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Enhancing immunity to nematode parasites in single-bearing Merino ewes through nutrition and genetic selection

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Abstract

The effectiveness of protein supplementation and genetic selection to enhance the resistance of periparturient Merino ewes to infection from gastrointestinal parasites was tested in a replicated grazing experiment. One hundred and twenty ewes from lines selected for increased resistance (R) to *Haemonchus contortus* or at random (C) were subjected to one of three supplement groups that provided 0 or 250 g/day cottonseed meal for 5 weeks prior to, or for 6 weeks after, the start of lambing. Faecal egg counts (FEC) of R ewes were consistently lower than those of C ewes but both groups exhibited a periparturient rise in FEC. Supplementation during the prepartum period reduced FEC and increased ewe body weight gain. The benefits of prepartum supplementation in reducing FEC continued to be apparent up to 10 weeks after supplementation ceased. There was a strong suggestion that the benefits to parasite resistance from protein supplementation were greatest in C ewes. Wool growth rates (15%) and birth weights (5%) were greater for C ewes but differences between the lines for lamb body weight had disappeared by day 97. The greatest benefit to resistance from protein supplementation was observed when ewes were experiencing a loss of maternal body weight. Conversely, no benefits to resistance were observed when ewes

had moderate (78-107 g/day) rates of maternal weight gain. These results suggest that increased resistance as a result of protein supplementation is dependent on the prevailing supply and demand for scarce nutrients such as metabolisable protein (MP). Both genetic selection and protein nutrition are effective strategies to enhance host resistance to nematode infection during the periparturient period.

Keywords: Sheep; Nematode parasites, Genetic resistance, Metabolisable Protein, Protein supplementation, Periparturient

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1. Introduction

In sheep, a transient diminution of immunity to intestinal parasites occurs around the time of lambing and results in a periparturient rise (PPR) in faecal egg count (FEC). Reduced immunity in the periparturient ewe is a major source of pasture larval contamination (Brunsdon, 1970; O'Sullivan and Donald, 1970; Connan, 1976; Lloyd, 1983). The consequences of increased pasture larval numbers are that lambs and young sheep are exposed to a greater level of infection, develop a greater worm burden, have reduced production and have an increased requirement for anthelmentic treatment.

Both selection of resistant genotypes (Woolaston, 1992) and improved protein nutrition (Donaldson et al., 1998; 2001; Kahn et al., 1999) can reduce the magnitude of the PPR. Woolaston (1992) reported reduced FEC in periparturient Merino sheep which had been selected for increased resistance to *Haemonchus contortus* at 4-6 months of age. In the same study, the rise in FEC after parturition in resistant ewes was less pronounced when compared to sheep selected at random. The PPR coincides with an increase in the nutritional requirements of the ewe due to the demands of late gestation and lactation. While requirements for both metabolisable energy (ME) and metabolisable protein (MP) increase

in reproductive ewes, the largest relative increase is for MP. Metabolisable protein requirements of a housed 50 kg single-bearing ewe fed a diet containing 11.5 MJ ME/ kg DM and maintaining maternal live weight typically increase from 60 g/day at mating to about 90 g/day two weeks prepartum and to 190 g/day three weeks post partum (Freer et al., 1997). Increasing the supply of MP but not ME to housed ewes during the periparturient period has been shown to reduce FEC and worm burdens (Donaldson et al., 1998; 2001) but beneficial effects of increased MP supply on worm burdens in periparturient ewes are not always observed (Houdijk et al., 2000).

The greatest increase in MP requirements is due to the demands of lactation, with peak requirements coincident with maximum milk production about 3 weeks post partum (Freer et al., 1997). Accordingly, the greatest benefits from increased MP supply on host resistance would be expected during the early post partum period. Houdijk and co-workers (2000) tested this proposition in housed sheep infected with *Ostertagia circumcincta* but were not able to demonstrate a beneficial effect of MP supply on worm burdens during either the pre or post partum period. Determining the benefits of increased MP supply during the periparturient period in grazing ewes has yet to be systematically evaluated. In addition, determining the relative benefits of pre and post partum protein supplementation on host resistance of grazing ewes is an important consideration for the commercial application of this practice.

Therefore, this experiment was conducted to test the following hypotheses. Firstly, that supplementation to increase MP supply of grazing periparturient ewes would enhance host resistance and resilience to gastrointestinal nematode infection and reduce the extent of the PPR in FEC. Secondly, that post partum, rather than prepartum, supplementation would be most effective in reducing the PPR in FEC. Thirdly, that increased MP supply during the periparturient period would be less effective in ewes selected for increased resistance to *H.*

contortus than their random-bred counterparts. Fourthly, that resistant genotypes and protein supplementation would result in lower levels of infection in resultant progeny.

2. Materials and Methods

2.1. Experimental design

A schematic representation of experimental events is detailed in Fig. 1. Ewes were stratified within selection line on the basis of sire of progeny and then allocated at random to supplement group. The experiment was a 2 x 3 factorial with 2 levels of genetic resistance and 3 supplement groups with 4 replicates for each treatment combination. Treatments were allocated at random to 24 experimental plots (0.8 ha each with 5 ewes per plot) to constitute a completely randomised design. Five ewes per plot was considered to be insufficient to control pasture growth and, because more ewes were unavailable, four ewe hoggets were added to each plot. The hoggets were stratified on the basis of sire within selection line and allocated at random to graze in plots with ewes from the same selection line. Hoggets were treated with Ivomec Maximiser™ controlled release capsule (80 mg ivermectin released over 100 days; (Merck Sharp & Dohme (Australia)) 14 days after entering the plots to minimise their influence on pasture infectivity. Results from the hoggets are not presented here. All animals continuously grazed the same plots until lambs were on average 97 days of age by which stage the lambs would be exposed to larval numbers on pasture that reflected treatment effects on ewe faecal egg count (FEC).

2.2. Animals and conditions

One hundred and twenty mixed age (2-6 years of age) Merino ewes selected either for increased resistance (R) to *H. contortus* (n = 60) or at random (C) (n = 60) (Woolaston et al., 1990) were used in this experiment. Selection had been ongoing for the past 23 years.

The ewes had been single sire mated (5 rams per selection line) over a 4 week period and were confirmed to be single-bearing by ultrasonic scanning 10 weeks after the start of mating. In addition to the 120 ewes, 96 ewe hoggets (10 months of age) from the same R (n = 48) and C (n = 48) selection lines were used in this experiment to ensure an appropriate stocking rate in experimental plots. Hoggets were drenched with Scanda® (8 mg/kg levamisole hydrochloride and 4.5 mg/kg oxfendazole; Schering-Plough Animal Health) one week prior to allocation to plots.

2.3. Supplement group

Animals were subjected to one of three supplement groups designed to provide on an as fed basis, 0 or 250 g/day cottonseed meal (CSM; 92% DM; 396 g CP/kg DM) pellets fed for either 5 weeks prior to the start of lambing or 250 g/day CSM pellets fed for 6 weeks after the start of lambing. Animals were fed three times per week on Monday, Wednesday and Friday mornings with the supplement being fed in a trough 3 m in length. Following inclusion of the hoggets to each plot, the amount of supplement was increased to provide an additional 50 g/day for each hogget.

2.4. Infection details

Prior to the start of the experiment all ewes had grazed together with the exception of a 4 week mating period some 4 months earlier. Nematode infections in the animals at the start of the experiment were considered to be part of the experimental worm burden. To supplement the existing nematode infection each ewe was infected with 3000 L_3 *Trichostrongylus colubriformis* and 1000 L_3 *H. contortus* larvae (both McMaster strain) on 3 occasions, those being 16, 9 and 1 day prior to allocation to experimental plots.

2.5. Sampling details

Timing of animal measurements is quoted with reference to the mid-point of lambing (day 0), 15 days after the start of lambing when approximately 50% of lambs had been born (Fig. 1).

2.5.1. Tracer sheep

Prior to the start of the experiment pasture larval numbers were assessed on the experimental plots. Weaner rams (n = 48) from the *H. contortus* susceptible line were stratified on the basis of sire and 2 rams were allocated at random to each plot to act as tracer sheep. Tracers grazed in the plots for a 2 week period prior to the start of the experiment (days –66 to –53 inclusive), were then removed and housed indoors in group pens (n = 12) and fed 500 g/head/day lucerne chaff (*Medicago sativa*). Four weeks after removal from the plots, faeces were collected from the tracers to determine FEC. Faeces from tracers which had grazed the same treatment combination were pooled and cultured to determine the contribution of infective species to FEC.

2.5.2. Pasture sampling

Pasture was sampled from each plot on days -51, 5 and 76 to assess herbage mass (kg DM/ha), and the percentage of green and dead matter. Sampling for herbage mass was conducted using a median quadrat containing 5 subquadrats (subquadrat area of 0.283m^2). In each plot, a total of 3 sampling locations were chosen using a stratified random sampling procedure. At each location the subquadrat which contained the median herbage mass was cut and the herbage from each location bulked together within plots and dried at 55ºC for 5 days, to determine dry matter content. A representative and well mixed sample (*c.* 6%) of

the dried herbage was used to determine the fraction of green and dead matter in the sample. Pasture species composition was estimated on day -21 from the frequency of pasture species calculated from 50 locations chosen at random from within each plot.

2.5.3. Live weight and parasitology

Ewes were moved to yards and weighed on days -71, -21, 28, 55 and 97. Lambs were weighed at birth and on day 97. Faeces were collected from the rectum of ewes on the weighing days to estimate FEC using a modification of the McMaster method (lower level of detection 100 epg). Faeces were collected from the rectum of lambs to estimate FEC on day 97. At each sampling, approximately equal amounts of faeces from each ewe within each plot were combined and cultured to facilitate microscopic identification of nematode larvae. Identification of nematode larvae was to genus level to determine genus contribution to FEC.

2.5.4. Supplement intake

To estimate supplement intake of individual sheep, CSM pellets were sprayed with lithium chloride (LiCl; 0.83 mg Li/g pellet) and fed on days -21 and 28. Animals were fed the lithium-containing supplement at 7:00 h, allowed 1 h access to the supplement, and then residual supplement was removed. Blood was sampled (10 ml) by jugular venepuncture 2-9 h later, into vacutainers that contained sodium heparin and then stored on ice. Blood samples were centrifuged (3000 *g* for 10 min) and plasma separated and immediately frozen at -20ºC. Plasma lithium concentrations were determined according to the procedure of Kahn (1994). Estimated individual supplement intake was calculated using the method of Suharyono et al., (1991) in which plasma concentration of lithium adjusted for live weight is used to predict the fraction of the supplement on offer eaten by an individual animal.

2.5.5. Wool growth

A 15 cm dyeband was placed on the midside position on the left side of ewes on day -21 followed by a second dyeband on day 28. The dyeband was removed (Oster clippers No. 40) on day 55. Ewes were shorn on day 68 and fleece weights, including belly wool, of individual sheep were recorded. Wool growth rate (g clean wool/day) was determined from dyeband wool samples according to the method of Langlands and Wheeler (1968). Clean wool growth was calculated after determination of washing yield using standard procedures. Briefly, wool was placed in nylon mesh bags (pore size 44μM) washed twice at 60ºC in a wool scouring machine (Goodwin Industries) using Lissapol Detergent (at *c*. 1 g/l) and then rinsed in warm water. Average fibre diameter was determined on 2000 x 2 mm wool snippets using an Optical Fibre Diameter Analyser (OFDA, BSC Electronics, Western Australia).

2.6. Statistical analysis

Data was analysed using a number of generalised linear models with the computer program SAS (SAS Institute Inc, 1990). The effects included in the models used to analyse the experimental variables are detailed in Table 1. The experimental unit for all analyses was defined as the experimental plot. Analysis of data was conducted in four discrete periods to avoid confounding of time with supplement group. The discrete periods were (1) prior to supplementation; (2) during the prepartum supplementation period; (3) during the postpartum supplementation period; and (4) after the postpartum supplementation period. Repeated measures analysis was used in period 4 when multiple samples were taken.

Contrasts were constructed within supplement group to examine the significance of the main effect of supplemented (either pre or post partum) versus unsupplemented and the interaction of supplemented versus unsupplemented in R and C animals. Ewe body weight

data is presented as wool-free body weight. Wool-free body weight was calculated by deduction of the estimated weight of fleece at each weighing date, as determined by reference to annual greasy fleece weight at shearing (day 68) and wool growth rate during the experimental period. Body weight immediately prior to entry to plots was included as a covariate for the analysis for body weight during the experimental period. Birth date was included as a covariate for the analysis for lamb body weight at day 97 and lamb growth rate from birth to day 97.

Faecal egg counts (expressed as the number of eggs per gram of faeces (epg)) were subjected to square root transformation to normalise the data prior to analysis to determine statistical significance and backtransformed means are presented in this paper. In all other cases least squares means (l.s.mean) ± standard error (se) are used throughout the results.

3. Results

3.1. Tracer sheep

Tracer rams developed patent nematode infections (backtransformed mean FEC 2181 epg) suggesting that there were reasonable numbers of infective nematode larvae on the plots at the start of the experiment. Faecal egg count of tracer rams which grazed plots allocated to different treatment combinations did not differ indicating that there were no initial differences in larval numbers on pasture.

3.2. Herbage mass and species frequency

Total, green and dead herbage mass did not differ between supplement group or selection line plots at any sampling period and the interaction between these effects was not significant. Least squares mean (± se) green and total herbage mass at days –51, 5 and 76

were 1200 ± 174.2 kg green and 2250 ± 169.7 kg total, 1560 ± 119.2 kg green and 2150 ± 153.3 kg total and 3190 ± 283.5 kg green and 4350 ± 375.4 kg total DM/ha respectively. The dominant pasture species were, in descending order, *Lolium perenne* (26%), *Phalaris aquatica* (19%), *Holcus lanatus* (18%), *Trifolium repens* (10%), *Anthoxanthum odoratum* (9%), *Hypochoeris radicata* (7%), *Vulpia* spp (6%) and *Bromus* spp (5%).

3.3. Supplement intake of experimental ewes

Mean individual intake (± se) of supplement by ewes during the pre and post partum periods was estimated to be 206 ± 44.8 and 175 ± 34.4 g/day respectively. Including supplement intake as a covariate in the analysis of variance for FEC did not improve the fit of the model.

3.4. Parasitology of ewes

Faecal egg counts of R ewes were significantly lower than those of C ewes throughout the experiment ($P<0.01$ at days -71, 28 and 55; $P<0.05$ at days –21 and 97) (Fig. 2). Ewes supplemented during the prepartum period had lower FEC at day -21 ($P=0.05$) but supplementation was ineffective in reducing FEC at other times. There was no significant residual benefit of prepartum supplementation on FEC. Interactions between supplement group and selection line for FEC were not statistically significant ($P=0.15$) but there was a suggestion that FEC of C but not R ewes at day -21 was reduced by supplementation during the prepartum period (Fig. 2).

The majority of larvae cultured from faeces of ewes were identified as *Trichostrongylus* spp but at day 97 *Oesophagostomum* spp accounted for 42% of larvae (Table 2). Faecal egg count attributable to *T.* spp and *Ostertagia* spp was significantly lower ($P<0.01$) in R ewes than C ewes at days 28 (231 v 534 epg) and 55 (256 v 739 epg) for *T.*

spp and at day 55 (37 v 186 epg) for *Ostertagia* spp. Differences for other nematode species and other times were not significant. The effects of supplement group and the interaction between the effects of supplement group and selection line were not significant.

3.5. Body weight of ewes

Wool-free body weight (WFwt) at day –71 (preexperimental) did not differ among supplement groups and selection lines and the interaction between supplement group and selection line was not statistically significant. WFwt at day -71 was a significant covariate ($P<0.01$) for all subsequent measures of WFwt. Supplementation during the prepartum period increased ($P<0.05$) WFwt of ewes at day –21. There was a suggestion ($P=0.08$) that WFwt of supplemented ewes (regardless of timing of supplementation) at day 28 was greater than that of unsupplemented ewes (Fig. 3). Up until day –21 of the prepartum period the wool-free weight gain (± se) of unsupplemented and supplemented ewes was 18 ± 5.6 and 34 ± 6.8 g/d respectively. At all other times WFwt was unaffected by supplementation. Throughout the experiment there were no differences in WFwt between R and C ewes, and the effect of ewe parity and the interaction between the effects of supplement group and selection line were not significant.

3.6. Wool growth of ewes

Clean wool growth (g/day) from day –21 to 28 of C ewes was greater ($P<0.01$) than that of R ewes (Table 3). Average fibre diameter of wool grown from day –21 to 28 of C ewes was greater ($P<0.01$) than that of R ewes. The effects of supplement group and the interaction between the effects of supplement group and selection line were not significant for clean wool growth and fibre diameter. Annual clean fleece weight of C ewes was greater ($P<0.01$) than that of R ewes (Table 3) but there was no difference in mean fibre diameter

for the entire fleece (20.2 ± 0.17 μM for R ewes and 20.4 ± 0.16 μM for C ewes).

3.7. Parasitology of lambs

Faecal egg count (backtransformed mean epg) of R lambs (237 epg) was significantly lower (P<0.001) than that of C lambs (858 epg) at day 97. Ninety three percent of the larvae cultured from faeces collected from lambs was *T. spp*. The effects of dam parity, gender, birth date, supplement group and the interaction between the effects of supplement group and selection line were not significant for lamb FEC.

3.8. Birth weight and growth of lambs

Birth date was a significant (P<0.01) covariate for body weight of lambs at day 97 and for change in body weight from birth to day 97 but was not significant for birth weight. Birth weight (± se) of lambs born to multiparous ewes (4.1 ± 0.06 kg) was greater (P<0.001) than that of lambs born to primiparous ewes (3.3 ± 0.13 kg). Lambs born to C ewes were heavier (P=0.04) than those born to R ewes. Least squares mean (± se) birth weights were 3.8 ± 0.07 kg and 3.6 ± 0.07 kg for C and R lambs respectively. Differences in weight between the selection lines had disappeared by day 97. The effects of supplement group, gender and the interaction between the effects of supplement group and selection line were not significant for birth weight.

Body weight at day 97 and body weight gain from birth to day 97 was greater (P<0.001) in male lambs. Least squares mean (± se) body weight at day 97 was 21.9 ± 0.33 and 19.4 ±0.34 kg for male and female lambs. Least squares mean (± se) body weight gain from birth to day 97 was 17.3 ± 0.47 and 16.0 ± 0.42 kg for male and female lambs respectively. The use of birth date as a covariate indicated that at day 97, lambs were an extra 165 ± 50.1 g heavier for every extra day of age. There was a suggestion that R lambs

tended (P=0.09) to grow faster from birth to day 97 with body weight gain being 17.3 ± 0.47 and 16.0 ± 0.42 kg for R and C lambs respectively. Differences due to supplement group, dam parity and the interaction between the effects of supplement group and selection line were not significant for body weight at day 97 or body weight gain to day 97.

4. Discussion

The transient diminution of immunity to intestinal parasites in the periparturient ewe contributes significantly to the epidemiology of infection which encourages the unsustainable use of anthelmintics. In this experiment the potential for and interactions between two alternative parasite control measures, namely protein supplementation and resistant genotypes was investigated.

Neither green herbage mass nor the initial level of natural infection from pasture differed among experimental treatments. These were critical measures because they demonstrate that parasitological and production effects discussed in this paper were attributable to experimental treatments.

In support of hypothesis 1, supplementation with CSM reduced FEC in periparturient ewes. Supplementation during the prepartum period resulted in lower FEC 3 weeks prior to the mid-point of lambing (ie. 6 days prior to the first lamb being born). Beneficial effects of prepartum supplementation on FEC did not continue to accrue after supplementation ceased but the benefits that had been obtained, were still largely present 8 weeks after the mid-point of lambing, when peak FEC was observed for all treatments. The persistence of effects on FEC should reduce pasture larval counts and may reduce future anthelmintic requirements. When averaged over the entire experimental period of 21 weeks, the 5 week period of prepartum supplementation reduced FEC by 43% (204 epg) relative to

the unsupplemented control. Despite the reduction in egg output from supplemented ewes, FEC of lambs at day 97 was unaffected by supplement group of the ewe. This probably indicates that prior pasture contamination overwhelmed the beneficial effects of CSM supplementation.

The benefits to host resistance obtained by supplementation of grazing periparturient ewes with CSM support and extend the observations of Donaldson and co-workers (1998; 2001) who demonstrated that increased MP supply, in the form of fishmeal, during the periparturient period greatly reduced burdens of *O. circumcincta* and *T. colubriformis* in housed ewes. Donaldson and co-workers (1998) concluded that the benefits from increased levels of fishmeal may not be attributable to changes in MP supply *per se* but may arise through changes in the intestinal supply of specific amino acids or polyunsaturated fatty acids. The benefits on host resistance demonstrated in this experiment, suggests that benefits to parasite resistance from protein supplementation may not necessarily be associated with the provision of specific amino acids as the amino acid composition of fishmeal and CSM differ (McDonald et al., 1995).

Post partum supplementation was ineffective in reducing FEC leading to the rejection of hypothesis 2. The framework underpinning the hypotheses tested in this experiment is the causal link between MP requirement and supply with the diminution of immunity as proposed by Coop and Kyriazakis (1999). In this framework the greatest benefit of increasing MP supply would be expected during the post partum period when milk yields, and hence MP requirements are greatest. The fact that prepartum and not post partum supplementation was effective in reducing FEC suggests that, in this experiment, the gap between MP requirement and supply (hereafter referred to as the MP pressure) was at its greatest during the prepartum period. Alternatively these results suggest that the framework proposed by Coop and Kyriazakis (1999) may require modification.

In order to determine the likely MP pressure operative during the pre and post partum periods maternal body weight gain, free of foetal fluids, foetus and placenta was calculated. Body weight change between days –71 to –21 (the prepartum supplementation period) was 18 and 34 g/day for unsupplemented and CSM supplemented ewes respectively. At days –71 and –21 ewes were on average 79 and 129 days into gestation. At these times, the combined weight of foetal fluid, foetus and placenta was likely to be 1.5 and 3.9 kg respectively (Black, 1983; Arthur et al., 1989) indicating that foetal tissues had been increasing in weight by 48 g/day during this period. Deduction of this non-maternal component from ewe body weight indicates that maternal body weight change during the prepartum period was –30 and –14 g/day for unsupplemented and CSM supplemented respectively. Similar calculations for the post partum period (days –21 to 28) revealed that all supplement groups experienced positive maternal body weight gains of 80, 78 and 107 g/day for unsupplemented and pre and post partum supplement groups respectively.

Calculation of maternal body weight change indicated the prepartum period as being the time when ewes were experiencing the most severe MP pressure. Consequently, the greatest improvement to immunity, in this experiment, as a consequence of increasing MP supply, would be expected during the prepartum period and would account for the ineffectiveness of CSM supplementation to enhance resistance during the post partum period. In support of this, Kahn and co-workers (2000) demonstrated that resistance to *T. colubriformis* in young sheep could not be enhanced by increasing MP supply over and above levels supporting moderate weight gain.

Faecal egg counts of R ewes were significantly less than those of C ewes at all times during pregnancy and lactation and exceeded 200 epg at only one sampling event. Such a low FEC indicates that anthelmintic treatment would have been unnecessary in R ewes and that these animals maintained sufficient resistance to nematode parasites during the

periparturient period. This is not to suggest that R ewes did not exhibit a PPR. Indeed the timing for the increase in FEC and of peak FEC were very similar to that exhibited by C ewes but the magnitude of the PPR was greatly reduced. This supports the observations of Woolaston (1992) who demonstrated that peak FEC of R ewes was significantly lower than that of C ewes 6-7 weeks after lambing.

Increased resistance to nematode infection was also evident in R lambs at 97 days of age at which stage FEC of R lambs was 72% (621 epg) lower than that of C lambs. That R lambs exhibited increased resistance at that age indicates that young sheep are not immunologically incompetent, as has been suggested by Smith and Angus 1980, and are capable of expressing protective immunity to nematode infection. Indeed, Ward and co-workers (1999) demonstrated that R lambs from these selection lines exhibited increased resistance by as early as 65 days of age.

While statistical analysis showed no significant interaction between supplement group and selection line for FEC during the prepartum period ($P=0.15$), the data (Fig. 2) suggests that CSM supplementation was most effective in reducing FEC of C ewes. At 3 weeks prior to the mid-point of lambing, FEC of supplemented C ewes (99 epg) was 77% lower than unsupplemented ewes (435 epg). In contrast, effects on R ewes at this time were trivial, providing some support for hypothesis 3, that increasing MP supply would be least effective in enhancing resistance in resistant genotypes.

The apparent interaction between genotype and supplementation for FEC of periparturient ewes is consistent with the greatly reduced immunoresponsiveness of young sheep to protein supplementation in resistant breeds (Abbott et al., 1985) and bloodlines (MacFarlane, 1999). The mechanisms by which increased MP supply and genetic selection enhance resistance of the periparturient ewe to nematode infection are not fully understood but are undoubtedly associated with enhanced mucosal immune function mediated through

mast cell hyperplasia and generation of globule leucocytes in association with goblet cell hyperplasia (Balic et al., 2000).

In support of the importance of mucosal immune responses in the resistance of periparturient ewes to nematode infection O'Sullivan and Donald (1973) demonstrated that globule leucocyte populations in the gut are reduced by pregnancy and lactation at a time of elevated protein requirements. Conversely, improved MP supply can increase numbers of globule leucocytes during pregnancy and lactation (Houdijk et al., 2000) providing support that globule leucocyte hyperplasia is dependent on the duodenal supply of MP.

It is possible that genetic differences in resistance to nematode infection may also be associated with changes in protein metabolism such that the gut, and gut-based immune effectors, have a higher priority for use of absorbed amino acids at the expense of other tissues. The data from this experiment provide some support for changes in protein metabolism as a result of selection for increased host resistance. Wool growth of R ewes was 15% lower than that of C ewes during the prepartum period (days –28 to 21), annual clean fleece weight of R ewes was 8% lower and lamb birth weights of R ewes were 5% lower.

If the gut immune system of R ewes does have a higher priority of amino acids it may be expected that these animals would have a diminished immune response to increased MP supply. The diminished response to increased MP supply with reduced MP pressure and with enhanced genetic resistance suggests that there are threshold effects operative which preclude additional responses to MP supply once a certain level of mucosal immunity has been achieved.

In conclusion, both genetic selection and protein nutrition are effective strategies to enhance host resistance to nematode infection during the periparturient period. Increased resistance as a result of protein supplementation is, however, dependent on the prevailing

MP supply and demand. That resistant animals have a diminished response to increased MP supply presents the possibility that the partitioning of amino acids between the gut and other tissues has been altered as a consequence of selection. Both genetic selection and protein nutrition offer sheep managers alternatives to the control of nematode parasites using anthelmintics alone.

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Table 1

Independent effects and respective error terms used in statistical analysis of experimental variables.

Effects	DF	Ewe			Lamb		
		FEC	body weight	wool traits	FEC	birth weight	weight and growth
Selection line	1	X	X	X	X[A]	X[A]	X[A]
Supplement group	2	X	X	X	X[B]	X[B]	X[B]
Selection line x supplement group	2	X	X	X	X[B]	X[B]	X[B]
Ewe parity	1	NS	NS	NS	NS	X[B]	NS
Ewe age	4	NS	NS	X	-	-	-
Initial ewe weight	1	-	X				
Lamb gender	1	-	-	-	NS	NS	X[B]
Birth date	1	-	-	-	NS	NS	X[B]
Error terms							
Sire (selection line) [A]	8	-	-	-	X	X	X
Selection line x supplement group (replicate)[B]	18	X	X	X	X	X	X

X – Effect included in final statistical model.

NS – Effect was not statistically significant and was removed from the final statistical model.

[A] Variation between sires within selection line used as the error term.

[B] Variation between replicates within the interaction between the effects of selection line x supplement group used as the error term.

Table 2

Overall mean species contribution to faecal egg count determined from culture of faeces pooled within plots.

Day	Nematode species (%)			
	Haemonchus contortus	*Trichostrongylus spp*	*Ostertagia spp*	*Oesophagostomum spp*
-21	23	64	11	2
28	0	80	5	15
55	0	67	15	18
97	0	58	0	42

Table 3

Annual clean fleece weight, clean wool growth rate (day –28 to –21) and mean fibre diameter (day –28 to 21) of Merino ewes selected for increased resistance to *H. contortus* or at random and either unsupplemented or supplemented with cottonseed meal.

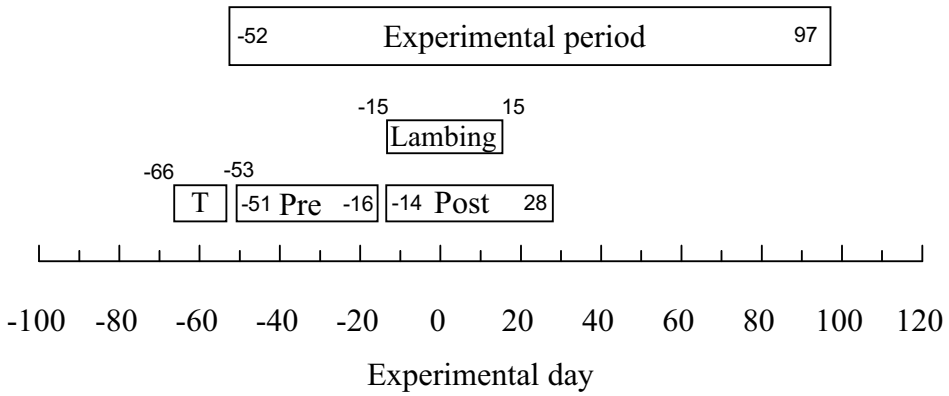
Selection line	Annual clean fleece weight (kg)		Clean wool growth (g/d)		Mean fibre diameter (μM)	
	Mean	se	Mean	se	Mean	se
Control	2.6[a]	0.05	7.1[a]	0.17	20.0[a]	0.15
Resistant	2.4[b]	0.05	6.2[b]	0.18	19.3[b]	0.16
P value	0.01		0.01		0.01	

Fig.1. Timing of experimental events relative to lambing mid-point which is defined as day 0 on 10th September 1998. Numbers indicate start and end day for each activity. T, Tracer sheep grazed in plots. Pre, period of prepartum supplementation. Post, period of postpartum supplementation.

Fig. 2. Faecal egg counts (epg) (backtransformed least square means) of R (open symbols and dashed lines) and C (filled symbols and solid line) Merino ewes fed either nil supplement (circle) or 250g CSM/day for 5 weeks prior to (square; prepartum) or 6 weeks after (triangle; postpartum) the start of parturition. Significant main effect of supplementation at day –21.

Fig 3: Main effect of supplementation on wool-free body weight (adjusted least squares mean ± pooled se) of Merino ewes fed either nil supplement (circle) or 250g CSM/day for 5 weeks prior to (square) or 6 weeks after (triangle) the start of parturition. Note significant ($P<0.05$) differences between unsupplemented v's pre partum supplementation at day –21 and a trend ($P=0.08$) for differences between unsupplemented v's pre and post partum supplementation at day 28.

1



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4 Fig.1. Kahn et al.

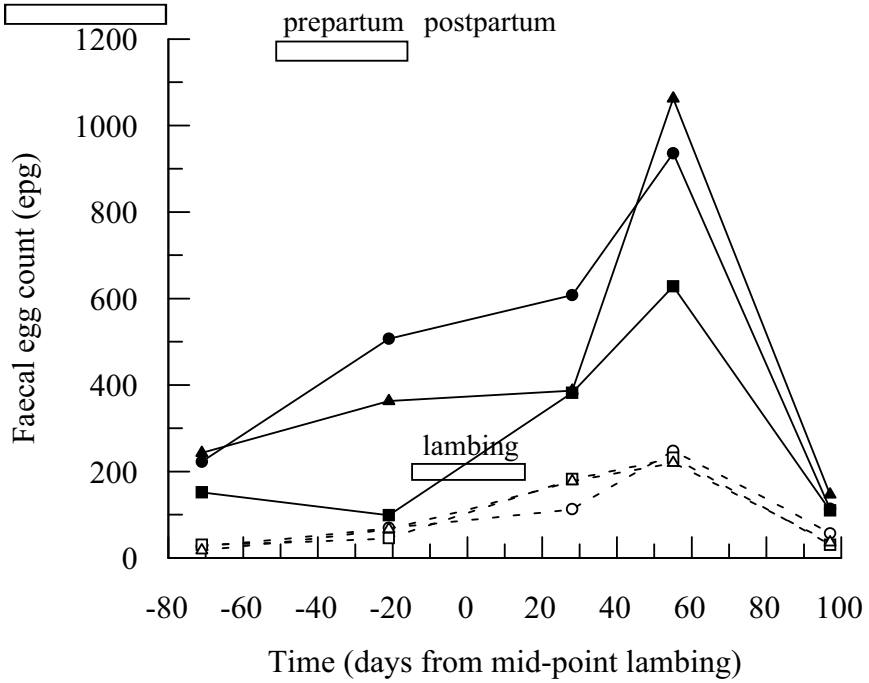

Fig. 2. Kahn et al.

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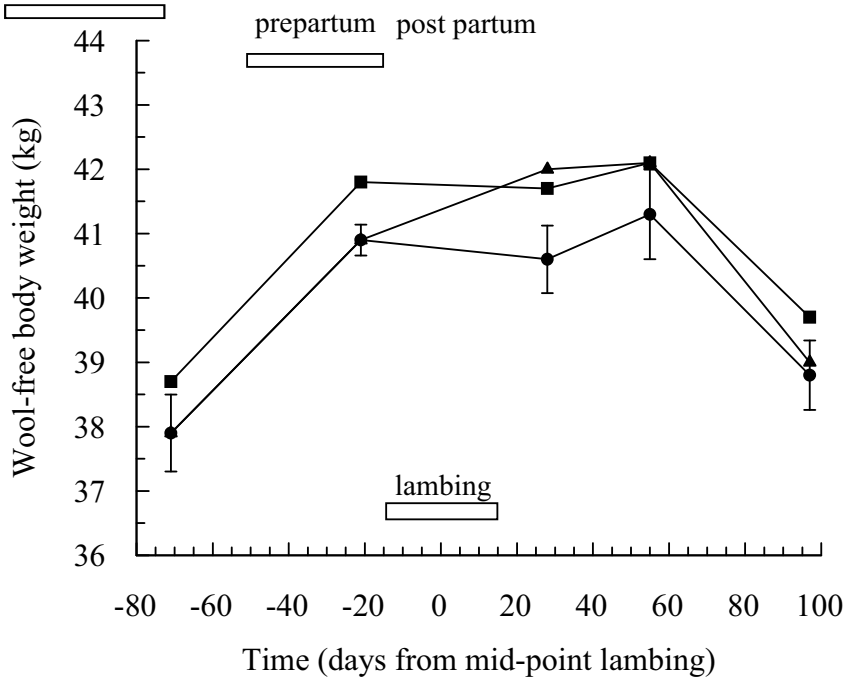

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3 Fig. 3. Kahn et al.

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