reach

levels similar to animals which are more susceptible and have higher parasite burdens. Resistant animals have no significant production advantage over their susceptible counterparts.

2.6 Regulating host response by nutrition

2.6.1 Effect of protein and energy

Gastrointestinal parasitism in growing sheep impairs protein metabolism. The damage to mucosa, caused by adult and developing worms, results in increased plasma leakage and endogenous protein loss into the lumen (Steel *et al.* 1980, Poppi *et al.* 1986, 1990). Repair and replacement of damaged mucosa and the induction of an immune response to infection increases the protein requirements of the parasitised animal (Kimambo *et al.* 1988). A high protein diet (~170 g CP/kg DM) has been shown to alleviate the clinical signs of haemonchosis in young animals, such as anaemia, hypoproteinaemia, hypoalbuminaemia, inappetence, weight loss, high worm egg counts and worm burdens (Abbott, *et al.* 1985ab, 1986a, 1988, Wallace *et al.* 1995, 1996, Datta, *et al.* 1998).

High dietary protein intake enabled weaners artificially infected with 1,500 *H. contortus* larvae/week, to maintain normal haematocrit values and improve erythropoiesis (Datta *et al.* 1998). A reduction in WEC was evident with as little as 80 g/hd/d of protein-rich pellets (28% CP) three times a week (Shaw *et al.* 1995).

Bown *et al.* (1991a) reported that the effect of parasitism with *T. colubriformis* infection in young sheep was markedly reduced by the supply of digestible protein, but not energy. Post-ruminal infusion into the abomasum with either 61 g/d of sodium caseinate (to increase MP supply) or 79 g/d glucose (to increase ME supply) resulted in significantly reduced worm burdens in animals receiving protein. Animals infused with casein also had nitrogen retention values in the carcass similar to those of uninfected animals, while values for glucose infused animals were 40% lower. Kahn *et al.* (2000) revisited the use of metabolisable energy, as a nutritional means to combat the effects of parasitism, by using a dietary source rather than abomasal infusions. Diets were formulated to offer low and moderate digestible energy and low and moderate metabolisable protein supply. Bodyweight gain of Merino weaners increased in both

high DE and MP diets, compared to gains on the low diets. However, in contrast to the results reported by Bown *et al.* (1991a), animals with a greater DE intake had lower worm burdens than those fed the low DE diet and dietary MP content did not affect WEC or worm burden. In support of Kahn *et al.* (2000) findings, Valderrábano, *et al.* (2002) reported that animals consuming high levels of energy have enhanced immune responses to *T. circumcinta* infection, compared to weaners kept on a restricted diet. Consequently worm burdens were reduced by a decrease in female worm size and fecundity.

These results suggest that in young growing animals improved nutrition rather than a particular component of the diet will enhance resistance to parasite infection (Kahn *et al.* 2000). Nevertheless, the host demand for protein relative to energy is greatest in young growing animals (van Houtert & Sykes, 1996) and the change in protein metabolism will be markedly influenced by metabolisable protein supply.

2.6.2 Sources of protein

Improved protein nutrition will reduce the pathogenic effects of nematode infection (Abbott *et al.* 1986b) however, increasing the dietary crude protein (CP) supply does not always denote an increase in the supply of metabolisable protein (MP) to the animal. The degradation of dietary protein in the rumen and the efficiency of conversion of dietary CP to MP can be variable (van Houtert & Sykes 1996). The residence time of the diet in the rumen and level of feed intake will influence the digestion of CP, both of which will be affected by the presence of a nematode infection (Roseby 1973, 1977). For example, Siddons *et al.* (1985) showed that sheep fed grass hay (115 g CP/kg DM) or grass silage (203 g CP/kg DM) consumed 68 or 108 g CP/kg DM, whilst the flow of MP through the duodenum was 97 and 88 g CP/d respectively. Animals fed the grass silage consumed 59% more CP than sheep given hay, yet this converted into a 10% lower MP supply.

A source of non-protein nitrogen (NPN), such as urea, when animals are fed a low quality roughage diet (5% CP) can also influence the effect of parasitism on the host. Feeding oaten chaff containing 3% urea increased feed consumption of Merino weaners infected with either *H. contortus, T. colubriformis* or both species and was accompanied

by greater weight gains and wool production, than weaners fed oaten chaff alone. Sheep with urea supplementation also had lower worm egg counts and reduced worm burdens (Knox *et al.* 1999). In contrast, Wallace *et al.* (1998) supplemented Hampshire Down weaners with urea and infected with *H. contortus*, but found no difference in worm egg count, worm burdens and fecundity of adult female worms, compared to unsupplemented weaners. However, in agreement with the results of Knox *et al.* (1999), weaners supplemented with urea did show better resilience, with increased bodyweight gain, greater packed cell volumes (PCV) and higher plasma albumin concentrations, compared to weaners fed the basal diet.

Bypass or protected protein will also assist young animals to expel worm burdens and reduce production losses associated with infection. van Houtert *et al.* (1995a) showed that weaner wethers infected with *T. colubriformis* and supplemented with fish meal, (relatively resistant to microbial proteolysis in the rumen), had increased levels of circulating eosinophils and intestinal mast cells and a higher rate of worm expulsion and reduced WEC. The use of legumes containing condensed tannins, such as *Hedysarum coronarium* (sulla), have also been shown to assist in reducing WEC and worm burdens of sheep infected with *T. colubriformis* (Niezen *et al.* 1995). Condensed tannins (CT) result in less plant protein degraded in the rumen and may increase availability of post-ruminal protein, thereby maintaining productivity in parasitised weaners. Infected animals grazing sulla had greater daily weight gain, wool growth and fibre diameter than those grazing *Medicago sativa* (lucerne), which does not contain CT.

2.6.3 Effect of protein on immunity

The immunological competence of a host is influenced by nutrition. Poor nutrition or dietary deficiencies, especially low protein, will lower protective immunity to nematode parasite infections (Dobson & Bawden 1974, Kambara *et al.* 1993, van Houtert *et al.* 1995a, Strain & Stear 2001). Equally, a high protein diet will enhance immunity, which will allow the host to reject adult worms and reduce the fecundity of female worms (van Houtert & Sykes 1996).

Strain & Stear (2001) showed that weaners fed a high protein diet (173 g MP/kg DM) and artificially infected with *H. contortus*, had an increased plasma IgA response

against third stage larvae, compared to weaners fed a low protein diet (98 g MP/kg DM). The authors suggested that IgA in the gut to be a major mechanism controlling the fecundity of *H. contortus*. A high protein diet can also have long-term effects on the immune response of infected animals. Crossbred sheep previously fed a high protein diet (22% CP) for a period of 9 weeks had higher antibody responses to *T. colubriformis* whilst grazing infective pastures, than animals exposed to short-term provision of a lower protein diet (10-13% CP) (Datta *et al.* 1999). Concentrations of eosinophils and mucosal globule leucocytes have also been shown to increase in response to an increase in protein supplementation (Dobson & Bawden 1974, van Houtert *et al.* 1995a, Datta *et al.* 1998, 1999, Valderrábano, *et al.* 2002).

Fish meal, as a source of bypass protein, has been reported to mediate some immunological responses of sheep challenged with *T. colubriformis* infection. van Houtert *et al.* (1995a) demonstrated increased concentrations of sheep mast cell proteases and numbers of eosinophils in animals supplemented with 100 g/d fish meal. However, circulating antibody levels and lymphocyte proliferation were not enhanced by supplementation with the protected protein. The lack of an antibody and lymphocyte response may not have been the effect of bypass protein, but rather the age (3-month-old Merino wethers) of the animals used in the study. Kambara *et al.* (1993) suggest that lymphocyte responsiveness to larval antigen is affected by age and may be involved in resistance to parasitism by older (12-months) animals. The $CD4^{+}$ T cell sub population, was shown to be involved in protective immunity to infection in older animals (Kambara *et al.* 1996).

2.6.4 Effect of protein on productivity - resilience

GI nematode infection in young sheep can result in reduced productivity and sometimes mortality. However supplementation with dietary protein can prevent losses in animal productivity. In a study of the effects of varying levels of crude protein, Datta *et al.* (1998) found weaner wethers fed 19 and 22% CP continued to gain bodyweight throughout a *H. contortus* infection at a similar rate to uninfected controls. Infected weaner wethers fed 10, 13 and 16% CP lost weight during this period.

Exposure to a short-term provision of a protein-enriched diet can have long-term effects on an animal's response to infection. In a study comparing weaner sheep fed either 10 or 22% CP for 9 weeks, Datta *et al.* (1999) showed sheep previously fed a higher protein diet had less than half the WEC from a *T. colubriformis* paddock infection than animals fed a low protein diet. This suggests a "carry-over" effect of protein supplementation on subsequent resistance to infection of young grazing sheep. Findings reported by van Houtert *et al.* (1995b) were similar in sheep previously supplemented with 75% sunflower meal. Weight gain was 12% and wool growth 7% greater in animals fed sunflower meal compared to unsupplemented counterparts.

2.6.5 Effect of protein on periparturient rise

The periparturient rise in WEC occurs due to a breakdown in the expression of acquired immunity to gastrointestinal nematodes in ewes during late pregnancy and lactation (Barger 1993). The rise in WEC and consequently an increase in pasture contamination exposes lambs to greater parasite challenge. Parasitised periparturient ewes also have lower wool growth and lose more bodyweight during lactation (Leyva *et al.* 1982).

An increase in dietary metabolisable protein (MP) supply has been demonstrated to reduce WEC of parasitised periparturient ewes (Donaldson *et al.* 1998, Houdijk *et al.* 2000, Kahn *et al.* 2003). Protein supply was found to have a greater impact on resistance to infection in periparturient ewes, as opposed to metabolisable energy (ME) (Donaldson *et al.* 1998). MP requirements increase during lactation (Kahn *et al.* 2003) and for the expression of immunity (Houdijk *et al.* 2000). In addition, MP is necessary during parasitic infection to counteract the endogenous protein losses into the gut in the form of blood, plasma, mucin and sloughed cells (Coop & Holmes 1996). A reduction in voluntary feed intake of 16% in lactating ewes infected with *T. circumcincta* (Leyva *et al.* 1982) accentuates the demand for a higher MP supply in periparturient ewes.

Supplementation with 250 g/d cottonseed meal 5 weeks prepartum was shown to decrease WEC, while reducing the maternal body weight loss from -30 to -14 g/d compared to unsupplemented ewes. Interestingly, supplementation postpartum (days -21 to 28, day 0 lambing midpoint) had no effect on WEC, but improved maternal bodyweight gain by 27 g/d (Kahn *et al.* 2003). The authors demonstrated in an earlier

study that resistance to nematode infection can not be enhanced by increasing MP supply above levels supporting moderate weight gain (Kahn *et al.* 2000). Therefore, the MP supply prepartum improved immunity of ewes, indicated by WEC, when the gap between MP requirement and supply was greatest. Houdijk *et al.* (2000) have demonstrated that improved protein nutrition during late pregnancy enhanced immunity in periparturient ewes which was associated with increased plasma albumin concentrations and globule leukocytes.

2.6.6 Diet selection

The benefits of feeding animals protein to combat the subclinical effects of parasite infection have been discussed previously. Animals also have the ability to select a high protein diet, when given a choice between two feeds differing in crude protein (CP) content (Kyriazakis *et al.* 1994, 1996). Growing lambs offered diets with the same energy content (11 MJ ME/kg feed) but differing in CP, have selected a diet that best meets their CP requirements and will avoid diets with excess protein. Sheep have also discriminate against feed with added urea, when the feed is offered as a choice with a high CP diet (Kyriazakis & Oldham, 1993). Arsenos & Kyriazakis (2001) also revealed that when foods were supplemented with the nitrogen sources urea, casein and formaldehyde-treated casein, diet selection was characterised by an avoidance of these foods. However, if animals were previously consuming a low protein diet, selection of these supplemented foods was greater than in animals fed a high protein diet. Animals have also given preference to rumen undegradable protein (UDP) when given a choice with rumen degradable protein (RDP) (Arsenos *et al.* 2000).

Selection of diets with preference for energy content has also been investigated (Wang & Provenza, 1996, Kyriazakis & Oldham 1997). Lambs fed a basal diet, selected novel foods that complement the macronutrient composition of their basal diet. For example, when lambs were fed a high energy diet, such as barley, they preferred novel foods lower in energy and higher in protein, such as alfalfa (Wang & Provenza, 1996). Kyriazakis & Oldham (1997) demonstrated that diet selection for slowly fermentable carbohydrates was lower, compared to rapidly fermentable carbohydrates. However, when sheep were exposed to low rumen degradable protein, selection for slowly fermentable carbohydrates was higher than for rapidly fermentable carbohydrate. The

authors suggested that diet selection may be affected by the degree of synchrony of energy and protein to the rumen (Kyriazakis & Oldham, 1997).

Diet selection can also be modified by the animal in response to the level of parasitic infection and protein requirement. Whilst growing sheep were artificially challenged with *T. colubriformis* and had a reduced feed intake, Kyriazakis *et al.* (1994) demonstrated that animals given a choice between two feeds differing in crude protein content selected a diet which met their increased protein requirements. The daily intake of CP was similar in infected animals compared to that of their uninfected counterparts (254 vs. 245 s.d. 21.5 g/d for nil and infected lambs respectively). A similar result was shown with a long-term infection with *T. colubriformis*, where voluntary feed intake declined from week 5 of infection. Diet selection changed so that the proportion of the high protein feed in the selected diet started to increase from week 6 of infection. However, there were no differences in diet selection between uninfected controls or parasitised animals beyond week 18 of infection, suggesting that no obvious nutritional penalties were incurred once animals became resistant to the parasite infection (Kyriazakis *et al.* 1996).

Hutchings *et al.* (2000) has also demonstrated that grazing animals choose a diet high in protein in response to parasite infection. Sheep grazing perennial ryegrass/white clover pastures and infected with *T. circumcincta* consumed a higher proportion of clover in their diet than uninfected controls, which resulted in lower worm burdens. Therefore diet selection may play a role in determining the severity of the worm burden.

2.6.7 Nutrition and resistance

The magnitude of the pathogenic effects to the host and development and establishment of the parasite is influenced by the plane of nutrition. The interaction between resistance and high protein diets was demonstrated by Abbott *et al.* (1988). Three-month old weaners fed a high protein diet and repeatedly infected with 200 *H. contortus* larvae 3 times week for 17 weeks developed resistance to further infection. In contrast, animals of the same age fed a low protein diet and similarly infected did not develop resistance to infection. The animals from the high protein group not only had lower WEC and

worm burdens, but suffered less severe hypoalbuminaemia, anaemia, anorexia and weight loss.

Supplementary protein appears to improve the development of resistance in breeds of sheep susceptible to haemonchosis. Wallace *et al.* (1995) demonstrated that supplementation of Hampshire down weaners (a breed relatively susceptible to haemonchosis), with soyabean meal (172 g CP/kg DM) reduced WEC and pathogenic effects of infection, such as anaemia and hypoproteinaemia. By comparison, in a later study by Wallace *et al.* (1996) with Scottish blackface weaners (a comparatively resistant breed), supplementation with additional protein did not influence WEC or worm burdens (Wallace *et al.* 1996).

2.7 Conclusion

The consequences of GI nematode infection in sheep is attributed to a reduction in voluntary feed intake, impaired rumen function and metabolism and reduced utilisation of nutrients for growth and wool production. There is a suggestion that components of the developing immune response account for the increase in metabolic costs of parasitism. Liu *et al.* (2005c) calculated the costs of parasite infection and showed the extra demand for protein to be equivalent to 1.0-1.4 g N/d. Parasitised resistant sheep required an additional 4% of daily ME and 5% of metabolisable CP. Comparable or lower productivity in resistant genotypes may reflect the changes in the physiological responses to infection, required to mount a strong immune response. Studies using the Rylington selection flock were conducted concurrently with the experimentation presented in this thesis. These studies begin to develop some understanding of the mechanisms involved in resistant genotypes when infected with *T. colubriformis* and *T. circumcincta* parasites. To my knowledge there are no reports which investigate if divergent selection for resistance to *H. contortus* has led to correlated physiological changes to the host.

CHAPTER 3

FEED INTAKE AND DIET SELECTION BY SHEEP SELECTED FOR GENETIC DIFFERENCE IN RESISTANCE TO NEMATODE INFECTION

3.1 Introduction

Reduction in voluntary food intake has been described as one of the main features of gastrointestinal nematode infections, which can range from a progressive decrease in intake to almost complete anorexia in acute cases (Coop 1981). Reduced feed intake is more equivocal in *H. contortus* infections, as some studies show no change in feed intake between infected and uninfected animals (Wallace *et al.* 1998). A consequence of depressed feed intake is the reduction in gross efficiency of energy utilisation for animal production. Gastrointestinal parasitism in growing sheep impairs protein metabolism, as the damage to mucosa caused by adult and developing worms results in increased plasma leakage and endogenous protein loss into the lumen (Steel *et al.* 1980, Poppi *et al.* 1986, 1990). Repair and replacement of damaged mucosa and the induction of an immune response to infection increase the protein requirements of the parasitised animal (Kimambo *et al.* 1988). During haemonchosis the blood loss into the gastrointestinal tract caused by haemorrhage in the abomasum can equate to 250 ml/d of blood or 2.6 g blood nitrogen/d in animals carrying a mean *H. contortus* burden of 5,000 adults (Rowe *et al.* 1988). The reduction in voluntary feed intake that accompanies gastrointestinal infection accentuates the need for a higher dietary protein intake (Symons 1985).

Abbott *et al.* (1986b) revealed that animals infected with *H. contortus* and given a low protein diet (88 g CP/kg DM) had lower daily feed intakes than animals fed a high protein diet (170 g CP/kg DM). Infected animals fed a low protein diet were also less able to withstand the pathogenic effects of *H. contortus* infection, showing increased oedema, anaemia, hypoproteinaemia and hypoalbuminaemia and weight loss (Abbott *et al.* 1985ab; 1986ab; 1988; Wallace *et al.* 1995; 1996). Dietary protein can influence the animal's ability to resist haemonchosis by reducing establishment and development of

incoming larvae. Abbott *et al.* (1988) showed a decrease of 30% in worm egg output in sheep fed a high protein diet (169 g CP/kg DM), compared to animals offered a low protein diet (88 g CP/kg DM). Enhancement of resistance by dietary protein appears to be demonstrated only in susceptible breeds, such as Hampshire Down (Abbott *et al.* 1985b, Wallace *et al.* 1995). Elimination of worm burdens in the known resistant breed, Scottish Blackface, was not dependent on diet (Abbott *et al.* 1985ab, Wallace *et al.* 1996). The authors concluded that increased dietary protein was not necessary to alleviate infection in more resistant breeds. Resilience to infection has also been demonstrated through the provision of dietary protein supplementation (19 and 22% CP), with reduced depression in growth rate and wool production in supplemented sheep (Datta *et al.* 1999). Whilst parasitised animals have a reduced feed intake, Kyriazakis *et al.* (1994) demonstrated that animals given a choice between two feeds differing in crude protein content have selected a diet which meets their increased protein requirements. The daily intake of CP was similar in infected animals compared to that of their uninfected counterparts (254 vs. 245 s.d. 21.5 g/d for nil and infected lambs respectively).

Given the importance of protein nutrition to the development of host resistance and resilience to nematode infection it was hypothesised that genetic differences in host resistance may be mediated through differences in protein supply. The aim of this experiment was to investigate if divergent selection for resistance to *H. contortus* has produced correlated changes in voluntary feed intake and diet selection. These effects were determined when animals were offered a high or moderate quality diet, in the absence and presence of *H. contortus* infection.

3.2 Materials and methods

3.2.1 Experimental design

The timing of experimental events is detailed in Table 3-1. The experiment was divided into two periods. In period 1 (day -36 to -1), animals were maintained worm-free (NIL) and in period 2 (day 0 to 88) they were given a trickle challenge with *H. contortus* (INF). Feed intake and diet selection measurements were made in both periods. The experiment was designed as a 3 x 2 factorial with three selection lines selected for genetic difference in resistance to nematode infection and two diets, a high quality (H)

(high metabolisable protein and metabolisable energy) and a moderate quality (M) (moderate metabolisable protein and metabolisable energy) diet. Animals were stratified within selection line on the basis of bodyweight measured following adjustment to experimental feed and worm egg counts (WEC), from a previous experimental challenge and were randomly allocated to diets and position of pen within the animal house.

Table 3-1 Timing of experimental events relative to initial bolus infection with *H. contortus* which is defined as day 0. Days indicate the start and end days for each activity.

Period	Time (days)	Experimental event
	-92 to -64	Covariate wool growth measurement in paddock
NIL period	-64	Animals enter animal house
	-63 to -57	Animals adjust to experimental conditions
	-56 to -47	Animals introduced to experimental diets
	-46 to -37	Diet selection training
	-36 to -23	Diet selection measurements
	-22 to -15	Animals fed High and Moderate diets
	-14 to -1	Feed intake measurements
INF period	0	Beginning of *H. contortus* infection
	0 to 63	Feed intake measurements
	64 to 68	Animals fed High and Moderate diets
	69 to 74	Diet selection training
	75 to 88	Diet selection measurements

3.2.2 Animals and housing

The study used 54 Merino weaner rams selected randomly from the CSIRO increased resistance to *Haemonchus* (IRH) (n = 19), decreased resistance to *Haemonchus* (DRH) (n = 15) and unselected control (C) (n = 20) selection lines (each line was represented by all 5 sire groups) (Woolaston *et al.* 1990). They were approximately 10 months of age at the beginning of the experiment with a mean ± s.d. bodyweight of 26.1 ± 2.85 kg. Animals were housed in individual pens at the University of New England animal house. Upon entering the animal house each animal was drenched with Scanda® (8 mg/kg levamisole hydrochloride and 4.5 mg/kg oxfendazole, Schering-Plough Animal Health Ltd) and Ivomec® (0.2 mg/kg ivermectin, Merial), to remove existing worm burdens, and given an intramuscular injection of vitamin B_{12} (1 ml/animal, hydroxocobalamin and cyanocobalamin, Novartis Animal Health). A worm egg count (WEC) was taken 5 d after drenching to confirm that all animals had zero counts.

3.2.3 Feed

The formulation and analysis of the experimental diets are given in Table 3-2. Diets were formulated to be balanced for major and trace minerals, and pelleted to reduce the ability of animals to select individual ingredients in the diet. Diet formulation was not constrained to make diets isoenergetic while differing in metabolisable protein (MP) content. Donaldson *et al.* (1998) have shown that the level of MP, rather than metabolisable energy (ME), has the most influence on host resistance of lactating ewes to nematode infection. Animals were offered quantities of fresh food once daily that was 10% greater than *ad libitum* intake on the previous day. A subsample of food refusals from each animal was taken weekly for later analysis of remaining contents. The high quality diet (H) was fed throughout the experimental period but two moderate quality diets were fed. Moderate quality diet 1 (M1) was fed in the non-infection period only and then the sheep were offered the moderate quality diet 2 (M2) at the start of the infection period. The change was instigated by unexpectedly high growth rates on M1, and was to ensure animals were fed diets with sufficient difference to enable genotype differences in two nutrient environments to be expressed.

3.2.4 Diet selection

Animals were offered a choice between two diets (H and M) and given the opportunity to experience both foods during a 'training period' as suggested by Kyriazakis & Oldham (1993). Each of the two feeds were offered alone in alternate periods of 2 d (4 times) and subsequently on alternate days (once), allowing the training period to last for 10 d. Following the training period the intake (g/d) of each diet was measured over 14 d. Two troughs, each containing either H or M diet, were placed in each pen with the trough position (i.e. left or right) randomised across sheep. In addition, the location of each feed was swapped between troughs for each animal every day, to avoid 'learning' of feed position rather than type. The intake of each feed was measured at 2, 8 and 24 h after initial provision. Diet selection was measured prior to infection and repeated during weeks 11 and 12 of infection.

Table 3-2 Ingredients[1] and chemical analysis of experimental diets.

Ingredients (% per kg fresh matter)	H diet	M1 diet	M2 diet
Cottonseed hulls	39.0	54.0	77.0
Barley	16.0	23.0	11.5
Lucerne chaff	15.0	10.0	5.0
Molasses	8.0	10.0	5.0
Cottonseed meal	22.0	3.0	1.5
Dry matter	90.8	92.3	89.9
Composition (per kg DM)			
Organic matter (g)	956.0	963.0	992.0
Digestibility[A] (g)	627.0	583.0	461.0
Ether extract (g)	30.0	20.0	16.0
Crude protein (g)	168.0	97.0	75.0
Calculated MP[B] (g)	90.0	43.0	30.4
Calculated ME[C] (MJ)	9.2	8.3	6.3
Sulphur (g)	2.1	1.7	2.0
Phosphorus (g)	3.3	2.2	2.4
Iron (mg)	147.2	154.5	160.3
Copper (mg)	7.1	5.4	6.0

[1]Major minerals and trace elements were added to make diets similar in concentrations. Averaged across diets these (per kg dry matter) were 3.25 g Ca, 2.6 g Mg, 12.8 g K, 1.213g Na, 42 mg Zn, 48 mg Mn and 2 mg Mo. Feed was analysed for mineral concentrations using the Vista MPX radially viewed, simultaneous Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES).
[A] Based on pepsin cellulase wet chemistry.
[B] Calculated from equations of Freer *et al.* (1997).
[C] Calculated from algorithm supplied by FeedTest, Victorian Department of Primary Industries, Hamilton Vic 3300 .

3.2.5 Infection

Animals had previously been artificially infected with 10,000 L_3 *H. contortus* at approximately six months of age (four months prior to experiment) and subsequently exposed to natural field infection. This infection history was important to ensure animals had the opportunity to acquire immunity to nematode infection and reflect genetic merit. All animals remained parasite free for the first period of the trial. In the second period, all animals were infected with McMaster strain *H. contortus* L_3. The infective dose was determined using four random bred Merino weaners of similar age, with two infected with 100 L_3/kg bodyweight and two with 150 L_3/kg bodyweight. The infection was maintained for seven weeks and WEC determined from week 3. The WEC from weaners infected with 100 L_3/kg bodyweight was considered too low to display the genetic differences of the selection lines. An initial dose of 150 L_3/kg

bodyweight was chosen as a more appropriate level, to ensure sufficient challenge. An initial infective dose of 150 L_3/kg bodyweight was orally administered at the beginning of the infection period, followed by a trickle infection for 12 weeks of 250 L_3 three times per week on Monday, Wednesday and Friday (average total dose ± s.d. over the infection period of 12,570 ± 565 L_3/sheep).

3.2.6 Animal measurements

Animals were weighed in the morning prior to feeding, commencing at the start of the experiment and weekly thereafter. A single worm egg count (WEC) per animal was measured at day 0 and 21 after the initial dose of infective larvae and weekly thereafter. A modified McMaster technique was used to count worm eggs and potassium iodide was used rather than sodium chloride salt solution. The higher specific gravity (1.4) of potassium iodide was required to enhance flotation and therefore detection of eggs from animals consuming the pelleted diets rich in cottonseed hulls. Total faecal output and daily worm egg output were measured during week 9 of infection on 18 weaner rams. Three animals from each selection line and diet were chosen on the basis of WEC recorded in previous weeks and food intake. Animals with a high, low and average WEC and an average feed intake for their group were selected. Faecal collection bags were attached to each animal for a 24 h faecal collection over a 3 d period. The total collection was thoroughly mixed and a sub-sample taken for worm egg counts and dry matter calculation. Six worm egg counts were measured on each sample and the average used to multiply by the total daily faecal output, to calculate daily WEC output.

Animals were bled by jugular venepuncture into 2 x 10 ml and 1 x 5 ml K_3-EDTA Vacutainer® tubes at day -46 (base sample), at the end of the 14 d NIL diet selection (day -23) and feed intake (day -1) periods and weekly during the INF feed intake period and following the 14 d INF diet selection (day 88). Haematology parameters; red blood cell (RBC) count, haemoglobin, haematocrit, white blood cell (WBC) count, eosinophil concentration and mean corpuscular volume (MCV) were measured on whole blood using a Cell-Dyn 3500R haematology analyser, (Abbott Diagnostics Division). The 10 ml tubes were centrifuged at 2,500 *g* for 20 min and the plasma divided into several aliquots for later analysis. Total plasma protein and albumin concentrations were measured using a Dimension XL Clinical Chemistry system (DADE Behring Inc.,

Newark, USA). The assay ranges for total protein were 20-120 g/*l* and albumin 6-80 g/*l*. Globulin concentrations were calculated from the difference between total protein and albumin concentrations. Insulin-like growth factor-1 (IGF-1) assay was conducted by Ms. J.B. Briegel, CSIRO Livestock Industries, Wembley WA and determined on base samples using the technique described by Breier *et al.* (1991). *Haemonchus* specific antibodies (IgG_1, IgG_2, IgA, IgE) in plasma were determined using enzyme-linked immunosorbant assay (ELISA). The procedure was similar to that described by Windon *et al.* (2002). The method was as follows:

Microtitre plates (96 well, Maxisorb, Nunc, Roskilde, Denmark) were coated with 100 µl/well carbonate coating buffer (pH 9.6) containing 3.6 mg/ml of soluble protein from 3rd stage *H. contortus* larvae and incubated at 4°C for 15 h. The *H. contortus* antigen was made by Ms. S.K. Burgess, University of New England, Armidale NSW. The coating solution was removed and 200 µl/well of blocking solution (phosphate buffered saline (PBS) containing 0.1% (w/v) sodium casein) was added and the plates incubated at room temperature for 2 h. Plates were then washed once with an automatic plate washer (Tecan CE, Austria) in PBS containing 0.05% Tween 20 (PBST) to remove blocking solution. Each plate was divided into two sections to allow for 6 (half plate) doubling serial dilutions. The plasma samples were diluted into ELISA diluent (PBST containing 1% (w/v) sodium casein) in the first and seventh wells. Each plate included a standard and 15 samples, which were randomised for animal and sample day. The starting dilutions to test isotype IgG_1 and IgG_2 were 1:100, IgE 1:2 and IgA was undiluted. The plates were incubated at room temperature for 1 h and then washed 4 times with PBST. 100 µl of monoclonal antibody (as culture supernatant) against sheep IgG_1, IgG_2 (Beh 1987), IgE (Bendixsen *et al.* 2004) and IgA (Beh 1988), diluted 1:10 in ELISA diluent was added to each well and incubated at room temperature for 1 h. The plates were washed a following 4 times with PBST and 100 µl of anti-mouse, affinity isolated, conjugated to horseradish peroxidase (HRP) raised in sheep (DAH, Chemicon, Australia), diluted 1:2000 in ELISA diluent, was added to each well and incubated at room temperature for a further 1 h. The plates were washed 6 times with PBST and 100 µl of tetramethyl benzidine (TMB, T2885-5G Sigma Aldrich, MQ, USA) substrate was added to each well. After 30 min at room temperature 50 µl of 0.5 M H_2SO_4 was added to each well to stop chromophore production. The optical density of each well was measured using a Thermomax microplate reader (Molecular Devices, CA, USA) at wavelength 450nm. Individual titres were calculated from the midpoint on a double log

scale of the straight line section of the curve using linear regression. The results, expressed as titres, represent the inverse of the dilution of these points.

Wool growth rate was measured using dyebands (Durafur black flakes, Imperial Chemical Industries, Sydney) placed vertically on the left midside position of the animal (Chapman & Wheeler 1963). The dyebands were applied on days -92 and -64 as a covariate wool growth measurement whilst animals were grazing on pasture. Corresponding 28 d measurements were applied on days -14 and 14 for a NIL measurement and on days 36 and 64 during weeks 5 to 9 of infection. Time restrictions did not allow a longer wool growth measurement during the NIL period. Nagorcka (1977) explained that a lag of 25 d occurs between a change in nutrient intake and a wool growth response and therefore it was expected that day 0 to 14 of the INF period would reflect the NIL measurement. The dyeband sample was removed using Oster clippers No. 40 on day 88. Animals were shorn 2 d later and fleeces weighed to enable calculation of wool growth rate (g/d). Each dyeband period was cut from the staple and analysed separately for fibre diameter (FD) and yield by New England Fibre Testing, Walcha NSW.

3.2.7 Statistical analysis

All statistical analyses were performed using the SAS computer program (SAS Institute Inc 1999-2001). General Linear Models (GLM) were used to analyse the significance of selection line (IRH, DRH and C), diet (H and M) and the interaction (line x diet) between these effects. The models used repeated measures analysis of variance for daily feed intake, bodyweight, WEC and haematology parameters. Initial bodyweight was not a significant covariate for subsequent bodyweight measures. Change in bodyweight during the NIL period was not of primary interest and, because this was only of 2 weeks duration, statistical analysis was not considered reliable. Analysis was performed separately for each period (with and without infection) and also because of the change from M1 to M2 diet. Least square means (ls mean) ± standard errors (s.e.) are presented for all measured parameters. Diet selection was measured by calculating the percentage of H diet selected from the whole diet (H/(H+M)x100). The percentage was arc-sine (ASIN) transformed and the average of 14 d measurement analysed. The data was presented as SIN back-transformed means with back-transformed 95% confidence

intervals (*ci*). Worm egg counts and circulating white blood cells (WBC) were cube-root transformed to normalise data prior to analysis and are presented as back-transformed means with back-transformed 95% confidence intervals (*ci*). Eosinophil counts and mean corpuscular volume were transformed using $\log_{10}$ with antilog means and antilog 95 % confidence intervals. Red blood cell (RBC) counts and haematocrit were square-root transformed with back-transformed means and 95 % confidence intervals. The base sample for all haematological measures was not a significant covariate for subsequent samples. The base sample for IgG_1, IgG_2 and IgA was a significant covariate for antibody analysis. The wool measurement made whilst animals were grazing on pasture (days -92 to -64) was a significant covariate for greasy and clean wool growth rates and fibre diameter.

3.3 Results

3.3.1 Feed intake

During the 2 week NIL period, daily feed intake differed significantly (P = 0.030) among selection lines. Weaner rams from the IRH line had greater feed intake than the C line but DRH animals were intermediate and not different from the other lines (Table 3-3). Daily feed intake was unaffected by diet (P = 0.344) and there was no significant interaction between the effects of selection line and diet. In contrast, daily feed intake did not differ (P = 0.242) among selection lines during the infection period (Figure 3-1) and feed intake was significantly ($P<0.0001$) lower in animals fed the M2 diet (Figure 3-2). Interactions between the effects of selection line and diet were not significant during the infection period.

Table 3-3 Average daily feed intake (g DM/day) of weaner rams, selected for increased resistance to *Haemonchus* (IRH), decreased resistance to *Haemonchus* (DRH) and random-bred controls (C), maintained worm free.

Selection line	ls mean	s.e.
IRH	1613^{a}	51.6
DRH	1508^{ab}	58.2
C	1415^{b}	50.3

Least square means with different suffix differ significantly ($P<0.05$).

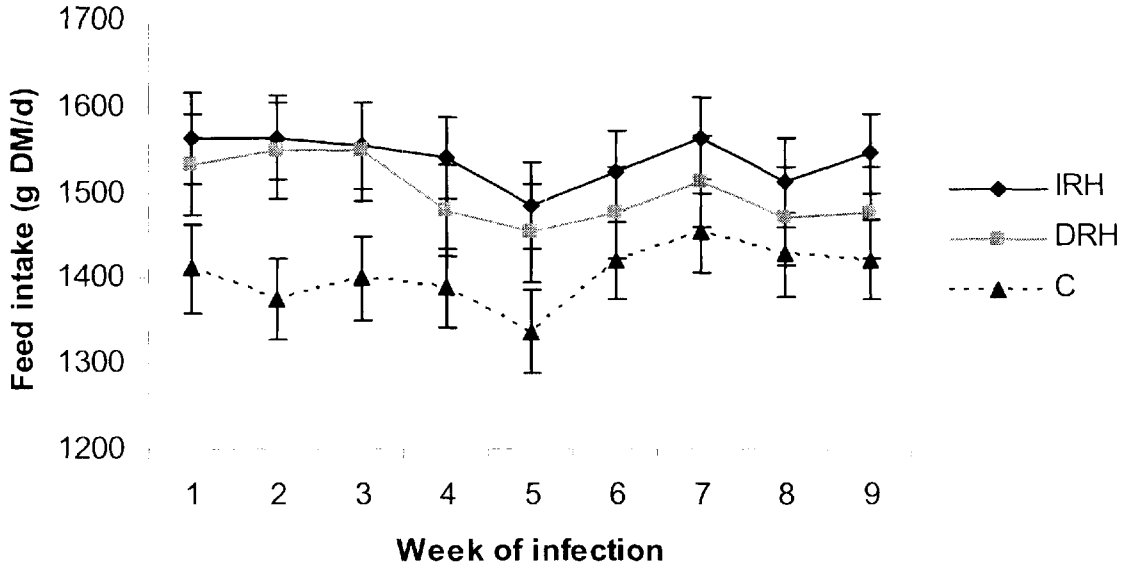


Figure 3-1 Daily feed intake (ls mean ± s.e.) of weaner rams selected for either increased resistance to *Haemonchus* (IRH), decreased resistance to *Haemonchus* (DRH) or random-bred control (C) and trickle infected with *H. contortus*.

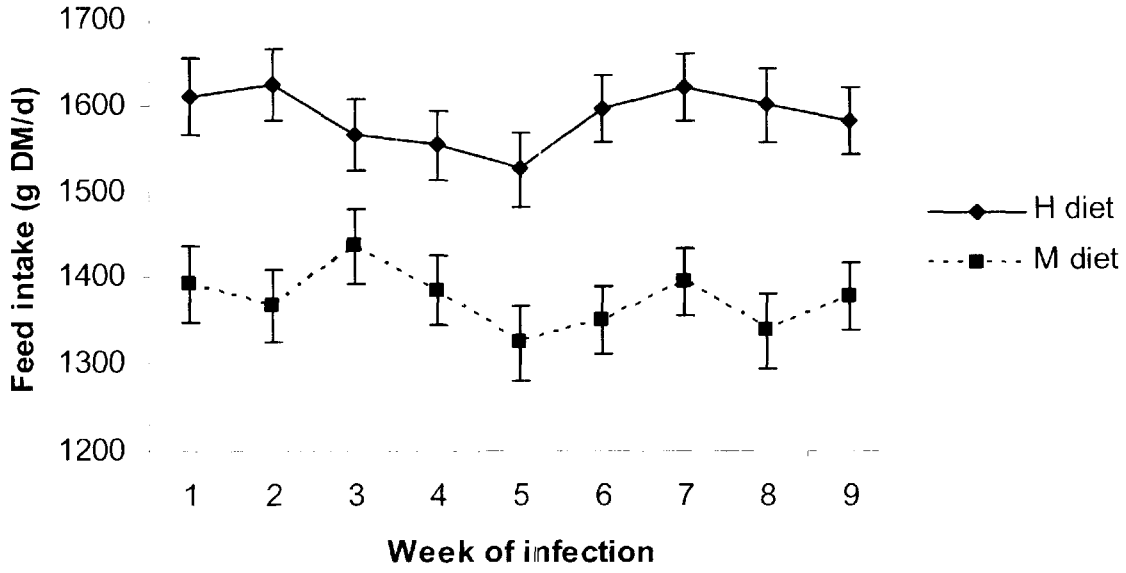


Figure 3-2 Daily feed intake (ls mean ± s.e.) of weaner rams fed either a high (H) or moderate quality diet (M2) and trickle infection with *H. contortus*.

3.3.2 Diet Selection

The consumption of diet H, expressed as a percentage of total daily intake, did not differ among selection lines, when animals were maintained worm free (P = 0.198) or infected with *H. contortus* (P = 0.175) (Figure 3-3). There were no differences in intake of H diet at 2, 8 or 24 hours after the initial provision of each feed, among the lines. The percentage of H diet eaten was higher during the INF period compared to NIL, but this cannot be statistically compared, because of the change in M1 to M2. The percentage of diet H eaten decreased over the course of each day in NIL animals, but was maintained above 95 % during the 24 h period in INF animals. Contrasts between selection lines for daily feed intake during the 14 d diet selection periods showed IRH animals had higher intakes compared to DRH and C lines for NIL (P = 0.038) and INF (P = 0.016) (Table 3-4).

Table 3-4 Daily feed intake (g DM/d), during diet selection periods, of weaner rams selected for increased resistance to *Haemonchus* (IRH), decreased resistance to *Haemonchus* (DRH) and random-bred controls (C) and either maintained worm free (NIL) or infected with *H. contortus* (INF).

	NIL		INF	
Selection line	ls mean	s.e.	ls mean	s.e.
IRH	1606[a]	40.7	1542[a]	46.8
DRH	1520[b]	47.0	1414[b]	53.0
C	1473[b]	40.7	1377[b]	46.8

Least square means, within columns, with different suffix differ significantly ($P<0.05$).

3.3.3 Parasitology

Worm egg counts (WEC) of IRH animals remained significantly lower throughout the 12 week infection period, compared to the DRH and C lines (P< 0.0001). The DRH animals had higher WEC compared to C during this time (Figure 3-4). Diet had no effect (P = 0.198) on WEC over the course of the 9 week feed intake measurement, nor was there a significant interaction between the effects of selection line and diet. There was a suggestion (P = 0.197) that WEC was reduced on the H diet, with a 31, 30 and 27% reduction for IRH, DRH and C respectively (Figure 3-5). Daily faecal collection, calculated during week 9 of infection, also showed IRH animals had lower (P = 0.03) total daily worm egg output, compared to DRH and C lines (Figure 3-6).

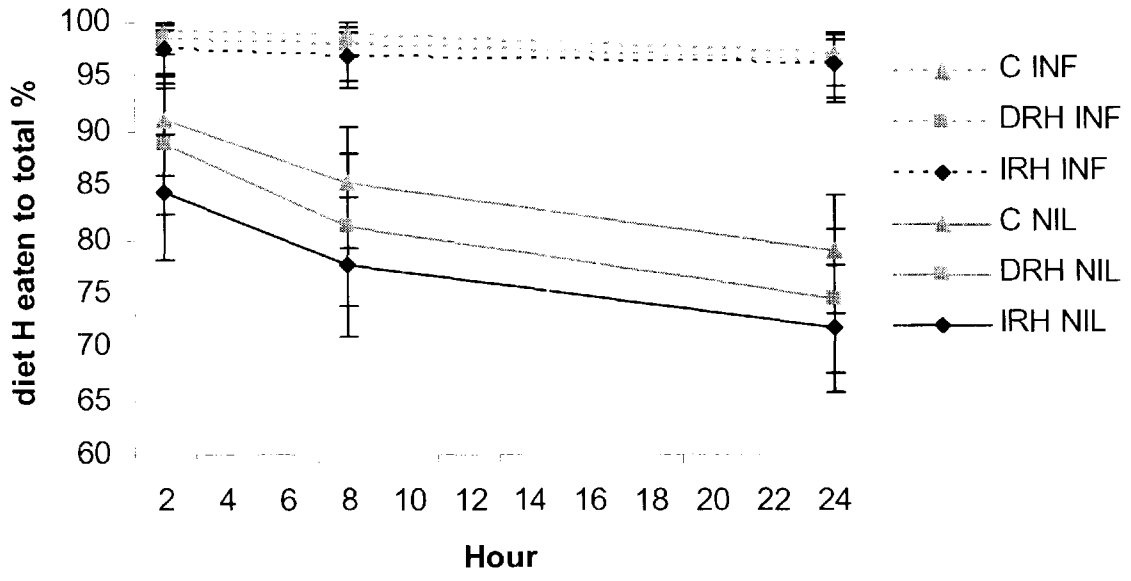


Figure 3-3 Diet H consumption expressed as a percentage of total daily feed intake (ls mean ± 95% c.i.) of weaner rams selected for either increased resistance to *Haemonchus* (IRH), decreased resistance to *Haemonchus* (DRH) or random-bred control (C) and either maintained worm free (NIL) or trickle infected with *H. contortus* (INF).

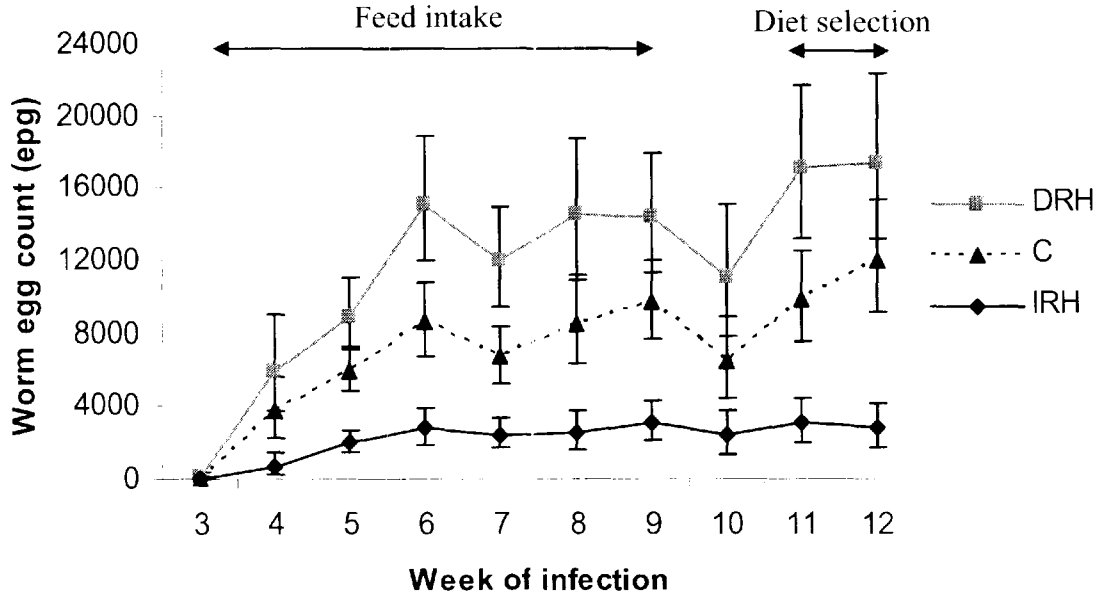


Figure 3-4 Back-transformed worm egg count (ls mean ± 95% *ci.*) of weaner rams from the increased resistance to *Haemonchus* (IRH), decreased resistance to *Haemonchus* (DRH) and random-bred control (C) selection lines, over a 12 week trickle infection.

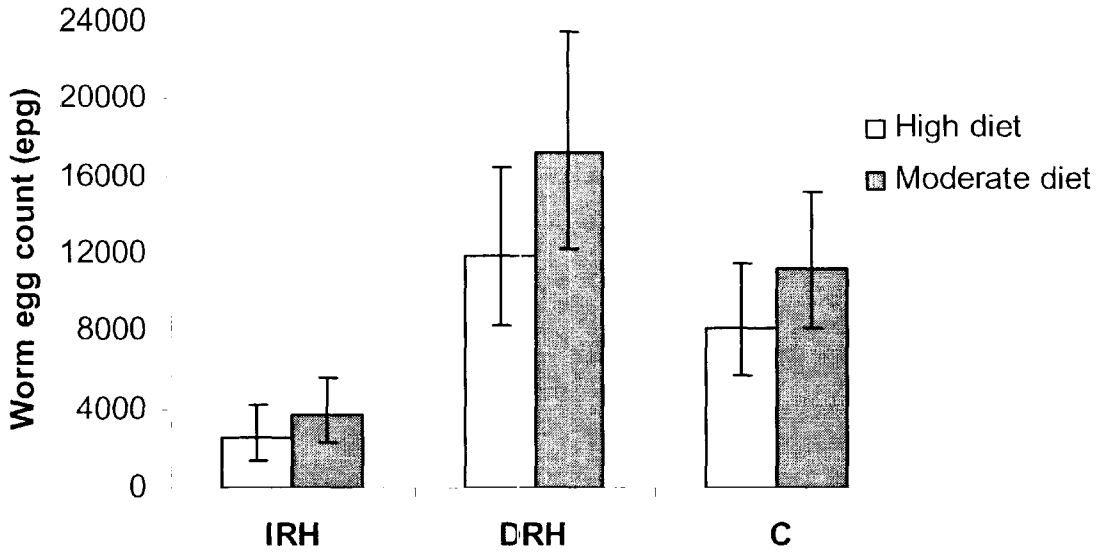


Figure 3-5 Back-transformed worm egg count (ls mean ± 95% *ci.*) of weaner rams from the increased resistance to *Haemonchus* (IRH), decreased resistance to *Haemonchus* (DRH) and random-bred control (C) selection lines, at week 9 of an *H. contortus* trickle infection, fed either a high or moderate quality diet.

3.3.4 Haematology

Red blood cell (RBC) numbers were higher in IRH animals (P = 0.013) compared to DRH and C lines from week 5 of infection. RBC numbers declined in the IRH animals until week 5 of infection and then slowly increased, while RBC numbers in the DRH and C lines continued to decline over the course of the infection (Figure 3-7). RBC numbers were lower (P = 0.020) in animals fed the moderate quality diet from week 5 of infection, compared to animals on the H diet (Figure 3-8). Haemoglobin (P = 0.042) and haematocrit (P = 0.041) responded similarly to RBC numbers among selection lines and were lower (P = 0.01) in animals fed the moderate quality diet (Table 3-5). Eosinophil counts were higher (P = 0.001) in IRH animals compared to DRH and C lines (Figure 3-9), but did not differ between diets. There was a significant (P = 0.041) interaction between the effects of selection line and diet, as IRH animals fed the moderate diet had lower eosinophil counts compared to IRH animals fed the H diet, while eosinophil counts were unaffected by diet in the DRH and C lines (Figure 3-10). Circulating white blood cells (WBC) were lower significantly (P = 0.022) in DRH animals from week 5 of infection compared to IRH and C lines (Figure 3-11). Animals

fed the moderate quality diet also had lower (P = 0.001) WBC counts from week 6, compared to the H diet. Mean corpuscular volume (MCV) increased (P = 0.010) to a greater extent in animals fed the H diet, over the course of the infection. DRH and C lines had a higher MCV (P = 0.007) compared to IRH, from week 5 of infection (Figure 3-12). IGF-1 tended to be higher (P = 0.100) in DRH animals compared to C lines during the NIL period before diet treatments were implemented. IGF-1 concentration for IRH, DRH and C lines were 107.6^{ab} ± 14.65, 144.4^{a} ± 16.49 and 97.8^{b} ± 14.28 μg/l respectively.

Table 3-5 Blood haemoglobin and haematocrit levels in blood samples taken (ls mean ± s.e.) at week 9 of *H. contortus* infection, from weaner rams selected for increased resistance to *Haemonchus* (IRH), decreased resistance to *Haemonchus* (DRH) and random-bred control (C) and fed either a high (H) or moderate (M) quality diet.

Group	**Haemoglobin g/dl**		**Haematocrit (%)**		
	ls mean	s.e.	ls mean	± *ci*	
IRH	9.9^{a}	0.22	29.8^{a}	1.26	1.21
DRH	8.7^{b}	0.25	26.5^{b}	1.61	1.51
C	9.0^{b}	0.22	27.4^{b}	1.33	1.27
H diet	9.8^{x}	0.19	29.9^{x}	1.05	1.01
M diet	8.6^{y}	0.19	25.8^{y}	1.24	1.18

Least square means within treatment effect and columns, with different suffix differ significantly (P<0.05).

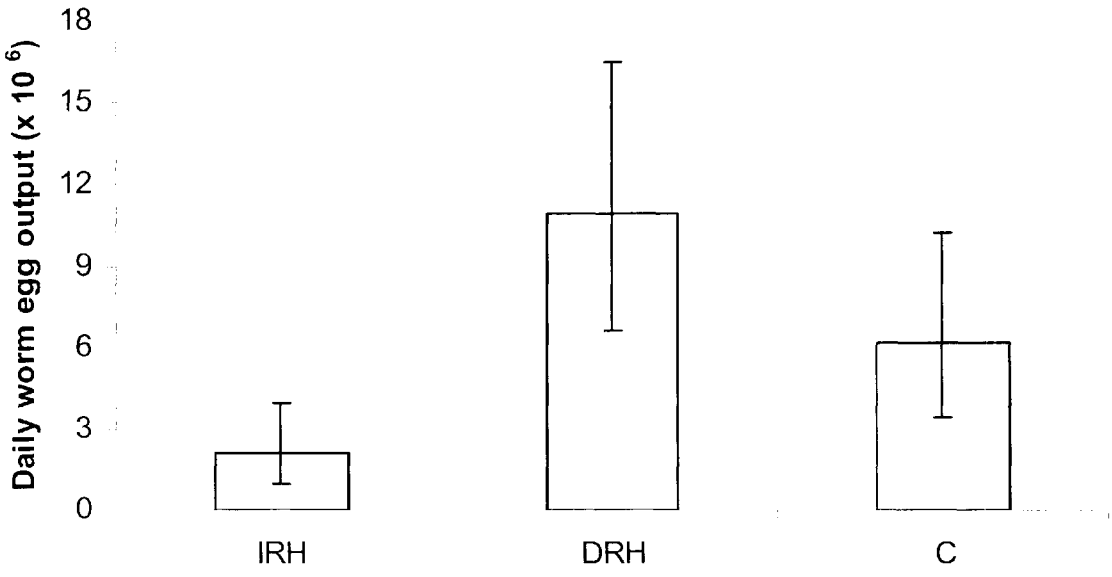


Figure 3-6 Back-transformed daily worm egg output (ls mean ± 95% *ci.*) of weaner rams from the increased resistance to *Haemonchus* (IRH), decreased resistance to *Haemonchus* (DRH) and random-bred control (C) selection lines, at week 9 of a *H. contortus* trickle infection.

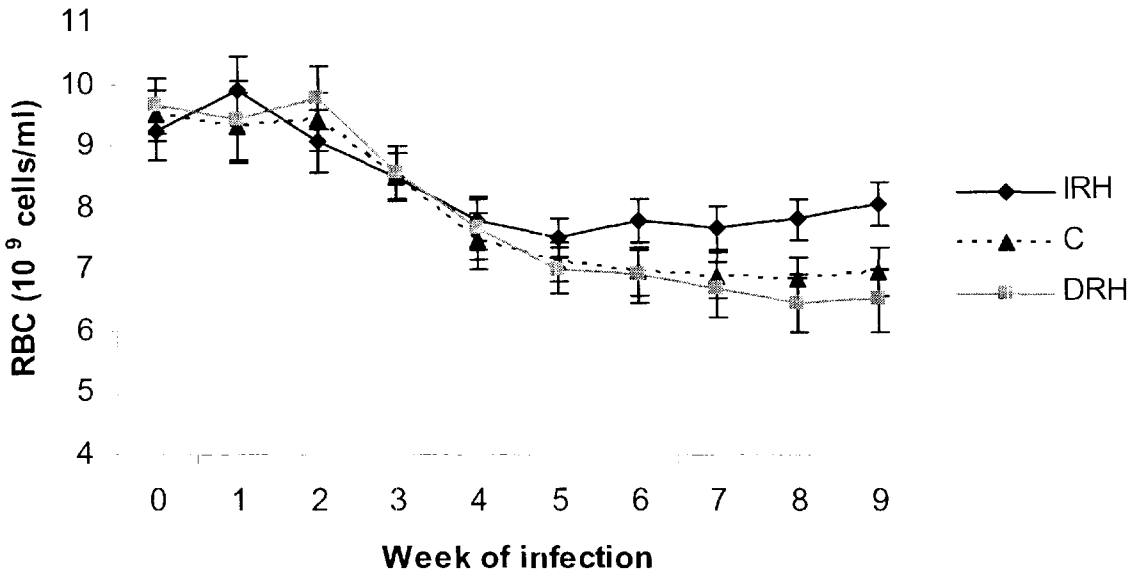


Figure 3-7 Red blood cell (RBC) count (back-transformed ls mean ± 95% *ci.*) in blood samples taken from weaner rams selected for either increased resistance to *Haemonchus* (IRH), decreased resistance to *Haemonchus* (DRH) or random-bred control (C).

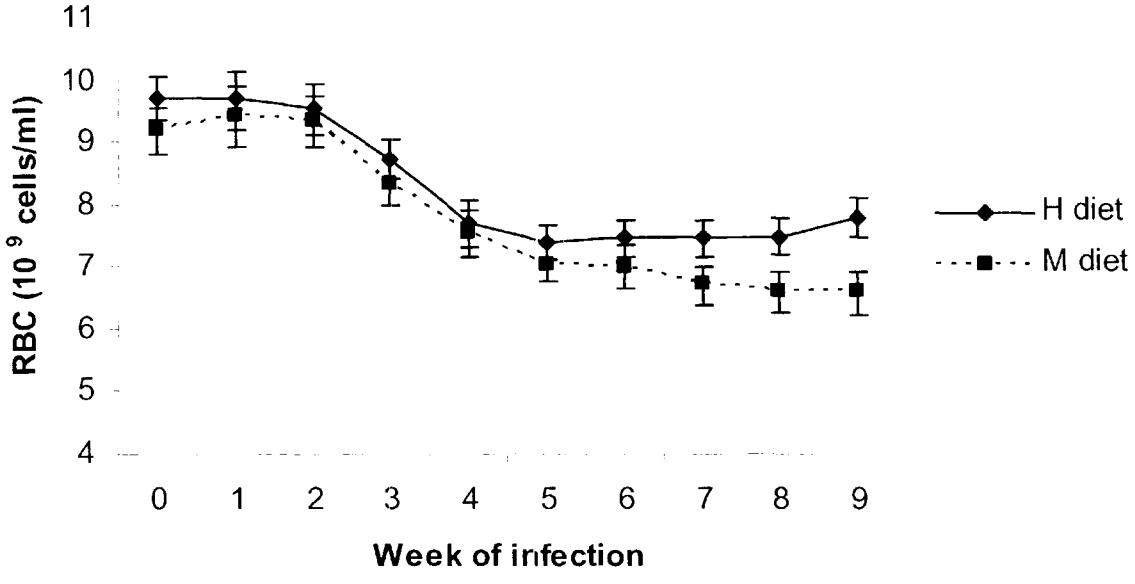


Figure 3-8 Red blood cell (RBC) count (back-transformed ls mean ± 95% *ci.*) in blood samples taken from weaner rams fed either a high (H) or moderate quality diet (M) and trickle infection with *H. contortus*.

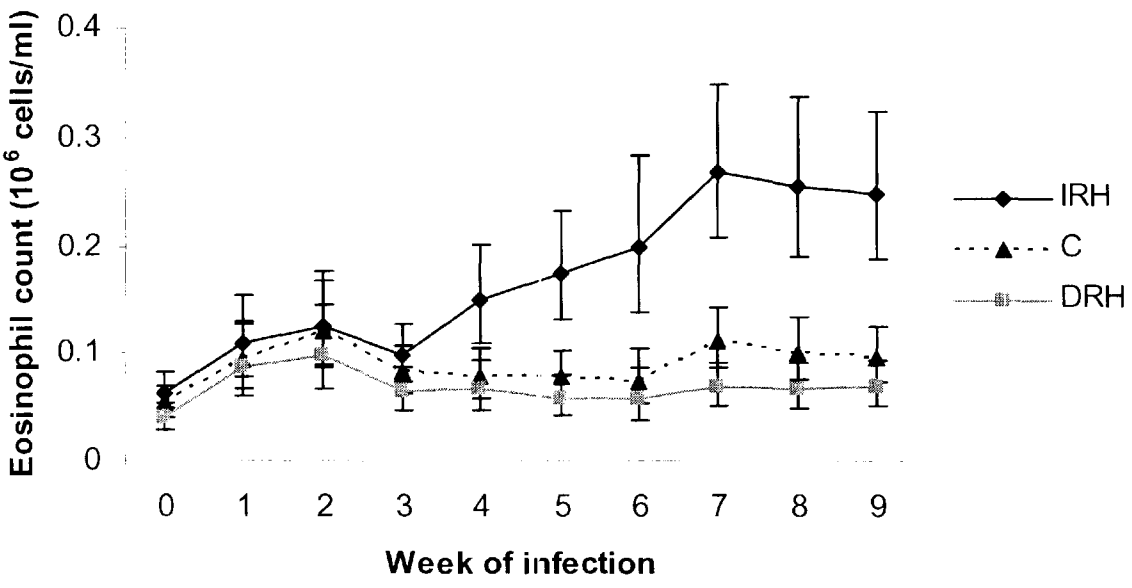


Figure 3-9 Eosinophil count (back-transformed ls mean ± 95% *ci.*) in blood samples taken from weaner rams selected for either increased resistance to *Haemonchus* (IRH), decreased resistance to *Haemonchus* (DRH) or random-bred control (C).

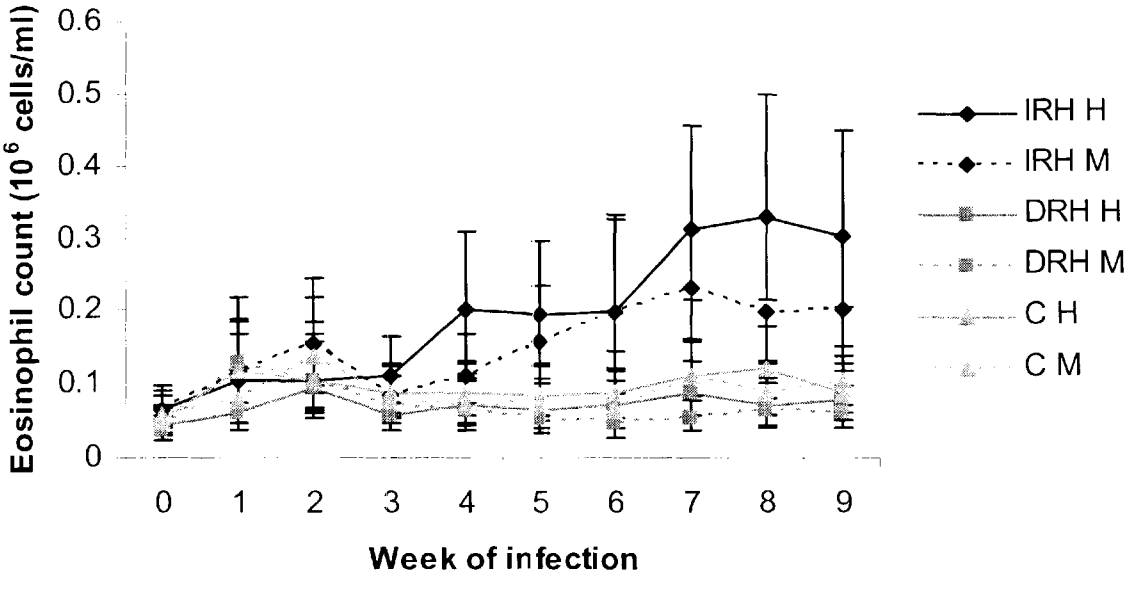


Figure 3-10 Eosinophil count (back-transformed ls mean ± 95% *ci.*) in blood samples taken from weaner rams selected for either increased resistance to *Haemonchus* (IRH), decreased resistance to *Haemonchus* (DRH) or random-bred control (C) and fed either a high (H) or moderate quality diet (M).

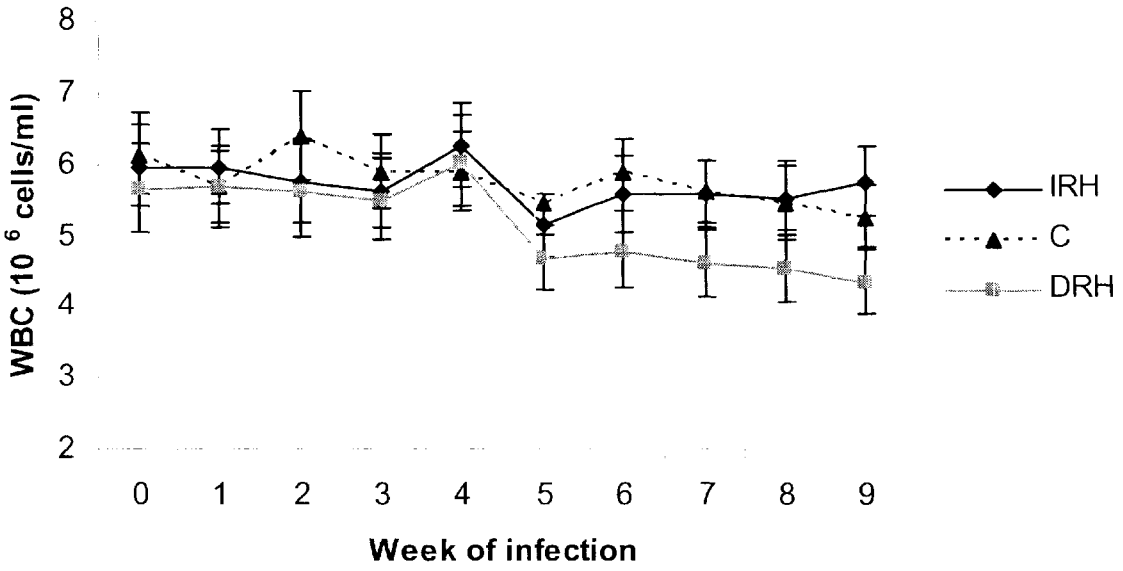


Figure 3-11 White blood cell (WBC) coun: (back-transformed ls mean ± 95% *ci.*) in blood samples taken from weaner rams selected for either increased resistance to *Haemonchus* (IRH), decreased resistance to *Haemonchus* (DRH) or random-bred control (C).

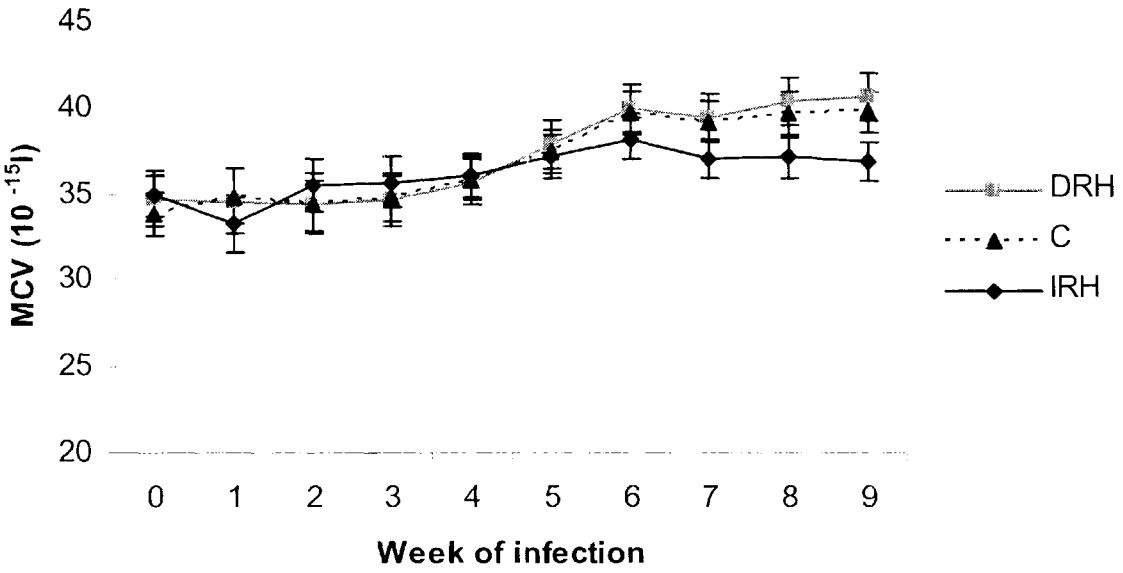


Figure 3-12 Mean corpuscular volume (back-transformed ls mean ± 95% *ci.*) in blood samples taken from weaner rams selected for either increased resistance to *Haemonchus* (IRH), decreased resistance to *Haemonchus* (DRH) or random-bred control (C).

3.3.5 Plasma protein

Total plasma protein was higher (P = 0.001) in animals fed the H diet, compared to the M diet, during the feed intake period (week 0-9) (Figure 3-13). Albumin (P = 0.0004) and globulin (P = 0.002) concentrations were also higher in animals fed the H diet. IRH animals had higher concentrations (P = 0.007) of plasma albumin from week 4 of infection, compared to the DRH and C lines (Figure 3-14). However total protein and globulin concentrations did not differ among selection lines. The interaction between the effects of selection line and diet was not significant for total protein, albumin or globulin concentrations, during the feed intake period.

After the INF diet selection period (week 13), the IRH animals had significantly higher total protein (P = 0.001), albumin (P <0.0001) and globulin (P = 0.011) concentrations than DRH animals, while there were no differences after the NIL diet selection period (Table 3-6).

Table 3-6 Total plasma protein, albumin and globulin concentration (ls mean ± s.e.) of weaner rams selected for increased resistance to *Haemonchus* (IRH), decreased resistance to *Haemonchus* (DRH) and random-bred controls (C), measured following 14 d diet selection period when animals were either maintained worm free (NIL) or infected with *H. contortus* (INF).

	Total protein (g/l)		**Albumin (g/l)**		**Globulin (g/l)**	
Group	ls mean	s.e.	ls mean	s.e.	ls mean	s.e.
NIL period						
IRH	69.8	1.93	18.6	0.38	51.2	1.64
DRH	71.2	2.17	18.5	0.43	52.7	1.85
C	69.2	1.88	19.0	0.37	50.2	1.60
INF period						
IRH	63.0^{a}	1.34	19.1^{x}	0.27	44.0^{a}	1.27
DRH	54.0^{b}	1.53	16.5^{y}	0.31	37.5^{b}	1.45
C	59.0^{c}	1.26	17.3^{y}	0.26	41.6^{a}	1.20

Least square means within period, with different suffix differ significantly ($P<0.01$). Values with no suffix do not differ significantly (P>0.05).

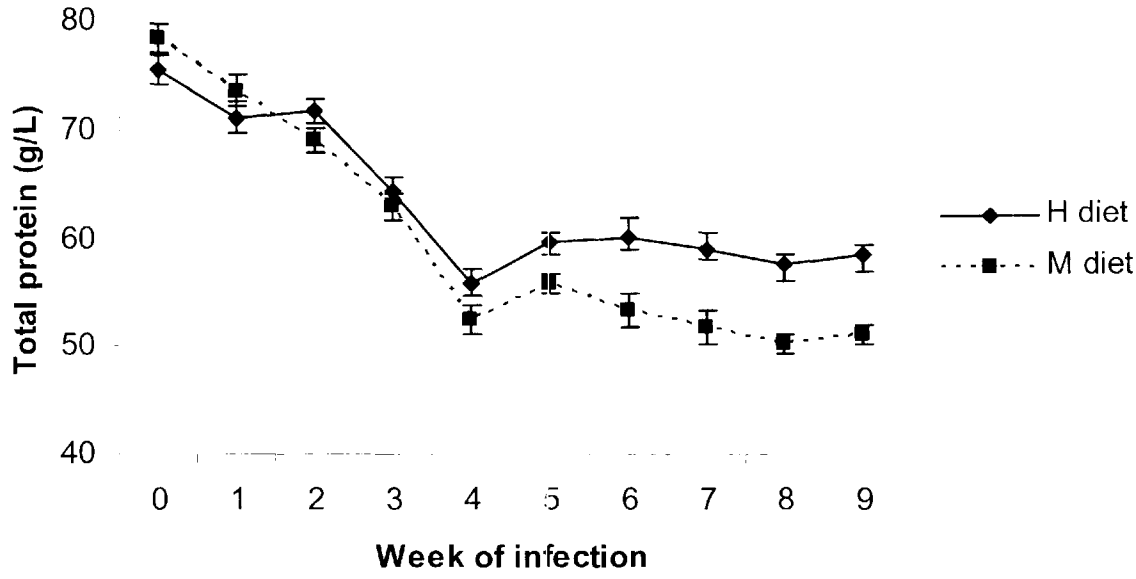


Figure 3-13 Total plasma protein concentrations (ls mean ± s.e.) of weaner rams fed either a high (H) or moderate quality diet (M) and given a trickle infection with *H. contortus*.

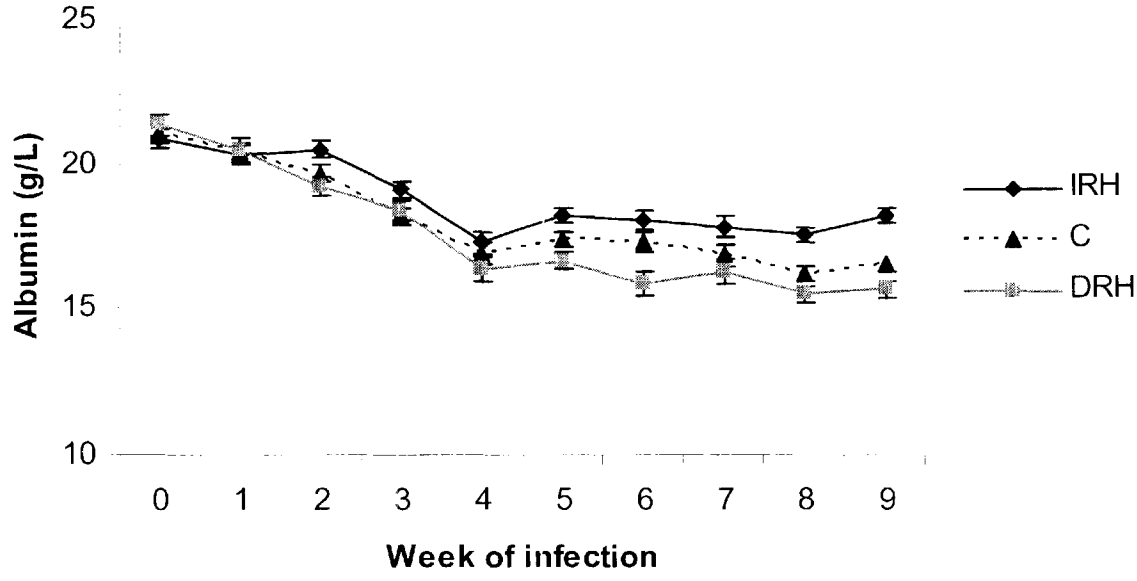


Figure 3-14 Albumin concentrations (ls mean ± s.e.) of weaner rams selected for either increased resistance to *Haemonchus* (IRH), decreased resistance to *Haemonchus* (DRH) or random-bred control (C).

3.3.6 Antibody responses

IgG_1 antibodies in plasma were higher (P = 0.002) in IRH animals from week 2 of infection compared to DRH and C lines (Figure 3-15), but did not differ between diets. IgG_2 antibody level followed a similar pattern to IgG_1, peaking at week 2 of infection, but was unaffected by selection line and diet IgE antibody levels did not differ among selection lines or between diets, but tended (P = 0.070) to be higher in C animals compared to IRH and DRH lines over the course of infection, due to the larger response at week 2 of infection (Figure 3-16). IgA antibody levels were higher (P = 0.035) in animals fed the H diet, but did not differ among selection lines (Figure 3-17). The interaction between the effects of selection line and diet was not significant for IgG_1, IgG_2 and IgE. IRH and C animals fed the H diet had higher (P = 0.047) IgA titres than their M diet counterparts.

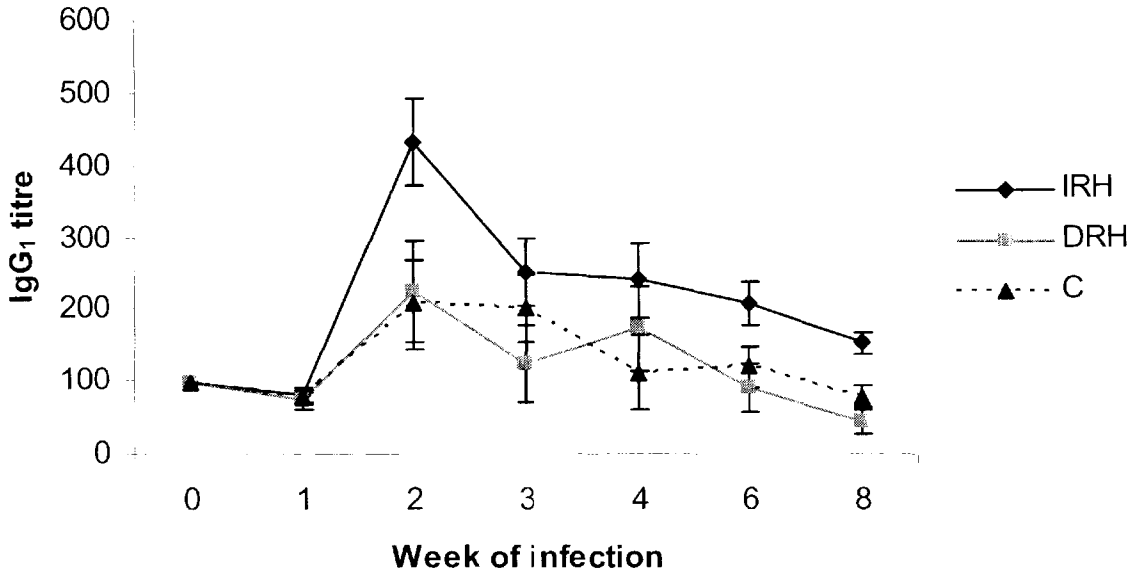


Figure 3-15 IgG_1 antibody response (ls mean ± se) in blood samples taken from weaner rams selected for either increased resistance to *Haemonchus* (IRH), decreased resistance to *Haemonchus* (DRH) or random-bred control (C) and trickle infected with *H. contortus*.

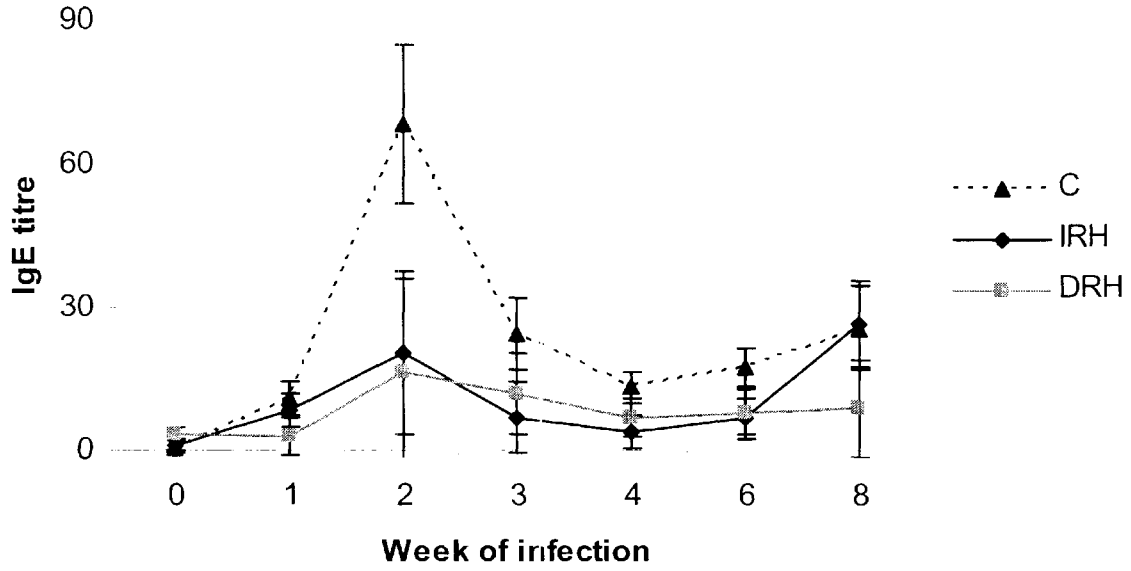


Figure 3-16 IgE antibody response (ls mean ± se) in blood samples taken from weaner rams selected for either increased resistance to *Haemonchus* (IRH), decreased resistance to *Haemonchus* (DRH) or random-bred control (C) and trickle infected with *H. contortus*.

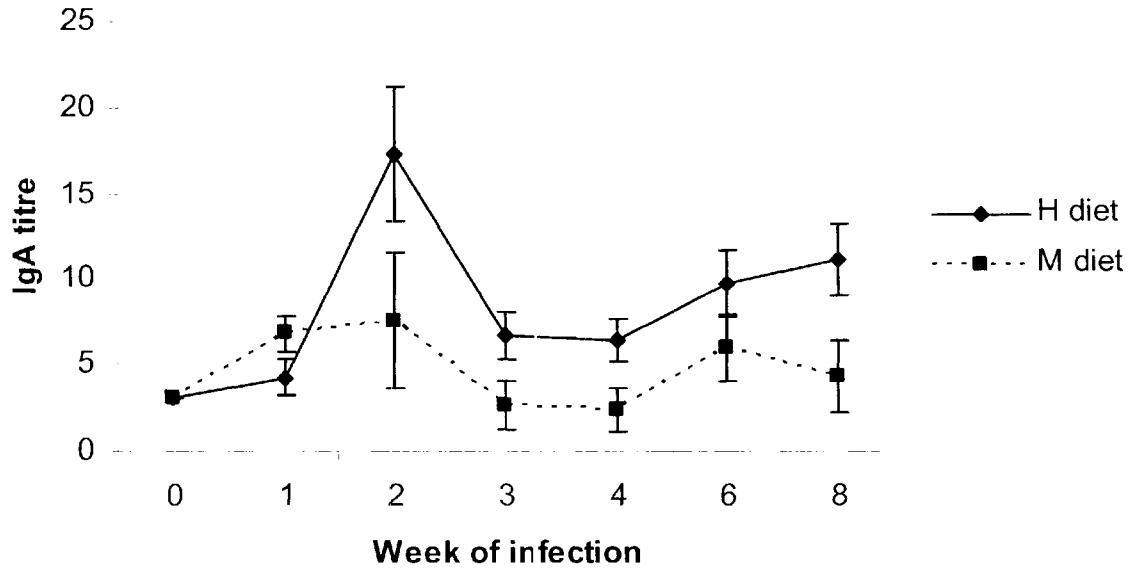


Figure 3-17 IgA antibody response (ls mean ± s.e.) in blood samples taken from weaner rams fed either a high (H) or moderate quality diet (M) and trickle infected with *H. contortus*.

3.3.7 Bodyweight

Animals on both the H and M2 diets gained bodyweight throughout the infection period. There was a significant difference (P <0.0001) in bodyweight due to diet, as weaner rams fed the H diet gained (ls mean ± s.e.) 11.8 ± 0.41 kg, while animals fed the M2 diet gained 5.9 ± 0.41 kg (Figure 3-18). Bodyweight gain did not differ (P = 0.488) among selection lines throughout the infection period (Table 3-7). There was also no significant interaction between the effects of selection line and diet.

Selection lines did not differ (P = 0.639) in bodyweight gain during the NIL diet selection period (average bodyweight change ± s.d. of 4.6 ± 0.98 kg); however the IRH animals gained more (P = 0.037) than the DRH and C lines, when contrasted during the INF diet selection. Weight change for IRH, DRH and C lines was 1.4^{a} ± 0.38, 0.42^{b} ± 0.43 and 0.29^{b} ± 0.37 kg respectively, over the 14 d period (Least square means with different suffix differ significantly $P<0.05$).

Table 3-7 Bodyweight gain (kg) of weaner rams, selected for increased resistance to *Haemonchus* (IRH), decreased resistance to *Haemonchus* (DRH) and random-bred control (C) and fed either a high (H) or moderate (M) quality diet.

	IRH		**DRH**		**C**	
Diet	ls mean	s.e.	ls mean	s.e.	ls mean	s.e.
H	11.9	0.71	11.5	0.75	12.0	0.67
M	5.7	0.67	7.0	0.80	4.8	0.67

3.3.8 Feed conversion ratio

Feed conversion ratio (kg feed/kg bodyweight gain) differed significantly between diets (P <0.0001), with values of 8.8 ± 0.58 kg feed per kg bodyweight gain for the H diet, compared to 15.6 ± 0.59 kg feed /kg gain for M diet. Feed conversion ratio did not differ among selection lines (P = 0.118), however there was a strong suggestion (P = 0.063) of an interaction between the effects of selection line and diet, such that DRH animals fed the M diet required less feed than IRH and C animals, to gain equivalent bodyweight (Figure 3-19).

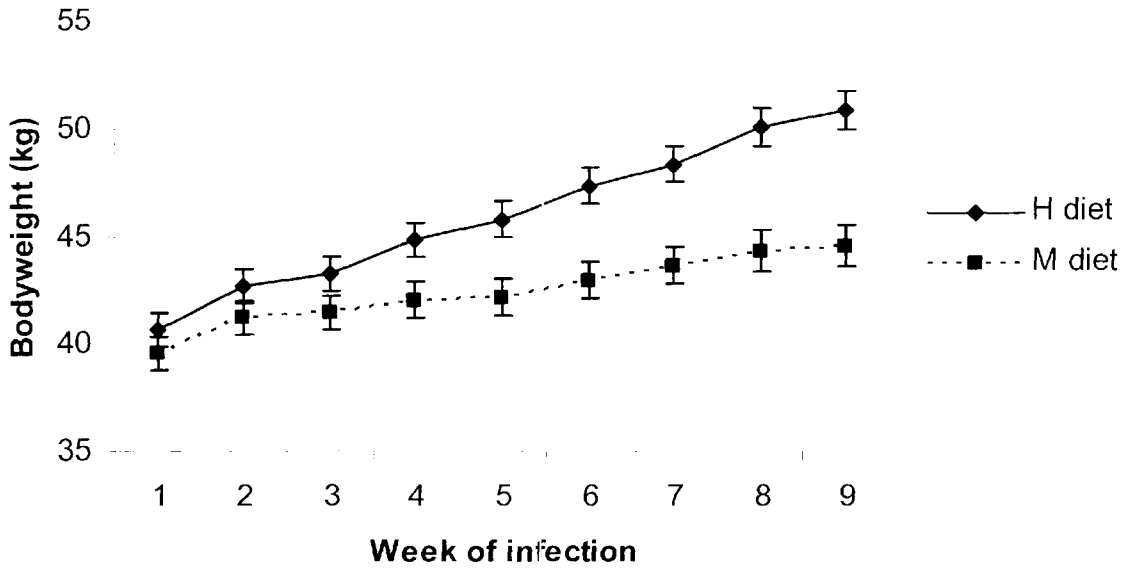


Figure 3-18 Bodyweight (ls mean ± s.e.) of weaner rams either fed a high (H) or moderate (M) quality diet and trickle infected with *H. contortus*.

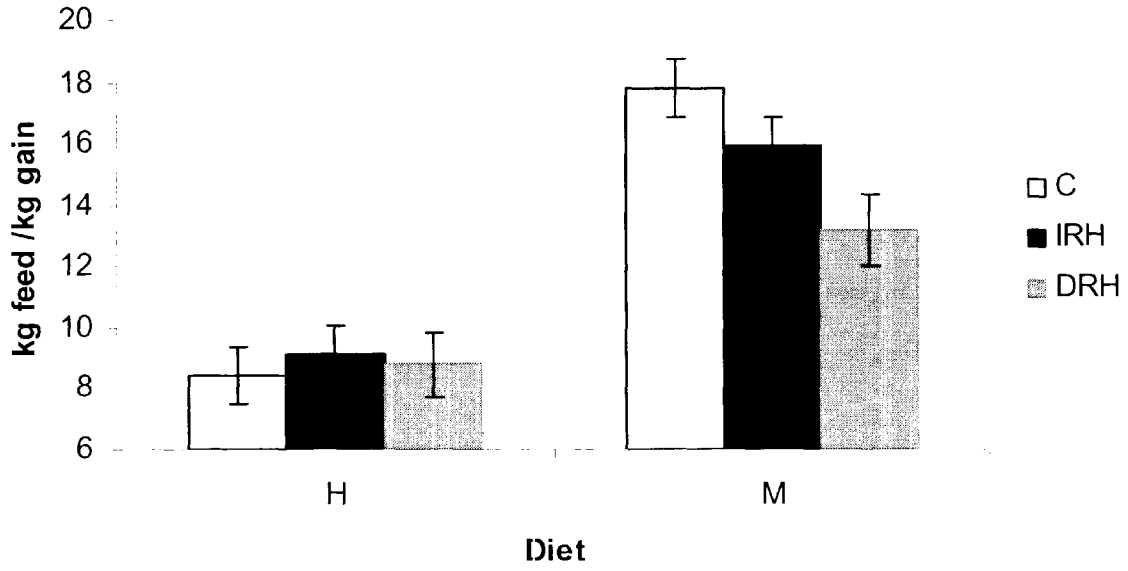


Figure 3-19 Feed conversion ratio (ls mean ± s.e.) of weaner rams, selected for increased resistance to *Haemonchus* (IRH), decreased resistance to *Haemonchus* (DRH) and random-bred control (C) and fed either a high (H) or moderate (M) quality diet.

3.3.9 Wool growth rate

The pre-experimental measurement of clean wool growth rate (g/d ± s.e.), conducted under grazing conditions, for the IRH, DRH and C lines was 6.4 ± 0.33, 6.2 ± 0.37 and 7.0 ± 0.32 g/d respectively and for fibre diameter 16.8 ± 0.36, 17.2 ± 0.41 and 16.9 ± 0.35 µm respectively. Greasy and clean wool growth rates, adjusted for pre-experimental differences, did not differ among selection lines during the NIL or INF measurements. H diet increased significantly greasy (P = 0.007) and clean (P = 0.024) wool growth rates in both NIL and INF (P <0.0001) periods (Table 8). Fibre diameter was significantly lower in animals fed the M diets during the NIL (P = 0.002) and INF (P<0.0001) periods, but was unaffected by selection line (Table 3-8).

Table 3-8 Greasy and clean wool growth rates and fibre diameter (ls mean ± s.e.) of weaner rams, selected for increased resistance to *Haemonchus* (IRH), decreased resistance to *Haemonchus* (DRH) and random-bred control (C) and fed either a high (H) or moderate (M) quality diet, measured when animals were maintained worm free (NIL) or infected with *H. contortus* (INF).

	Greasy wool growth (g/d)		**Clean wool growth (g/d)**		**Fibre diameter (µm)**	
Group	ls mean	s.e.	ls mean	s.e.	ls mean	s.e.
NIL period						
IRH	13.2	0.47	7.9	0.29	20.8	0.25
DRH	12.6	0.53	7.8	0.33	20.7	0.29
C	12.5	0.46	7.6	0.29	20.7	0.25
H diet	13.6^{x}	0.39	8.2^{x}	0.25	21.2^{x}	0.21
M diet	12.0^{y}	0.40	7.3^{y}	0.25	20.2^{y}	0.21
INF period						
IRH	12.9	0.50	7.9	0.32	20.4	0.26
DRH	12.0	0.57	7.6	0.37	20.7	0.29
C	12.3	0.50	7.8	0.32	20.5	0.25
H diet	14.1^{x}	0.42	8.6^{x}	0.27	21.6^{x}	0.22
M diet	10.7^{y}	0.43	6.9^{y}	0.27	19.4^{y}	0.22

Least square means with different subscripts differ significantly ($P<0.005$). Values with no suffix do not differ significantly (P>0.05).

3.4 Discussion

3.4.1 Nutrient intake and production

IRH animals had significantly higher daily feed intake during the NIL period, compared to the C line. However, when artificially challenged with *H. contortus* the effect of selection line on intake was not apparent. Experimentation conducted by Liu *et al.* (2005) concurrently using the Rylington Merino flock (Karlsson *et al.* 1991) found similar results. There were no differences in voluntary feed intake between resistant and control lines during both NIL and INF periods (Liu *et al.* 2005b). Selection lines had similar bodyweight gains and wool growth rates during the infection period on both H and M2 diets, despite a significant difference in WEC. This result validates previous work with pregnant and lactating ewes (Kahn *et al.* 2003) and weaners (Eady and Smith 2001) from the same *Haemonchus* selection lines.

This study has shown few differences in feed intake or diet selection between selection lines and hence there would have been a similar nutrient supply. From that basis the response in terms of growth and wool production did not differ between lines, yet WEC was vastly different. The IRH and DRH lines had similar bodyweight gains and wool growth, while differing five-fold in WEC. Similar voluntary feed intakes, body weight change, body composition and fleece weights were also measured between resistant and control animals from the Rylington flock, whilst control animals carried a four-fold greater worm burden (Liu *et al.* 2005). These results suggest that genetic resistance to parasites does not cause any unfavourable effects on productive performance. But one would expect a reduced worm burden to produce higher weight gains and wool growth. Moreover, susceptible animals did not demonstrate the expected reduction in feed intake and bodyweight gain following infection (Abbott *et al.* 1986b), indicating a strong resilience to *Haemonchus* challenge. Not only was productive performance of DRH animals similar to IRH animals, but feed conversion ratios showed that DRH animals gained more bodyweight on lower feed requirements when fed the M2 diet, compared with IRH and C lines (75.8, 62.9 and 56.2 g bodyweight gain/kg DM respectively). Recent observations may assist in explaining these findings; Greer *et al.* (2005) reported that animals infected with the abomasal parasite, *T. circumcincta* and immuno-suppressed with corticosteroid methylprednisolone acetate, had greater feed conversion

efficiency (FCE) than infected animals. The authors suggest that the infected animal's reduction in FCE may be a consequence of increased cell proliferation during the development of the immune response (Greer *et al.* 2005). It is assumed that IRH animals have a highly developed immune response, which is supported by greater circulating eosinophil counts, plasma globulin concentrations and IgG_1 titres. Therefore a strong host immune response associated with nematode parasitism may reduce feed conversion efficiency. Poppi *et al.* (1990) described the nutrient demand created by parasitism as similar to that of lactation and foetal growth. More importantly the development of resistance may impose as big a demand on nutrients as the consequences of parasitism, lactation or pregnancy.

DRH animals demonstrated a strong resilience to *H. contortus* infection, with equal bodyweight gain and wool growth and greater FCE than IRH animals with smaller worm burdens. The DRH line also tended to have a greater concentration of plasma IGF-1 than C animals suggesting the possibility of greater whole body protein metabolism (Lobley 1992). An increase in IGF-1 concentration in skeletal muscle results in elevated protein synthesis, rather than protein degradation (Lobley 1998). This effect was observed by Oddy *et al.* (1995) who demonstrated that sheep selected for a greater weaning weight (W+) had higher plasma IGF-1 concentrations and FCE than unselected counterparts (W-). Reduced rates of protein degradation in muscle has also been reported in sheep selected for susceptibility to nematode parasites (Adams & Liu 2003). These observations lend support to the notion that greater FCE in DRH animals may have been associated with an increase in protein synthesis and reduction of protein degradation in skeletal muscle, which was hormonally regulated by IGF-1.

The beginning of the infection regime coincided with the change from the M1 to the M2 diet, for reasons discussed earlier. Feed intake for all selection lines declined uniformly with the simultaneous introduction of M2 and the beginning of infection. The decrease in feed intake on the M2 diet occurred within one week of its introduction, suggesting that the change from M1 to M2 diets rather than the beginning of the *H. contortus* infection was the underpinning factor for the intake decline. A reduction in feed intake as a consequence of infection would generally occur beyond week 3 of infection, as demonstrated by Abbott *et al.* (1986b).

Diet significantly influenced the daily feed intake of each selection line during the infection period with higher intakes recorded when animals were fed the H diet. The effect of diet on feed intake of animals infected with *H. contortus* has previously been demonstrated by Datta *et al.* (1998), who found that voluntary feed intake of infected lambs increased with increasing dietary crude protein. Abbott *et al.* (1986b) also showed that animals infected with *H. contortus* and given a high protein diet had higher daily feed intakes than animals fed a low protein diet.

During the 14 d INF diet selection period (week 11 and 12) IRH animals consumed a higher daily feed intake and were able to maintain higher weight gain, compared to DRH and C lines, but did not select a higher proportion of diet H. Daily feed intake and weight gain did not differ among selection lines early in infection (week 1-9), but decreased in DRH and C animals later in infection. This coincided with an increase in WEC of 21 and 23% in DRH and C animals respectively and a decrease of 8% in WEC of IRH animals, from week 9 to week 12 of infection. The establishment of worms may have been higher in DRH and C animals, resulting in a larger worm burden and thus reduced feed intake and bodyweight gain. Alternatively, the fecundity of female worms may have been higher in DRH and C lines, causing the greater WEC. Worm counts and calculation of fecundity are determined in later studies.

The design of the experiment did not allow statistical comparison of NIL and INF diet selection periods. However, selection of diet H during the INF period (week 11 and 12) was 97%, compared to 75% in NIL. This agrees with the findings of Kyriazakis *et al.* (1996) who found the proportion of H diet selected remained higher from weeks 10-18 of a trickle infection with *T. colubriformis*, compared to uninfected controls.

The H diet supported larger weight gains (187 g/d) than did the M2 diet (93 g/d) and a 1.7 g/d increase in clean wool growth rate, during infection. The importance of diet on resilience to infection has previously been shown in European breeds of sheep also infected with *H. contortus*, where animals fed high protein diets gained more weight than those fed low protein diets (Abbott *et al.* 1985a; Wallace *et al.* 1995, 1996).

3.4.2 Parasitology

WEC and daily worm egg output were lower in IRH animals compared to DRH and C lines. Woolaston *et al.* (1990) previously observed the distinct selection line effect on WEC. The non-significant effect of diet quality on WEC, at least in the IRH line, supports earlier work by Abbott *et al.* (1985a,b) and Wallace *et al.* (1996) who found that supplementation with protein did not improve the development of immunity in breeds of sheep (e.g. Scottish Blackface) which are relatively resistant to nematode infection. However, Abbott *et al.* (1985a;b) and Wallace *et al.* (1995) also found that breeds susceptible to nematode infection benefited from dietary protein supplementation. In this experiment animals from the DRH and C lines had 31 and 27% lower WEC respectively on the H diet, but differences were not significant. The benefit of dietary protein may not have been displayed in DRH and C lines, due to the quality of the M2 diet being sufficiently high to allow expression of resistance.

3.4.3 Haematology

RBC numbers declined in IRH animals by 1.69 x 10^9 cells/ml from day 0 to week 5 of infection and then began to rise. In contrasts, RBC numbers decreased in DRH and C lines with the continued rise in WEC. DRH animals had a 3.18 x 10^9 cells/ml reduction from day 0 to week 8 of infection. This was associated with the apparently greater abomasal haemorrhage created by the blood-sucking *H. contortus* adults. The higher level of blood loss associated with infection was more severe in animals fed the M2 diet, which supports the findings of Abbott *et al.* (1985b). The fact that mean corpuscular volume (MCV) was higher in DRH and C animals indicates that these animals were capable of RBC regeneration to compensate for the higher RBC loss.

Plasma leakage resulting from the damage caused by the blood-sucking activities of the *Haemonchus* parasite, contributes to an even greater loss of plasma into the gut, than ingestion of blood by the parasite (Steel 1978). The DRH and C lines had lower plasma albumin concentrations than IRH animals, suggesting greater damage in the abomasum due to larger parasite burdens. Abbott *et al.* (1986b) showed that albumin concentration was 66% lower in INF animals compared to their pre-infection level and significantly lower on low protein diets compared to H diet.

The blood eosinophil count was higher in IRH animals compared to DRH and C lines and eosinophil count increased in response to the H diet but only within the IRH line. Datta *et al.* (1998) demonstrated weaner wethers infected with *H. contortus* and fed increasing levels of dietary crude protein tended to have increased circulating eosinophil count. This result agrees with studies on the immunological response of high and low responders in the *Trichostrongylus* selection line (Windon 1989, Dawkins *et al.* 1989). These authors suggest that eosinophil count is a measure of an immune response to infection.

The WBC measurement is an indication of cells present in the circulating blood pool. Detection of lower circulating WBC in DRH animals from week 4 of infection may indicate the mobilisation of circulating cells from the blood to the inflamed tissue in the GI tract (Balic *et al.* 2000). The maintenance of circulating WBC throughout infection in IRH animals, suggests that expulsion of larvae before development into adults and damage to gut mucosa resulted in the absence of a local cellular inflammatory response.

3.4.4 Antibodies

IgG_1 plasma antibodies were higher in IRH animals compared to DRH and C lines. Elevated plasma IgG concentrations have been associated with resistance to *H. contortus* in the Golden Ram flock (Gill 1991) and elevated IgG_1 titres were reported in resistant animals from the Wallaceville Romney breeding line (Bisset *et al.* 1996), when infected with mainly *T. colubriformis*. IgA antibody titres were higher in animals fed the H diet. IgA has been found to be a major mechanism suppressing worm growth and controlling fecundity of *H. contortus* (Strain & Stear 2001). The magnitude of this mechanism was influenced by the crude protein content of the diet (Strain & Stear 2001). Interestingly, the IRH and C lines had significantly higher IgA titres on the H diet, while the DRH line had no IgA response to diet. The concentration of plasma antibody titres is a measure of a local antibody response that has peaked and flowed into the blood circulation. Gill *et al.* (1992) measured the local antibody response, by taking serial biopsies of the wall of the abomasum to determine titres of immunoglobulin in abomasal cells. IgA cells were the most frequent immunoglobulin isotype found in the abomasal tissue and increased 6-fold following *H. contortus* infection (Gill *et al.* 1992). Therefore, the increased plasma IgA titres in IRH animals fed the H diet may indicate

an increased local immune response in the abomasum. The increase in plasma IgG_1 and IgA concentrations demonstrates a greater immune response in the IRH line and may play a role in the ability to prevent establishment and/or development of a nematode infection. An increase in immunoglobulin response in IRH animals and a reduction in establishment and development of infection may have eliminated the need for a local inflammatory response, indicated by an absence of local WBC at the time of measurement.

This experiment implies that with a similar nutrient supply and response in growth and wool production, but significantly different WEC, the use of dietary nutrients, i.e. metabolisable protein and energy may have differed among selection lines. The IRH line had higher concentrations of circulating eosinophil, plasma globulin and IgG_1 titres. The increase in the immune responsiveness of resistant animals would require a greater partitioning of nutrients toward the gut immune response. In contrast, DRH animals had greater endogenous protein loss, indicated by reduced albumin concentrations, red blood cell loss and increased erythropoiesis. The increase in protein synthesis required to repair the gastrointestinal tract will reduce protein synthesis in other tissues. There is an indication that the metabolism and utilisation of nutrients differs between resistant and susceptible genotypes. The succeeding chapters will investigate whether divergent selection lines partition nutrients differently between skeletal muscle and skin, organs and the gut immune response.

3.5 Conclusion

In the absence of infection IRH animals had a significantly higher daily feed intake compared to C. However, there were no differences in daily feed intake, diet selection or production measurements between selection lines during infection, despite a significant difference in WEC.

The mechanisms that allow resistant animals to effectively prevent establishment and/or development of nematode infection and allow susceptible animals to have productivity equal to or higher than those carrying smaller worm burdens, did not appear to involve a change in nutrient intake or selection of diet. Investigation into whether divergent selection for resistance to nematode infection has produced correlated changes in

nutrient digestion and absorption, and protein metabolism and partitioning may help to identify the mechanisms that influence resistance and resilience.

CHAPTER 4

RUMEN FUNCTION AND METABOLISM OF SHEEP SELECTED FOR GENETIC DIFFERENCE IN RESISTANCE TO NEMATODE INFECTION

4.1 Introduction

Supply of metabolisable energy (ME) and metabolisable protein (MP) to ruminants is dependent on normal rumen function. However, studies have shown GI parasites affect rumen function and metabolism (van Houtert & Sykes 1996). Steel (1972) found infection with *T. colubriformis* reduced acetate production by 30% compared to uninfected pair-fed controls. Microbial protein synthesis and production of amino acids for tissue protein synthesis may also be reduced (Steel 1972). A comprehensive study by Rowe *et al.* (1988) on the effects of haemonchosis in Merino weaner animals showed a decrease in acetate: propionate ratio, increase in rumen fluid outflow rate, decrease in apparent digestion of organic matter across whole tract and an increase in N lost into the GI tract. Impaired feed digestion, energy and nitrogen utilisation in the parasitised animal are apparent factors contributing to reduced performance (Symons & Steel 1978, Parkins & Holmes 1989).

Given the affect GI parasites have on rumen function and metabolism it was hypothesised that genetic differences in host resistance may have altered rumen fermentation and hence nutrient supply. Therefore the aim of this experiment was to investigate if divergent selection for resistance to *H. contortus* has produced correlated changes in rumen function and absorption in the absence and presence of *H. contortus* infection. This information will allow a better understanding of the physiological changes associated with different levels of resistance to *H. contortus*.

4.2 Materials and methods

4.2.1 Experimental design

The 8-week experiment was designed as a 3 x 2 factorial with 3 selection lines, selected for genetic difference in resistance to nematode infection and 2 infection levels, namely a trickle infection of McMaster strain *H. contortus* (INF) or maintained worm-free (NIL). Animals were given 2 weeks to adjust to animal house conditions and feeding before undergoing surgery to fit a rumen cannula (Godwin & Chaffey 1988). The animals were allowed 4 weeks to recover from the surgical procedure. The experimental diet was fed for the final 2 weeks of recovery prior to the experimental period. Animals were stratified within selection line on the basis of bodyweight (measured following recovery from surgery and adjustment to experimental feed) and then randomly allocated to infection treatment and position of pen within the animal house. Animals were housed in individual pens until 5 weeks post infection (*p.i*) then moved to metabolic crates for a further 24 d. After 14 d, when animals were assumed to have adjusted to metabolic crates, rumen measurements and daily faecal and urine outputs were taken over a 10 day collection period. Subsequent to sampling, animals were euthanased to determine abomasal worm counts.

4.2.2 Animals and housing

The study used 29 Merino weaner wethers selected randomly from the CSIRO increased resistance to *Haemonchus* (IRH) (n = 10), decreased resistance to *Haemonchus* (DRH) (n = 9) and unselected control (C) (n = 10) selection lines (each line was represented by at least 3 of the 5 sire groups) (Woolaston *et al.* 1990). They were approximately 8 months of age at the beginning of the experiment with a mean ± s.d. bodyweight of 22.8 ± 2.93 kg. Animals were housed in individual pens and later in metabolic crates at the University of New England animal house. Upon entering the animal house each animal was drenched with Scanda® (8mg/kg levamisole hydrochloride and 4.5 mg/kg oxfendazole, Schering-Plough Animal Health Ltd) and Cydectin® (0.2 mg/kg moxidectin, Fort Dodge), to remove existing worm burdens. A worm egg count (WEC) was taken 5 d after drenching confirming all animals had zero counts.

4.2.3 Surgery

Animals were pre-medicated with an intramuscular injection of 0.15 ml of Rompun® (20 mg/ml xylazine, Bayer). Once the animals were sedated they were moved to an adjacent surgery where an area of wool was closely clipped to the skin with Oster clippers No. 40 on the left-hand side of the animal behind the last rib. Animals were then given an inverted "L" block local anaesthetic with 10 ml of Lignocaine 20® (20 mg/ml lignocaine hydrochloride, Troy laboratories). Skin was prepared for surgery using Hibitane surgical scrub and Povidone Iodine. Before surgery commenced, animals were given pre-operative intramuscular injections, 1 ml of Temgesic® (324 µg buprenorphine hydrochloride, Reckitt Benckiser), 1 ml of Tolfedine® CS Injection (40 mg/ml tolfenamic acid, Vētoquinol) and 2 ml of Bivatop®200 (200 mg/ml oxytetracycline, Boehringer Ingelheim). Post-operative treatment with Temgesic, Tolfedine and Bivatop continued until animals returned to normal state.

4.2.4 Feed

The formulation and analysis of the experimental diet is given in Table 4-1. The diet was formulated to be balanced for major and trace minerals and pelleted to reduce the ability of animals to select individual ingredients in the diet. The digestibility and crude protein content was similar to that for pastures, outside the growing season, in many regions of Australia (Milford 1959). Animals were fed on a restricted basis (30 g fresh feed /kg bodyweight) to maintain bodyweight throughout the experimental period. Fresh food was offered once daily for the first 5 weeks, then twice daily (9am and 3pm) during the final 3-week sampling period when housed in metabolic crates.

4.2.5 Infection

Animals had previously been artificially infected with 10,000 L_3 *H. contortus* at approximately 6 months of age and subsequently exposed to natural field infection. This infection history was important to ensure animals had prior opportunity to acquire immunity to nematode infection and reflect genetic merit. Half of the animals from each selection line were infected with Mc Master strain *H. contortus* L_3. An initial infective dose of 150 L_3/kg bodyweight was orally administered at the beginning of the infection period, followed by a trickle infection of 250 L_3 three times per week on Monday,

Wednesday and Friday (average total dose ± s.d. over the infection period of 8679 ± 520 L_3/sheep).

Table 4-1 Ingredients[1] and chemical analysis of experimental diet.

Ingredients (% per kg fresh matter)	Formulated diet
Cottonseed hulls	75.0
Barley	8.0
Oaten straw chaff	8.5
Molasses	7.0
Cottonseed meal	1.5
Dry matter	91.0
Composition (per kg DM)	
Organic matter (g)	970.0
Digestibility[A] (g)	431.0
Ether extract (g)	11.0
Crude protein (g)	68.0
Calculated MP[B] (g)	28.6
Calculated ME[C] (MJ)	5.9
Sulphur (g)	1.4
Phosphorus (g)	1.8
Iron (mg)	204.7
Copper (mg)	9.8

[1]Major minerals and trace elements were added to reach daily requirements. Minerals (per kg dry matter) were 2.5g Ca, 1.6g Mg, 11.3g K, 788 mg Na, 41 mg Zn, 50 mg Mn and 0.6 mg Mo. Feed was analysed for mineral concentrations using the Vista MPX radially viewed, simultaneous Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES).

[A] Based on pepsin cellulase wet chemistry.

[B] Calculated from equations of Freer *et al.* (1997).

[C] Calculated from algorithm supplied by FeedTest, Victorian Department of Primary Industries, Hamilton, VIC.

4.2.6 Animal measurements

Animals were weighed in the morning prior to feeding, commencing at the start of the experiment and weekly thereafter. A single worm egg count per animal was measured at day 0 and 21 after the initial dose of infective larvae and weekly thereafter. A modified McMaster technique was used to count worm eggs and potassium iodide was used rather than sodium chloride solution. The higher specific gravity (1.4) of potassium iodide was required to enhance flotation and therefore detection of eggs from animals consuming the pelleted diet rich in cottonseed hulls. Animals were bled by jugular venepuncture into 5 ml K_3-EDTA Vacutainer® tubes at day 0, weeks 1, 6 and 8. Haematology parameters; red blood cell (RBC) count, haematocrit, white blood cell

(WBC) count and eosinophil concentration were measured on whole blood using a Cell-Dyn 3500R haematology analyser, (Abbott Diagnostics Division).

4.2.7 Apparent whole tract digestibility and nitrogen balance

Daily urine and faecal outputs were collected to determine the apparent whole tract dry matter, organic matter, nitrogen digestibility, N balance and urinary purine excretion. Daily urine output was collected into buckets containing 10 ml of 50% v/v sulphuric acid, to maintain pH < 3. A daily subsample (40%) was taken, pooled over the 10 d of collection and stored at –20 °C for later analysis. Daily faecal output was collected in plastic bags, weighed and well mixed, then subsampled (10%) into a pooled sample and stored at –20 °C for later analysis.

4.2.8 Determination of dry matter, organic matter and total N

Dry matter was determined by drying experimental feed and faecal subsamples to a constant weight in a forced draught oven at 105°C for 24 h. Ash content was determined by combusting dried samples in a muffle furnace (Carbolite CWF 1200, Carbolite, England) at 550°C for 3 h. Total nitrogen was measured using an automated organic nitrogen analyser (Leco FP2000, Leco Corporation, USA). Feed and faecal subsamples for nitrogen analysis, were oven dried at 80°C for 48 h and then ground through a 1 mm sieve.

4.2.9 Purine derivatives in urine

The concentration of purine derivatives; xanthine, hypoxanthine, uric acid and allantoin were analysed by Mr M. Nielsen, University of Queensland, Brisbane QLD, using a high performance liquid chromatograph (HPLC) according to the method by Balcells *et al.* (1992), with modifications described below. The acidified frozen urine samples were thawed and filtered through 0.22 µm Millex-GV PVDF Durapore syringe driven filter unit, followed by a C_{18} 300 mg pack size Maxi-clean cartridge (Alltech). Purine derivatives were separated using Bondiclone C_{18} reverse phase column (3.9 x 300 mm) (Phenomenex). The chromatogram analysis and calculation of results used Shimadzu

VP5.3 software. The sample volume was 20 µl with absorbance measured at 205 nm and flow rate through the column of 0.8 ml/min.

The estimation of microbial nitrogen supply could not be calculated using the relationship derived by Chen *et al.* (1990), as daily purine derivative excretion rates were lower than those included in the predictive relationship. Data analysis and results were based on purine derivatives, assuming they represent the same ratio of microbial nitrogen supply.

4.2.10 Rumen measurements

4.2.10.1 in sacco dry matter digestibility

Dry matter (DM) digestibility was measured using the nylon bag incubation method described by Ørskov *et al.* (1980). The nylon bags were 5 x 10 cm with a pore size of 44 µm (Bar Diamond Inc., Parma, USA). Nylon bags contained approximately 5 g of whole pelleted feed and a small glass marble. Preliminary studies found that feed ground through a 2 mm sieve had similar disappearance rates as whole pellets. The bags were soaked in cold tap water for 30 min prior to use. All 6 bags were introduced into the rumen one h before morning feed. Bags were removed after 6, 9, 12, 24, 36 and 48 h of incubation and placed in a bucket of cold water to arrest microbial activity. All bags, including 12 zero incubation bags, were cleaned under a cold running tap to remove feed particles from the outside of the bag, particularly the neck of the bag. Bags were then immediately placed into a washing machine (Hoover Commodore 792) on a "woollens/delicates" standard wash. Each wash contained zero incubation samples. The bags were then oven dried at 60°C for 48 h, cooled in a desiccator and weighed. The DM disappearance in mg/g DM was multiplied by 100 for percentage units. The sampling was collected over two periods, 7 d apart. The correlation coefficient from the regression between sampling periods was r = 0.95.

4.2.10.2 Rumen fluid sampling

Rumen fluid samples were taken by suction using a sampling probe inserted through the rumen cannula. The sampling probe consisted of a metal frame (5 cm x 1 cm x 1 cm) covered with a nylon bag and attached to a curved stainless steel metal tube (25 cm

long). The probe was placed in the middle of the ventral sac of the rumen and held in position through the rubber stopper in the cannula.

4.2.10.3 Rumen volume and outflow rates measured using Chromium-EDTA

The Cr-EDTA complex was prepared by the method of Binnerts *et al.* (1968). One h before the morning feed, a single dose of Cr-EDTA (2.8 mg Cr/ml) was injected into the rumen through the sampling probe using a 20 ml syringe (weighed before and after injection) and washed through with approximately 50 ml of tap water. The Cr-EDTA solution was injected at a dose rate of 1.12 mg Cr/kg bodyweight.

Background rumen fluid samples were collected from each animal just before marker injection. A 10-15 ml sample of rumen fluid was collected from each animal 3 h after injection then hourly to 12 h and at 21 h and 24 h. Samples were acidified with 0.3 ml of concentrated sulphuric acid and immediately frozen for later analysis.

Rumen fluid samples were defrosted and mixed before being centrifuged at 3,000 *g* for 10 min. Samples were analysed using an atomic absorption spectrometer (Perkin-Elmer 360, Norwalk, Connecticut, USA) with nitrous oxide-acetylene flame. A series of potassium chromate standards of known concentration were used to produce a standard curve and to recalibrate the spectrometer. The original dose sample was also used every 12^{th} sample to monitor the sensitivity of the instrument. Two measurements were made on each sample and the average of these used for the linear regression. the $\log_{10}$ of Cr concentration against time. The Cr concentration declined over time, according to first order dilution kinetics, described by the following equation:

$$A_t = A_0 e^{-kt}$$

where A_t and A_0 are the Cr concentrations at time t and 0 respectively and k is the fractional disappearance rate (h^{-1}) estimated from the regression coefficient of the regression line.

Rumen fluid volume and outflow rate were calculated using the following equations:

$$\text{Rumen fluid volume (ml)} = \frac{\text{Dose injected (µg Cr)}}{A_0 \text{ (µg Cr/ml)}}$$

Rumen outflow rate (*l*/d) = Rumen fluid volume (*l*) x k x 24 (h)

4.2.10.4 Volatile fatty acid concentration in the rumen

Rumen fluid samples were taken before morning feed and 4 h after the morning and afternoon feeds. A 10 –15 ml sample of rumen fluid was collected from each animal, acidified (0.3 ml of concentrated sulphuric acid) and immediately stored at –20 °C for later analysis. Samples were collected on two days (3 and 5 d). Rumen fluid was centrifuged (3,000 g for 10 min) and supernatant analysed for volatile fatty acid (VFA) concentration using a gas chromatograph (Varian CP-3800, California, USA) with isocaproic acid as an internal standard.

4.2.10.5 Ammonia nitrogen concentration in the rumen

Rumen fluid samples were collected and prepared to determine ammonia nitrogen concentration as described for VFA samples. To determine ammonia concentration, undiluted rumen fluid supernatant was analysed colorimetrically using a Technicon autoanalyser (Technicon Instruments Comp. New Jersey, USA), according to the method of Crook and Simpson (1971) and modified by Beitz (1974).

4.2.10.6 Rumen fluid pH

Rumen fluid pH was determined immediately after sample collection with a portable pH meter (Ecoscan, Eutech Instruments, Singapore). The pH was measured before the morning feed and 4 h after the morning and afternoon feeds. The pH meter was re-calibrated before each sampling event with standard buffers pH 4.0 and 7.0.

4.2.11 Euthanasia

Animals were euthanased at the beginning of week 8 of infection, using a captive bolt pistol followed by exsanguination. Following euthanasia, the abomasum was removed ensuring total contents were maintained. The abomasum was cut along the greater curvature and laid flat, the contents were collected and the abomasum thoroughly washed. Aliquots (1%, 20 ml) from the total washed volume were taken in triplicate (total of 3%) and preserved in iodine. All worms present in the 1% subsample were differentiated and counted into sexually mature adult male, adult female and larvae, the

average of the counts were multiplied by the aliquot factor (100) for total count. The fecundity of female worms was calculated from worm egg count (epg) multiplied by average faecal output, divided by the average female worm count of each animal.

4.2.12 Statistical Analysis

All statistical analyses were performed using the SAS computer program (SAS Institute Inc 1999-2001). General Linear Models (GLM) were used to analyse the significance of selection line (IRH, DRH and C), infection (NIL and INF) and the interaction (line x infection) between these effects. The models used repeated measures analysis of variance for body weight, worm egg count and haematology parameters. Initial bodyweight was not a significant covariate for subsequent bodyweight measurements. Least square means (ls mean) ± standard errors (s.e.) are presented for all measured parameters. Worm egg count, worm counts, fecundity of female worms and circulating white blood cell (WBC) counts were cube-root transformed to normalize data prior to analysis and presented as back-transformed means with back-transformed 95% confidence intervals (*ci*). Ammonia nitrogen measurements and eosinophil counts were transformed using $\log_{10}$ with antilog means presented with antilog 95% confidence intervals. The base sample for haematology parameters, WBC, eosinophils and haematocrit percentage was a significant covariate for subsequent samples at week 1, 6 and 8. Analysis for VFA and ammonia nitrogen concentration and *in sacco* dry matter digestibility was performed on the average of the two sample periods.

4.3 Results

4.3.1 Feed intake and apparent whole tract digestibility

Daily feed intake did not differ significantly among selection lines or in response to infection (Table 2). Dry matter (DM) and organic matter (OM) digestibility were significantly ($P = 0.050$) higher in IRH and DRH lines compared to C. The interaction between the effects of selection line and infection was not statistically significant for feed intake or digestibility.

4.3.2 Apparent whole tract Nitrogen digestibility and N balance

Nitrogen digestibility and N balance did not differ among selection lines and infection. The interaction between the effects of selection line and infection was not statistically significant (Table 4-2).

Table 4-2 Average daily feed intake (g/kg BW), dry matter digestibility (DMD %), organic matter digestibility (OMD %), nitrogen digestibility (ND %) and nitrogen balance (g N/d) of Merino weaner wethers. from lines selected for either increased resistance to *Haemonchus* (IRH), decreased resistance to *Haemonchus* (DRH) and random-bred control (C) with (INF) or without (NIL) a trickle infection with *H. contortus*.

Group	Feed Intake		DMD		OMD		ND		N balance	
	ls mean	s.e..	ls mean	s.e.	ls mean	s.e.	ls mean	s.e.	ls mean	s.e.
IRH	27.6	1.56	54.6[a]	1.69	54.6[a]	1.74	32.7	2.12	1.3	0.26
DRH	24.5	1.65	54.9[a]	1.80	54.9[a]	1.84	31.7	2.24	1.2	0.28
C	27.3	1.56	49.2[b]	1.69	49.1[b]	1.74	30.4	2.12	1.4	0.26
NIL	25.8	1.27	54.3	1.39	54.4	1.42	30.0	1.73	1.2	0.22
INF	27.1	1.32	51.5	1.44	51.4	1.48	33.3	1.80	1.4	0.23
Statistics										
Line (L)	P = 0.336		P = 0.049		P = 0.047		P = 0.741		P = 0.927	
Infection (I)	P = 0.487		P = 0.172		P = 0.163		P = 0.198		P = 0.458	
L x I	P = 0.442		P = 0.315		P = 0.324		P = 0.822		P = 0.889	

Within columns, values with a common suffix or no suffix do not differ significantly (P>0.05).

4.3.3 Rumen measurements

4.3.3.1 in sacco dry matter digestibility

Dry matter digestibility (*in sacco*) at early incubations (6, 9 and 12 h) was significantly lower ($P < 0.05$) in animals infected with *H. contortus*. At the 12 h incubation, IRH and DRH animals had significantly ($P = 0.005$) higher digestibility compared to C animals (Table 4-3).

4.3.3.2 Rumen volume and flow rates

Rumen fluid volume, outflow rate and turnover rate did not differ between selection lines and were not affected by *H. contortus* infection. The interaction between the effects of line and infection for rumen outflow rate was significant ($P = 0.032$). The form of the interaction was such that only the IRH line increased outflow rate in response to infection. In contrast, infection reduced outflow rate in C animals. The

interaction between the effects of line and infection for rumen turnover rate tended (P = 0.072) to follow a similar pattern to rumen outflow rate (Table 4-4).

Table 4-3 Comparison of *in sacco* DM digestibility (%) over 48 h in weaner wethers selected for increased resistance to *Haemonchus* (IRH), decreased resistance to *Haemonchus* (DRH) and random-bred controls (C), either maintained worm free (NIL) or infected with *H. contortus* (INF).

	Incubation time (h)											
	6		9		12		24		36		48	
Group	ls mean	s.e.	ls mean	s.e.	ls mean	s.e.	ls mean	s.e.	ls mean	s.e.	ls mean	s.e.
IRH	21.1	0.70	23.0^{ab}	0.69	25.4^{a}	0.67	31.3	1.01	37.2	1.28	43.1	1.12
DRH	21.7	0.74	24.1^{a}	0.73	26.2^{a}	0.71	33.4	1.07	38.3	1.36	44.5	1.19
C	19.8	0.70	21.5^{b}	0.69	22.8^{b}	0.67	30.1	1.01	37.5	1.28	42.8	1.12
NIL	21.8^{x}	0.57	23.7^{x}	0.56	25.8^{x}	0.54	31.9	0.82	38.5	1.05	43.3	0.92
INF	20.0^{y}	0.59	22.0^{y}	0.59	23.8^{y}	0.57	31.4	0.86	36.8	1.09	43.6	0.95
Statistics												
Line (L)	P = 0.175		P = 0.049		P = 0.005		P = 0.102		P = 0.832		P = 0.548	
Infection (I)	P = 0.037		P = 0.050		P = 0.014		P = 0.677		P = 0.285		P = 0.865	
L x I	P = 0.933		P = 0.796		P = 0.955		P = 0.781		P = 0.855		P = 0.633	

Within treatment effect and columns, values with a common suffix or no suffix do not differ significantly (P>0.05).

Table 4-4 Rumen fluid volume (*l*), outflow rate (*l*/d) and turnover rate (/d) measured using Chromium-EDTA in weaner wethers selected for increased resistance to *Haemonchus* (IRH), decreased resistance to *Haemonchus* (DRH) and random-bred controls (C), either maintained worm-free (NIL) or trickle infected with *H. contortus* (INF).

		Fluid volume		Outflow rate		Turnover rate	
Line	**Infection**	ls mean	s.e.	ls mean	s.e.	ls mean	s.e.
IRH	NIL	3.76	0.51	3.28^{a}	0.58	0.89	0.13
IRH	INF	4.28	0.51	5.11^{b}	0.58	1.21	0.13
DRH	NIL	4.02	0.51	3.27^{a}	0.58	0.82	0.13
DRH	INF	4.26	0.57	4.63^{a}	0.65	1.06	0.14
C	NIL	4.68	0.51	5.47^{b}	0.58	1.29	0.13
C	INF	4.26	0.51	4.25^{a}	0.58	1.02	0.13
Statistics							
Line (L)		P = 0.665		P = 0.300		P = 0.293	
Infection (I)		P = 0.795		P = 0.185		P = 0.385	
L x I		P = 0.645		P = 0.032		P = 0.072	

Within columns, values with a common suffix or no suffix do not differ significantly (P>0.05).

4.3.3.3 Rumen pH

Rumen pH was significantly higher in INF compared to NIL animals, before 9am feed (P = 0.005), 4 h after 9am feed (P = 0.008) and 4 h after 3pm feed (P = 0.020) (Figure

4-1). Selection line had no effect on rumen pH and all selection lines behaved similarly in response to infection.

4.3.3.4 Rumen ammonia nitrogen

Rumen ammonia nitrogen levels were extremely low in all experimental animals. INF animals had significantly (P = 0.032) lower ammonia nitrogen than NIL animals 4 h after the 9am feed; however there were no differences between selection lines (Table 4-5). The interaction between the effects of line and infection for rumen ammonia was significant (P = 0.026), such that the IRH group had a five-fold reduction in rumen ammonia, after feeding, in response to infection. The effect of infection on rumen ammonia in DRH and C animals was not significantly different.

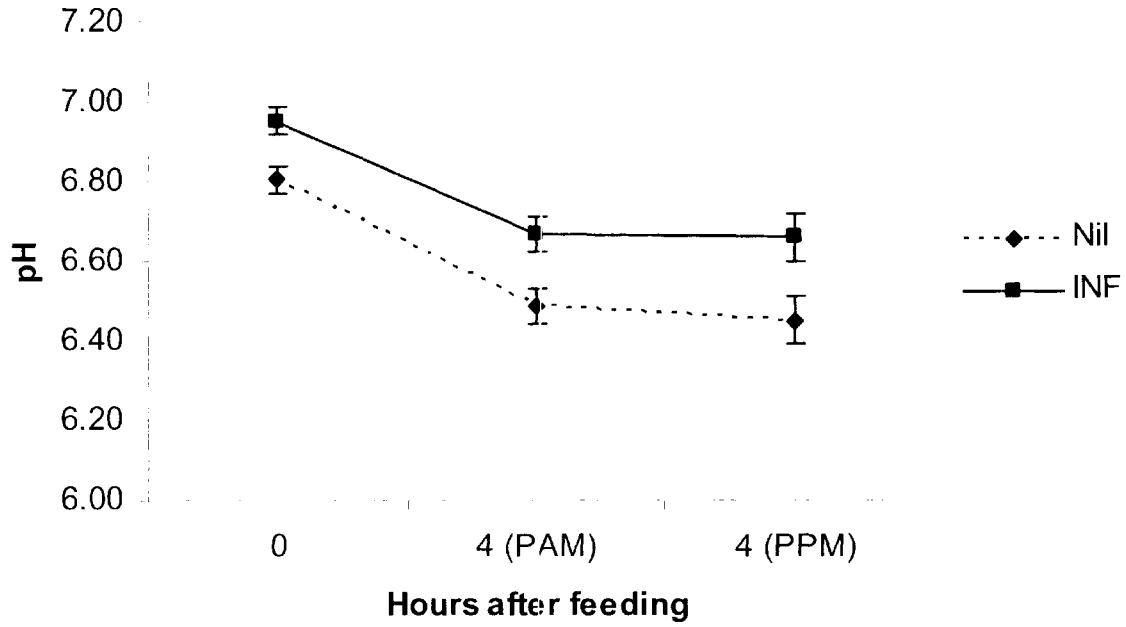


Figure 4-1 Rumen pH (± s.e.) before 9am feed (0 h), 4 h after 9am feed (4 PAM) and 4 h after 3pm feed (4 PPM) in weaner wethers, either maintained worm-free (NIL) or infected with *H. contortus* (INF).

Table 4-5 Rumen ammonia nitrogen (mg NH_3- N/*l* ± 95% *ci.*) measured before 9am feed (0 h), 4 h after 9am feed (4 h PAM) and 4 h after 3pm feed (4 h PPM) in weaner wethers selected for increased resistance to *Haemonchus* (IRH), decreased resistance to *Haemonchus* (DRH) and random-bred controls (C), either maintained worm free (NIL) or infected with *H. contortus* (INF).

		0 h			4 h PAM			4 h PPM		
Line	**Infection**	ls mean	± *ci*		ls mean	± *ci.*		ls mean	± *ci*	
IRH	NIL	14.1	6.36	11.58	14.0	5.47	8.99	10.2^{a}	3.45	5.20
IRH	INF	2.8	1.24	2.26	1.4	0.54	0.88	1.6^{b}	0.52	0.79
DRH	NIL	8.7	3.92	7.14	6.0	2.36	3.88	3.2^{ab}	1.07	1.61
DRH	INF	11.8	5.72	11.16	3.9	1.66	2.88	4.4^{ab}	1.62	2.56
C	NIL	3.4	1.53	2.78	2.6	1.02	1.68	2.0^{b}	0.69	1.04
C	INF	4.1	1.84	3.34	2.4	0.95	1.56	2.3^{b}	0.77	1.17
Statistics										
Line (L)			P = 0.273			P = 0.391			P = 0.290	
Infection (I)			P = 0.444			P = 0.032			P = 0.173	
L x I			P = 0.212			P = 0.074			P = 0.026	

Within columns, values with a common suffix or no suffix do not differ significantly (P>0.05).

4.3.3.5 Volatile fatty acids

Total VFA concentrations were significantly (P = 0.003) lower in infected animals, however there were no differences among selection lines. There was a trend (P = 0.078) for IRH animals to have a lower total VFA concentration in response to infection, while infection had no effect on the DRH and C lines. Propionic acid concentration was significantly (P = 0.033) lower in IRH animals in response to infection (Figure 4-2). In contrast, infection increased propionic acid concentration in DRH and C animals. The decrease in total VFA concentration in infected animals was a consequence of a decline in acetic and butyric acids. Isobutyric and isovaleric acids did not vary among selection lines or in response to infection (Table 4-7). The molar percentage of individual VFA did not differ among selection lines or due to infection and the interaction between the effects of line and infection was non-significant at any time point.

4.3.4 Purine derivatives

There were no differences in total daily purine derivative excretion among selection lines (P = 0.743) or in response to infection (P = 0.311). The interaction between the effects of selection line and infection (P = 0.719) was not statistically significant. There

were no significant differences between the main effects for individual purine derivatives. The results of the interaction are presented in Table 4-6. The purine derivatives increased by 27% in IRH animals in response to infection, this effect will be discussed later.

Table 4-6 Total purine derivatives (mmol/d ± s.e.) of weaner wethers selected for increased resistance to *Haemonchus* (IRH), decreased resistance to *Haemonchus* (DRH) and random-bred controls (C), either maintained worm-free (NIL) or trickle infected with *H. contortus* (INF).

Group	IRH		DRH		C	
	ls mean	s.e.	ls mean	s.e.	ls mean	s.e.
NIL	1.8	0.30	1.8	0.30	1.9	0.30
INF	2.3	0.30	1.8	0.34	2.2	0.30

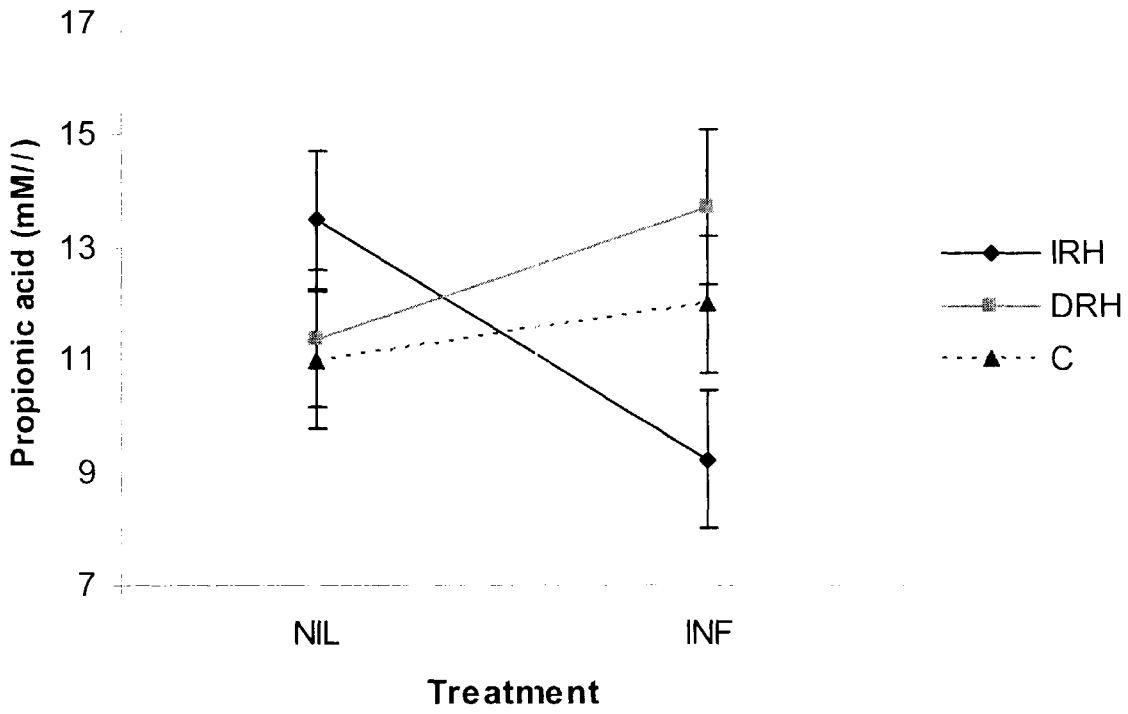


Figure 4-2 Propionic acid concentration in rumen fluid (ls mean ± s.e.) measured 4 h after 9am feed in weaner wethers selected for increased resistance to *Haemonchus* (IRH), decreased resistance to *Haemonchus* (DRH) and random bred controls (C), either maintained worm free (NIL) or infected with *H. contortus* (INF).

Table 4-7 Volatile fatty acid concentration in rumen fluid (mM/*l* ± s.e.) measured before 9am feed (0 h), 4 h after 9am feed (4 h PAM) and 4 h after 3pm feed (4 h PPM) in weaners selected for increased resistance to *Haemonchus* (IRH), decreased resistance to *Haemonchus* (DRH) and random-bred controls (C), either maintained worm free (NIL) or infected with *H. contortus* (INF).

Group	**0 h**		**4 h PAM**		**4 h PPM**	
Total VFA concentration	ls mean	s.e.	ls mean	s.e.	ls mean	s.e.
IRH	55.0	3.15	61.8	2.99	72.1	3.71
DRH	53.0	3.34	59.1	3.17	71.6	3.93
C	49.2	3.15	57.6	2.99	68.6	3.71
NIL	58.4^{x}	2.57	62.9	2.44	74.2	3.03
INF	46.3^{y}	2.68	56.1	2.54	67.3	3.15
Statistics						
Line (L)	P = 0.429		P = 0.609		P = 0.779	
Infection (I)	P = 0.003		P = 0.069		P = 0.130	
Acetic acid	ls mean	s.e.	ls mean	s.e.	ls mean	s.e.
IRH	40.7	2.32	43.7	2.20	50.4	2.73
DRH	38.1	2.46	40.4	2.33	48.8	2.90
C	36.3	2.32	40.7	2.20	47.4	2.73
NIL	42.7^{x}	1.90	44.1	1.80	51.7	2.23
INF	34.0^{y}	1.97	39.1	1.87	46.1	2.32
Statistics						
Line (L)	P = 0.418		P = 0.525		P = 0.741	
Infection (I)	P = 0.004		P = 0.070		P = 0.092	
Propionic acid	ls mean	s.e.	ls mean	s.e.	ls mean	s.e.
IRH	8.7	0.65	11.4	0.87	13.5	0.95
DRH	9.7	0.69	12.5	0.92	15.1	1.01
C	8.8	0.65	11.5	0.87	14.4	0.95
NIL	9.7	0.53	12.0	0.71	14.1	0.78
INF	8.4	0.55	11.6	0.74	14.5	0.81
Statistics						
Line (L)	P = 0.529		P = 0.601		P = 0.525	
Infection (I)	P = 0.104		P = 0.766		P = 0.721	
Butyric acid	ls mean	s.e.	ls mean	s.e.	ls mean	s.e.
IRH	4.9	0.47	6.0	0.58	7.2	0.77
DRH	4.4	0.50	5.4	0.61	6.8	0.82
C	3.4	0.47	4.6	0.58	5.8	0.77
NIL	5.2^{x}	0.38	6.1^{x}	0.47	7.4	0.63
INF	3.3^{y}	0.40	4.6^{y}	0.49	5.8	0.66
Statistics						
Line (L)	P = 0.102		P = 0.239		P = 0.440	
Infection (I)	P = 0.002		P = 0.048		P = 0.097	
Iso acids	ls mean	s.e.	ls mean	s.e.	ls mean	s.e.
IRH	0.5	0.07	0.3	0.09	0.4	0.09
DRH	0.5	0.08	0.3	0.09	0.4	0.10
C	0.4	0.07	0.4	0.09	0.5	0.09
NIL	0.5	0.06	0.3	0.07	0.4	0.07
INF	0.4	0.06	0.3	0.07	0.4	0.08
Statistics						
Line (L)	P = 0.947		P = 0.629		P = 0.724	
Infection (I)	P = 0.125		P = 0.992		P = 0.818	

Within columns, values with a common suffix or no suffix do not differ significantly (P>0.05).

4.3.5 Parasitology

WEC of the IRH animals remained significantly (P = 0.003) lower throughout the 7-week infection period, than for the DRH and C lines. The DRH and C lines did not differ significantly during this time (Figure 4-3). Adult male and female worm counts and larval (L4) counts recovered from the abomasum were not significantly different among selection lines (P = 0.558) at the beginning of week 8 of infection (Figure 4-4). However, total worm counts of IRH animals were 36 and 43% of DRH and C animals respectively. In addition the fecundity of female worms did not differ among selection lines (P = 0.578), despite more than double the eggs per DRH female worm compared to IRH females (Figure 4-5).

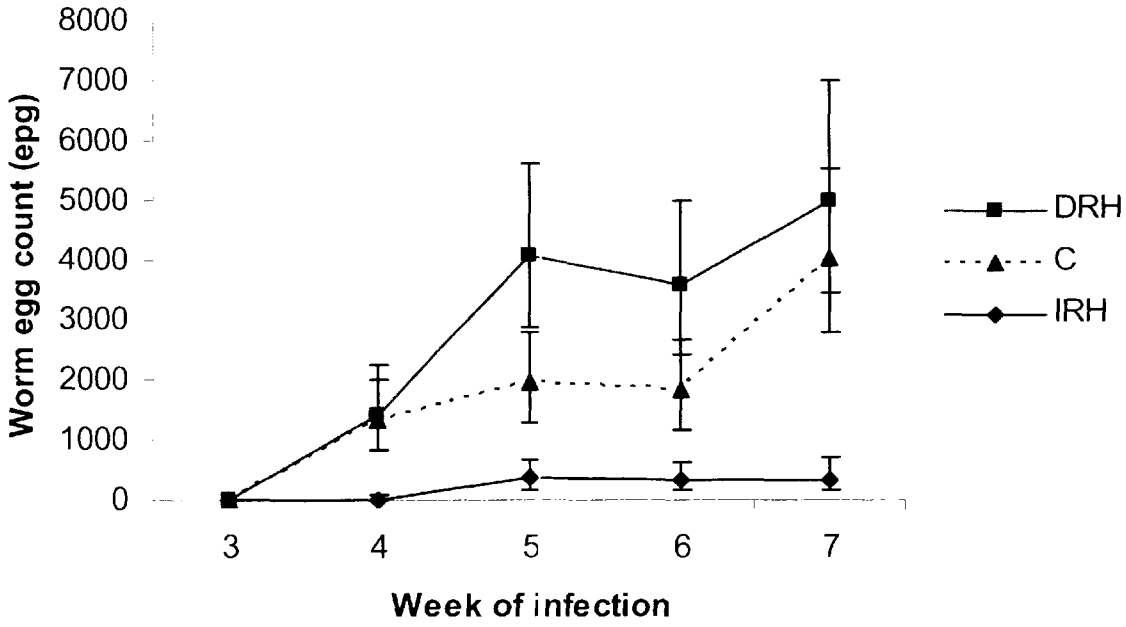


Figure 4-3 Back-transformed worm egg counts (ls mean ± 95% *ci.*) of weaner wethers from the increased resistance to *Haemonchus* (IRH), decreased resistance to *Haemonchus* (DRH) and random-bred control (C) selection lines, over a 7-week trickle infection.

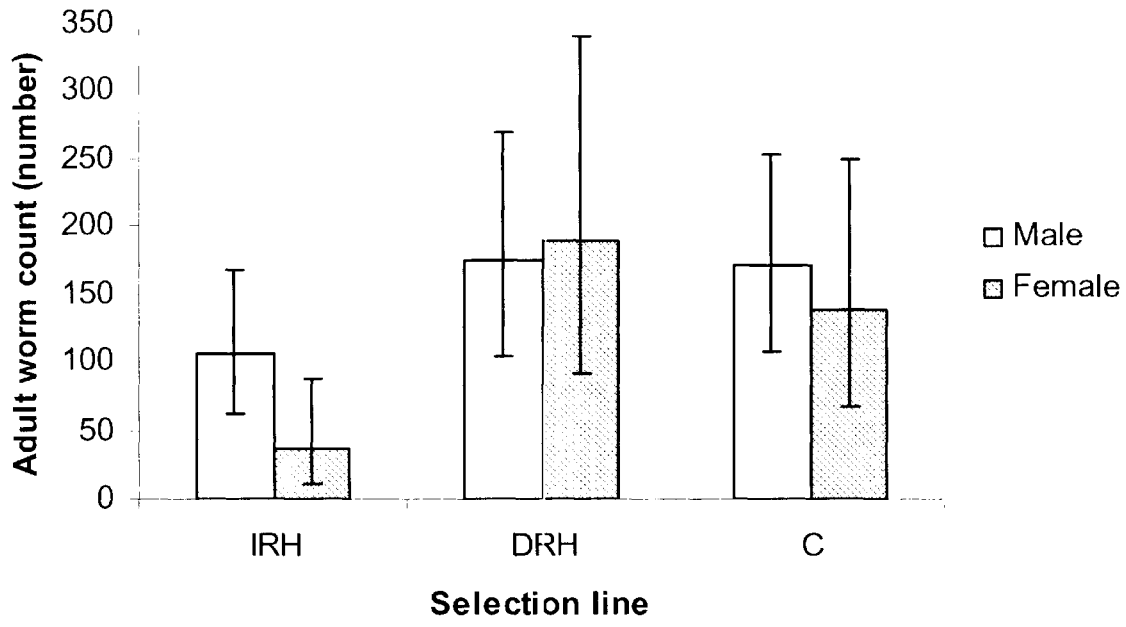


Figure 4-4 Total adult male and female back-transformed worm counts (ls mean ± 95% *ci.*) of weaner wethers from the increased resistance to *Haemonchus* (IRH), decreased resistance to *Haemonchus* (DRH) and random-bred control (C) selection lines, at the beginning of week 8 of infection.

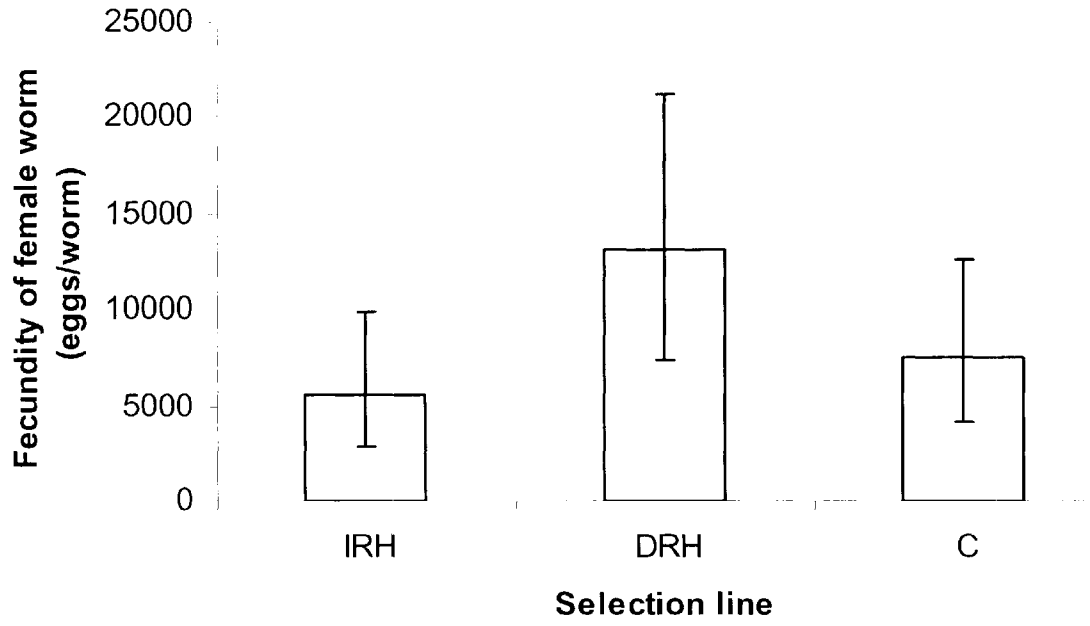


Figure 4-5 Fecundity of female worms (ls mean ± 95% *ci.*) of weaner wethers from the increased resistance to *Haemonchus* (IRH), decreased resistance to *Haemonchus* (DRH) or random-bred control (C) selection lines at the beginning of week 8 of infection. .

4.3.6 Haematology

H. contortus infection significantly decreased red blood cell (RBC) numbers (P = 0.036) and haematocrit (P = 0.011) when averaged over time (Figure 4-6 and Figure 4-7). Eosinophil counts were higher in INF animals compared to NIL (Figure 4-8) when averaged over time (P = 0.052); groups differed significantly (P = 0.035) at week 6 of infection. RBC numbers, haematocrit and eosinophil count did not differ among selection lines over the infection period. RBC numbers at week 8 of infection for IRH, DRH and C were 9.6 ± 0.36, 9.0 ± 0.38 and 9.8 ± 0.36 (10^9 cells/ml) respectively. Haematocrit at week 8 of infection for IRH, DRH and C were 30.0 ± 0.84, 29.2 ± 0.88 and 30.9 ± 0.84% respectively. Eosinophil count at week 8 of infection for IRH, DRH and C were 0.12 ± 0.11, 0.13 ± 0.11 and 0.14 ± 0.10 (10^6 cells/ml) respectively. In contrast white blood cell (WBC) count did differ among selection lines (P = 0.025) (Figure 4-9), but was unaffected by infection. WBC decreased in all groups at week 1 then steadily increased in the IRH and C groups, while continuing to decline in DRH. The decline at week 1 resulted from a consistent decrease in all WBC types.

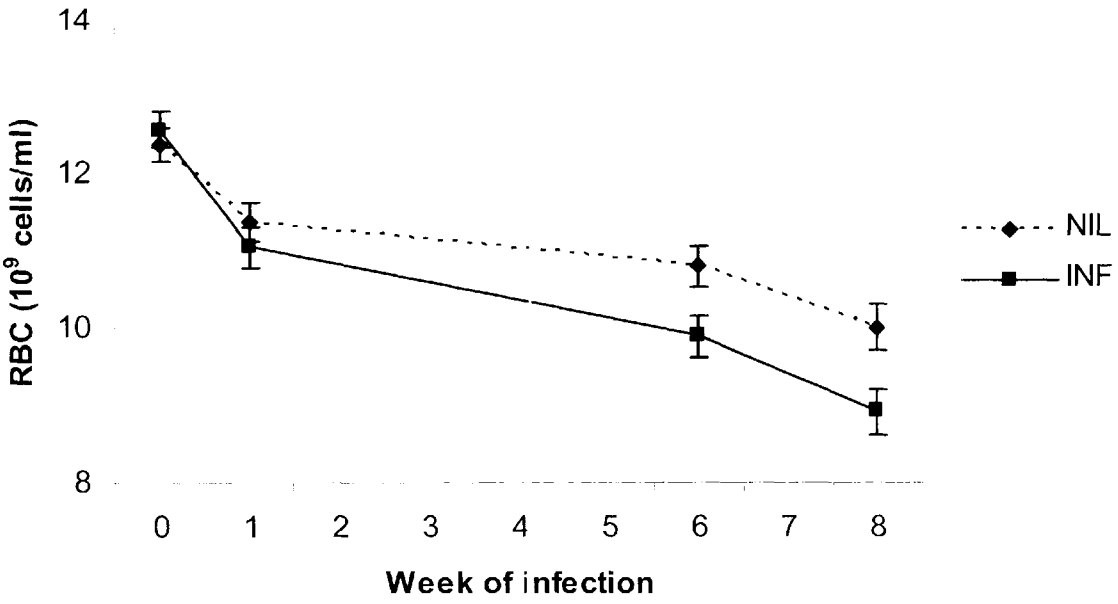


Figure 4-6 Red blood cell (RBC) count (ls mean ± s.e.) in blood samples taken from weaner wethers maintained worm free (NIL) or trickle infected with *H. contortus* (INF).

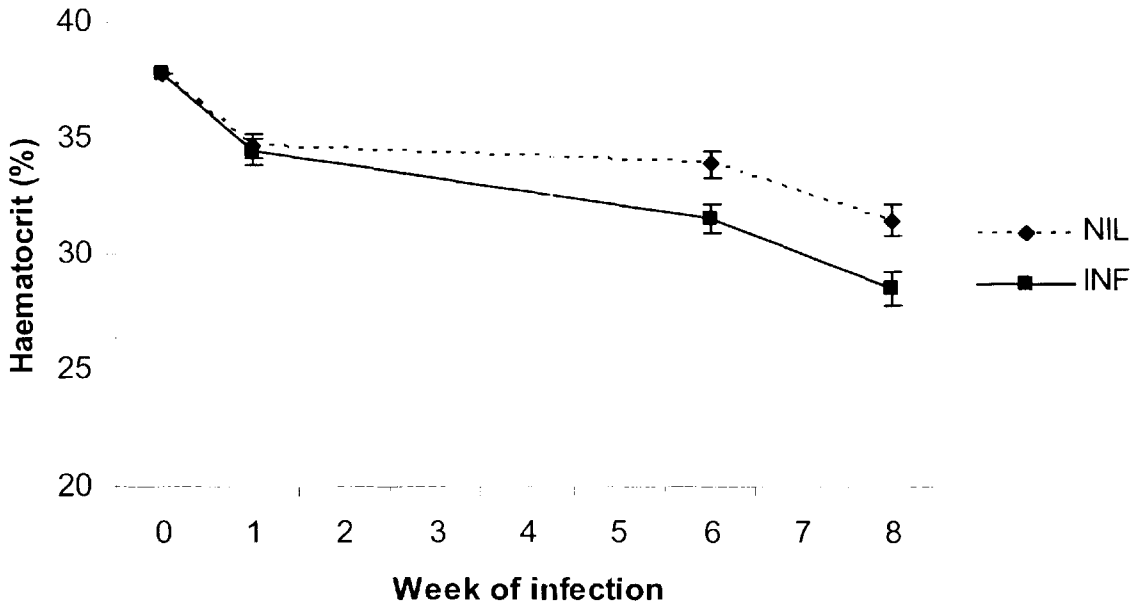


Figure 4-7 Haematocrit (ls mean ± s.e.) in blood samples taken from weaner wethers maintained worm free (NIL) or trickle infected with *H. contortus* (INF).

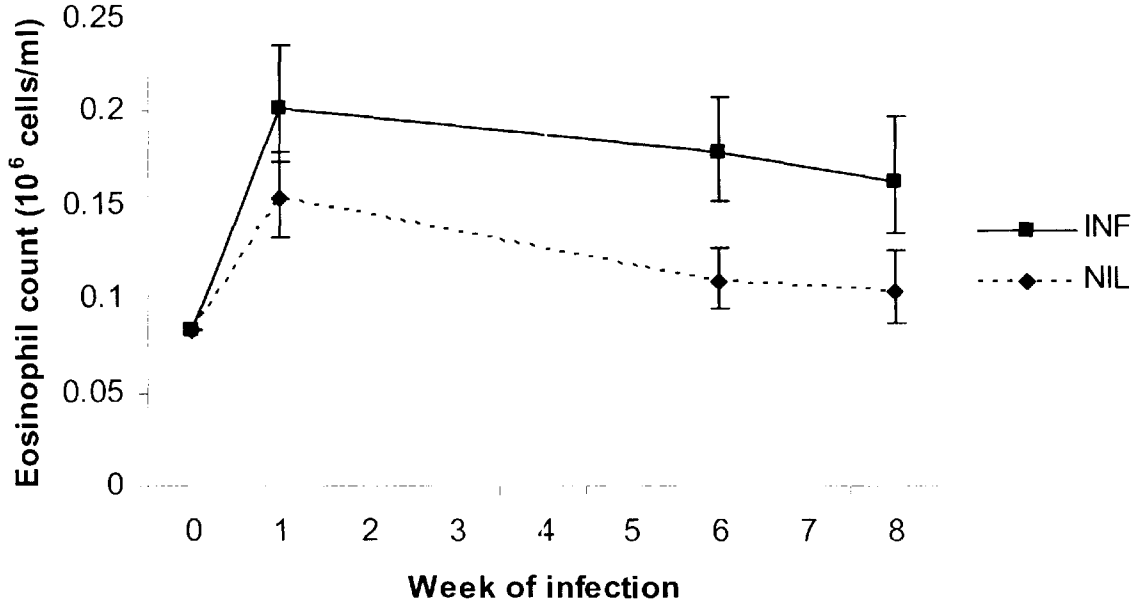


Figure 4-8 Eosinophil count (ls mean ± 95% *ci.*) in blood samples taken from weaner wethers maintained worm free (NIL) or trickle infected with *H. contortus* (INF).

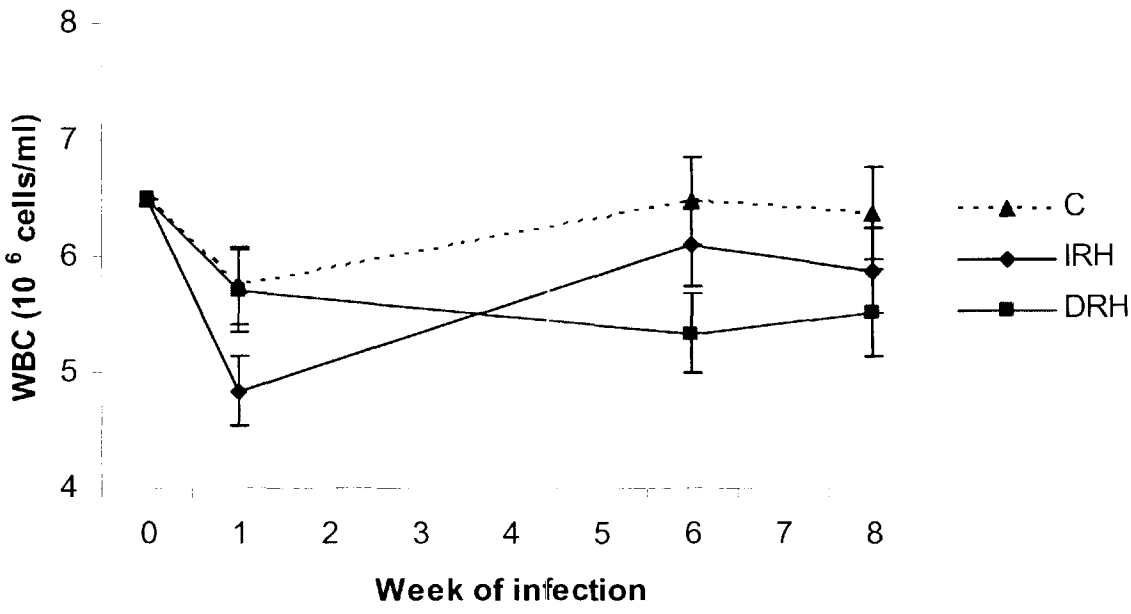


Figure 4-9 White blood cell (WBC) count (ls mean ± 95% *ci.*) in blood samples taken from weaner wethers from the increased resistance to *Haemonchus* (IRH), decreased resistance to *Haemonchus* (DRH) or random-bred control (C) selection lines.

4.3.7 Bodyweight

Bodyweights were maintained throughout the experimental period. Bodyweight (kg) was not affected by nematode infection (P = 0.168) and did not differ among selection lines (P = 0.572). Bodyweight (kg ± s.e.) at week 7 of infection for IRH, DRH and C lines was 22.6 ± 1.18, 22.4 ± 1.25 and 23.4 ± 1.18 respectively. The interaction between the effects of infection and selection line was not statistically significant (P = 0.934).

4.4 Discussion

4.4.1 Change in fermentation

Selection for differences in resistance to nematode infection does not appear to have consistently affected indicators of rumen function in the presence or absence of *H. contortus* infection. DM and OM digestibility and *in sacco* degradability of IRH and DRH selection lines were greater than in C animals, but differences between resistant and susceptible lines were not apparent.

More importantly the interaction between selection line and response to infection in some of the measured indicators of rumen function, suggests a notable shift in fermentation in IRH animals. The IRH line may have altered rumen function to favour the synthesis of microbial protein at the expense of propionic acid. The relative change in response to infection of these indicators of rumen function were calculated and presented for each selection line in Table 4-8.

Total volatile fatty acid concentration declined by 26% in response to infection in IRH animals, which is specifically associated with a 32% decline in propionic acid. At the same time purine derivatives increased by 29%. These changes were accompanied by an increase in rumen outflow rate, which may assist IRH animals to increase microbial protein supply, in response to infection (Roseby 1977). In addition, IRH animals had a large (90%) decrease in rumen ammonia nitrogen in response to infection. This may have been due to an increased utilization of ammonia by the microbial population, which in turn increased microbial protein supply, as shown by the rise in excretion of purine derivatives. In contrast, total volatile fatty acid concentration of DRH and C animals was unaffected by infection, but the acetate: propionate ratio decreased. These changes in protein and energy supply correspond with previous studies using animals unselected for resistance and infected with *H. contortus*. Rowe *et al.* (1988), found no change in total VFA concentration between INF and NIL Merino wethers of the same age, given a bolus infection of *H. contortus* and rumen fluid measurements made at week 6 *pi*. Steel (1975) also found no difference in VFA concentration in mature sheep infected with a trickle infection of *T. circumcincta*, compared to NIL sheep.

Table 4-8 The relative change (%) in indicators of rumen function in weaner wethers selected for increased resistance to *Haemonchus* (IRH), decreased resistance to *Haemonchus* (DRH) and random-bred controls (C) in response to *H. contortus* infection.

Measurement	IRH	DRH	C
Rumen flow rate	↑ 56*	↑ 42	↓ 22*
in sacco DM digestibility (9 h)	↓ 9*	↓ 8*	↓ 4
Rumen ammonia (4 h PAM)	↓ 90*	↓ 35	↓ 7
Total VFA (4 h PPM)	↓ 26	↓ 1	↓ 2
Propionic acid (4 h PAM)	↓ 32*	↑ 21	↑ 9
Purine derivative	↑ 29	0	↑ 13

(* denotes significance P>0.05)

The observed decrease in acetic acid concentration in response to infection in this experiment is supported by previous studies. Steel (1972) reported a 30% decrease in acetic acid concentration in *T. colubriformis* infected animals, compared to NIL counterparts. Production of acetic acid during microbial fermentation of carbohydrate in the rumen is a major source of energy to ruminants (Steel 1972). This result is consistent with effects of nematode infection, i.e. depression in bodyweight gain and wool growth. The difference in rumen fermentation in IRH animals in response to infection appears to be caused by the significant reduction in propionic acid concentration. This change was not apparent in the DRH and C lines in this experiment and in the INF wethers used by Rowe *et al.* (1988) the concentration of propionic acid increased. Leng *et al.* (1967) and Bergman *et al.* (1966) found that 50-60% of glucose is synthesised from propionate and Cridland (1984) proposed propionate may contribute up to 80-90% of glucose synthesised in sheep on roughage diets. If the lower concentration of propionic acid is assumed to represent a lower rate of production, then it is possible that glucose entry rate may be lower in IRH animals in response to infection. Amino acids catabolised from muscle protein could potentially be used for gluconeogenesis, to supply glucose to IRH animals. Glucose is a major requirement of growth in young sheep. IRH animals may have reduced growth rates, compared to expected growth when carrying a low *H. contortus* infection, due to changes in rumen fermentation pattern, arising from their response to *H. contortus* larvae.

It is acknowledged that the relative change in purine derivatives in response to infection is the only non-significant interaction. However the low concentration of purine derivatives, compared to those shown by Chen *et al.* (1992) and Kahn *et al.* (2000;

2001), may have affected significant effects of infection on microbial protein supply in IRH animals. The low values are not unreasonable, considering the dry matter intake and digestibility of the feed, however small values are difficult to distinguish differences between selection lines. The inability to calculate microbial protein supply using the predictive relationship of Chen *et al.* (1990), may not only have been due to a low concentration of daily purine excretion but may also have been influenced by breed, age and diet. The Chen (1990) study based measurements on 1 to 3 year old Blackface x Suffolk animals and not Australian Merinos and the diet was almost double the crude protein content (19.4g nitrogen /kg DM) of this study.

4.4.2 Parasitology

IRH average WEC remained below 1000 epg throughout the infection, demonstrating the benefit of selection. WEC of DRH and C animals did not differ throughout this period and exceeded levels where chemical control would generally be applied in a commercial situation. Adult worm and larval counts were numerically lower in IRH animals but differences among selection lines were not significant. This result may have been influenced by the small number of animals used for this study and large standard errors of each group. One would expect the differences in worm egg counts among selection lines to translate into differences in worm counts, as indicated by Stear *et al.* (1995b). However, the *Haemonchus* selection lines are selected for WEC, with no published data on the correlation between WEC and adult worm counts.

4.4.3 Haematology

The number of red blood cells declined from day 0, by 2.4 x 10^9 cells/ml in NIL and 3.7 x 10^9 cells/ml in INF animals, over the course of the infection period. This indicates that the *Haemonchus* infection accounted for only 35% of the loss in RBC numbers over the course of the infection period. The level of anaemia (haematocrit 29%) in infected animals would also not be considered severe for a *Haemonchus* infection, with normal haematocrit ranging from 27-45% (Radostits *et al.* 2000). Nutritional factors were most likely involved in the decline in red cells in both groups of animals (Abbott *et al.* 1986a).

Eosinophil count at the start of the experiment was similar in both NIL and INF animals and the rise post-infection suggest that all animals had been immunologically primed before the artificial infection. Selection line had no effect on eosinophil count, which supports the findings reported by Woolaston *et al.* (1996) who suggested that circulating eosinophils were not a suitable indicator of resistance to *Haemonchus* infection. In contrast, chapter 3 presented eosinophil counts significantly higher in IRH animals and in response to a high quality diet. Eosinophil count has been shown to be a measure of an immune response to *T. colubriformis* infection (Windon 1989, Dawkins *et al.* 1989). In this study the immune response of IRH appears to be similar to that of DRH and C animals.

The WBC measurement is an indication of cells present in the circulating blood pool. However, when an immune host is exposed to larvae, cells will migrate and accumulate at the site of infection, in this case the gut mucosa and lymph nodes surrounding the gut. This is shown in the IRH animals by a fall in the blood concentration of WBC one week post-infection. Circulating WBC in the DRH animals took longer to respond to infection and remained lower, in response to incoming larvae into the gut.

The small differences among selection lines in worm count may have diminished differences in measures of circulating immunity such as eosinophil count and loss of red blood cells. As a result the hypothesised correlated changes in rumen fermentation between divergent selection lines in response to infection may have been reduced and difficult to detect.

4.4.4 Bodyweight

Bodyweight was maintained in all groups of animals throughout the experimental period. The feeding regime used in this experiment was designed to maintain bodyweight. Interestingly, bodyweight did not differ among selection lines despite a significant difference in WEC. The lack of benefit accruing to IRH animals as a result of decreased WEC supports previous work using the *Haemonchus* selection lines. The effect of infection on bodyweight of pregnant and lactating ewes (Kahn *et al.* 2003), weaners (Eady and Smith 2001) and weaner rams reported in Chapter 3 was similar among selection lines.

The effect of *H. contortus* infection on bodyweight gain in young Merino sheep has been investigated by Albers *et al.* (1989). Weaners infected with 11,000 *H. contortus* larvae had a 38% reduction in bodyweight gain over an 8-9 week infection period. The efficacy of the infection in this study may not have caused the reduced weight gains associated with parasite infection. Observations made at slaughter indicated little fat deposition. It is possible that the level of feed intake imposed in this experiment may have reduced differences between treatments.

4.4.5 Feed intake and digestibility

The experiment was designed to restrict feed intake to support weight maintenance, aimed at exacerbating the effect of infection under pen conditions. Differences in feed intake were minimized by the restriction in food offered. Apparent whole tract DM and OM digestibility were not significantly altered by *H. contortus* infection in this study, but there was a suggestion that infection reduced DM and OM digestibility. In support of this trend Rowe *et al.* (1988) infected Merino weaners with a bolus dose of *H. contortus* and fed lucerne (490 g) and oaten chaff (480 g) and reported a significant decrease in apparent whole tract digestion. Furthermore, measurements of *in sacco* digestibility in this experiment at incubations under 12 h showed a decline of 7.6% in digestibility in response to infection. It is likely that compensatory digestion occurred in the large intestine, reducing differences in whole tract digestion. Leng (1981) stated that an increase in fermentation in the large intestine would compensate for a 30% reduction in fermentation in the rumen. However, since microbial protein synthesised in the large intestine is unavailable, the net result would be a decreased availability of amino acids to the animal.

4.4.6 Apparent whole tract nitrogen digestibility and N balance

Nitrogen digestibility did not differ among treatment groups, but was 20% lower than DM digestibility. This provides evidence that nitrogen was poorly available for digestion in this experimental diet, as nitrogen digestibility is usually greater than organic and dry matter digestibility (Barger 1973, Roseby 1973). Hypoproteinaemia is also a commonly reported symptom of nematode infection, due to increased endogenous

nitrogen loss into the gastrointestinal tract (Rowe *et al.* 1988). Therefore, it is perhaps surprising that the animals did not display more pronounced subclinical effects of infection, such as a decrease in haematocrit and weight gain, due to poor nitrogen metabolism.

There was no significant difference among selection lines or effect of *H. contortus* infection on urine and faecal nitrogen excretion. Rowe (1982) also found no difference in faecal and urine nitrogen excretion with *H. contortus* infected sheep. In contrast, increased faecal nitrogen excretion has been associated with infections with *H. contortus* (Dargie 1973), *T. circumcincta* (Sykes & Coop 1977) and *T. colubriformis* (Steel 1974). Increased urinary nitrogen has also been linked to the above nematode infections (Poppi *et al.* 1981, Sykes & Coop 1977, Dargie 1973). A decrease in nitrogen retention has been demonstrated with *T. colubriformis* (Poppi *et al.* 1981, 1986 Steel *et al.* 1980) and *H. contortus* (Rowe *et al.* 1988) infections. This study showed no change in nitrogen balance between NIL and INF animals. Previous investigators (Steel 1975, Rowe *et al.* 1988, Bown *et al.* 1991) concluded that the input of endogenous nitrogen into the abomasum in abomasal infections is reabsorbed in the small intestine. Steel (1975) showed 80% of endogenous non-ammonium nitrogen secreted in the abomasum of *Teladorsagia* infected animals was reabsorbed in the small intestine. Bown (1991) also found that the major site of digestion and absorption of amino acids occurred in the distal small intestine beyond the site of *Trichostrongylus* infection. It could be possibly that faecal and urine excretion of nitrogen did not represent endogenous nitrogen loss from the abomasal infection as reabsorbed nitrogen in the small intestine masked differences.

4.4.7 Rumen pH

Rumen pH was significantly higher in INF compared to NIL animals, which is associated with the lower VFA concentration in INF animals. This result is not consistent with the study by Rowe *et al.* (1988), where no significant difference in rumen pH was found between NIL, INF or sham-infected, (transfer of blood from the jugular vein to the abomasum) animals. However, the fact that Rowe *et al.* (1988) did not observe a difference in pH was probably because infection did not affect VFA concentration.

4.4.8 Rumen volume and flow rates

The present study showed no difference in rumen fluid volume or outflow rates between animals infected with *H. contortus* and those maintained worm-free. This agrees with results observed in both *Trichostrongylus* (Steel 1972) and *Teladorsagia* infections (Steel 1975), but not in *H. contortus*. However, IRH animals had higher outflow rates in response to infection. Increased outflow rates were also found in *H. contortus* infected animals studied by Rowe *et al.* (1988). They suggested that the faster outflow of liquid from the rumen may be caused by altered reticulorumen motility. Bueno *et al.* (1982) studied the effects of motor and transit disturbances associated with *H. contortus* infection and concluded that an increased rate of duodenal flow was a consequence of ionic permeability changes in the gastrointestinal mucosa and altered abomasal gastric acid secretions. Stewart *et al.* (1994) examined the local nervous system for changes correlated with the development of a protective immune response to infection with *T. colubriformis*. The study found that susceptible sheep had no increase in immunoreactive nerve fibres in the jejunum, while immune sheep had a significant increase in fibres after challenge. This suggests nerve hyperplasia occurs as a physiological reaction in immune animals. Stewart (1994) also suggested that a primary worm infection acts to not only prime the immune system but also to stimulate the nervous system. These changes described by Bueno (1982) and Stewart (1994) may be mechanisms employed by resistant animals to assist in expulsion and prohibit development of incoming larvae.

4.5 Conclusion

In conclusion, there were few differences in rumen function as a consequence of divergent selection for *H. contortus* in response to infection. Most notably, dry matter and organic matter digestibility were greater in IRH and DRH animals, as was *in sacco* disappearance at 12 h. There was some evidence that IRH animals had an altered rumen function to favour the synthesis of microbial protein at the expense of propionic acid. Further quantitative evidence is required to determine if rumen fermentation differs between resistant and susceptible genotypes. The following chapter will investigate whether divergent selection lines partition nutrients differently between skeletal muscle and skin, organs and the gut immune response.

CHAPTER 5

NUTRIENT PARTITIONING OF SHEEP SELECTED FOR GENETIC DIFFERENCE IN RESISTANCE TO NEMATODE INFECTION

5.1 Introduction

Utilisation of nutrients for growth and wool production is usually impaired in sheep infected with nematode parasites. The decrease in productivity is thought to be due to a number of factors including a repartitioning of nutrients to the gastrointestinal tract for the immune response and tissue repair (Parkins & Holmes 1989), while body protein reserves mainly in the muscle, are broken down (MacRae 1993).

Symons & Jones (1975) reported that infection of sheep with *T. colubriformis* resulted in increased protein synthesis in the liver, decreased protein synthesis in muscle and reduced incorporation of amino acids to wool follicular homogenates. More recently, Yu *et al.* (2000) showed a 24% increase in leucine sequestration in the whole gastrointestinal tract and increased oxidation losses of leucine of 22 to 41% associated with infection of *T. colubriformis*. As a consequence of the repartitioning, availability of absorbed amino acids for peripheral tissue metabolism was reduced by 30%.

An increase in amino acid utilisation by the gut is likely to have a greater effect on essential amino acids such as cysteine which is required for synthesis of immunoglobulins, mucins and wool follicles (Colditz 2002, 2003). When supplemented with 2 g/d cysteine via abomasal infusion animals selected for high fleece weight had increased peripheral eosinophil concentrations and globule leukocytes in the abomasum, while infected with *H. contortus* and *T. colubriformis* (Miller *et al.* 2000). The increased demand for cysteine toward the gut immune response during a GI nematode infection, limits the availability for wool synthesis (MacRae *et al.* 1993). Barger *et al.* (1973) demonstrated that an infection with *T. colubriformis* depressed wool growth by 18%, but with cysteine supplementation wool growth increased by 33% in both infected and

uninfected sheep. The net result of repartitioning towards the gut immune response has been estimated to be up to 15% loss in productivity (Sykes 1994).

Given the impairment GI infection has on the utilisation of nutrients for production, it was hypothesised that the partition of nutrients between skeletal muscle, skin, internal organs and the gut immune response may differ among genotypes when challenged with *H. contortus*. Resistant genotypes may divert more metabolisable protein (MP) to the gut immune response and susceptible genotypes may have an increased supply of MP for tissue repair and production of plasma protein. The consequence of the effect of selection for parasite resistance on the tissue availability of amino acids could provide the basis for the small differences in production that resistant animals exhibit (Eady & Smith 2001, Kahn *et al.* 2003). The aim of this experiment was to investigate if divergent selection for resistance to *H. contortus* has produced correlated changes in partitioning of amino acid-nitrogen between tissues in the absence and presence of *H. contortus* infection.

5.2 Materials and methods

5.2.1 Experimental Design

The experiment was designed as a 3 x 2 factorial with 3 selection lines, selected for genetic difference in resistance to nematode infection and 2 infection levels, namely a trickle infection of Kirby strain (only available strain) *H. contortus* (INF) or maintained worm-free (NIL). Animals were stratified within selection line on the basis of bodyweight measured following adjustment to experimental conditions and then randomly allocated to infection treatment, position of pen within the animal house and experimental group. Two experimental groups (group 1; n =24, group 2; n = 18) were established, with day of infection delayed by 1 week in group 2. Creation of experimental groups was necessitated by a limited number of metabolic crates. Animals were housed in individual pens for 8 weeks post infection (*pi*) then moved to metabolic crates for 3 d, where animals were injected with a single dose of ^{15}N labelled duckweed directly into the abomasum by laparoscope technique. Nitrogen partitioning within the body was investigated with a bolus abomasal injection of ^{15}N duckweed, which has been demonstrated to uniformly label amino acids (Liu *personal communication*).

Faecal and urine outputs were measured following injection. Animals were euthanased at either 6 or 24 h after the injection to collect tissue samples for calculation of percentage recovery of ^{15}N in tissue and to determine abomasal worm counts.

5.2.2 Animals and housing

The study used 42 Merino weaner wethers selected randomly from the CSIRO increased resistance to *Haemonchus* (IRH) (n = 14), decreased resistance to *Haemonchus* (DRH) (n = 14) and unselected control (C) (n = 14) selection lines, (each line was represented by all 5 sire groups) (Woolaston *et al.* 1990). They were approximately 5 months of age at the beginning of the experiment with a mean ± s.d. bodyweight of 24.8 ± 3.42 kg. Animals were housed in individual pens and metabolic crates at the University of New England animal house. Before entering the animal house, each animal was drenched with Cydectin® (0.2 mg/kg moxidectin, Fort Dodge) and left in yards overnight to remove existing worm burdens. A faecal egg count was taken 7 days after drenching, confirming that all animals had zero counts. Animals were given 2 weeks to adjust to animal house conditions and then fed the formulated diet for a further 2 weeks prior to the experiment commencing.

5.2.3 Feed

The formulation and analysis of the experimental diet is given in Table 5-1. The diet was formulated to be balanced for major and trace minerals and pelleted to reduce the ability of animals to select individual ingredients in the diet. Animals were fed on a restricted basis (40 g fresh feed /kg bodyweight) calculated to allow a gain of 125 g/d bodyweight throughout the experimental period (Freer *et al.* 1997). Fresh food was offered once daily; there were no refusals.

5.2.4 Infection

Half of the animals from each selection line were infected with Kirby strain *Haemonchus contortus* L_3. An initial infective dose of 150 L_3/kg bodyweight was orally administered at the beginning of the infection period, followed by a trickle

infection of 250 L_3 three times per week on Monday, Wednesday and Friday (average total dose ± s.d. over the infection period of 9837 ± 499 L_3/sheep).

Table 5-1 Ingredients[1] and chemical analysis of experimental diet.

Ingredients (% per kg fresh matter)	Formulated diet
Oaten straw chaff	47.0
Lucerne chaff	40.0
Molasses	8.0
Cottonseed meal	5.0
Dry matter	91.8
Composition (per kg DM)	
Organic matter (g)	929.0
Digestibility[A] (g)	651.0
Ether extract (g)	43.0
Crude protein (g)	176.0
Calculated MP[B] (g)	86.0
Calculated ME[C] (MJ)	9.8
Sulphur (g)	2.1
Phosphorus (g)	3.0
Iron (mg)	338.3
Copper (mg)	3.0

[1] Minerals per kg dry matter were 4.2 g Ca, 2.4 g Mg, 22.0 g K, 1167 mg Na, 23 mg Zn, 52 mg Mn and 4 mg Mo. Feed was analysed for mineral concentrations using the Vista MPX radially viewed, simultaneous Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES).
[A] Based on pepsin cellulase wet chemistry.
[B] Calculated from equations of Freer *et al.* (1997).
[C] Calculated from algorithm supplied by FeedTest, Victorian Department of Primary Industries, Hamilton, VIC.

5.2.5 Animal Measurements

Animals were weighed in the morning prior to feeding, commencing at the start of the experiment and weekly thereafter. A single worm egg count per animal was measured at day 0 and 21 after the initial dose of infective larvae and weekly thereafter. A modified McMaster technique was used to count worm eggs. A sample of faeces (approximately 6 g) was taken weekly from each animal and immediately stored at -20°C for analysis of faecal blood loss. Animals were bled by jugular venepuncture into 5 ml K_3-EDTA Vacutainer® tubes at day 0 and weekly thereafter. Haematology parameters; red blood cell (RBC) count, haemoglobin, haematocrit, white blood cell (WBC) count, eosinophil concentration and mean corpuscular volume (MCV) were measured on whole blood using a Cell-Dyn 3500R haematology analyzer, (Abbott Diagnostics Division). Wool growth rate was measured using dyebands (Durafur black flakes, Imperial Chemical

Industries, Sydney) placed vertically on the left midside position of the animal. The dyebands were 10 cm in length and applied on day 1 and 28 (week 4 of infection). The dyeband sample was removed using Oster clippers No. 40 on day 42. Animals were shorn the following day and greasy fleeces with bellies weighed to calculate wool growth rate (g/d).

5.2.6 ^{15}N labelled duckweed injection

5.2.6.1 Pilot study

A small pilot study was initially conducted to develop the single dose labelling technique employed in this experiment. Three animals from the *Haemonchus* selection flock (one from each selection line), were housed in individual pens, maintained worm-free and fed the experimental diet for a period of 3 weeks. Animals were injected with 7.2 g of dried ^{15}N - duckweed (described in 5.2.6.3) in 100 ml of 9% NaCl solution, (8.7 mol ^{15}N/mol N and 29% crude protein), directly into the abomasum using a technique developed by Mr. G.C. Uphill, CSIRO Livestock Industries, Armidale NSW (description of the technique provided in section 5.2.6.4). To determine the optimum time for experimental sampling, animals were euthanased at either 4, 7 or 24 h after the injection. The sampling and processing of samples is described in section 5.2.8 and analysis of ^{15}N enrichment described in section 5.2.9. To ensure samples were homogenous, repeated measures of each sample were made on the TracerMass, Stable Isotope Analyser. The correlation coefficient from regression between the replicates was high (r = 0.999).

5.2.6.2 ^{15}N - duckweed

Duckweed (*Spirodela polyrrhiza*) was grown in a synthetic growth medium in a galvanized tank (diameter = 180 cm, height = 65 cm) filled to a depth of 25 cm with one litre stock solution (Table 5-2) to 80 litres tap water (Plate 1). The tank was placed outside with average minimum and maximum temperatures of 9°C and 23°C respectively. The stock solution contained $^{15}NH_4$ $^{15}NO_3$ with ^{15}N enrichment of 99.23% (Shanghai Research Institute of Chemical Industry Shanghai, China). The solution was adjusted to pH 7.0 with NaOH, before being mixed with tap water. A sparse covering of mature duckweed was placed into the tank of labelled solution. The tank was

replenished with stock solution one week after the first stocking and the duckweed allowed to grow for a further week. The harvested ^{15}N-labelled duckweed was freeze-dried for a period of 4-5 d and stored at 4°C (Plate 2). The ^{15}N enrichment of the labelled duckweed from the 1st and 2nd harvest was 7.4 and 11.3 mol ^{15}N/mol N and crude protein content of 35.5 and 33.3% respectively. Duckweed from the 1st and 2nd harvests were used for group 1 and group 2 animals respectively.

5.2.6.3 Preparation of ^{15}N - duckweed injection

The day prior to injection, freeze-dried duckweed was ground through a 1 mm sieve (Cyclotec 1093 sample mill, FOSS Tectator, Hoganas, Sweden) (Plate 3). The ground sample was then weighed (approximately 13.5 g/dose, average 57.6 mg ^{15}N group 1 and 86.4 mg ^{15}N group 2) into individual 250 ml bottles and 200 ml of pyrogen-free physiological saline solution (9% NaCl) was added. The solution was thoroughly mixed and kept at 4°C overnight.

Table 5-2 Chemical composition of the stock medium used to grow and label duckweed.

Substance	Concentration	Volume required for 1 L stock solution. (ml)
Macronutrients	g/*l*	
$MgSO_4.7H_20$	246	10
$CaCl_2.2H_20$	147	25
KH_2PO_4	135	5
$(^{14}NH_4)_2SO_4 + {}^{15}NH_4\,{}^{15}NO_3$*	96 + 10*	50
KCl	75	25
Micronutrients	mg/*l*	5
H_3BO_3	35.8	
$MnCl_2.4H_2O$	22.8	
$ZnSO_4.7H_2O$	2.8	
$Na_2MoO_4.2H_2O$	1.1	
Fe-EDTA	mg/*l*	200
$FeCl_3.6H_2O$	484	
EDTA	1500	
Ultra high purity water		680

* ^{15}N enrichment of 99.23%

5.2.6.4 Laparoscope injection into the abomasum

The afternoon prior to injection, feed and water were withheld from animals to reduce abdominal content. The following morning starting at 8.30 am animals were individually moved to an adjacent surgery and given a subcutaneous injection of local anaesthetic with 5 ml of Lignocaine 20® (20 mg/ml lignocaine hydrochloride, Troy laboratories) approximately 10 min prior to the procedure. A modified artificial insemination cradle was used to restrain the animal. The cradle was elevated at the anterior end, to encourage the gut to fall and make the abomasum easier to locate. The laparoscope cannulae were inserted 5 cm below the base of the sternum and 2 cm from either side of the midline. The abdominal cavity was slightly inflated with carbon dioxide for a larger field of view. Once the abomasum was located a modified insemination pipette (14 gauge IV catheter needle) was introduced through the cannula. The abomasum was pierced using a stabbing action with the pipette needle (Plate 4). The duckweed solution was then drawn into four 50 ml syringes and administered through the pipette (Plate 5). The bottle and syringes were rinsed with a further 50 ml of the physiological saline solution and injected. Any bleeding wounds were closed with michel clips. Animals were given access to feed and water immediately following the procedure. The majority (>90%) of animals consumed all of the experimental diet upon return to the metabolic crates.

5.2.7 Euthanasia and animal dissection

Animals within treatment groups were allocated at random to be euthanased either 6 or 24 h after injection. Prior to euthanasia, a sample of blood was taken by jugular venepuncture into a 10ml K_3-EDTA Vacutainer® tube. Animals were euthanased using a captive bolt pistol followed by exsanguination. Following bleeding out, skin was removed firstly around the neck, brisket and forelegs. The brisket and neck were carefully cut along the midline to remove the thymus, located at the base of the neck and overlying the heart. Following removal of the thymus, the skin around the hindlegs was removed to expose the tendons of the hocks, used to hang the carcass. The legs were removed at the joint immediately proximal to the long cannon bone. Once the animal was hanging the entire skin was removed and the body was prepared for evisceration. The head was removed along the lower jaw and the rectum was cut away to allow it to fall into the pelvic cavity. The abdominal wall was carefully cut along the

midline and diaphragm severed to expose the viscera to allow it to be collected into a tray.

Plate 1 Galvanised tanks for growth and labelling of duckweed (*Spirodela polyrrhiza*).

Plate 2 Harvesting of duckweed after two weeks of growth

Plate 3 Freeze-dried sample of duckweed and sample ground through a 1 mm sieve

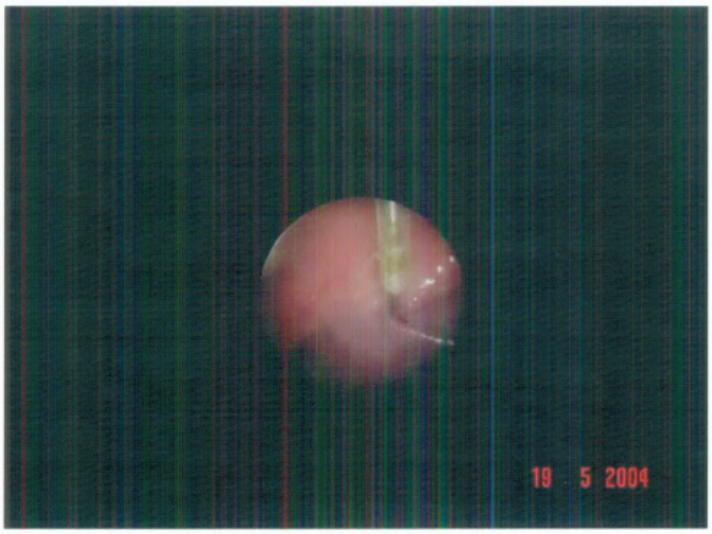


Plate 4 View through the laparoscope cannula, the pipette needle inserted through the abomasal wall for duckweed injection

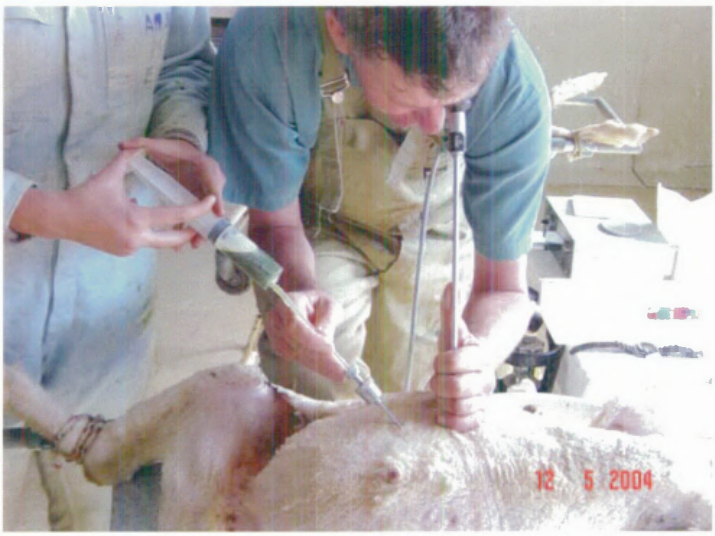


Plate 5 Administering labelled duckweed solution through pipette

5.2.8 Sampling and processing

The carcass was weighed for hot weight, the front legs tied upwards against the body and the carcass hung overnight in a cooled room at 10°C. Cold carcass weight was measured the following day. Dressing percentage was calculated from cold carcass weight divided by bodyweight.

Total collection of urine and faecal output was taken from all animals in the period between injection of ^{15}N duckweed and euthanasia. Collection occurred at 6 h for all animals and again at 12 and 24 h for those animals euthanased at 24 h. A subsample of approximately 60 g of wet faeces and 50 ml of urine from each collection period was immediately stored at -20°C and kept for ^{15}N analysis. Blood was taken by jugular venepuncture at the same time points as faeces and urine collection. Whole blood was centrifuged (2,500 *g* for 20 min) and plasma collected and stored at -20°C to measure ^{15}N enrichment. A subsample (approximately 40 g) of *semimembranosus* muscle was taken from each hind limb to represent ^{15}N in muscle. The *semimembranosus* muscle was chosen for its fast uptake of nutrients and aerobic, red muscle type. The entire skin was weighed and then laid flat to subsample 4 regions, one sample (5 x 5 cm) from each shoulder and a sample from the left and right midside position. These sites were chosen for their known variation in wool fibre diameter (Whiteley, 1972). The remaining wool was clipped closely to the skin (leaving approximately 3 mm of wool), using Oster clippers No. 40 discarded and the four samples combined for ^{15}N analysis in skin.

The gastrointestinal tract was separated and the required organs weighed individually for total wet organ weight and then sub-sampled (approximately 10 g). The spleen and liver were sampled from the centre of the organ, each kidney was sampled from the inferior and superior segments and the heart was sampled at the apex. The largest mesenteric lymph node and the total abomasal lymph nodes were collected for analysis. The contents of the small intestine were removed and the organ carefully washed and weighed. The first metre of the duodenum was cut open and the mucosa stripped using a glass slide. Weights of mucosa and smooth muscle were taken to calculate the proportion of mucosa to smooth muscle. The ratio of mucosa to smooth muscle in the duodenum was assumed to be the same for the total small intestine. In a separate

sample, equal portions of duodenum, jejunum and ileum were taken and weighed collectively for total small intestine ^{15}N analysis. The abomasum was removed ensuring total contents were maintained and the organ weighed with contents. The contents were then carefully collected and sub-sampled (approximately 50 ml) for ^{15}N measure and the remaining kept for total worm count. The abomasum was then rinsed with 100 ml of warm tap water. The empty abomasum was cut along the greater curvature and laid flat before being thoroughly washed to remove larvae and adult worms and washings added to worm count collection. The corners of the abomasum were removed to leave a central section (6 x 6 cm) for sampling. The mucosa was stripped using a glass slide and weighed to calculate the proportion of mucosa to smooth muscle.

Muscle, skin and organ samples were immediately placed on ice followed by storage at -20°C. The frozen samples were chosen at random to be freeze-dried in groups of 50 for 5-7 days. On removal from the freeze drier, samples were placed into a 40°C oven for 48 h and then weighed for approximate dry matter (freeze drying was ~ 95% dry matter). The samples were then ground into a fine powder using a blunt blade grinder (Yellowline, Kika-Werke, Germany). The grinder blade and stainless steel bowl were thoroughly cleaned between each sample with 95 % ethanol.

5.2.9 ^{15}N enrichment analysis

The ground tissue samples were carefully weighed (approximately 1.50 mg) into tin capsules (8 x 5 mm, Alpha Resources Australia Pty Ltd), folded and rolled into tight balls for analysis of ^{15}N enrichment. The samples (96 per run) were loaded into an automatic N analyser (Carlo Erba Instruments, NA 1500 Nitrogen, Carbon Analyser), which oxidizes the sample to nitrogen oxide in a combustion furnace at 1030°C, then reduces the N_2O to N_2 through a reduction column at 500°C. The N_2 is passed through a filter which absorbs the moisture from combustion. The elemental nitrogen enters a chromatographic column for separation according to molecular size and is then transferred to a mass spectrometer (TracerMass, Stable Isotope Analyser, Europa Scientific, Pfeiffer/Balzers), used to estimate ^{15}N abundance in the nitrogen sample.

The samples were randomly loaded into the autosampler following a series of wheat flour samples used to prepare the instrument. This begins with a 'bypass' sample, which

acts as a system stabiliser, a 'scan' sample used to calibrate the high voltage signal of the instrument, three empty tin capsules (blanks) which act as a baseline (0.365 mol ^{15}N/mol N) and subtracted from each sample prior to calculating the final value, followed by two condition samples which prime the instrument before standards. The five standards (wheat flour 1.70 % N and 0.365 ^{15}N enrichment) were weighed to contain about 17, 85, 170, 255 and 340 μg N. Similar sets of standards were processed in the middle of the 96 sample run and at the end. These standards were used to account for any 'drift' in the instrument resulting from changes in conditions throughout the day. To detect any day to day variation of the instrument, 2 samples were used as internal controls. The coefficients of variation (n = 12) for ^{15}N enrichment of these samples were 1.5 and 0.87 mol ^{15}N/mol N and for nitrogen % 3.24 and 2.32. Six animals (one from each treatment group) were free from ^{15}N label and euthanased at 6 and 24 h and sampled as base measurements. These values were subtracted from each organ enrichment percentage.

5.2.10 Kjeldahl nitrogen analysis

To determine nitrogen content of urine and plasma, samples were processed by the Kjeldahl method (AOAC, 1980) and the end product, ammonia salt, used for ^{15}N enrichment analysis. The procedure in brief, was as follows.

5.2.10.1 Digestion

Approximately 0.2 ml of urine and plasma sample were weighed into distillation flasks with one Kjeldahl catalyst titanium tablet (1g potassium sulphate, 0.03 g hydrated cupric sulphate, 0.03 g titanium dioxide, VWR International Ltd, England) and 3 ml of concentrated H_2SO_4 acid. The flasks were then placed into heated blocks (Digestion system – 1007 Digester, Tacator) at 350°C for 2 h. The samples were removed from heat and allowed to cool before approximately 5 ml of ultra high purity water was added.

5.2.10.2 Steam distillation

Ammonia–nitrogen was recovered from each sample by steam distillation under alkaline conditions. Before distillation, 3 drops of universal indicator was added to each

flask and a sufficient volume of 50 % (w/v) NaOH added to create a highly alkaline sample. A water blank and 5 ml standard of $(NH_4)_2 SO_4$ (0.2 mg N/ml) were distilled before every 18 samples. The sample was collected into 3 ml of 0.025 M H_2SO_4 for a period of 3.5 min. The distillation apparatus was cleaned between each sample by distilling 15 ml of 95 % ethanol.

5.2.10.3 Titration

The distillate was back-titrated to pH 5, using an auto-titrator (PHM82 Standard pH meter, TTT80 Titrator, ABU80 Autoburette, Radiometer, Copenhagen), with standard solution of 0.025 M NaOH. If the sample was not acidic (pH 2-3) before titration a further 3 ml of 0.025 M H_2SO_4 was added to the distillate. After titration 200 µl of 0.025 M H_2SO_4 was added to acidify the sample before drying at 80°C for 48 h in an ammonia-free oven.

5.2.10.4 Preparation of samples for ^{15}N analysis

The dried ammonia salt sample was re-suspended in 500 µl of ultra high purity water and mixed. A 50 µl sample (calculated to contain approximately 200 µg N) was transferred into tin capsules and dried in a vacuum desiccator using concentrated H_2SO_4. The tin capsules were then prepared for ^{15}N analysis as described for the tissue samples. The N % used for ^{15}N calculation of urine and plasma was calculated from the Kjeldahl method.

5.2.11 Calculation of percentage recovery of ^{15}N

^{15}N enrichment of the whole organ and percentage recovery were calculated using the following equations:

$$\text{Mass of } ^{15}N = \text{total N (g) of organ (DM)} \times ^{15}N \text{ percent}$$

where ^{15}N percent was adjusted for a background value of 0.365%

$$\text{Percentage recovery} = \frac{\text{mass of } ^{15}N \text{ in organ (mg)}}{\text{apparent absorbed } ^{15}N \text{ (mg)}} \times 100$$

where the apparent absorbed ^{15}N was calculated from the 24 h apparent digestibility of the ^{15}N abomasal dose.

5.2.12 Computer-aided tomography

Carcass composition was estimated using computer-aided tomography (CATSCAN Hitachi CT-W400; 120 kV at 100 mA, 420 mm field of view and 4.5 sec scan time). The distance between each image was 40 mm and captured in 512 x 512 pixel grey scale bitmap and edited using Micrographics Picture Publisher. AUTOCAT and AUTOCALC software (Neville Jopson, Agresearch, Invermay NZ, 1996) were used to determine the areas of fat, muscle and bone by the grey scale values (fat 30-125, muscle 126-200 and bone 201-255) and calculate the total mass of fat, muscle and bone from the sum of all images.

5.2.13 Plasma volume

To calculate the pool size of plasma nitrogen, the plasma volume was determined for each animal using Evan's Blue (dye T1824) marker (MacFarlane *et al.* 1959). Animals were initially bled by jugular venepuncture into a 10 ml K_3-EDTA tube for base values. The Evan's Blue (EB) solution (10 mg/ml) was injected into the jugular, through a 21 G winged infusion set (Surflo® Terumo Pty Ltd, Australia) and washed through with approximately 5 ml of physiological saline. Time was recorded when the entire 2 ml dose was injected and animals re-bled at 15, 35 and 60 min following injection. All blood samples were centrifuged at 2,500 g for 20 min and the plasma collected. Standards were made from a bulk sample of plasma with Evans blue (20 µg/ml) and serially diluted to 2.5 µg/ml. A spectrophotometer (Shimadzu UV-1201) was used to measure absorbance of the standards and unknowns at 620 nm. The absorbance of the pre-injection plasma was subtracted from the dose samples and the concentration read from the standard curve (r^2 = 0.9924). The concentrations were then $\log_{10}$ transformed and plotted against time.

$$\text{Plasma volume (ml)} = \frac{EB_{concentration} \times EB_{volume}}{\text{Plasma concentration}_{t=0}}$$

5.2.14 Worm counts

Four 3 % (60 ml) aliquots were taken from the well-mixed total abomasum (2 *l*) wash and stored at -20°C until analysis. The samples were filtered through a 200 µm sieve with a steady flow of tap water to remove unwanted digesta. All worms present in the 3 % sub-samples were differentiated and counted into adult male, adult female and larvae. The average of the counts were multiplied by the aliquot factor (100/3) for total count. The fecundity of female worms was calculated from the worm egg count (epg) multiplied by the faecal output, collected prior to euthanasia, divided by the average female worm count of each animal.

5.2.15 Faecal haemoglobin loss

Faecal blood loss was determined indirectly following conversion of the haemoglobin in faeces to a fluorescent coproporphyrin. The procedure was adapted from the "HemoQuant" test (Schwartz, *et al.* 1983). The method was as follows:

Faecal samples were thawed and well mixed and approximately 30 mg weighed into two 5 ml polypropylene tubes. 2 ml of oxalic acid reagent (2.5 M oxalic acid, 90 mM iron sulphate, 50 mM uric acid and 50 mM mannitol) was added to one tube, for total haemoglobin determination and 2 ml of 1.5 M citric acid to the other tube to determine the fraction of porphyrins already converted in the gastrointestinal tract. The tubes were placed into an 80°C water bath for 2 min then vortex-mixed for 15 s. Following mixing, the samples were heated for 30 min in a 100°C water bath and vortex-mixed for a further 15 s and allowed to settle for 2 min in the 80°C water bath. The samples were subsequently run through a 3-step purification process using a solvent-extraction system. The steps were; (1) 250 µl of supernatant was transferred into a glass tube with 1500 µl of ethyl acetate/acetic acid (10/1, v/v) and 500 µl of 3 M potassium acetate and vortex-mixed for 15 s between each addition: (2) 625 µl of the upper phase from step 1 was transferred to a glass tube containing 250 µl of *n*-butanol and 1900 µl of 3 M potassium acetate in 1 M potassium hydroxide solution and vortex-mixed for 15 s; and (3) 250 µl of the upper phase from step 2 was transferred to another glass tube with 700 µl of 2 M orthophosphoric acid and acetic acid (9/1, v/v). The sample was finally vortex mixed for 15 s. The bottom phase from step 3 was carefully transferred, using a glass pipette, into a clean glass tube. The samples were covered and stored at 4°C overnight. The following morning 100 µl/well of sample was dispensed in triplicate into a 96 well

plate (NUNC® Black Maxisorp SH). Each plate included a sample blank (orthophosphoric acid:acetic acid) and a standard blank (1.5 M HCl) in triplicate. The standards were made using 50 µg/*l* solution of coproporphyrin (Porphyrin Products, Logan, USA) in 1.5 M HCl at a 1/20 dilution (25 ng/ml). The standards were serially diluted to 0.39 ng/ml. For purposes of quality control, haemoglobin was also assayed in a stable form, as cyanmethemoglobin, by converting and storing haemoglobin in Drabkin's solution (Sigma Aldrich, St Louis, USA). A 40 µl sample was assayed at three concentrations, 0.5 g/*l*, 1 g/*l* and 2 g/*l* with faecal samples and placed on each plate in triplicate. The plates were read using a Spectra Max Gemini fluorescence spectrophotometer, set at excitation wavelength of 402 nm and 653 nm fluorescence emission. To calculate coproporphyrin concentrations from fluorescence emission values, the sample concentration was read from the standard curve (ng/ml), multiplied by the dilution factor (6300) and by 0.7 for total coproporphyrin (µg). This value is equivalent to hemoglobin concentration in faeces.

5.2.16 Calculation of protein turnover

The whole body protein turnover; flux, synthesis rate and degradation rate were calculated using the following equations (Waterlow *et al.* 1978):

$$\text{Flux (Q) (g/d)} = (D/G) \times 6.25$$

$$\text{Synthesis Rate (SR) (g/d)} = Q - D$$

$$\text{Degradation rate (DR) (g/d)} = SR - \text{protein retention}$$

where D is the N excretion in urine (g/d) and G the cumulative recovery of ^{15}N dose in urine at 24 h (dose is apparent absorbed ^{15}N).

5.2.17 Statistical Analysis

All statistical analyses were performed using the SAS computer program (SAS Institute Inc 1999-2001). General Linear Models (GLM) were used to analyse the significance of selection line (IRH, DRH and C), infection (NIL and INF) and the interaction (line x infection) between these effects. The models used repeated measures analysis of variance for bodyweight, WEC, faecal blood loss and haematology parameters. Initial

bodyweight was a significant covariate for subsequent bodyweight measures (P = 0.0002). The one week difference between groups was significant for bodyweight (P <0.0001) and remained in the analysis.

Least square means (ls mean) ± standard errors (s.e.) are presented for all measured parameters. Data for WEC, worm counts and circulating white blood cell (WBC) counts were cube-root transformed to normalise prior to analysis and are presented as back-transformed means with back-transformed 95 % confidence intervals (*ci*). Faecal blood loss, eosinophil counts and mean corpuscular volume were transformed using $\log_{10}$ with antilog means and antilog 95 % confidence intervals. Red blood cell (RBC) counts, haemoglobin and haematocrit were square-root transformed with back-transformed means and 95 % confidence intervals. The base sample (day 0) for white blood cells and eosinophils were significant covariates for subsequent samples. Organ weights were analysed as dry matter weights and also scaled for bodyweight.

The percentage recovery of ^{15}N was arc-sine (ASIN) transformed and analysed. Data presented as SIN back-transformed means with back-transformed 95% confidence intervals (*ci*). Data from six animals were removed from the analysis as the animals either had duckweed solution present in the body cavity, a high percentage of ^{15}N enrichment in abomasal contents or a low total recovery (< 75% recovery). The mean total recovery of ^{15}N for the animals used in the analysis was 90%.

5.3 Results

5.3.1 Organ weights

Dried organ weights were proportional to fresh weights and presented because they were used in the calculation of ^{15}N recovery. Fresh weights are provided in Appendix 1 and organ weights adjusted for bodyweight in Appendix 2. Dried organ weights were similar among selection lines, with the exception of spleen weight which was significantly (P = 0.002) lower in IRH animals compared to C and DRH lines (Table 5-3). The difference remained after adjustment for bodyweight. Skin weight was higher (P =0.002) in the C line, than in DRH and IRH lines, when adjusted for bodyweight. Liver and abomasum weights were heavier in DRH animals however this difference was removed by adjustment for bodyweight. *H. contortus* infection affected both abomasum and abomasal lymph node weights. Both organs increased in weight significantly (P =

0.004, <0.0001 respectively) in response to infection, while other organ weights were unaffected (Table 5-4). The interaction between the effects of selection line and infection was not significant for organ weights, apart from a lower spleen weight in C animals (P = 0.011) from 13.9 ± 0.74 g in NIL to 10.6 ± 0.74 g in INF animals. While the spleen weight in DRH animals tended to increase in response to infection and the IRH line remained unchanged.

5.3.2 Carcass composition

Cold carcass weight tended (P = 0.084) to be lower in C animals compared to DRH animals, but did not differ in response to infection or the interaction between the effects of selection line and infection. Dressing percentage was higher (P = 0.030) in IRH animals compared to C, but did not differ significantly from DRH. Dressing percentage was unaffected by infection and the interaction between the effects of selection line and infection was not significant. Muscle mass was lower (P = 0.018) in C compared to DRH and IRH animals (Table 5-5). Bone mass tended (P = 0.072) to be higher in DRH animals, compared to IRH and C lines, but fat mass did not differ between selection lines. Expression of muscle, bone and fat as a proportion of carcass weight showed no significant difference among selection lines, indicating effects were driven by differences in growth. Carcass composition did not differ in response to infection or the interaction between the effects of selection line and infection.

Table 5-3 Dried organ weights (g) of Merino weaner wethers, from lines selected for either increased resistance to *Haemonchus* (IRH), decreased resistance to *Haemonchus* (DRH) or random-bred control (C)

	IRH	DRH	C		
Organ	ls mean	ls mean	ls mean	pooled s.e.	P value
Spleen	9.4[a]	11.5[b]	12.2[b]	0.52	0.002
Thymus	10.3	11.4	9.6	0.87	0.360
Liver	166.2[ab]	177.6[a]	153.0[b]	6.60	0.041
Kidney	21.0	21.6	20.6	0.83	0.683
Heart	30.2	31.1	30.5	1.17	0.842
Abomasal lymph node	0.51	0.56	0.47	0.05	0.370
Mesenteric lymph node	5.1	5.2	5.3	0.38	0.945
Abomasum	31.0[a]	37.3[b]	31.2[a]	1.81	0.030
Abomasal mucosa	1.2	1.4	1.1	0.12	0.288
Abomasal smooth muscle	29.8[a]	36.0[b]	30.1[a]	1.82	0.037
Small intestine (SI)	99.3	100.9	105.2	5.62	0.747
SI mucosa	22.6	23.1	26.6	2.53	0.487
SI smooth muscle	81.0	92.0	91.4	4.03	0.109
Skin	715.7[a]	782.5[a]	838.7[b]	40.68	0.116

Within rows, values with a common suffix or no suffix do not differ significantly (P>0.05).

Table 5-4 Abomasum and abomasal lymph node weights (g) of Merino weaner wethers either maintained worm free (NIL) or trickle infected with *H. contortus* (INF).

	NIL	INF		
Organ	ls mean	ls mean	pooled s.e.	P value
Abomasal mucosa	1.1[x]	1.4[y]	0.10	0.041
Abomasal smooth muscle	28.8[x]	35.1[y]	1.49	0.005
Abomasal lymph node	0.3[x]	0.7[y]	0.04	< 0.0001

Within rows, values with a common suffix do not differ significantly (P>0.05).

Table 5-5 Cold carcass weight (kg) dressing (%) and muscle, fat and bone weights (kg) of Merino weaner wethers, from lines selected for either increased resistance to *Haemonchus* (IRH), decreased resistance to *Haemonchus* (DRH) or random-bred control (C)

	IRH	DRH	C		
Measurement	ls mean	ls mean	ls mean	pooled s.e.	P value
Carcass weight	13.2	13.9	12.3	0.52	0.084
Dressing %	41.3[a]	40.6[ab]	39.5[b]	0.27	0.030
Muscle	7.7[a]	8.1[a]	7.0[b]	0.25	0.018
Fat	3.4	3.4	3.0	0.25	0.453
Bone	2.2	2.3	2.1	0.07	0.072

Within rows, values with a common suffix or no suffix do not differ significantly (P>0.05).

5.3.3 Partitioning of ^{15}N

Percentage recovery of ^{15}N in various organs was similar among selection lines, with the exception of SI mucosa and smooth muscle which had a significantly (P = 0.026, 0.036 respectively) higher recovery in C animals compared to IRH (Table 5-7). Recovery of ^{15}N in urine tended (P = 0.117) to be higher in IRH animals compared to DRH and C with the contrast between IRH and DRH reaching statistical significance (P = 0.040). The contrast between IRH with DRH, also indicated that IRH animals had a higher (P = 0.095) percentage recovery of ^{15}N to the thymus. *H. contortus* infection tended to reduce recovery of ^{15}N to kidney (P = 0.089) and SI smooth muscle (P = 0.028). Infected animals had a higher ^{15}N recovery in abomasal lymph node (P <0.0001) (Table 5-8). Percentage recovery of ^{15}N from 6 to 24 h did not change in spleen, thymus, abomasal and mesenteric lymph nodes and skin but decreased over time in other measured organs (Table 5-8). The interaction between the effects of selection line and infection was not significant for measured parameters, except for spleen (P = 0.014). The recovery of ^{15}N in the spleen in response to infection decreased in C animals, increased in DRH and remained unchanged in IRH. The contrast which examined ^{15}N uptake to the abomasal smooth muscle among the lines in response to infection indicated that IRH animals tended to have a greater (P = 0.076) percentage recovery of ^{15}N and significantly (P = 0.046) greater total recovery. Total ^{15}N uptake for IRH NIL and IRH INF was 0.60 ± 0.10 and 0.87 ± 0.11 mg ^{15}N respectively, while DRH and C were unchanged in response to infection with an average for both of 0.79 ± 0.12 mg ^{15}N.

5.3.4 Protein turnover and N balance

Protein flux, synthesis and degradation rate did not differ significantly among selection lines or between nil and infected animals (Table 5-6). N balance tended to differ between selection lines (P = 0.068). The contrast between DRH and IRH lines, indicated that DRH animals had a significantly higher (P = 0.025) N balance. N balance did not differ in response to infection (Table 5-6). N digestibility was significantly (P = 0.009) higher in DRH than IRH and C lines (Table 5-6).

Table 5-6 Protein flux (g/d), synthesis rate (g /d), degradation rate (g /d), N balance (g N/d) and N digestibility (%) of Merino weaner wethers, from lines selected for either increased resistance to *Haemonchus* (IRH), decreased resistance to *Haemonchus* (DRH) or random-bred control (C) and either maintained worm free (NIL) or trickle infected with *H. contortus* (INF).

Group	**Flux**		**Synthesis**		**Degradation**		**N balance**		**Digestibility**	
	ls mean	s.e.	ls mean	s.e.	ls mean	s.e.	ls mean	s.e.	ls mean	s.e.
IRH	270.5	16.61	259.7	16.77	251.2	16.39	8.5[a]	1.34	69.2[a]	0.94
DRH	298.9	18.30	288.9	18.48	275.4	18.06	13.4[b]	1.48	74.7[b]	1.05
C	263.2	16.61	253.8	16.77	244.3	16.39	9.4[ab]	1.34	71.4[a]	0.94
NIL	292.8	13.56	282.3	13.70	271.0	13.38	11.2	1.10	72.0	0.77
INF	262.2	14.50	252.6	14.64	242.9	14.30	9.7	1.17	72.0	0.83
Statistics										
Line	P = 0.351		P = 0.361		P = 0.439		P = 0.068		P = 0.009	
Infection	P = 0.146		P = 0.163		P = 0.175		P = 0.347		P = 0.988	

Within columns, values with a common suffix or no suffix do not differ significantly (P>0.05).

Table 5-7 Percentage recovery (± 95% *ci.*) of ^{15}N in organs urine output in Merino weaner wethers, from lines selected for either increased resistance to *Haemonchus* (IRH), decreased resistance to *Haemonchus* (DRH) or random-bred control (C)

	IRH			DRH			C			
Organ	ls mean	± *ci*		ls mean	± *ci*		ls mean	± *ci*		P value
Spleen	0.31	0.034	0.035	0.38	0.040	0.043	0.40	0.043	0.046	0.194
Thymus	0.47	0.053	0.056	0.35	0.048	0.052	0.39	0.053	0.057	0.228
Liver	7.96	0.284	0.289	7.69	0.301	0.306	7.21	0.306	0.312	0.226
Kidney	1.11	0.106	0.111	1.24	0.120	0.126	1.20	0.121	0.130	0.722
Heart	0.77	0.113	0.122	0.50	0.097	0.107	0.57	0.109	0.120	0.216
Abomasal lymph node	0.017	0.002	0.002	0.018	0.002	0.003	0.016	0.002	0.003	0.766
Mesenteric lymph node	4.53	0.390	0.406	4.57	0.420	0.439	4.50	0.437	0.458	0.995
Abomasal mucosa	0.105	0.011	0.012	0.114	0.013	0.014	0.115	0.013	0.014	0.815
Abomasal smooth muscle	1.30	0.144	0.153	1.35	0.158	0.167	1.39	0.168	0.178	0.929
Small intestine (SI)	8.50	0.468	0.480	7.67	0.479	0.493	9.18	0.545	0.560	0.132
SI mucosa	2.98[a]	0.314	0.331	3.39[ab]	0.359	0.379	4.52[b]	0.434	0.458	0.026
SI smooth muscle	5.73[a]	0.483	0.503	5.62[a]	0.541	0.537	7.63[b]	0.622	0.646	0.036
Skin	20.33	1.422	1.460	19.57	1.505	1.550	20.01	1.590	1.639	0.936
Muscle	15.31	1.075	1.106	14.90	1.141	1.178	14.18	1.170	1.212	0.779
Plasma	8.22	0.742	0.773	10.11	0.876	0.911	8.86	0.863	0.903	0.281
Urine	17.85	1.661	1.727	12.80	1.548	1.632	15.20	1.718	1.835	0.117

Within rows, values with a common suffix or no suffix do not differ significantly (P>0.05).

Table 5-8 Percentage recovery (± 95% *ci.*) of ^{15}N in organs and urine output in Merino weaner wethers, either maintained worm free (NIL) or trickle infected with *H. contortus* (INF) and euthanased either 6 or 24 h after injection.

Organ	NIL		INF			6 h		24 h		
	ls mean	± ci	ls mean	± ci	P value	ls mean	± ci	ls mean	± ci	P value
Spleen	0.37	0.030 0.031	0.36	0.035 0.037	0.896	0.38	0.037 0.039	0.35	0.028 0.029	0.438
Thymus	0.43	0.041 0.043	0.37	0.044 0.047	0.360	0.40	0.048 0.051	0.40	0.038 0.040	0.983
Liver	7.92	0.228 0.231	7.32	0.261 0.265	0.102	8.91^{x}	0.295 0.300	6.42^{y}	0.200 0.203	<.0001
Kidney	1.31	0.093 0.096	1.06	0.099 0.104	0.089	1.70^{x}	0.130 0.135	0.76^{y}	0.068 0.071	<.0001
Heart	0.55	0.077 0.083	0.67	0.101 0.109	0.352	0.82^{x}	0.115 0.124	0.43^{y}	0.066 0.071	0.008
Abomasal lymph node	0.01^{x}	0.001 0.001	0.03^{y}	0.003 0.003	<.0001	0.02	0.002 0.002	0.02	0.002 0.002	0.845
Mesenteric lymph node	4.89	0.326 0.337	4.19	0.358 0.373	0.173	4.40	0.378 0.395	4.68	0.309 0.319	0.581
Abomasal mucosa	0.10	0.009 0.010	0.12	0.012 0.012	0.204	0.16^{x}	0.014 0.015	0.07^{y}	0.007 0.008	<.0001
Abomasal smooth muscle	1.33	0.118 0.123	1.37	0.141 0.149	0.849	1.88^{x}	0.171 0.179	0.90^{y}	0.091 0.099	<.0001
Small intestine (SI)	8.75	0.382 0.389	8.13	0.437 0.448	0.301	10.37^{x}	0.504 0.516	6.69^{y}	0.326 0.333	<.0001
SI mucosa	3.65	0.281 0.291	3.55	0.327 0.342	0.816	4.91^{x}	0.396 0.412	2.49^{y}	0.224 0.235	<.0001
SI smooth muscle	7.06^{x}	0.430 0.443	5.57^{y}	0.454 0.473	0.028	7.61^{x}	0.545 0.563	5.10^{y}	0.356 0.369	0.001
Skin	19.99	1.138 1.163	19.95	1.346 1.381	0.982	18.76	1.358 1.397	21.20	1.126 1.148	0.187
Muscle	14.77	0.853 0.874	14.82	1.011 1.040	0.966	18.08^{x}	1.134 1.163	11.78^{y}	0.748 0.769	<.0001
Plasma	8.79	0.617 0.638	9.31	0.749 0.778	0.602	5.66^{x}	0.612 0.644	13.14^{y}	0.715 0.732	<.0001
Urine	14.05	1.213 1.259	16.45	1.534 1.593	0.237	8.73^{x}	1.192 1.271	23.14^{y}	1.435 1.466	<.0001

Within rows, values with a common suffix or no suffix do not differ significantly (P>0.05).

5.3.5 Parasitology

Worm egg counts of the IRH animals were lower than the DRH line throughout the 8 week infection period with differences reaching significance at week 8 (P = 0.038). The DRH and C lines did not differ during this time (Figure 5-1). Adult male and female worm counts and larval (L_4) counts recovered from the abomasum differed significantly among selection lines (P = 0.005) at week 8 of infection (Figure 5-2). The correlation coefficient of the regression between worm egg count and total worm count was 0.9 (P < 0.0001). In contrast to worm counts, the fecundity of female worms did not differ significantly between selection lines (P = 0.577), even though female worms in DRH and C animals had more than twice the fecundity of female worms in IRH animals. Fecundity of female worms for the IRH, DRH and C lines was 1308, 2850 and 3782 (eggs/worm/day) respectively.

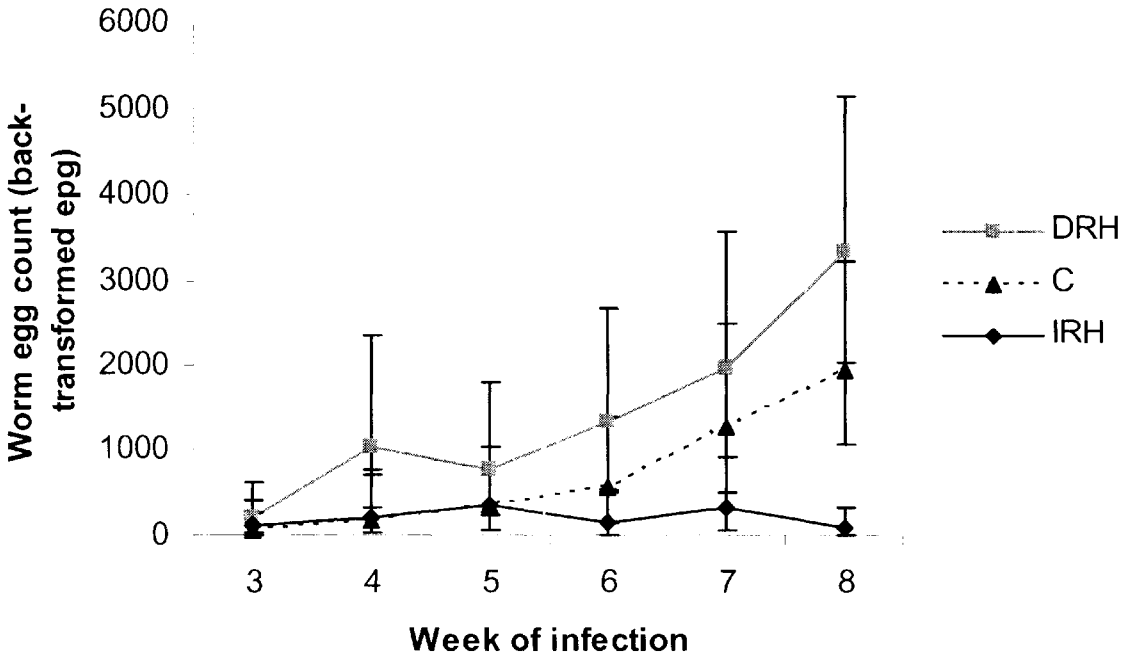


Figure 5-1 Back-transformed worm egg counts (ls means ± 95% *ci.*) of weaner wethers from the increased resistance to *Haemonchus* (IRH), decreased resistance to *Haemonchus* (DRH) and random-bred control (C) selection lines, over an 8 week trickle infection.

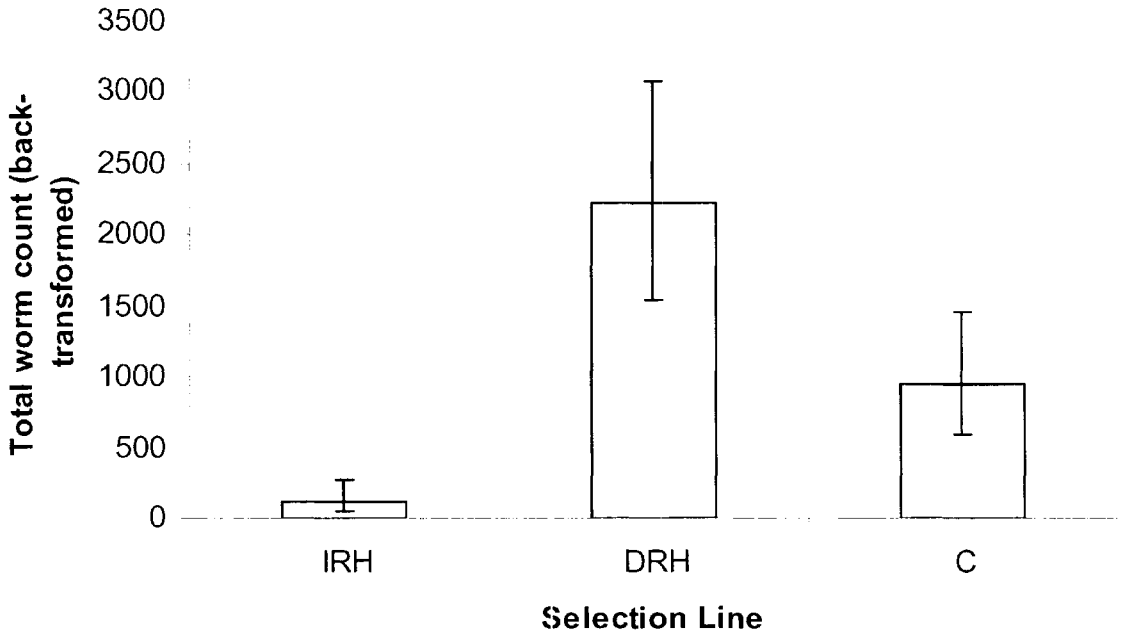


Figure 5-2 Total back-transformed worm counts (± 95% *ci.*) from the increased resistance to *Haemonchus* (IRH), decreased resistance to *Haemonchus* (DRH) and random-bred control (C) selection lines, at week 8 of infection.

5.3.6 Haematology

H. contortus infection decreased red blood cell (RBC) counts (P = 0.011) (Figure 5-3), haematocrit (P = 0.020) and haemoglobin (P = 0.009) (Table 5-9). Selection lines differed for RBC (P = 0.017) (Figure 5-4), haematocrit (P = 0.037) and haemoglobin (P = 0.008). Haematocrit and haemoglobin concentration were lower in DRH animals than IRH and C lines (Table 5-9). The interaction between the effects of selection line and infection was significant for haematocrit (P = 0.032) and haemoglobin (P = 0.034), such that when DRH and C animals were infected there was a dramatic decline in values, compared to no effect of infection in IRH group. Eosinophil counts were higher (P < 0.0001) in INF animals compared to NIL (Figure 5-5) and differed among selection lines. Eosinophil counts of IRH animals were higher than DRH and C lines, when averaged over time (P = 0.004) (Figure 5-6). In contrast circulating white blood cells (WBC) did not differ among selection lines (P = 0.153) or in response to infection (P = 0.221). Mean corpuscular volume increased (P = 0.049) in response to infection, but did not differ among selection lines (P = 0.351) (Figure 5-7).

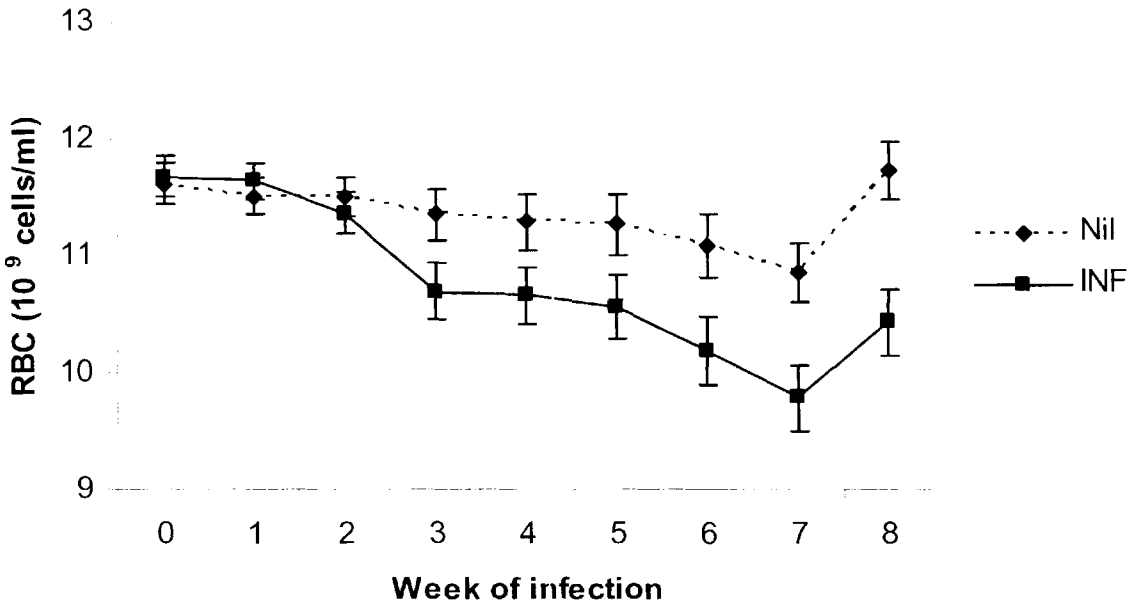


Figure 5-3 Red blood cell (RBC) count (back-transformed ls mean ± 95% *ci.*) in blood samples taken from weaner wethers either maintained worm free (NIL) or infected with *H. contortus* trickle infection (INF).

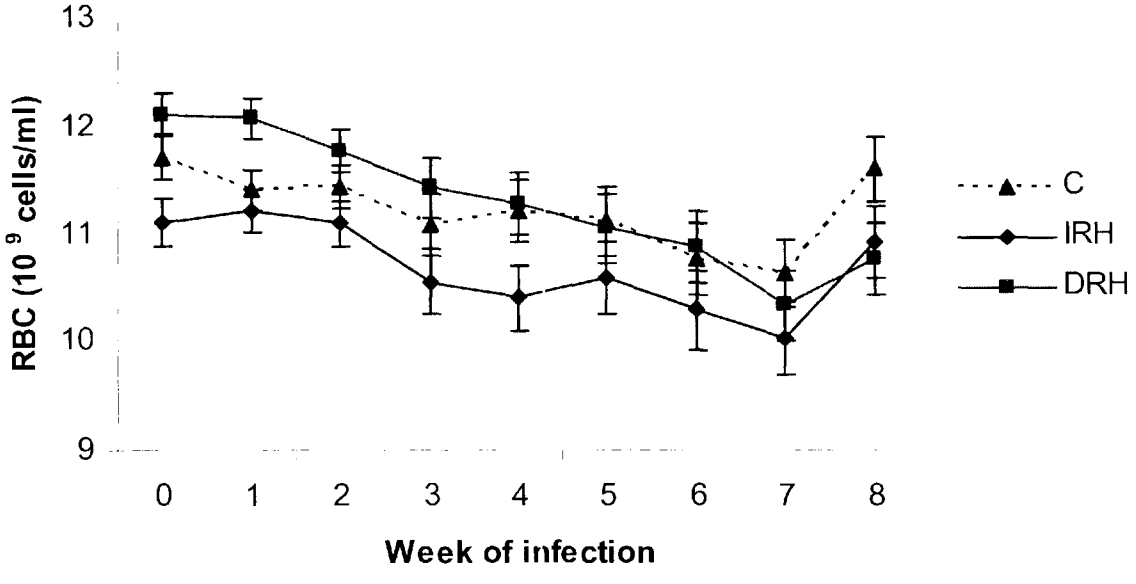


Figure 5-4 Red blood cell (RBC) count (back-transformed ls mean ± 95% *ci.*) in blood samples taken from weaner wethers selected for either increased resistance to *Haemonchus* (IRH), decreased resistance to *Haemonchus* (DRH) or random-bred control (C).

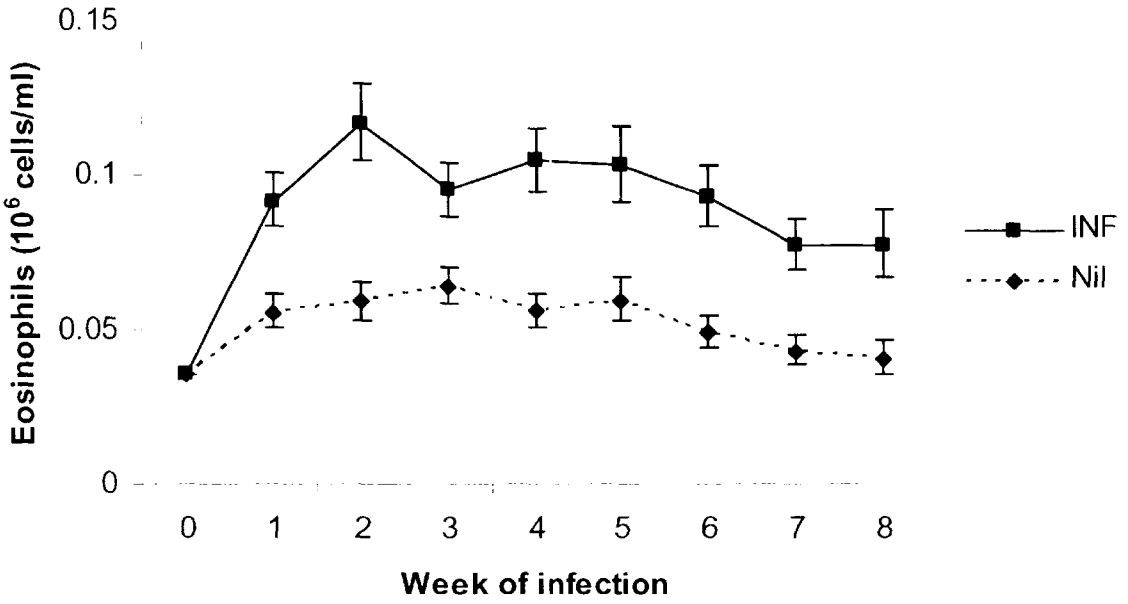


Figure 5-5 Eosinophil count (back-transformed ls mean ± 95% *ci.*) in blood samples taken from weaner wethers either maintained worm free (NIL) or trickle infected with *H. contortus* (INF).

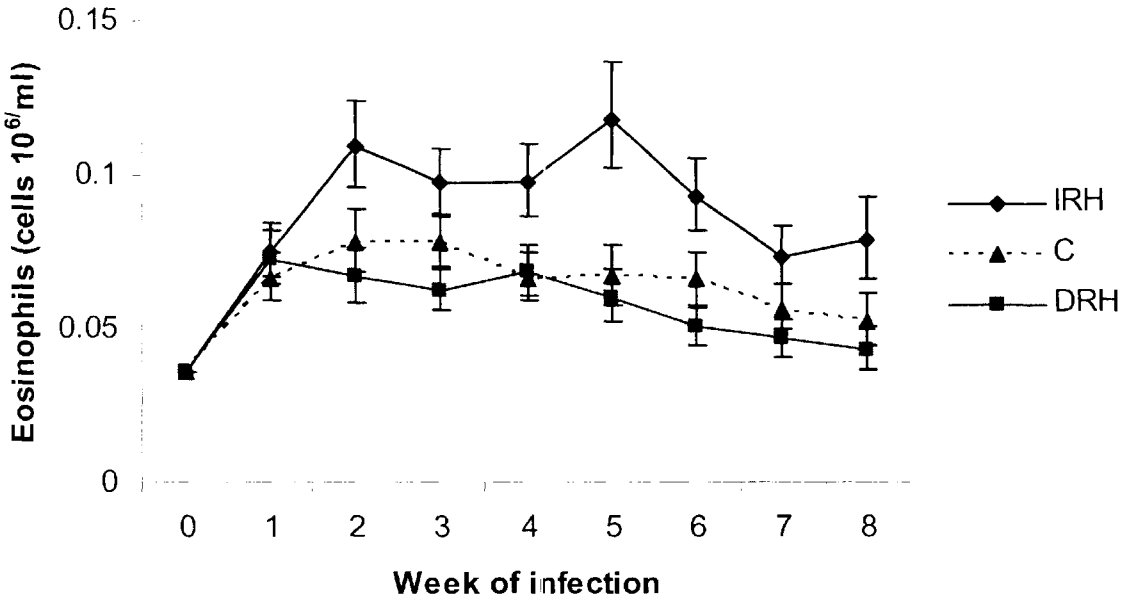


Figure 5-6 Eosinophil count (back-transformed ls mean ± 95% *ci.*) in blood samples taken from weaner wethers selected for either increased resistance to *Haemonchus* (IRH), decreased resistance to *Haemonchus* (DRH) or random-bred control (C).

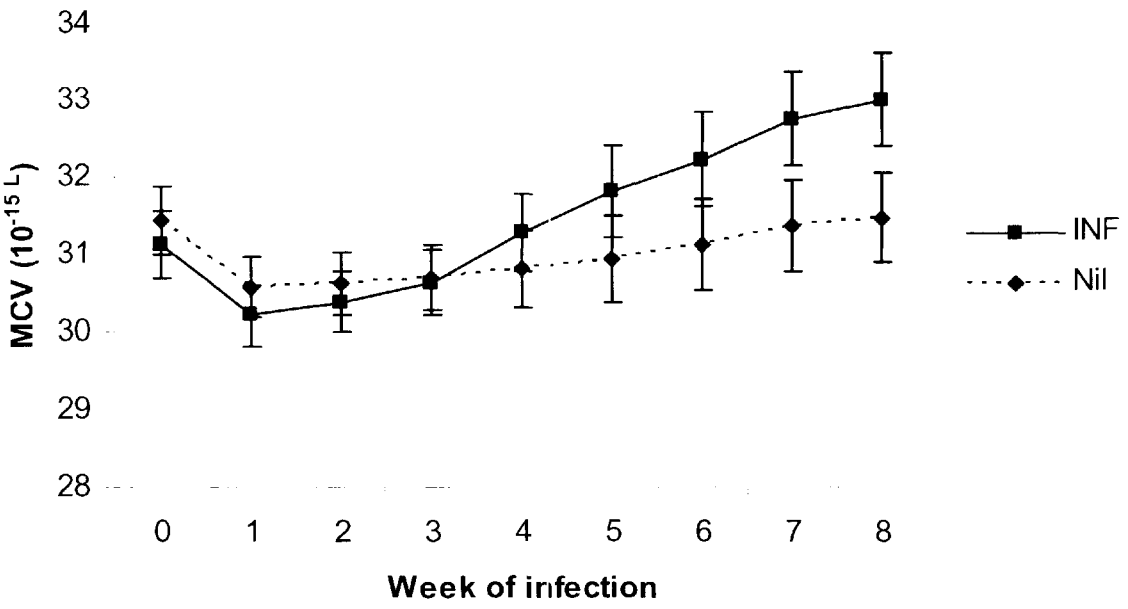


Figure 5-7 Mean corpuscular volume (back-transformed ls mean ± 95% *ci.*) in blood samples taken from weaner wethers either maintained worm free (NIL) or trickle infected with *H. contortus* (INF).

Table 5-9 Haemoglobin and haematocrit (ls mean ± 95% *ci.*) in blood samples taken at week 8 of *H. contortus* infection, from weaner wethers selected for increased resistance to *Haemonchus* (IRH), decreased resistance to *Haemonchus* (DRH) and random-bred control (C) and either maintained worm free (NIL) or trickle infected with *H. contortus* (INF).

Group	Haemoglobin g/dl			Haematocrit %		
	ls mean	± *ci*		ls mean	± *ci*	
IRH	12.7^{a}	2.13	2.57	35.4^{ab}	0.82	0.084
DRH	11.8^{b}	2.26	2.82	33.9^{a}	0.86	0.88
C	13.2^{a}	2.06	2.44	37.1^{b}	0.78	0.80
NIL	13.1	2.07	2.70	37.0	0.78	0.80
INF	12.1	2.23	2.74	33.8	0.86	0.89

Within treatment effect and columns, values with a common suffix or no suffix do not differ significantly (P>0.05).

5.3.7 Feed intake

Daily feed intake did not differ significantly between selection lines or in response to infection. Animals were fed an average (± s.d.) of 1150 ± 127.7 g (40g/kg BW) daily and the total amount was consumed by all animals throughout the experimental period, with the exception being the presence of feed refusals by some animals (n = 4) on the day ^{15}N dose was administered.

5.3.8 Bodyweight

Bodyweight was higher in DRH compared to IRH and C animals throughout the experimental period (P = 0.036) (Figure 5-8). Bodyweight was not affected by infection (P = 0.976) and the interaction between the effects of selection line and infection was not significant. Difference in bodyweight change between selection lines was significant (P < 0.0001), with DRH gaining $8.2^{a} \pm 0.24$ kg over the 8-week period and IRH and C lines, $7.3^{b} \pm 0.24$ and $6.4^{c} \pm 0.24$ kg respectively.

5.3.9 Wool growth rate

Wool growth rate measured between week 1 and 4 of the infection period did not differ between selection lines (P = 0.442) or in response to infection (P = 0.330). The interaction between the effects of selection line and infection was not significant (P = 0.981). Wool growth rate (g/d ± s.e.) for the IRH, DRH and C lines was 10.7 ± 0.57, 11.4 ± 0.57 and 11.8 ± 0.57 g/d respectively. The wool growth rate of NIL animals was 11.6 ± 0.47, compared to 11.0 ± 0.47 g/d in INF animals.

5.3.10 Faecal haemoglobin loss

Faecal blood loss estimated from haemoglobin concentration in faeces increased significantly (P = 0.013) in response to infection (Figure 5-9). Faecal haemoglobin loss did not differ (P = 0.324) between selection lines until week 8 of infection when selection line was significant (P = 0.037) with faecal haemoglobin loss for IRH, DRH and C of $0.99^{a} \pm 0.05$, $1.45^{b} \pm 0.05$ and $1.19^{ab} \pm 0.05$ mg/g faeces respectively. The interaction between the effects of selection line and infection was not significant. The correlation coefficients between untransformed worm egg count and total worm count with faecal haemoglobin loss were 0.8 (P < 0.0001) and 0.6 (P = 0.002) respectively. There was no effect of infection or selection line on the proportion of porphyrins (haemoglobin) converted in the gastrointestinal tract.

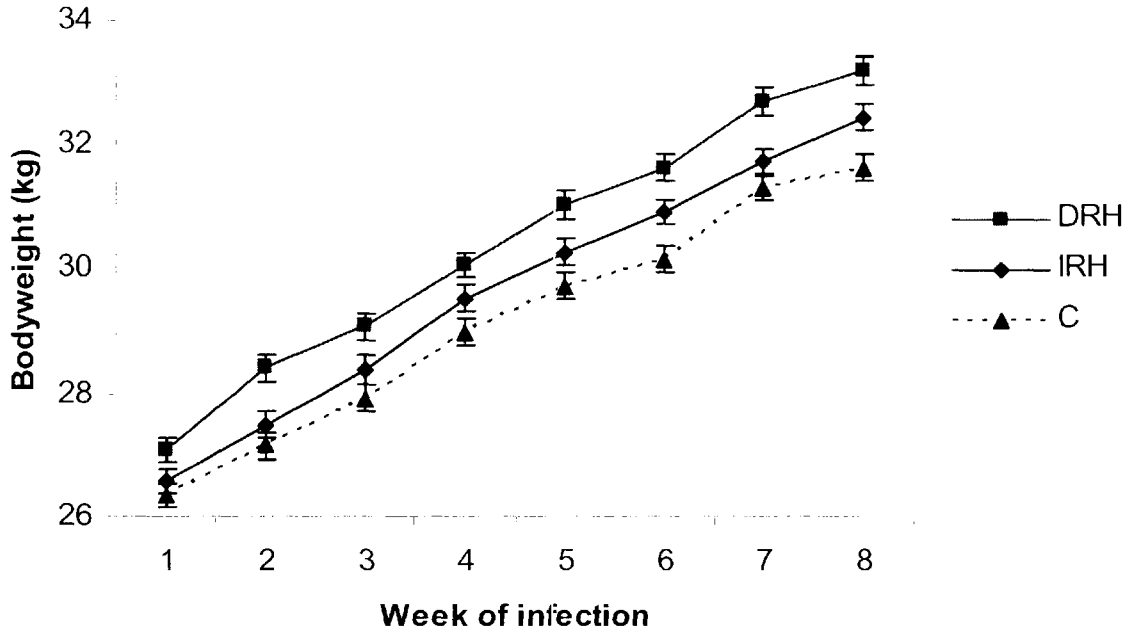


Figure 5-8 Bodyweight (ls mean ± s.e.) of weaner wethers from the increased resistance to *Haemonchus* (IRH), decreased resistance to *Haemonchus* (DRH) and random-bred control (C) selection lines.

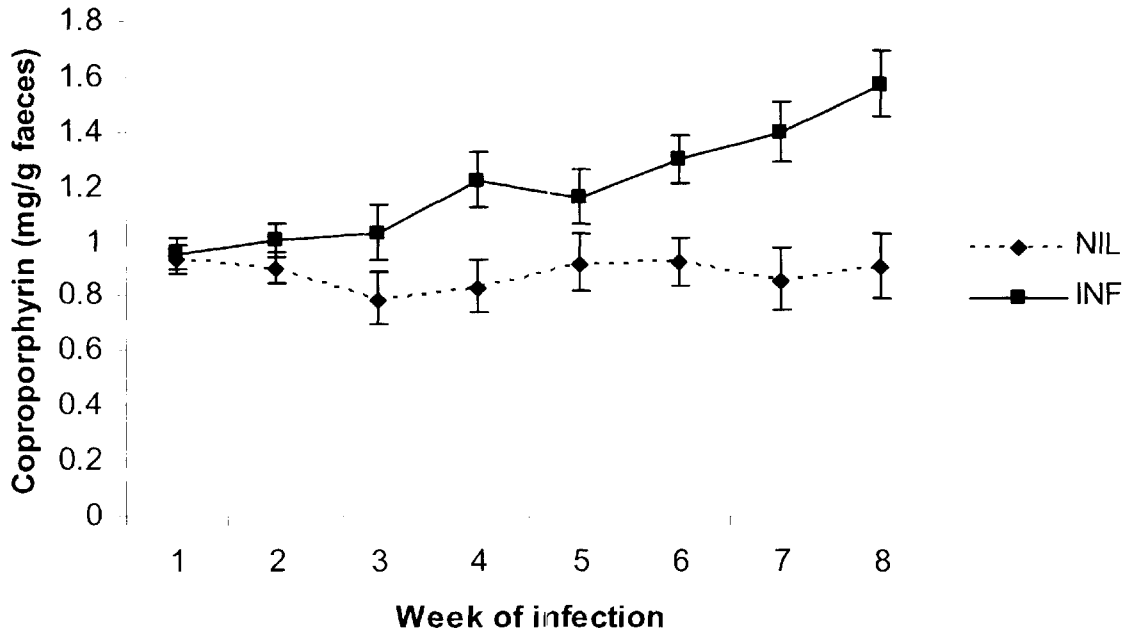


Figure 5-9 Faecal haemoglobin loss (back-transformed ls mean ± 95% *ci.*) of weaner wethers either maintained worm free (NIL) or trickle infected with *H. contortus* (INF).

5.4 Discussion

5.4.1 ^{15}N partitioning and organ weights

Divergent selection for resistance to *H. contortus* has produced some correlated changes in apparent N digestibility and in the partitioning of N among organs, which likely represent changes to amino acid metabolism. IRH had a lower digestibility and an increased oxidation of amino acids, as indicated by a higher percentage recovery of ^{15}N in urine output, when contrasted to animals from the DRH line. It is possible that the increased amino acid oxidation in resistant animals may be related to an immune-associated response to infection. ^{15}N uptake in the abomasal smooth muscle of IRH animals increased by 31% in response to infection, while uptake remained unchanged in DRH and C. Greater proportional uptake in IRH as opposed to DRH animals occurred despite lower mass of abomasal smooth muscle. Le Floc'h *et al.* (2004) suggest that immunological stress, such as that induced by *H. contortus*, results in the redistribution of amino acids toward tissues involved in the immune response. It is uncertain if this process accounts for the increased partitioning of amino acids towards abomasal smooth muscle in IRH, as examination of local gut immune responses were not taken. However, increased immunity to the intestinal parasite *T. colubriformis* is associated with increased numbers and activity of gut immune effector cells, such as mast cells and globule leucocytes. It has been demonstrated that infection with *T. colubriformis* leads to an increase in oxidation of amino acids (leucine) by the gastrointestinal tract (Yu *et al.* 2000). Thus a link between gut effector cell activity and abomasal uptake of ^{15}N in the current experiment may be plausible.

The net effect of the removal of amino acids from the circulation by intestinal oxidation was a reduction in available absorbed amino acids to other tissues, i.e. muscle and skin. Yu *et al.* (2000) calculated that an extra 3.0 to 3.5 mmol/d of gastrointestinal tract leucine oxidation during infection equates to 1 g/d of metabolic N not available for protein deposition. The consequence of a lower digestibility of amino acids coupled with an increase in the rate of amino acid oxidation in IRH animals was detected in a significantly lower N balance compared to the DRH line. A lower N retention in IRH animals would indicate a reduced N availability for protein deposition in skeletal muscle and therefore may explain the lower bodyweight and growth in IRH animals throughout the infection period compared to the DRH line.

The percentage recovery of ^{15}N to the thymus was greater in IRH than in DRH animals. The increased partitioning of nitrogen to the thymus may reflect the IRH immune response, such as an increase in T cell production (Roitt *et al.* 1985). The liver is also an important immune-associated organ and may play a role in the gut immune response of IRH animals. The oxidation of amino acids in the liver results in the production of urea, which is eventually excreted from the body in urine (Meijer *et al.* 1999). It is possible that the liver may have contributed to the increase in ^{15}N in the urine of IRH animals. The liver is responsible for the synthesis and secretion of acute phase proteins (Colditz 2002), which can override the normal control of nutrient utilisation, such that amino acid resorption from muscle is diverted toward the immune system (Colditz 2003). MacRae *et al.* (1993) explain that because the concentrations of amino acids, such as cysteine are proportionally lower in muscle than in the liver and gut, more muscle protein needs to be mobilised to supply the demand of these organs. A heightened immune response in IRH animals may place a greater demand on muscle protein, thus reducing their potential growth.

The percentage recovery of ^{15}N in small intestine smooth muscle decreased in response to infection and was lower in IRH and DRH animals compared to the C line. In contrast, Symons & Jones (1983) reported a 24% increase in protein synthesis in the small intestine of animals infected with the intestinal parasite *T. colubriformis*. The authors suggested that proliferation of intestinal epithelial cells and inflammation of the lamina propria in response to the intestinal infection could explain a faster rate of protein synthesis. In this experiment the infection was not at the site of the small intestine but rather the abomasum. The consequence of lower recovery of ^{15}N in small intestine smooth muscle of IRH and DRH animals may indicate altered amino acid metabolism as a result of divergent selection. One benefit could be accounted by a nutrient-sparing effect because the gastrointestinal tissues dominate energy costs (Liu *et al.* 2005a), with the highest rate of oxygen consumption (23%) and protein synthesis (28%) in the whole body (Lobley 1994). Experimentation using the Rylington selection flock also showed that the fractional synthesis rate in the jejunum was lower in the resistant line compared to control (52.3 vs. 58.2%/d), when trickle infected with *T. colubriformis and T. circumcincta* (Liu *et al.* 2006).

The reduced utilisation of N in the small intestine smooth muscle creates a greater availability for other functions. For example, spleen weight of DRH animals was greater than for IRH and C lines. The DRH animals also had an increased uptake of ^{15}N by the spleen in response to infection. The DRH animals carried worm burdens of almost 2,500 adults and a faecal haemoglobin loss measured at week 8 of 1.9 g /d (calculated from an average faecal output of 1315g/d), both greater than IRH animals. Rowe *et al.* (1988) reported that 9 month old Merino wethers carrying a mean *H. contortus* burden of 5,000 adults had a blood loss into the gastrointestinal tract of 253 ml/d, which equated to a loss of 2.6 g blood nitrogen /d. The spleen is a lymphoid organ which contains lymphocytes and red blood cells (Abramoff & La Via 1970) and acts as a blood reservoir (Dooley *et al.* 1971). Turner and Hodgetts (1959) demonstrated that the weight of the spleen was directly proportional to its red blood cell content. The greater spleen capacity and increased N uptake in DRH animals may be a possible adaptation to combat the loss of blood associated with a *H. contortus* infection. The IRH animals after 8 weeks of infection, had fewer established adult worms, less faecal blood loss, but lower RBC numbers, which allows resources such as amino acids to be available for other functions.

IRH and DRH animals did not differ in recovery of ^{15}N to muscle as measured in the *semimembranosus* muscle at slaughter. However, the recovery of ^{15}N in muscle at a single time point was the net amount of amino acid nitrogen that was deposited at 24 h and may not reflect the processes of synthesis and degradation which contribute to the recycling of amino acids. Numerically greater carcass weight and muscle mass of DRH as opposed to IRH animals may have occurred due to differences in protein degradation rate in skeletal muscle, rather than protein synthesis. Oddy *et al.* (1995) explain that genetic differences between sheep in protein deposition appear to be achieved by differences in protein degradation rate rather than protein synthesis. An increased rate of protein degradation would also contribute to amino acid catabolism, greater urinary N excretion and lower N retention, as observed in IRH animals. Future studies should consider including measurement of protein degradation in skeletal muscle as a means to better understand the increased rate of amino oxidation in IRH animals.

H. contortus infection and selection line had no affect on whole body protein flux, which conforms with observations made investigating protein flux in response to

intestinal infection with *T. colubriformis* (Yu *et al.* 2000, Bermingham *et al.* 2000, Hoskin *et al.* 2002). Whole body protein flux may not change as the increase in protein synthesis in some tissues may be balanced by an increase in protein degradation in others (Bermingham *et al.* 2000). The higher amino acid oxidation and lower N retention in IRH animals indicates an alteration in amino acid metabolism, such that an increase in protein synthesis in the immune response may be associated with an increase in degradation in tissues, such as skeletal muscle.

The weight of the abomasal lymph node in INF animals was more than double that in NIL. An increase in the weight of the abomasal lymph node also occurred in Merino lambs given a bolus dose of 50,000 *H. contortus* infective larvae (Balic *et al.* 2000a). Interestingly, Balic *et al.* showed the lymph node had not changed weight 3 days post infection, but was double the weight by day 5 and at week 5 of infection. In this experiment the abomasal lymph node did not vary in size among selection lines, measured at week 8 of infection, which probably reflects the different time course over which the selection lines acquired resistance. The IRH immune response appears to have occurred more quickly to incoming larvae and maintained a lower WEC from week 3 of infection. A consequence of a greater rate of acquisition of immunity may be that measurement at week 8 of infection would selectively underestimate abomasal lymph node weight in IRH animals. The animals in the IRH line may have had a heavier abomasal lymph node, due to increased cell population at an earlier stage of infection. At week 8 of infection the local response to infection, as indicated by abomasal lymph node weight, was similar among selection lines. Liu *et al.* (2005a) also showed no differences in weight of abomasal and mesenteric lymph nodes at week 18 of a trickle infection with *T. circumcincta* and *T. colubriformis,* in resistant and control Merino rams from the Rylington selection flock.

The abomasum increased in weight in response to infection, but did not differ among selection lines. The increase in mucosa weight is reflective of the infiltration of lymphocytes from the enlarged abomasal lymph node (Balic *et al.* 2000a). Some acute phase proteins are also synthesised in the gut mucosa (Wang *et al.* 1998), which may account for some of the increased protein sequestration in gut tissue reported during parasitism (Yu *et al.* 2000). Thus the lymph nodes and mucosa are immunologically

active tissues at the site of infection, but do not indicate the level of resistance to infection when measured at week 8 in infection.

5.4.2 Parasitology

WEC did not differ among selection lines during the first 7 weeks of infection, but was lower in the IRH than in DRH and C lines at week 8. The typical divergence in WEC as reported by Woolaston *et al.* (1991) was not evident during this infection. The WEC in DRH animals began to rapidly increase from week 7 of infection, producing the significant difference from IRH animals at week 8 of infection. The establishment rate may have been low in infected animals following the bolus dose and the accumulation of adult worms in the DRH animals over time increased the worm egg output. The worm counts and WEC at week 8 were significantly different between IRH and DRH and C lines, which would indicate that by week 8 of infection IRH animals were displaying a stronger resistance to infection. The fecundity of female worms did not differ between lines, which also occurred in the previous experiment (Chapter 4). Resistant animals in the *Haemonchus* selection flock do not display the ability to significantly reduce fecundity of the parasite and therefore a reduction in worm burden is presumably due to a lower establishment rate.

5.4.3 Haematology

Red blood cell numbers declined in INF animals, as did haematocrit and haemoglobin concentration over the 8 week infection period. These signs of *H. contortus* infection are due to the blood-sucking adult worms causing haemorrhage in the abomasum (Albers *et al.* 1990). The mean corpuscular volume increased during this period, indicating that INF animals were regenerating RBC to replace losses due to infection. Circulating eosinophils were elevated in all INF animals, with IRH animals significantly higher than DRH and C lines. This has been demonstrated in the high responder (resistant) animals in the *Trichostrongylus* selection line (Dawkins *et al.* 1989) and in Chapter 3.

5.4.4 Faecal haemoglobin loss

INF animals had greater faecal haemoglobin loss than NIL animals, throughout the infection. Selection lines did not differ in faecal haemoglobin loss until week 8 of infection, when WEC and worm burden differed significantly between lines. This agrees with the positive linear relationship between blood loss and WEC and worm burden (Le Jambre 1995). A greater blood loss generally indicates a greater production loss, with lower weight gains and wool growth (Albers *et al.* 1990). However, the greater faecal haemoglobin loss in DRH animals in this experiment was not associated with lower productivity. This would indicate that the DRH animals were resilient to infection, as they had the ability to maintain production, despite the effect of parasitism (Albers *et al.* 1987).

5.5 Conclusion

Divergent selection for resistance to *H. contortus* produced some correlated changes in partitioning of nitrogen among organs and differences in N digestibility. IRH animals had a lower N digestibility, increased oxidation of amino acids and lower N balance but whole-body protein flux was unaffected. Alteration of amino acid metabolism, as assessed from ^{15}N uptake and excretion in response to *H. contortus* infection, appears to differ between IRH and DRH animals. In IRH animals a greater recovery of ^{15}N in the thymus and abomasal smooth muscle indicates greater partitioning of amino acids towards the immune response. In DRH animals an increased recovery of ^{15}N in the spleen, in response to infection, may be a possible adaptation. A different rate of acquisition of immunity and the time after infection may confound differences among selection lines. Nevertheless, the differences in amino acid metabolism were associated with lower growth and N retention in IRH animals, despite having significantly lower worm burdens. It appears that divergent selection for WEC has not been associated with symmetrical changes in amino acid metabolism, but rather the partitioning of amino acid resources reflects each selection line response to infection. If one were to assume that a lower worm burden results in higher growth rates, these results suggest that selection for increased resistance has been accompanied by a larger nutritional 'cost' than that observed with selection for decreased resistance.

CHAPTER 6

GENERAL DISCUSSION

6.1 Introduction

Breeding for resistance to GI worms is a long-term worm management practice that has been adopted by some leading sheep breeders. The benefits of selection for resistance are lower worm burdens, reduced frequency of anthelmintic use and reduced pasture contamination by infective larvae (Gray 1997). However correlated responses with production traits, such as growth rate and wool production, appear to have undesirable consequences. The expectation is that resistant sheep should suffer less production loss due to a reduced parasite infection. Yet productivity is comparable or lower than animals carrying a large parasite burden (susceptible genotype).

The main hypothesis which was tested in this thesis was that divergent selection for resistance to *H. contortus* has produced correlated changes in nutrient availability associated with an immune response. Specifically the hypothesis embraced the notion that nutrient availability may have been altered through indirect effects of selection in either of three ways. 1) voluntary feed intake or diet selection which regulates dietary intake, 2) ruminal digestion, which regulates nutrient supply and 3) amino acid partitioning within the body associated with immunological responses to gastrointestinal nematode infection. These aspects of the host-parasite relationship were tested in three experiments, reported in Chapters 3, 4 and 5.

6.2 Physiological responses to parasitism in divergent selection lines

The magnitude of the pathogenic effects on the host and development and establishment of the parasite can be influenced by dietary supply and more specifically the supply of metabolisable protein. The reduction in voluntary feed intake that generally accompanies gastrointestinal infection results in depressed animal productivity. Studies have demonstrated that young animals infected with *H. contortus* and fed a high protein diet (170 g CP/d) have higher feed intakes, are more able to withstand the pathogenic

effects of infection showing reduced anaemia, hypoproteinaemia, hypoalbuminaemia and weight loss (Abbott *et al.* 1986ab) and have the ability to resist haemonchosis by reducing establishment and development of incoming larvae (Abbott *et al.* 1988). Chapter 3 reports on an experiment which tested if divergent selection for resistance had produced changes in voluntary feed intake or diet selection. This hypothesis was rejected as divergent selection for resistance to *H. contortus* has produced no differences in daily feed intake or diet selection between IRH and DRH animals in response to an *H. contortus* infection, despite a significant difference in WEC. Interestingly, feed intake of C animals was always numerically lower than the selected animals. These results may be interpreted in two ways. Firstly, that IRH does not gain superior resistance through greater dietary intake. Secondly, DRH manage a higher worm burden without exhibiting restriction of feed intake, which eliminates an important and detrimental impact of GI worms on productivity. Partial support for the latter of these interpretations is expressed in feed intake of C animals tending to be 9.5% lower than DRH during the first 5 weeks of infection. Therefore it was concluded that the mechanisms that allow resistant animals to effectively prevent establishment and/or development of nematode infection do not appear to involve a change in dietary intake. In contrast, the ability of susceptible animals to have productivity equal to or higher than resistant animals, while carrying a greater worm burden may be the ability to maintain feed intake during infection.

Supply of metabolisable energy (ME) and metabolisable protein (MP) to ruminants is dependent on normal rumen function (van Houtert & Sykes 1996). However, studies have shown GI parasites affect rumen function and metabolism, contributing to reduced performance (Symons & Steel 1978, Parkins & Holmes 1989). Rowe *et al.* (1988) reported a decrease in acetate: propionate ratio, increase in rumen fluid outflow rate, decrease in apparent digestion of organic matter across whole tract and an increase in N lost into the GI tract, due to haemonchosis in Merino weaners. Infection with *H. contortus* altered rumen fermentation in a number of ways. The most notable effects include a reduction in the *in sacco* digestibility of the diet for up to 12 h, an elevated rumen pH and a reduction in total VFA concentration mostly accounted by effects on acetate and butyrate. The fact that infection had an impact on rumen fermentation suggests that different levels of host infection, arising from genetic differences in resistance, may alter the magnitude of these effects. There was some evidence in

Chapter 4 that the response of IRH animals to *H. contortus* infection was characterised by altered rumen function which favoured the synthesis of microbial protein at the expense of propionic acid. It is likely that such a change may reduce rates of glucose entry in IRH animals and lower body growth or result in the use of glucogenic amino acids as a source of glucose. A shift towards increased protein supply at the expense of glucose would convey an advantage to a stronger immune response and may, in part, account for the greater worm resistance in these animals. This shift in response to infection, may also explain why IRH animals do not translate lower WEC into greater bodyweight gain. Thus there is an indication that rumen fermentation and metabolism differs between resistant and susceptible genotypes. However, values for ammonia, VFA and purine derivatives were comparable lower than previous studies (Annison *et al.* 2002), which may have prevented significant differences.

Utilisation of nutrients for growth and wool production is usually impaired in sheep infected with nematode parasites. The decrease in productivity is thought to be due to a number of factors including a repartitioning of nutrients to the gastrointestinal tract for the immune response and tissue repair (Parkins & Holmes 1989), while protein synthesis in muscle and wool is decreased (Symons & Jones 1975). Yu *et al.* (2000) showed a 24% increase in leucine sequestration in the whole gastrointestinal tract and increased oxidation losses of leucine of 22 to 41% associated with infection of *T. colubriformis*. As a consequence availability of absorbed amino acids for peripheral tissue metabolism was reduced by 30%. Effects of divergent selection for resistance to *H. contortus* on nitrogen partitioning within the body were investigated with a bolus abomasal injection of ^{15}N duckweed, which has been demonstrated to uniformly label amino acids (Liu *personal communication*). Divergent selection was observed to have produced some correlated changes in the post-ruminal apparent digestion of amino acid nitrogen and in the partitioning of nitrogen among organs (Chapter 5). IRH animals had a lower apparent N digestion and an increased oxidation of amino acids, which were observed as a reduced N balance. N uptake in abomasal smooth muscle and thymus in response to infection was also greater in IRH. DRH animals had a greater N digestibility than for IRH and increased the percentage recovery of ^{15}N to the spleen in response to infection. This increase in N uptake may be a possible adaptation in DRH animals to combat the loss of blood associated with a *H. contortus* infection, as the spleen is able

to act as a reservoir of RBC. The percentage recovery of ^{15}N to muscle did not differ among selection lines or between NIL and INF animals. However, DRH lines had greater weight gain than IRH and C animals throughout the infection period, despite a worm burden 10-fold that of IRH. These results suggest that IRH animals partition a greater proportion of absorbed amino acids towards the response to infection. In contrast, DRH animals appear to partition amino acids towards the consequences of infection. Divergent selection for resistance has not been associated with symmetrical changes in amino acid metabolism, but rather the partitioning of amino acid resources reflects each selection lines response to infection. If one were to assume that a lower worm burden results in higher growth rates, these results suggest that selection for increased resistance has been accompanied by a larger nutritional 'cost' than that observed with selection for decreased resistance.

6.3 Increased resistance to *Haemonchus* (IRH) selection line display a greater immune response and a redirection of amino acids

The three experimental chapters have indicated that IRH animals have a heightened immune response, compared to DRH and C lines. IgG_1 plasma antibodies were higher in IRH animals compared to DRH and C lines (Chapter 3). Elevated plasma IgG concentrations have been associated with resistance to *H. contortus* in the Golden Ram flock (Gill *et al.* 1991) and elevated IgG_1 titres were reported in resistant animals from the Wallaceville Romney breeding line (Bisset *et al.* 1996), when infected with mainly *T. colubriformis*. The increase in plasma IgG_1 may play a role in the ability to prevent establishment and/or development of a nematode infection in IRH animals.

Eosinophil count was higher in IRH animals in Chapter 3 and 5 compared to DRH and C lines and responded to a high quality diet. A significant difference among the selection lines was not evident in Chapter 4, most likely the result of a lower quality diet fed at a level near maintenance requirements. It is suggested that a higher eosinophil count may play a role in the gut immune response of resistant animals, but is inferior to the use of WEC as a selection criterion for resistance to *Haemonchus* infection (Woolaston *et al.* 1996).

Total volatile fatty acid concentration declined by 35%, while purine derivatives (an indicator of ruminal microbial outflow) increased by 27% in IRH animals in response to infection (Chapter 4). An increase in supply of microbial protein has the potential to assist a gut immune response in IRH animals.

The percentage recovery of ^{15}N to the thymus in IRH animals was higher than DRH and C lines (Chapter 5). The increased partitioning of N to the thymus is likely to contribute to the IRH immune response, by increasing T cell production. ^{15}N uptake in the abomasal smooth muscle of IRH animals increased by 31% in response to infection, while uptake remained unchanged in DRH and C. Greater proportional uptake in IRH as opposed to DRH animals occurred despite lower mass of abomasal smooth muscle. An increase in partitioning of N towards abomasal smooth muscle in IRH animals may be associated with a redistribution of amino acids toward tissues involved in the immune response.

The efficiency of feed utilisation differed among selection lines, such that DRH animals gained more bodyweight with lower feed intake compared with IRH and C lines (75.8, 62.9 and 56.2 g bodyweight gain/kg DM respectively) (Chapter 3). Immuno-suppressed animals infected with nematode infection have a greater feed conversion efficiency (FCE) than infected animals (Greer *et al.* 2005a) It is suggested that the infected animals reduced FCE may be a consequence of the development of the immune response (Greer *et al.* 2005a). A lower efficiency of feed utilisation accompanied by a greater proportional use of amino acids in the organ associated with the immune response, suggests that differences among selection lines may mirror the observed response to immuno-suppression.

The implication of these experiments is that selection has been associated with an increase in the immune responsiveness of resistant animals which requires a greater partitioning of nutrients toward the gut immune response. Liu *et al.* (2006) suggests that the potential benefit of resistant genotypes of lower worm burden and WEC may be compromised by maintaining an altered immunity. The results discussed in this thesis support this view. The correlated changes in IRH animals in response to infection with *H. contortus* are summarised in Figure 6-1.

6.4 Decreased resistance to *Haemonchus* (DRH) selection line display resilience to nematode infection

DRH animals have greater endogenous protein loss, indicated by reduced albumin concentrations, red blood cell loss and increased erythropoiesis. The increase in protein synthesis required to repair the gastrointestinal tract should reduce protein synthesis in other tissues, such as the skeletal muscle. However the DRH animals demonstrate a strong resilience to *H. contortus* infection, with equal or greater bodyweight gain and greater FCE than animals carrying smaller worm burdens.

DRH animals carried worm burdens of almost 2,500 adults and a faecal haemoglobin loss measured at week 8 of 1.9 g blood/d (calculated from an average faecal output of 1315g/d) (Chapter 5), both values significantly greater than for IRH. DRH also had a heavier spleen than IRH animals and increased recovery of ^{15}N to the spleen in response to infection. A greater spleen capacity and increase in N uptake in DRH animals may be a possible adaptation to combat the loss of blood and plasma leakage associated with an *H. contortus* infection.

The physiological responses in DRH animals when infected with *H. contortus* may be due to the selection method applied to the line. Whilst DRH animals were selected for high WEC, the selection line was indirectly selected for survival and therefore adaptation mechanisms to combat the effects of *H. contortus* (Le Jambre *personal communication*). The *Haemonchus* selection flock was selected on the basis of WEC when given a bolus artificial challenge with 10,000 *H. contortus* infective larvae at 6 months of age. The animals were managed under commercial grazing practices and are exposed to natural challenge. In some years a proportion of DRH animals did not survive the artificial challenge and therefore a selection pressure for survival also existed within the line. This selection pressure existed for over 25 years, leading to the DRH line indirectly selected on the ability to survive while also carrying large *H. contortus* burdens.

DRH animals had equal or greater bodyweight gains and greater FCE than IRH and C animals. The DRH line also tended to have a greater concentration of plasma IGF-1 than C animals (Chapter 3). Oddy *et al.* (1995) demonstrated that sheep selected for

weaning weight (W+) had higher plasma IGF-1 concentrations and FCE than unselected counterparts (W-). Susceptibility to parasites is also linked with low protein degradation rate in muscle (Adams & Liu 2003). Measurement of protein degradation in skeletal muscle may have presented differences in protein turnover between DRH and IRH animals, as DRH animals had higher N retention than the IRH line (Chapter 5). The higher FCE in DRH animals may be regulated by IGF-1, resulting in reduced protein degradation in skeletal muscle and a greater weight gain. The correlated changes in DRH animals in response to infection with *H. contortus* are summarised in Figure 6-2.

Figure 6-1 Summary of correlated changes in animals selected for resistance to *H. contortus* (IRH) in response to infection.

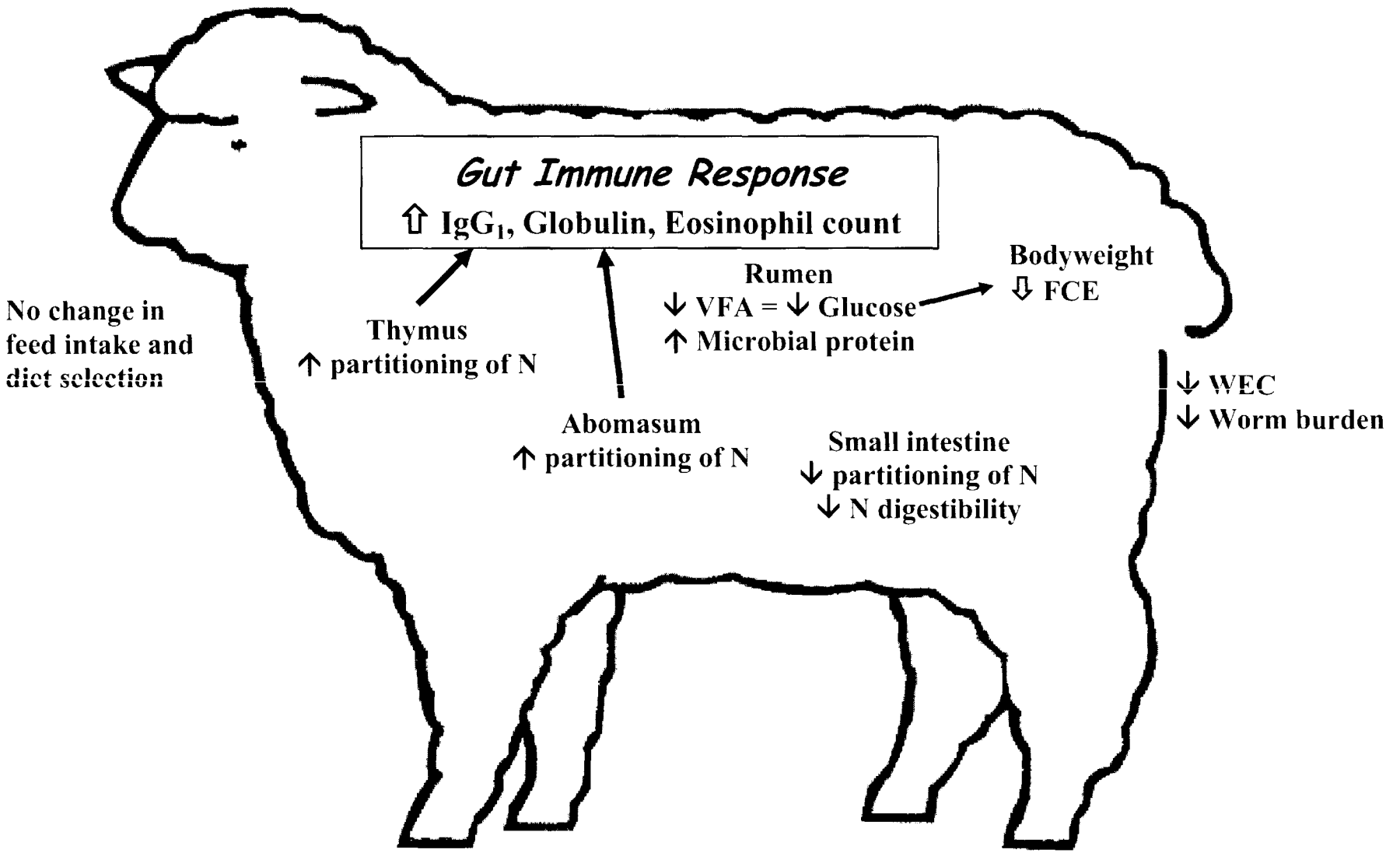
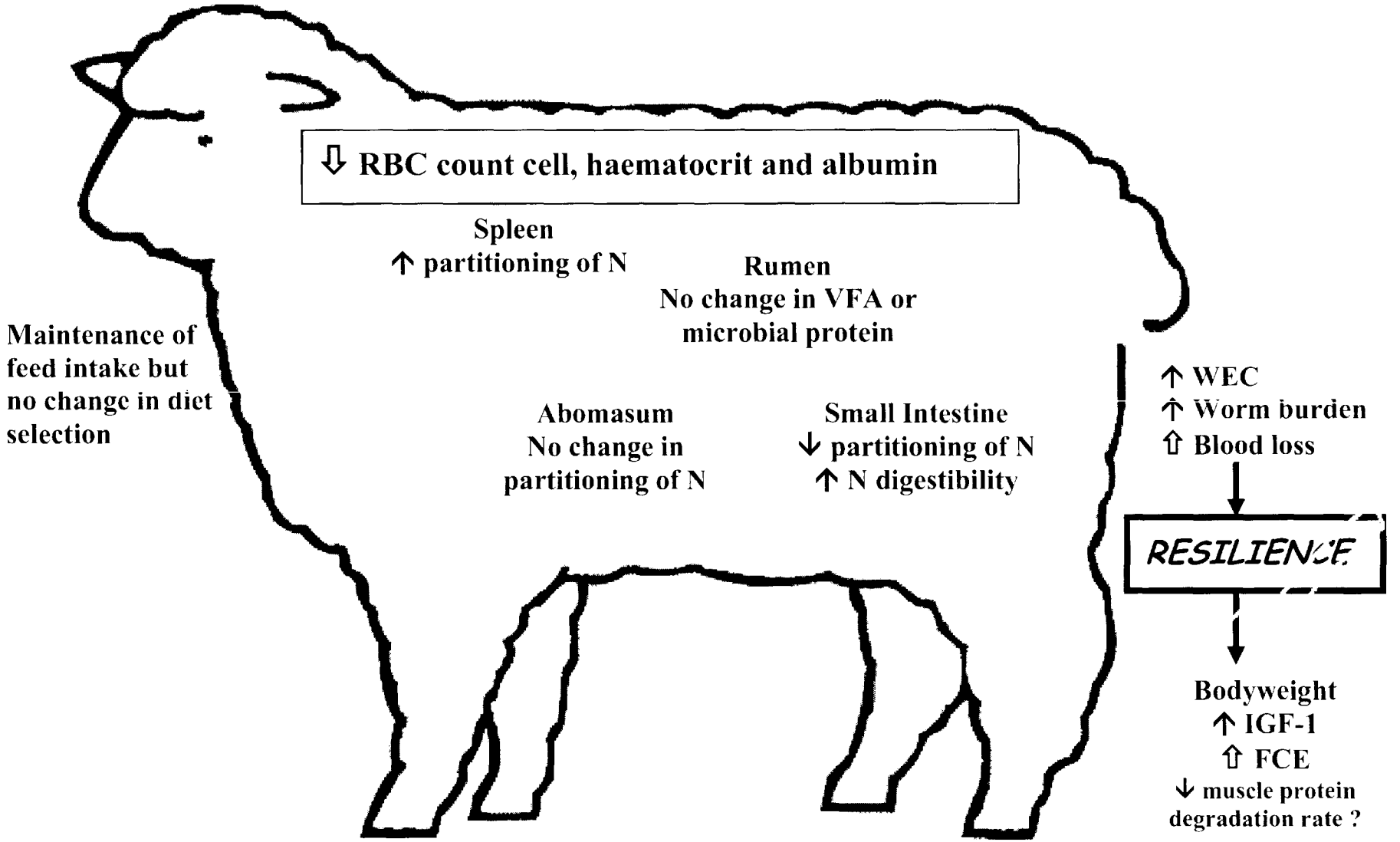


Figure 6-2 Summary of correlated changes in animals selected for susceptibility to *H. contortus* (DRH) in response to infection



6.5 Level of Infection

WEC, worm burden and fecundity of female worms were not consistent among selection lines in each of the three experiments. WEC was five times greater in Experiment 1 (Chapter 3) at week 8 of infection than in Experiment 3 (Chapter 5). The fecundity of female worms reported in Chapter 5 was very low, with an average of 2,600 eggs/worm. *H. contortus* is distinguished from other sheep roundworm parasites by their prolific egg laying capability (Gordon 1948). The lower fecundity may have been related to the strain of *H. contortus*, as McMaster strain *H. contortus* was used to infect animals in Experiment 1 and 2 and the Kirby strain in Experiment 3. There is no reason for the lower overall fecundity to be a consequence of the animal's response to infection, as animals within each line were sourced from the same selection flock.

WEC did not differ between DRH and C lines in animals reported in Chapter 4 and 5 and worm burden was not significantly different among lines in Chapter 4. The animals from each experiment were all infected with a similar bolus dose, based on bodyweight at the beginning of the infection period ($150L_3$/kg BW) and followed with a trickle infection of $750L_3$/week. The most likely explanation for the occasions when a similar response to infection by the divergent selection lines was observed is a sampling effect, whereby there is an overlapping WEC distribution of each genotype. If this was the case then the random selection of experimental animals from within line may at times result in an overlap in resistance among the lines. For example, some DRH animals at the resistant end of the DRH distribution, having lower WEC than C animals at the susceptible end of the C distribution and so forth. If animals were identified individually as a true representation of their selection line, the genotypes may have exhibited the divergent response to *H. contortus* as reported by Woolaston *et al.* (1990) and observed in Chapter 3.

The IRH animals displayed a strong level of resistance to *H. contortus* infection, by maintaining a low WEC throughout experimentation. Selection for resistance based on low WEC during artificial challenge at 6 months of age, can be a valuable tool for control of parasite burden (Woolaston *et al.* 1997) and particularly as resistance to chemical control increases in severity.

Ideally experimentation with these divergent selection lines should be conducted under paddock conditions as the magnitude of the effect of *H. contortus* infection on Merino weaners is often smaller in the animal house than reported in a field situation (Albers *et al.* 1987, Bisset *et al.* 1997). However, it was not practical to do intensive experimentation under paddock conditions.

6.6 The cost of resistance versus resilience

The difference in growth rate between DRH and IRH lines in Chapter 5 was 16g/d. The metabolic cost of the difference in growth rate between DRH and IRH lines, in terms of MCP and ME was calculated using equations from Freer *et al.* (1997). The daily cost of maintaining greater resistance in IRH animals was 0.8 MJ ME and 5 g MCP. These values are in accordance with values calculated by Liu *et al.* (2005c) of an extra 0.3 MJ ME/d and 3 g MCP/d required by resistant sheep. Calculation of these requirements in term of actual costs of feed will vary with feed prices. Assuming that the difference is met with cereal grain (assumed cost of grain at $200/t and 12 MJ ME/kg DM) the annual cost of resistance will amount to $4.87/head. If the difference is met by pasture (assume cost of pasture at $40/t and 9 MJ ME/kg DM) the annual cost of resistance will amount to $1.30/head.

Sackett *et al.* (2006) reported that the cost of internal parasites of sheep in summer high rainfall areas, such as the New England, with optimal control practices is $4.13/head annually. This value has taken into account the reductions in bodyweight gain and wool growth associated with a nematode infection. Resilient animals display the ability to maintain productivity, in spite of a nematode challenge. However, death can occur in more vulnerable classes of sheep, such as weaners and periparturient ewes.

The cost of host resistance to the producer appears to be more than the cost of controlling the parasite nematode infection. This is without consideration of the benefits of reduced pasture contamination by resistant genotypes, as this is difficult to calculate in monetary terms. However the information presented in this thesis is based on animals that have been selected solely for worm resistance. In reality, a selection index would be used in a commercial situation and animals would be selected for production traits with

less emphasis on resistance to parasites. Conversely, DRH animals produced heavier carcass weights despite carrying a worm burden five times greater than the IRH line. Carcass weight in DRH animals was 0.7 kg heavier after 8 weeks of *H. contortus* challenge than IRH animals. If the 0.7 kg advantage was maintained over the year, in terms of income to the producer this equates to an additional $1.34/head (191c/kg prime lamb carcass). These results highlight the question why is nematode infection such a major health cost? It may be that the animal house infections, while at times generating very high WEC, did not cause the same physiological stress as observed in the field. The generally high haematocrit values support this theory as values may fall to less than 15% (Kahn, *personal communication*) in the field with moderate *H. contortus* infections.

6.7 Comments on the experimental designs

Measurement of voluntary feed intake and diet selection (Chapter 3) were determined over two periods, NIL and INF. The experiment was divided into two periods to maximise the number of animals from each selection line in each infection regime. However, the design did not allow statistical analysis of the interaction of feed intake and diet selection in response to infection, due to the confounding effect of time. A 3 x 2 x 2 factorial design, with selection line (IRH, DRH and C), diet (H and M) and infection (NIL and INF) could have been implemented resulting in considerable less degrees of freedom.

Partitioning of amino acid-nitrogen between tissues was determined at week 8 of infection (Chapter 5). Liu *et al.* (2006) suggests the effect of infection on protein metabolism of the host varies with the stage of infection and the time-related responsiveness to the infection by divergent genotypes. In this study the WEC remained low in IRH animals throughout the infection. Thus, the acquisition of the immune response most likely occurred earlier in the infection than in DRH and C lines. Measuring protein metabolism at one time point may not be a sensitive method to describe genotype variation in response to parasite infection. The alteration of protein metabolism may have occurred earlier in the infection period. Ideally, with more experimental animals, measurement of the partitioning of nitrogen at day 0-7of infection and then at weeks 3-4 and 8 would be the optimal experimental design. A comparison

could then be made not only between genotypes but the change in protein metabolism at different stages of infection and response by the host. Bermingham (2004) proposed that week 3 of infection, when parasites are in the reproductive stage of development could potentially have a negative impact on the host and therefore detect a change in protein metabolism.

In the three experiments, animals were infected with a bolus dose of infective *H. contortus* larvae ($150L_3$/kg BW) and subsequently trickle infected ($750L_3$/week) for the entirety of the experimental period. A trickle infection is representative of a natural parasite challenge. However, the data relates to the physiological responses in the animal when challenged with parasite larvae, developing adults and reproductive adult worms. The continuous challenge with infective larvae may have clouded the assessment of the immune response among divergent selection lines due to the continuity of incoming third-stage larvae. To measure the acquisition of the immune response among selection lines and the physiological change over time, arising from a single infection, a single bolus dose may provide clearer assessment of selection line differences. To measure the acquisition of the immune response among selection lines, the use of naïve animals at the beginning of the experiment would also be optimal. However, animals were previously artificially infected with 10,000 L_3 *H. contortus* to ensure animals had the opportunity to acquire immunity to nematode infection and reflect genetic merit.

6.8 Future Research

The experiments presented in this thesis have shown that resistant animals most likely partition nutrients to mount a strong immune response against *H. contortus*. There is little information on the variation in the immune responses in divergent lines of the *Haemonchus* selection flock. Additional studies may help to clarify some of the immunological mechanisms of resistance and the acquisition of immunity. Histology of the abomasal tissue following *H. contortus* challenge and measurement of cell populations in the tissue and immunoglobulin isotypes and eosinophils, may establish differences in the gut immune response among divergent selection lines. Measurement of these parameters at day 0-10 post infection may also elucidate differences in the acquisition of the immune response among lines.

Supplementation with specific amino acids may reveal differences in utilisation among the divergent selection lines. Supplementing animals with 2 g/d cysteine via abomasal infusion increased peripheral eosinophil concentrations and globule leukocytes in the abomasum, while infected with *H. contortus* and *T. colubriformis* (Miller *et al.* 2000). Coop *et al.* (1997) also demonstrated with the addition of 0.2% protected methionine, animals infected with *T. colubriformis* had improved efficiency of feed utilisation. IRH animals may utilise the sulphur amino acids, cysteine and methionine more readily, to heighten their gut immune response and therefore reduce oxidation of dietary amino acids and improve the efficiency of feed utilisation. Supplementing with specific amino acids may ascertain the effect of individual amino acids on the immune response in nematode infection and reveal rate limiting amino acids.

Interestingly, digestive changes were shown in the rumen of IRH animals, which is earlier in the digestive tract from the actual site of infection. Abomasal cannulation during an *H. contortus* infection may detect correlated changes among divergent selection lines at the site of infection. Poppi *et al.* (1990) showed when animals were infected with the abomasal infection *T. circumcincta*, the pH in the abomasum increased reducing the release of copper and uptake by the liver. The abomasal smooth muscle in IRH animals had a greater ^{15}N uptake when infected with *H. contortus* than DRH and C lines. Measuring the utilisation of nitrogen in the abomasum may help to determine the physiological changes that are occurring in the abomasum of IRH animals in response to infection.

6.9 Conclusion

This study has elucidated the biological basis for genetic correlations between parasite resistance and production traits. It has provided a better understanding of the separate mechanisms that arise from divergent selection on the same trait. The studies have also quantified the differences in dietary intake, digestion and partitioning among divergent selection lines infected with *H. contortus*, which have not been previously reported. There has been a metabolic cost of single trait selection for increased resistance to *H. contortus*, which may be associated with a greater immune response.

Appendices

Appendix 1 Fresh organ weights (g) of Merino weaner wethers, from lines selected for either increased resistance to *Haemonchus* (IRH), decreased resistance to *Haemonchus* (DRH) or random-bred control (C)

Fresh weight	IRH		DRH		C		
Organ	ls mean	s.e	ls mean	s.e	ls mean	s.e	P value
Spleen	42.5^{a}	2.49	52.8^{b}	2.49	55.2^{b}	2.49	0.002
Thymus	46.7^{ab}	3.54	54.2^{a}	3.54	43.1^{b}	3.54	0.092
Liver	516.2^{ab}	20.48	567.2^{a}	20.48	490.0^{b}	20.48	0.035
Kidney	101.9	3.72	105.7	3.72	101.5	3.72	0.681
Heart	142.8	4.65	153.8	4.65	140.3	4.65	0.105
Abomasal lymph node	2.3	0.21	2.7	0.21	2.0	0.21	0.126
Mesenteric lymph node	29.2	1.58	30.7	1.58	28.5	1.58	0.621
Abomasum	146.6^{a}	10.66	179.2^{b}	10.66	150.0^{a}	10.66	0.073
Small intestine	539.6^{a}	13.50	570.0^{ab}	13.50	600.4^{b}	13.50	0.011
Skin	2618.6^{a}	135.05	2882.9^{ab}	135.05	3080.1^{b}	135.05	0.066

Within rows, values with a common suffix or no suffix do not differ significantly (P>0.05).

Abomasum and abomasal lymph node fresh organ weights (g) of Merino weaner wethers either maintained worm free (NIL) or trickle infected with *H. contortus* (INF)

	NIL		INF		
Organ	ls mean	s.e	ls mean	s.e	P value
Abomasum	149.1^{x}	8.70	168.1^{y}	8.70	0.1314
Abomasal lymph node	1.5^{x}	0.17	3.1^{y}	0.17	<0.0001

Within rows, values with a common suffix do not differ significantly (P>0.05).

Appendix 2 Dried organ weights (g/kg bodyweight) of Merino weaner wethers, from lines selected for either increased resistance to *Haemonchus* (IRH), decreased resistance to *Haemonchus* (DRH) or random-bred control (C)

	IRH		DRH		C		
Organ	ls mean	s.e	ls mean	s.e	ls mean	s.e	P value
Spleen	0.31[a]	0.02	0.35[a]	0.02	0.42[b]	0.02	0.0003
Thymus	0.34	0.03	0.35	0.03	0.33	0.03	0.897
Liver	5.47	0.13	5.46	0.13	5.20	0.13	0.273
Kidney	0.69	0.02	0.66	0.02	0.70	0.02	0.289
Heart	1.00	0.03	0.96	0.03	1.04	0.03	0.178
Abomasal lymph node	0.02	0.002	0.02	0.002	0.02	0.002	0.908
Mesenteric lymph node	0.17	0.01	0.16	0.01	0.18	0.01	0.538
Abomasum	1.03	0.06	1.15	0.06	1.07	0.06	0.391
Abomasal mucosa	0.04	0.004	0.04	0.004	0.04	0.004	0.824
Abomasal smooth muscle	0.99	0.06	1.11	0.06	1.03	0.06	0.395
Small intestine (SI)	3.28	0.18	3.10	0.18	3.64	0.18	0.120
SI mucosa	0.74	0.07	0.71	0.07	0.89	0.07	0.181
SI smooth muscle	2.71	0.18	2.85	0.18	3.18	0.18	0.169
Skin	23.58[a]	0.96	24.01[a]	0.96	28.32[b]	0.96	0.002

Within rows, values with a common suffix or no suffix do not differ significantly (P>0.05).

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