

The role of cysteine in the increased parasite susceptibility of Romney sheep selected for hogget fleece-weight

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ABSTRACT

To investigate the potential role of cysteine in the increased parasite susceptibility of sheep selected for increased wool production, ten sheep from both fleece-weight-selected (FW) and randomly-selected (C) Romney lines received either 2g of supplemental cysteine per day, or saline, via abomasal infusion. Following drenching to remove any pre-existing parasites, all sheep were dosed with *Haemonchus contortus* and *Trichostrongylus colubriformis* infective larvae. Cysteine infusion elevated plasma cysteine levels by an average of 65% ($P < 0.0001$). Off-pasture faecal egg counts (FEC) were higher in FW than C sheep (645 versus 200 eggs/g, $P < 0.01$). No significant effects of line or infusion treatment on FEC were observed following the trial infection, though on days 52, 57 and 58, FEC in sheep receiving saline were at least twice those of sheep receiving cysteine. Total *H. contortus* counts were greater in FW than C lines of sheep (1208 versus 1055, $P < 0.05$), while total numbers of *T. colubriformis* did not differ between lines or infusions. This study confirms the greater susceptibility of FW sheep to internal parasites, and suggests cysteine may influence certain aspects of immunocompetency in sheep.

Keywords: helminths; sheep; wool production; cysteine; immunocompetency

INTRODUCTION

Internal parasites represent a significant cost to the New Zealand sheep industry, both in terms of control costs and lost production (Brunsdon, 1988). Traditional anthelmintic drenches are rapidly losing favour, due to the increasing incidence of parasite resistance to anthelmintics (Vlassoff & McKenna, 1994), and the growing consumer awareness of the potentially harmful effects of chemical residues in food. For this reason, alternate avenues of parasite control such as breeding genetically resistant sheep are being explored. However, the nature of the relationships between parasite resistance and production traits needs to be established. Williamson *et al.* (1995) found that Romney sheep selected for increased hogget fleece-weight had a higher parasite burden than control (randomly-selected) lines, which suggests there may be an undesirable compromise in parasite resistance when selecting for improved fleece-weights.

A possible link between wool production and parasite resistance is the sulphur containing amino acid, cysteine. Cysteine is vital for wool growth and is usually the first-limiting amino acid for wool fibre synthesis. Sheep selected for high wool production tend to have lower levels of free cysteine and glutathione (GSH; a tripeptide containing a cysteine residue) in circulation than those selected for low wool production (Hopkins *et al.*, 1975; Williams, 1979). One possible explanation is that fleece-weight selected sheep are more efficient at utilising circulating cysteine for wool growth.

Cysteine and GSH are also emerging as key players in immune responses. Cysteine may act as an immunoregulatory signal between macrophages and

lymphocytes (Gmünder *et al.*, 1990). GSH has been shown to be important for certain T-cell functions (Dröge *et al.*, 1994) and GSH depletion leads to decreased numbers of CD4+ T cells (Kinscherf *et al.*, 1994). Little attempt has been made to determine whether cysteine affects immunocompetency in livestock species, though several studies have shown general protein supplementation to improve immune responsiveness in sheep (e.g., van Houtert *et al.*, 1995; Israf *et al.*, 1996).

This study tested the hypothesis that reduced availability of circulating cysteine is an important factor in the increased susceptibility of fleece-weight selected (FW) sheep to helminthic infection. The effects of supplemental cysteine (via infusion) on faecal egg counts and parasite burdens were assessed in both FW and control lines of Romney sheep.

MATERIALS & METHODS

Animals

This study used 1995-born Romney rams from the Massey University fleece-weight-selected (FW) and control (C) lines, which were approximately 18 months of age at the onset of the trial. A detailed description of these lines is given by Blair *et al.* (1985). Sheep were brought off pasture on day 1, then housed indoors in metabolism crates from day 2 of the trial, and fed a daily ration of 0.75kg pellets and 0.25kg lucerne chaff, which was intended to maintain live weight. Approximately 2g of mineral supplement (94% NaCl, 6% Na molybdate) was added to the feed three times a week, and water was available *ad libitum*. Live weights were monitored throughout the trial. All sheep were surgically fitted with an abomasal T-cannula on

days 9, 10, 12, 15, or 16 using standard surgical procedures, similar to those described by Hecker (1974). Wound hygiene was maintained throughout the trial by clipping the area, and washing with diluted Savlon at least twice weekly.

Experimental design

Twenty rams were used, ten each from the FW and C lines. FW rams were randomly selected from those that remained following selection of replacement breeding sires. Within each line, sheep were randomly allocated to an infusion treatment group, either cysteine (+cys), or saline (+sal) to serve as a control. All sheep received a combination drench (Leviben®, dosage 25mL) on days 22, 23 and 24. Faecal egg counts on days 29 and 31 confirmed that this drenching had removed any existing internal parasites (i.e., no eggs were found). Continuous abomasal infusions commenced on day 33, using peristaltic pumps that delivered 8.43 mL of infusate per hour to each sheep. Sheep in the +cys group were infused with 0.0826M cysteine in saline (0.9% NaCl), adjusted to pH 3.0-4.5, and thus received 2.00 ± 0.002 g of supplemental cysteine daily, whereas +sal sheep received saline only. On day 35, each sheep was infected with two species of infective (third stage) nematode larvae: *Haemonchus contortus* (n = 4000) and *Trichostrongylus colubriformis* (n = 25 000). These larvae were ovine-derived strains supplied by the Veterinary Parasitology Laboratory at Massey University. A drench gun was used to orally administer the larvae in a water medium. On day 58, all sheep were slaughtered using a captive bolt pistol, and the gastrointestinal tract removed for worm counts.

Blood preparation and analysis

Blood samples were collected from the jugular vein on days 29, 38, 45, 52 and 57. A small portion of the whole blood was used to estimate packed cell volumes, following centrifugation at 3800g for 15min. The remainder of the blood was processed as described by Lee *et al.* (1993) for determination of free cysteine concentration in plasma by HPLC.

Wool growth

On day 1, a left midside patch (approximately 15cm x 15cm) was clipped to skin level using a size 10 clipper blade. Subsequent measured patches (approximately 10cm x 10cm) were clipped on days 31 and 57, and the exact area of the patch determined by triangulation. Wool samples were weighed, scoured and re-weighed at 22°C and 65% humidity. For each period, clean wool growth (mg/cm²/day) was determined. Greasy hogget fleece-weights were obtained from flock records.

Parasitological techniques

Immediately after slaughter, the abomasum and small intestine were removed, ligated separately, and frozen for later parasite counts. Worm counts were carried out as described by Williamson *et al.* (1995). Faecal egg counts (FEC) were assessed on days 1, 29, 38, 45, 52 and 57 using a modified McMasters technique (Stafford *et al.*, 1994), where each egg counted represented 50 eggs/g of wet faeces.

Statistical Analysis

Analysis of variance (ANOVA) was used to examine the effects of selection line, infusion treatment and line by infusion treatment interactions. Multiple observations on single parameters over time were treated as repeat measures, with pre-infusion observations used as covariates where necessary (i.e., where they had a significant (P<0.10) effect on post-infusion measurements). To improve normality, FEC and abomasal worm counts were square root-transformed, while small intestinal worm counts were log-transformed. The figures and tables in the results section are expressed as untransformed values for ease of interpretation.

RESULTS

Flock records showed that FW sheep had significantly heavier greasy hogget fleece-weights than C sheep (4.96kg versus 3.90kg, P<0.001). However, the difference in wool growth rates expressed per unit area of skin between lines was not significant during the pre- or post-infusion periods. Cysteine infusion did not significantly affect wool growth rates, though it did tend to increase wool growth rates (+6.1% for FW sheep and +21.5% for

FIGURE 1: Pre- and post-infusion clean wool growth rates in fleece-weight-selected (FW) and randomly-selected (C) lines of Romney sheep receiving either supplemental cysteine (+cys), or saline (+sal), via abomasal infusion. Standard error bars are shown.

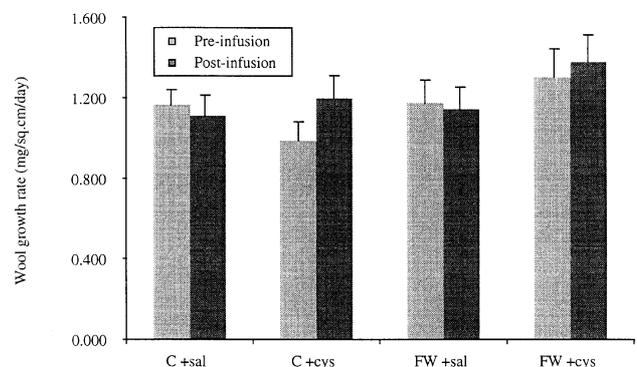
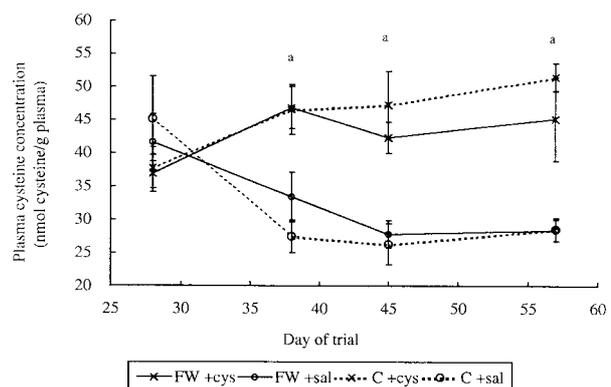


FIGURE 2: The effect of cysteine (+cys) or saline (+sal) infusion on the plasma cysteine levels of fleece-weight-selected (FW) and randomly-selected (C) Romney sheep. Infusions commenced on day 33. Standard error bars are shown.



a = significant difference between +sal and +cys sheep effect, P<0.0001

C sheep), while sheep receiving saline tended to show depressions in wool growth during the experiment (-2.6% for FW sheep and -4.6% for C sheep). Pre- and post-infusion wool growth rates are presented in Figure 2.1. FW sheep had significantly heavier live weights than C sheep on day 1 (63.8kg versus 56.9kg, $P<0.05$), and all animals lost an average of 5kg of live weight over the adjustment and surgery period. Following the surgical recovery period, there were no significant changes in live weight over time.

Cysteine infusion elevated plasma cysteine levels by an average of 65% ($P<0.0001$), relative to levels in sheep receiving saline infusions, which appeared to drop and then stabilise (Figure 2). There was no difference in initial plasma cysteine levels between the FW and C lines, nor were there any interactions between line and infusion following infusion. However, the C +cys group had consistently higher mean cysteine concentrations in plasma, up to 14% (day 57), following commencement of infusion than the FW +cys group.

The mean off-pasture (day 1) FEC of FW sheep was more than double that of C sheep (645 versus 200 eggs/g, $P<0.01$), but no significant line effects, nor differences between sheep receiving cysteine and those receiving saline, were observed on days 38 to 58, following artificial infection. However, within both lines, sheep receiving saline had FEC that were 2-4 times higher than sheep receiving cysteine. FW sheep had a greater *H. contortus* burden at slaughter than C sheep ($P<0.05$), but this was not affected by the provision of supplemental cysteine. *T. colubriformis* numbers did not differ between lines, nor between sheep receiving saline and those receiving cysteine. FEC and worm burden data are presented in Table 2.1. FEC on day 58 was positively correlated with both *H. contortus* numbers (+0.42, $P<0.10$), and *T. colubriformis* numbers (+0.54, $P<0.05$). Packed cell volumes (PCV)

tended to decrease over time, but did not differ between lines or infusion treatments (saline or cysteine) (data not shown).

DISCUSSION

This study has provided further evidence for an undesirable relationship between parasite susceptibility and wool production and, although the precise role of cysteine in this relationship is still unclear, cysteine appeared to influence certain aspects of the immune response to parasitic challenge.

Cysteine infusion had a substantial impact on plasma cysteine levels in both the FW and C sheep, elevating plasma cysteine levels by an average of 65% ($P<0.0001$). The decline in plasma cysteine levels from day 29 to 38 in sheep receiving saline may be due to an increased demand for cysteine for mounting an immune response against the challenge infection on day 35. The provision of supplemental cysteine may have met this increased demand, and thus prevented the decline being observed in sheep that received cysteine infusion.

There were no initial (pre-infusion) differences in plasma cysteine levels between the two lines, which does not support the hypothesis that reduced availability of cysteine in FW sheep contributes to their greater parasite susceptibility. This is in contrast to comparisons between high- and low-producing Merino sheep, where high-producing animals were observed to have lower free cysteine levels in the plasma (Williams *et al.*, 1972; Williams, 1976). However, as glutathione (GSH) is a major reservoir of cysteine, possible differences in erythrocyte GSH levels between the two lines may have altered the total amount of cysteine in circulation. Erythrocyte GSH concentrations have been observed to be lower in Merinos selected for high clean fleece-weight than those selected for low clean fleece-weight (Hopkins *et al.*, 1975).

TABLE 1: Faecal egg counts and worm burdens measured in fleece-weight-selected (FW) or randomly-selected (C) Romney sheep, receiving either 2g supplemental cysteine per day (+cys), or saline (+sal), via abomasal infusion (untransformed means). Infusions commenced on day 33, and sheep were artificially infected with larvae on day 35. Sheep were drenched on days 22, 23 and 24 to remove any pre-existing internal parasites.

	FW		C		PSE ¹	Significance ²		
	+cys	+sal	+cys	+sal		Line	Inf	Line*Inf
n	5	5	5	5				
Faecal egg counts (eggs/g wet faeces)								
Day 1	610	680	80	320	199	**	ns	ns
Day 29	0	0	0	0	0	ns	ns	ns
Day 38	0	0	0	0	0	ns	ns	ns
Day 45	0	0	0	0	0	ns	ns	ns
Day 52	10	80	20	40	33	ns	ns	ns
Day 57	240	980	280	650	282	ns	ns	ns
Day 58	390	1280	620	1020	448	ns	ns	ns
Worm burden (total number per sheep)								
<i>H. contortus</i>	1248	1168	1086	1024	105	*	ns	ns
<i>T. colubriformis</i>	676	2890	804	442	1392	ns	ns	ns

ns = not significant, * = $P<0.05$, ** = $P<0.01$

¹ Pooled standard error of untransformed data

² Significance of selection line (Line), infusion (Inf), or line by infusion interaction (Line*Inf) based on transformed data, where appropriate (refer text).

Despite no pre-infusion differences, FW sheep receiving cysteine during the infusion period had consistently lower plasma cysteine levels than C sheep receiving cysteine. Though this difference was not significant, it lends support to the original hypothesis that FW sheep are more efficient than C sheep at removing cysteine from circulation for use in wool fibre synthesis. A possible explanation for the seemingly conflicting findings in pre- and post-infusion levels is that differences may only become apparent when sufficient cysteine is available (i.e., during cysteine infusion) for differences in tissue entry rates to be reflected in plasma cysteine concentrations. Barger *et al.* (1973) also suggested that such a differential partitioning of sulphur amino acids occurred between relatively resistant and susceptible crossbred sheep. Resistant sheep, as defined by lower faecal egg counts, appeared to grow less wool in response to cysteine supplementation during parasitic infection, than susceptible sheep.

The greater off-pasture FEC, and greater *H. contortus* counts at slaughter, in FW sheep compared with C sheep, are in agreement with the findings by Williamson *et al.* (1995) with the same selection lines. FEC on day 57 was positively correlated with both abomasal (+0.42, $P < 0.10$) and small intestinal (+0.54, $P < 0.05$) worm numbers, indicating that FEC is a good predictor of worm burden. Williamson *et al.* (1995) also found that numbers of *Ostertagia circumcincta*, an abomasal parasite, were greater in FW than C sheep. Neither study found a difference in *T. colubriformis* numbers between selection lines. Parasitic establishment rates for *H. contortus* were similar to those observed by Williamson *et al.* (1995), while *T. colubriformis* establishment was much lower in the current study. Results in this trial may have been confounded by residual immune responses to the previous parasitic infection. Though the actual off-pasture parasite burden appeared to have been eliminated, it is possible that immune responses to this infection, such as antibody responses and cellular responses in the gut, were sufficiently high at the time of the trial infection (eleven days later) to impede larval establishment.

Cysteine infusion failed to have a significant impact on either faecal egg counts or parasite burdens, though, interestingly, faecal egg counts in sheep receiving saline were at least twice those of sheep receiving cysteine on days 52, 57 and 58. This suggests cysteine infusion may have reduced worm fecundity. Young sheep (three months of age) receiving protein supplementation have been found to have greater rates of *T. colubriformis* worm expulsion, and lower FEC, than sheep not receiving supplementation (van Houtert *et al.*, 1995). The acquisition of immunity with age may have prevented differences in worm establishment rates between cysteine and saline infusion treatments being observed in the present study.

In summary, the increased parasite susceptibility of FW sheep has been confirmed, as indicated by greater faecal egg counts and an increased abomasal parasite burden. Cysteine may influence certain aspects of the immune response, as shown by a tendency for supplemental cysteine to decrease faecal egg counts. Though cysteine may improve certain aspects of immunocompetency in

both FW and C sheep, the role of cysteine in the increased parasite susceptibility of FW sheep remains unclear, as no differences in pre-infusion plasma cysteine levels were observed.

REFERENCES

- Barger, I. A.; Southcott, W. H.; Williams, V. J. (1973). Trichostrongylus and wool growth. 2. The wool growth response of infected sheep to parenteral and duodenal cystine and cysteine supplementation. *Australian Journal of Experimental Agriculture and Animal Husbandry*, **13**: 351-359
- Blair, H. T.; Garrick, D. J.; Rae, A. L.; Wickham, G. A. (1985). Selection responses in New Zealand Romney sheep. 2. Selection for yearling greasy fleece weight. *New Zealand Journal of Agricultural Research*, **28**: 257-264
- Brunsdon, R. V. (1988). The economic impact of nematode infection in sheep: implications for future research. *New Zealand Society for Parasitology Inc. Miscellaneous Publication No.1* pp4-16
- Dröge, W.; Schulze-Ostohoff, K.; Mihm, S.; Galter, D.; Schenk, H.; Eck, H.; Roth, S.; Gmünder, H. (1994). Functions of glutathione and glutathione disulfide in immunology and immunopathology. *The FASEB Journal*, **8**: 1131-1138
- Gmünder, H.; Eck, H.; Benninghoff, B.; Roth, S.; Dröge, W. (1990). Macrophages regulate intracellular glutathione levels of lymphocytes. Evidence for an immunoregulatory role of cysteine. *Cellular Immunology*, **129**: 32-46
- Hecker, J. F. (1974). Experimental Surgery on Small Ruminants, Butterworths & Co (Publishers) Ltd
- Hopkins, G. J.; Roberts, J.; Evans, J. V. (1975). Red blood cell concentrations of reduced glutathione and potassium as biochemical markers of wool growth in Merino sheep. *Journal of Agricultural Science, Cambridge*, **84**: 481-486
- Israfi, D. A.; Coop, R. L.; Stevenson, L. M.; Jones, D. G.; Jackson, F.; Jackson, E.; MacKellar, A.; Huntley, J. F. (1996). Dietary protein influences upon immunity to Nematodirus battus infection in lambs. *Veterinary Parasitology*, **61**: 273-286
- Kinscherf, R.; Fischbach, T.; Mihm, S.; Roth, S.; Hohenhasu-Sievert, E.; Weiss, C.; Edler, L.; Bärtsch, P.; Dröge, W. (1994). Effect of glutathione depletion and oral N-acetyl-cysteine treatment on CD4+ and CD8+ cells. *The FASEB Journal*, **8**: 448-451
- Lee, J.; Harris, P.M.; Sinclair, B.R.; Treloar, B.P. (1993). Whole body metabolism of cysteine and glutathione and their utilization in the skin of Romney sheep: consequences for wool growth. *Journal of Agricultural Science, Cambridge*, **121**: 111-124
- Stafford, K. J.; West, D. M.; Pomroy, W. E. (1994). Nematode worm egg output by ewes. *New Zealand Veterinary Journal*, **42**: 30-32
- van Houtert, M. F. J.; Barger, I. A.; Steel, J. W.; Windon, R. G.; Emery, D. L. (1995). Effects of dietary protein intake on responses of young sheep to infection with Trichostrongylus colubriformis. *Veterinary Parasitology* **56**: 163-180
- Vlassoff, A.; McKenna, P.B. (1994). Nematode parasites of economic importance in sheep in New Zealand. *New Zealand Journal of Zoology*, **21**: 1-8
- Williams, A. J. (1976). Metabolism of cysteine by Merino sheep genetically different in wool production. 4. Rates of entry of cysteine in to plasma, measured with a single intravenous injection of L-[34S] cystine, and the subsequent incorporation of 35S into wool fibres. *Australian Journal of Biological Sciences*, **29**: 513-524
- Williams, A. J. (1979). Speculations on the biological mechanisms responsible for genetic variation in the rate of wool growth. Pp337-354 In: Physiological and Environmental Limitations to Wool Growth, Black, J. L.; Reis, P. J. ed. Armidale, NSW, The University of New England Publishing Unit
- Williams, A. J.; Leng, R. A.; Stephenson, S. K. (1972). Metabolism of cysteine by Merino sheep genetically different in wool production. 1. Comparison of the entry rates of cysteine in sheep from flocks selectively bred for high and low fleece-weight. *Australian Journal of Biological Sciences*, **25**: 1259-1276
- Williamson, J. F.; Blair, H.T.; Garrick, D. J.; Pomroy, W. E.; Douch, P. G. C.; Green, R. S. (1995). Parasitological characteristics of fleece-weight selected and control sheep. *New Zealand Journal of Agricultural Research*, **38**: 389-397