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COST OF IMMUNE RESPONSE TO *TRICHOSTRONGYLUS VITRINUS* INFECTION IN MEAT SHEEP

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Abstract

There is experimental evidence that reduced productivity of sheep infected with *Teladorsagia circumcincta* and *Trichostrongylus colubriformis* is due largely to host immune response to infection. To investigate this in Australian sheep infected with *Trichostrongylus vitrinus* under grazing conditions, an experiment was designed using 176 lambs sired by 22 Poll Dorset rams selected for extreme resistance and resilience to worm infection. For a period of 112 days following weaning at 3 months, lambs were immune suppressed with corticosteroid and trickle infected with *Trichostrongylus vitrinus* in a 2x2 factorial design. Comparison of weight gain between treatment groups enabled calculation of the cost of immune response in grams per day reduced growth rate. Selection for high resistance to internal parasites resulted in a cost of immune response approximately 5g/day greater than lambs with low resistance. Conversely, selection for high resilience to parasites resulted in a cost of immune response 21g/day lower than lambs selected for low resilience. Comparison across the four quadrants of resistance and resilience found the cost of immune response to be lowest in animals selected for low resistance/high resilience, and highest in animals selected for low resistance/low resilience.

Introduction

Gastro-intestinal nematode (GIN) parasites are a significant impediment to the prime lamb industry, with an annual estimated loss of $65.7m in the scour worm dominated regions of Australia (1). The costs of GIN parasites and escalating drench resistance have led industry to invest significantly in breeding sheep with genetic resistance to parasites. However, the correlation between resistance and productivity traits remains unclear with both favourable (2, 3) and unfavourable (4-7) relationships observed.

New Zealand studies with Coopworth ewe lambs have found that immune suppression with corticosteroid ameliorates the influence of chronic trickle infection with *Teladorsagia circumcincta* (*Tel. circumcincta*) or *Trichostrongylus colubriformis* (*T. colubriformis*) (8-10). This suggests that host immune response rather than parasite damage is the major cause of lost productivity. As genetic resistance is driven by host immune response (11), the lack of a clear favourable relationship between genetic selection for low worm egg count and productivity may result from increased resource allocation to immune response in favour of animal

growth. It is also possible that there is genetic variation in the cost of immunity, and co-selection for factors such as high growth reduces the cost of immune response. To investigate this an experiment was conducted on Struan Research Centre in the south-east of South Australia. Experimental design allowed the calculation of the cost of immune response to the small intestinal parasite *Trichostrongylus vitrinus* (*T. vitrinus*) in lambs sired by Poll Dorset rams with the most divergent Australian Sheep Breeding Values (ASBV) for resistance and resilience to internal parasites currently available in the Australian gene pool.

This paper contains preliminary analysis of live weight (LWT), faecal worm egg count (WEC) and total worm count (TWC) data from a large data set that will be further analysed and published separately.

Materials and methods

All research was conducted with approval from Primary Industry and Resources South Australia (18/11) and University of New England (AEC12-008) Animal Ethics Committees.

Animals

Semen from 22 Poll Dorset rams selected from each of four quadrants (n = 5 – 6) reflecting industry extremes for resistance and resilience to worm infection were used to generate lambs from unselected Border Leicester x Merino ewes. Resistance was based on ASBV for post-weaning worm egg count (PWEC) and resilience on ASBV for post-weaning weight (PWT) in the LambPlan database. Lambs grazed with their dams receiving natural exposure to worm infection until October, when lambs were weaned at 12 weeks of age. One hundred and seventy six representative lambs, 8 from each sire, were then drenched with 100mg of monepantel (Zolvix™, Novartis Animal Health) and 10mg abamectin (Abamax LV™, Biomac Pty Ltd) and moved to a "worm free" cell based grazing system. To prevent reinfection with worms, lambs were rotated twice weekly (Tuesday & Friday) to a "worm free" cell prepared by spelling for 9 months. To offset seasonal decline in pasture quality and quantity lambs were supplemented with barley, lupins, and worm free pasture hay.

Experimental Design

Lambs within each sire group were randomly allocated in a 2x2 factorial design to two levels of infection with *T. vitrinus* (yes/no) and two levels of immune suppression (yes/no) to provide four treatment combinations ('control', 'infect', 'immune suppress' and 'infect/immune suppress'). Infected lambs were orally administered a local isolate of *T. vitrinus* (Branxholme/Kalangadoo strain) twice weekly at the dose rates shown in Table 1. Immune suppressed lambs had weekly injections of 1.3mg/kg methylprednisolone acetate (Ilium Depredil Injection, 40mg/mL) calculated from their individual weights the previous week.

Table 1 L_3 *T. vitrinus* dose rate (L_3/kg liveweight/day)*

Days	1-31	35	38-42	45-56	59-84	87-112
Rate[1]	30	45	60	45	60	75

*Total larval bolus calculated from average infected animal weight in previous week. Bolus given orally twice weekly (Tuesday & Friday) in evenly divided doses.

[1]L_3 dose assessed on a continuous basis throughout the trial to provide strong exposure without clinical parasitism.

Throughout the 112 day experiment lambs were weighed and faecal sampled weekly, and blood collected fortnightly for haematology and serology (not reported). At the conclusion of the experiment lambs were slaughtered over an 8-day period from day 119 in two blocks of 4 days. Within each block each treatment group was randomly allocated a day, and 22 lambs from each group randomly allocated to slaughter. Lambs were held in lairage for 24 hours before humane slaughter by captive bolt/exsanguination. The abomasum, small intestine, liver and adrenal glands were harvested. Liver and adrenal glands were weighed (not reported), and a section of the small intestine 1m distal to the pylorus collected for histology. Sections were immediately fixed in 10% buffered formalin, and later sectioned and stained with haematoxylin and eosin and toluidine blue (not reported). The proximal 15m of small intestine was thoroughly washed in warm water, the contents sieved (75µm) and fixed in formalin for TWC. Cold carcase weight, eye muscle area and fat depth at GR site were recorded (not reported) on chilled carcases the following day.

Parasitology

Weekly faecal samples were collected rectally from each animal and placed on ice. WECs to a sensitivity of 25 eggs per gram of faeces (epg) were done using a modified McMaster Technique (12).

Estimates of total worm numbers were derived from counts of aliquots of small intestinal contents.

Statistical Analysis

WEC data of infected animals were square root transformed [$\sqrt{(n)}$] and analysed by restricted maximum likelihood (REML), fitting animal as a random effect and day, sex, rear type, immune suppression (yes/no), resistance (high/low) and resilience (high/low) sire genotypes as fixed effects. Tukey's HSD was used for post hoc comparison as required.

Body weight over the 112 day trial was analysed by REML with initial body weight as a covariate and fixed effects of day, sex, rear type, infection (yes/no), immune suppression (yes/no), resistance (high/low), and resilience (high/low) sire genotype. Tukey's HSD was used for post hoc comparison as required.

To enable generation of cost of immune response (CIR), least square (LS) means of average daily gains (ADG) over the course of the trial were calculated by ANOVA separately for infected and non-infected lambs. In both analyses mean square root WEC and initial body weight were fitted as covariates, with fixed effects of sex, rear type, immune suppression (yes/no), resistance (high/low) and resilience genotype (high/low).

All statistical analyses were conducted with JMP® 10.0.2 (SAS Institute, Cary, NJ, USA).

Results

Worm egg count

Genotype for resistance and resilience had no effect on worm egg count (P = 0.82 & 0.49 respectively), therefore average back-transformed LS means WEC of infected lambs over all genotypes is presented below (Fig 1).

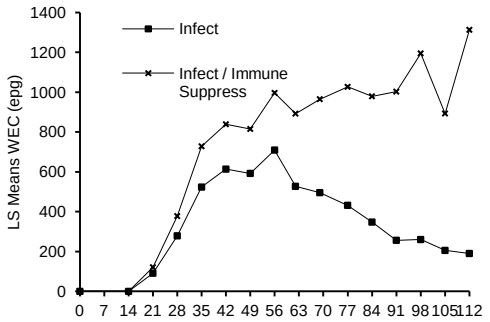

Fig 1 Mean back-transformed LS means WEC of infected groups (combined resistance and resilience genotypes)

WEC of non-infected 'control' and 'immune suppress' groups remained near 0 at all times, with group WEC averages never exceeding 3 epg.

Positive WEC was detected in 'infect' and 'infect/immune suppress' lambs from day 21. WEC of 'Infect' lambs increased to a plateau around day 42, after which a steady decline was observed. 'Infected/immune suppressed' lamb WEC continued to increase throughout the course of the trial. A significant day by immune suppression interaction (P<0.0001) led 'infect/immune suppress' lambs to have a WEC significantly exceeding 'infect' from day 62 until the end of the experiment.

Total worm count

To date TWCs from half of the 'infect' (n=22) and 'infect/immune suppress' (n=22) lambs have been completed. Some samples from uninfected lambs have also been processed with minimal or zero counts. Purity of *T. vitrinus* artificial infection has been confirmed by identification of 300 males from spicule morphology. Preliminary results for completed TWCs are presented in Table 2.

Table 2 Numbers of *Trichostrongylus vitrinus* in 'infect' and 'infect/immune suppress' lambs

Experimental group	Mean adult worm burden ($x10^3$)	Mean immature worm burden ($x10^2$)
'Infect'	35 (0.6 – 146)*	7 (0.25 – 35)
'Infect/immune suppress'	107 (36 – 147)*	36 (6 – 72)

*Range presented in parenthesis

Body weight

Average LS mean body weight by treatment group for all genotypes combined is presented in Fig. 2. The effect of day on body weight was significant (P<0.0001), with average body weight increasing throughout the trial.

Corticosteroid treatment significantly reduced body weight (P<0.0001). Significant interaction between day and immune suppression (P<0.0001) led 'immune suppress' lambs to be significantly lighter than 'control' from day 49. Likewise infection with *T. vitrinus* significantly reduced body weight (P<0.0001), and an interaction between day and infection (P<0.0001) drove infected non-suppressed and suppressed groups to be significantly lighter than their non-infected counterparts from day 62.

Lamb genotype for both resistance and resilience had a significant effect on body weight (P=0.041 & 0.009 respectively).

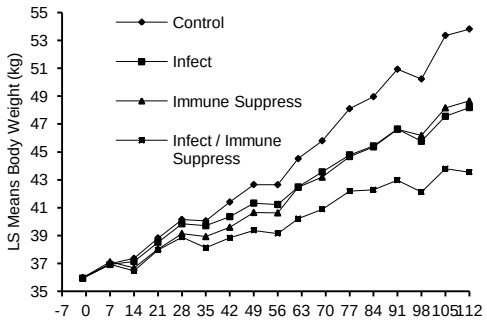

Fig 2 LS means body weight change over time by treatment group (combined resistance and resilience genotypes)

Cost of immune response

LS means generated by ANOVA of the average daily gain of infected and non-infected lambs enabled calculation of the cost of infection (CI) for suppressed and non-suppressed lambs in each genotype and quadrant. Cost of immune response (CIR) in each genotype or quadrant was calculated as below.

CI = (ADG uninfected) – (ADG infected)
CIR = (CI unsuppressed) – (CI suppressed)

Calculation of overall CIR for resistance and resilience genotypes is presented in Table 3.

Table 3 Cost of immune response (g/day) by genotype

Genotype	High	Low
Resistance	26.76	21.92
	(0.17)*	(0.14)*
Resilience	13.92	34.76
	(0.08)*	(0.23)*

*Proportion of total 'control' (non-infected/non-immune suppressed) growth rate lost due to immune response within each genotype.

CIR for each quadrant is presented in Table 4.

Table 4 Cost of immune response (g/day) by genotype quadrant

Genotype	High Resistance	Low Resistance
High Resilience	36.54	-8.7
	(0.21)*	(N/A)*
Low Resilience	16.96	52.55
	(0.12)*	(0.33)*

*Proportion of total 'control' (non-infected/non-immune suppressed) growth rate lost due to immune response within each quadrant.

Discussion

To our knowledge this is the first estimate of the cost of immune response in animals differing in genotype for resistance and resilience to infection with GIN. It is also the first estimate of the cost of immune response to GIN in Australian meat sheep under grazing conditions, and for the important southern Australian scour worm *Trichostrongylus vitrinus*.

In the current model, selection for high genetic resistance to internal parasites was approximately 5g/day more costly than selecting for low resistance (Table 3). As a proportion of uninfected unsuppressed growth rate this equated to a 3% higher cost. Conversely, low resilience animals had a CIR 21g/day higher than their high resilience counterparts, equating to a proportionately 15% increased CIR.

In light of these results, variation in cost of immune response across quadrants (Table 4) is partially but not entirely explained. As one would expect the lowest cost of immune response was observed in low resistance high resilience animals. However, the apparent higher cost of immune response in the high resistance/high resilience quadrant compared to the high resistance/low resilience quadrant is unexpected, as is the higher cost observed in the low resistance/low resilience quadrant compared to the high

resistance/low resilience quadrant. Further analysis is required to confirm these early results. Nevertheless, a simple proposition might be that disparity between quadrants is as a result of different mechanisms of immune response operating in each. The apparent variation in cost of immune response between genotypes and quadrants adds support to reservations of previous authors (8, 10, 13) that selection for animals with high genetic resistance to internal parasites may lead to an inadvertent reduction in productivity. Equally, results suggest that failure to select for animals with high resilience to infection may also lead to an inadvertent reduction in productivity as a result of increased cost of immune response.

The failure of methylprednisolone acetate to ameliorate reduced live weight gain in response to infection with the small intestinal parasite *T. vitrinus* (Fig. 2), is at odds with that previously reported by Greer et al. for infections with *T. colubriformis* (8) and the abomasal parasite *Tel. circumcincta* (10). The dose rate of corticosteroid used in this trial was identical to the Greer et al. model. Our work suggests that cost of infection with *T. vitrinus* is not driven entirely by the cost of immune response as previously observed (8, 10), but is at least partially due to direct effects of the parasite on the host. Whilst detailed analysis of small intestinal histology has not yet been undertaken, preliminary assessment suggests severe villus atrophy to be present in infected lambs both in the presence and absence of immune suppression.

Worm egg counts of non-infected lambs remained near zero throughout the trial period. This confirms the effectiveness of rotation to spelled pasture every 3 and 4 days at preventing reinfection, and allows valid comparison between infected and non-infected lambs. At trial completion WEC of infected immune suppressed lambs exceeded that of those infected alone by 5 fold. This higher WEC reflected a TWC in infected immune suppressed lambs 3 times that of those infected alone.

Preliminary TWC results also demonstrate that far greater numbers of immature worms persisted in immune suppressed lambs. The numbers of immature worms in immune suppressed lambs also comprise a greater proportion of the total worm burden (3%) than in non-immune suppressed lambs (1.9%). This evidence suggests a strong immunological assault against incoming immature worms in non-immune suppressed lambs, and apparent absence of arrested development of immature worms as an important component of the dynamics of natural infection.

Conclusions

The variation in cost of immune response observed between quadrants differing in genotype for resistance and resilience adds significantly to our understanding of the role of host immune response in the cost of scour worm infection in sheep. Based on these results it appears possible to minimise cost of immune response by selecting for animals which exhibit strong resilience to infection, but weak resistance to infection. Further research is currently underway to investigate if similar varying costs are observed following infection with the abomasal parasite *Tel. circumcincta*.

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