Variation in daily feed intake and growth between Merino sheep lines selected for differences in resistance to nematodes

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Summary

This trial was designed to characterise daily feed intake and growth responses of animals selected for genetic difference in resistance to nematode infection when fed different quality diets, with and without an artificial infection with *Haemonchus contortus*. Fifty four Merino 10–months old rams from the CSIRO flock with three divergent lines selected for either increased (IRH) or decreased (DRH) resistance to *Haemonchus*, or unselected (C) were stratified within selection line on the basis of liveweight measured following adjustment to experimental conditions and faecal egg counts from previous experimental challenge and individually housed during a two week non–infected period and nine weeks of trickle infection with McMaster strain of *H. contortus.*

Throughout infection, there were no significant differences in daily feed intake and body weight gains between selection lines, but rams on the high quality diet had significantly higher (P<0.0001) feed intakes and liveweight gains than those fed the moderate quality diet. Average faecal egg counts per gram at week 9 of infection followed the expected differences between selection lines; the counts were 3,102 for IRH, 14,417 for DRH and 9,674 for C. It is assumed from the lower faecal egg counts that resistant animals were carrying significantly lower worm infections. The results suggest a difference in supply and/or partitioning of peptides and amino acids between selection lines, the IRH compared with DRH and C directing more of their protein supply into immune functions and less to production functions such as weight gain.

Keywords: feed intake, resistance, genetic selection, nematode infection, *Haemonchus contortus*

Introduction

Breeding for resistance to gastrointestinal (GI) nematodes is a long–term method of control that has been demonstrated to be an effective strategy to reduce susceptibility to infection and frequency of anthelmintic use. Resistance is the ability of a host to reduce the establishment, survival or reproductive rates of the parasite, whereas with resilience the host continues to maintain productivity despite infection (Gray 1995).

There are a number of experimental flocks within Australia that have demonstrated the value of genetic selection for nematode resistance, one of which is the CSIRO *Haemonchus* selection flock (Woolaston *et al.* 1990). This flock consists of three divergent lines selected for either increased resistance to *Haemonchus* (IRH), decreased resistance to *Haemonchus* (DRH) and a random–bred line (C). The IRH selection line has displayed lower faecal egg counts (FEC) (Woolaston *et al.* 1990, 1997) and reduced pasture contamination (Eady *et al.* 2003) compared with susceptible counterparts.

There has been little work to elucidate the physiological and immunological responses that characterise the differing level of resistance against GI nematodes in the *Haemonchus* selection flock. There is evidence that selection for nematode resistance has correlated effects on the supply and/or partitioning of peptides and amino acids between the gut immune response and production functions, such as liveweight gain and wool growth. Doyle (1999) demonstrated that in the absence of GI parasites the IRH sheep have lower levels of wool production and liveweight gain than C sheep. More recently, Eady and Smith (2001) and Kahn *et al.* (2003) reported that IRH animals do not have greater levels of production compared to their C counterparts, despite having considerably lower burdens of GI nematodes as assessed from worm egg counts. The absence of a production advantage in the IRH line is unexpected because it could be presumed that nematode–induced pathology and hence endogenous protein loss are reduced in these sheep, allowing utilization of additional protein for production.

Therefore, this experiment was conducted to test two hypotheses: firstly, that animals from lines selected for differences in resistance to nematode infection would have different daily feed intakes when fed moderate and high quality diets in the absence and in the presence of infection; and secondly that animals from these selection lines would have similar liveweight gains during infection when fed moderate or high quality diets.

Materials and methods

Experimental design

The sheep were kept in individual pens in an animal house. Seven weeks were allowed for the animals to adapt to experimental conditions and to complete a diet selection study (results of which are not reported here). Animals were fed experimental diets for three weeks prior to the experimental design described below. The experiment was a 3 x 2 factorial with three selection lines selected for genetic difference in resistance to nematode infection and two diets, one a high quality (high metabolizable protein and metabolizable energy) and the other of moderate quality (moderate metabolizable protein and metabolizable energy). They were stratified within selection line on the basis of liveweight measured following adjustment to experimental conditions and faecal egg counts (FEC) from a previous experimental challenge, and randomly allocated to diets and position of pen. The experiment was conducted over two periods, firstly a period of two weeks where animals were maintained worm–free, followed by an infection period of nine weeks.

Animals and housing

The study used fifty–four Merino weaner rams selected randomly from the CSIRO *Haemonchus* IRH (n = 19), DRH (n = 15) and C (n = 20) selection lines (Woolaston *et al.* 1990). They were approximately 10 months of age at the beginning of the experiment with a mean ± SD liveweight of 26.1 ± 2.85 kg. They had previously been artificially infected with 10 000 L_3 *H. contortus* at approximately six 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.

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 Ivomec® (0.2 mg/kg ivermectin, Merial), to remove existing worm burdens, and were given an intermuscular injection of vitamin B_{12} (1 mL/animal, hydroxocobalamin and cyanocobalamin, Novartis Animal Health). A faecal egg count was taken 5 days after drenching to ensure all animals had zero counts.

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

	High quality	Mod quality 1	Mod quality 2
Ingredients (% per kg fresh matter)			
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 (% as fed)	90.8	92.3	89.9
Organic matter (% as fed)	86.8	88.9	89.2
Digestibility[A] (% DM)	62.7	58.3	46.1
Ether extract (% DM)	3.0	2.0	1.6
Crude protein (% DM)	16.8	9.7	7.5
Calculated MP[B] (g/kg DM)	90.0	43.0	30.4
Calculated ME[C] (MJ/kg DM)	9.2	8.3	6.3
Sulphur (g/kg DM)	2.1	1.7	2.0
Phosphorus (g/kg DM)	3.3	2.2	2.4
Iron (ppm)	147.2	154.5	160.3
Copper (ppm)	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, 1.213 g 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
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Feed

The composition and analysis of the experimental diets are given in Table 1. Diets were formulated to be non–limiting and balanced for minerals, and were pelleted to reduce selectivity of each feed component. Diet formulation was not constrained to make diets isoenergetic while differing in metabolizable protein (MP) content. Donaldson *et al.* (1998) have shown that it is the level of MP, not metabolizable energy (ME), which has the most influence on host resistance to nematode infection.

Animals were offered quantities of fresh food once daily that were 10% greater than *ad libitum* intakes on the previous day. A sub–sample 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. Two moderate quality diets were used. Moderate quality diet 1 (M1) was fed in the non–infection period only and then changed to the moderate quality diet 2 (M2) at the start of the infection; this change was instigated by unexpectedly high growth rates on M1, and was to ensure animals would be able to differentiate between the moderate and high quality diets for the diet selection study.

Infection

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* L3. An initial infective dose of 150 L3/kg liveweight was administered orally at the beginning of the infection period and followed by a trickle infection of 250 L3 three times per week on Monday, Wednesday and Friday (average total dose ± SD over the infection period of 12 570 ± 565 L3/sheep).

Animal measurements

Animals were weighed in the morning prior to feeding at the start of the experiment and then weekly during both periods that followed. A faecal egg count per animal was measured at days 0 and 21 after the initial dose of infective larvae and weekly thereafter. A modified McMaster technique was used to count worm eggs, but potassium iodine was used rather than sodium chloride salt solution. The higher specific gravity (1.4) of potassium iodine was required to enhance flotation and therefore detection of eggs from animals consuming the pelleted diets rich in cottonseed hulls.

Statistical analysis

All statistical analyses were performed using the SAS computer program (SAS Institute Inc 1999–2001). Generalised Linear Models (GLM) were used to analyse the significance of diet quality (H and M), selection line (IRH, DRH and C) and the interaction (diet x line) between these effects. The models used repeated measures analysis of variance for daily feed intake, body weight gain and faecal egg counts. Initial liveweight was not a significant covariate for subsequent liveweight gain measures. Change in liveweight during the uninfected period was not of primary interest and, because this was only 2 weeks long, 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 squares means ± SE are presented for daily feed intake and weight gain. Faecal egg counts were cube–root transformed to normalize data prior to analysis and are presented as back–transformed means.

Results

Daily feed intake

During the 2 week non–infection period, selection line significantly ($P = 0.03$) affected daily feed intake. Weaner rams from the IRH line had greater feed intake than the C line but there was no difference in intake between C and DRH (Table 2). Diet had no affect on daily feed intake and there was no significant interaction between the effects of selection line and diet.

The effect of the independent variables selection line and diet on the daily feed intake (g DM/day) of the weaner rams with an artificial infection of *Haemonchus contortus* is displayed in Figure 1.

During the infection period the daily feed intake did not differ between selection lines despite a large and significant ($P<0.0001$) difference in faecal egg counts. Conversely, diet did significantly ($P<0.0001$)

Table 2 Average daily feed intake (g DM/day) of weaner rams, from lines that had been selected for either increased resistance to *Haemonchus* (IRH), decreased resistance to *Haemonchus* (DRH) and random–bred control (C), without nematode infection.

Selection line	Least squares means	Standard error
IRH	1613^{a}	51.6
DRH	1508^{ab}	58.2
C	1415^{b}	50.3

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

affect daily feed intake of infected animals (Figure 2). Interactions between the effects of selection line and diet were not statistically significant during the infection period.

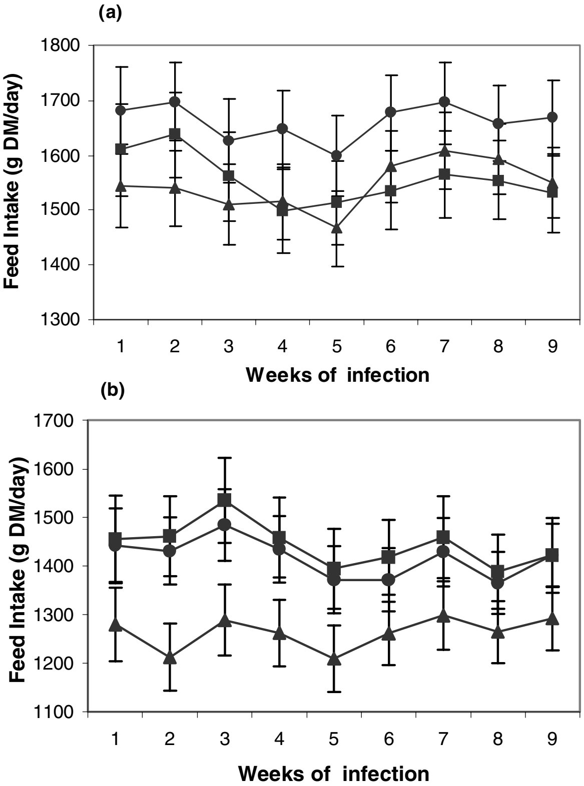

Figure 1 Daily feed intake (g DM/day ± SE) of IRH (—●—), DRH (—■—) and C (—▲—) weaner rams differing in their genetic resistance to *Haemonchus contortus*, with an artificial infection of *H. contortus* and fed high quality (a) and moderate quality (b) diets.

Liveweight change

Animals on both the H and M quality diets gained liveweight throughout the infection period (Table 3). There was a significant difference ($P<0.0001$) in liveweight gain between diets during the infection period. On average weaner rams fed H gained 11.8 kg during the 9 week infection period, while animals fed M2 gained an average of 5.8 kg.

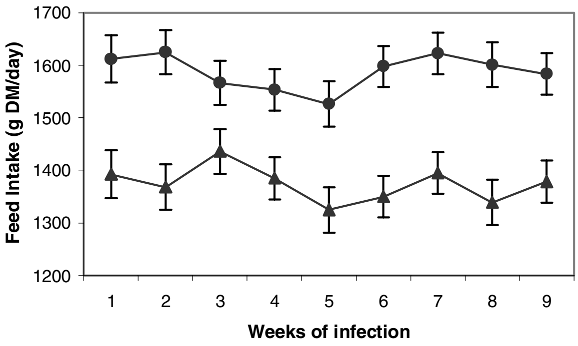

Figure 2 Daily feed intake (g DM/day ± SE) of weaner rams artifically infected with *Haemonchus contortus* and fed different diets of high (—●—) and moderate (—▲—) quality.

The effect of selection line on liveweight change was not significant during the infection period. There was also no significant interaction between the effects of selection line and diet for liveweight gain.

Faecal egg count

Faecal egg counts followed the expected differences between selection lines; the average FEC per gram of faeces at week 9 of infection was 3,102 for IRH, 14,417 for DRH and 9,674 for C rams. Analysis showed a significant ($P<0.0001$) selection line effect throughout the infection period. There was no effect of diet on FEC throughout the infection, nor was there a significant interaction between the effects of selection line and diet.

Discussion

Daily feed intake

The first objective of this study was to investigate whether animals from lines selected for differences in resistance to nematode infection would have different daily feed intakes when fed two different quality diets in the absence and presence of infection. Results found the IRH line had significantly higher daily feed intakes during the non–infected period, compared to the C line, with the DRH line being intermediate. However, when

Table 3 Liveweight gain (kg) of weaner rams, from lines that had been selected for either increased resistance to *Haemonchus* (IRH), decreased resistance (DRH) and random–bred control (C) calculated over the 9 week period of artificial infection with *H. contortus*.

Quality of diet	Selection line	Least squares mean	Standard error
High	IRH	11.9	0.71
	DRH	11.5	0.75
	C	12.0	0.67
Moderate	IRH	5.7	0.67
	DRH	7.0	0.81
	C	4.9	0.68

the same animals were artificially challenged with *H. contortus* the selection line effect on intake was not apparent. The start of the infection regime coincided with the introduction of the M2 diet for reasons discussed earlier. Feed intake for all selection lines declined uniformly with the introduction of M2 and the start 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. Further experimentation is required to determine if the significant difference in feed intake between selection lines during the non–infected period would have remained apparent during the infection period if M1 had continued to be fed.

Diet significantly influenced the daily feed intake of each selection line during the infection period with higher intakes recorded for 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. The non–significant difference between the diets for feed intake in the non–infection period may have been because differences between dietary levels of MP and ME were not sufficient to allow expression of feed intake differences.

Liveweight change

Animals from lines selected for differences in resistance to nematode infection had similar liveweight gains during the infection period on both high and moderate quality diets despite a significant difference in FEC. One would expect the IRH line to have had higher weight gains than the animals from the DRH and C lines, as these animals had considerably lower FEC. However 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; weight changes of IRH and C lines were similar whether uninfected or infected. A possible explanation for this recurring observation is that divergent selection for resistance has produced changes in supply and/or partitioning of peptides and amino acids between the gut mucosal immune response and production functions, such as weight gain and wool growth, in each selection line. The basis of these changes may originate in the selection process used to identify resistant animals, which have been selected on their ability to more effectively prevent establishment and/or development of nematode infection. The IRH animals may continue to redirect their protein supply away from body growth into immune functions to prevent larval establishment and maintain resistance, even in the absence of infection. It is possible that resistant animals may not be able to effectively ‘down regulate’ their mucosal immune response, resulting in excessive activity. In contrast, the DRH line showed similar liveweight gains as the IRH line while carrying an infection almost five–fold that of the IRH line. Clearly these susceptible animals are displaying strong resilience to *Haemonchus* challenge.

In further support for this working hypothesis, weight gains of animals eating the M2 diet varied between selection lines, although differences were not significant. The DRH line gained 1.3 kg more than the IRH line over the infection period, despite the two selection lines having similar daily feed intakes. Analysis showed a trend ($P = 0.091$) of dry matter intake adjusted for body weight (DMI/BW), showing the IRH line did eat a higher proportion of dry matter per unit body weight than the DRH. This may suggest that with lower quality diets the metabolic cost of resistance is greater than the metabolic cost of infection (Walkden–Brown and Eady 2003).

Liveweight gain during the infection was far greater than predicted (Freer *et al.* 1997). The reasons are currently unknown, but the cottonseed hulls used in the experimental diets may play a role in the greater weight gains. The H diet supported larger weight gains (187 g/d) during infection compared to the M2 diet (93 g/d). 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).

Faecal egg count

Woolaston *et al.* (1990) have observed the distinct selection line affect on FEC. The non–significant effect of diet quality on FEC 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 there was a trend for animals from the DRH and C lines to have lower FEC on the H diet. One reason dietary effects on FEC may not have been greater, is the quality of the M2 diet was sufficiently high to allow expression of resistance.

In conclusion we have found firstly that in the absence of infection the IRH line has significantly higher daily feed intakes compared to the C selection line. Secondly, there were no differences in daily feed intake or weight gain between selection lines during infection, despite a significant difference in faecal egg count. Experimentation continues into the possible changes in supply and/or partitioning of peptides and amino acids between the gut mucosal immune response and production functions, such as weight gain and wool growth in each selection line.

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