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## The relationship between internal parasite burden, faecal egg count, and mucosal mast cells in fleeceweight-selected and control sheep

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### ABSTRACT

Research has shown that sheep from a Massey University flock selected for increased fleece weight (FW) for thirty-six years have higher faecal egg counts (FEC) than unselected controls (C). The purpose of this trial was to determine whether differences in FEC between FW and C sheep accurately reflected differences in adult worm burdens. In addition levels of mucosal mast cells, and their relationship with worm burden in each line were examined.

Thirteen one year-old rams from each flock were housed indoors and artificially infected with larvae of three species of nematode parasite: *Haemonchus contortus*, *Ostertagia circumcincta*, and *Trichostrongylus colubriformis*. FECs were monitored and adult worm burdens, and levels of mucosal mast cells, determined following slaughter 28 days after infection.

FECs were higher in FW than C sheep (4204 vs 300 epg,  $p < 0.0001$ ), as were numbers of adult *Haemonchus* (1151 vs 249,  $p < 0.01$ ) and *Ostertagia* (2268 vs 600,  $p < 0.05$ ). Numbers of *Trichostrongylus* (5838 vs 5266) and total worm burden (9257 vs 6115) were not significantly different between lines. A 'Total Pathogenic Index', calculated from the number of parasites of each species present in the total burden, was higher in FW than C sheep (3.8 vs 1.5,  $p < 0.005$ ).

The regression coefficient relating FEC to worm burden was larger in FW than C sheep (0.35 vs 0.05 eggs per g faeces/worm,  $p < 0.01$ ). Levels of mucosal mast cells did not differ significantly between lines, but were inversely related to number of worms in FW sheep. There was no such relationship in C sheep.

It is concluded that while the large difference in FEC between lines overestimates the difference in total worm burden, FW sheep do carry significantly more worms of the abomasal species *H. contortus* and *O. circumcincta* than C sheep. FW sheep are able to maintain superior wool production in spite of this potentially more pathogenic worm burden. The mucosal mast cell response to parasitic infection also appears to have been changed by selection.

**Keywords:** sheep; selection; internal parasites; faecal egg count; mucosal mast cells.

### INTRODUCTION

The sheep industry is showing increasing interest in alternative methods of controlling internal parasites as a consequence of increasing anthelmintic resistance by parasites and as a response to consumer concerns regarding chemical residues in food. One method currently being evaluated is to select sheep for low faecal egg count, thereby reducing the worm burden in the gastro-intestinal tract. However in a flock which has undergone long-term single trait selection for high fleece weight (FW) at Massey University, faecal egg count has increased relative to unselected controls (C) (Howse *et al.*, 1992). A similar increase in faecal egg count has been reported in sheep selected for high production at Woodlands Research Station in Southland (McEwan *et al.*, 1992). If the reverse is true, such that selection for low faecal egg count results in decreased production, sheep breeders may inadvertently decrease the productivity of their sheep.

McKenna (1981) showed that faecal egg count was closely correlated with worm burden in young outbred sheep (up to 12 months of age), with a weaker correlation in sheep greater than 12 months of age. However it was also found that faecal egg count was a good indicator of the potential

pathogenicity of the burden in both age groups. It can be argued that the pathogenicity of a mixed infection, as determined by the relative proportion of species present, is potentially of more significance than the number of worms alone.

The main objective of this trial was to confirm that the higher faecal egg counts of the fleeceweight-selected sheep actually reflect higher worm burdens, or greater potential pathogenicity of the burden, in the gastro-intestinal tract.

In addition, levels of mast cells in the gastro-intestinal mucosa were determined following infection, and the relationship between cell numbers and parasite numbers within each line examined. The level of these cells in infected outbred sheep has been shown to be inversely related to numbers of parasites (Rothwell, 1989), suggesting a functional role in worm expulsion.

This paper presents preliminary findings on the relationship between faecal egg count and worm burden (and potential pathogenicity) in fleeceweight-selected and control sheep, and the possible role of mucosal mast cells in worm expulsion in these two lines.

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## MATERIALS AND METHODS

### 1. Sheep

The Romney sheep used in this trial were drawn from the Massey University fleece weight selection lines. One line is a randomly bred control (C), the other (FW) has undergone single trait selection for greasy fleece weight for thirty-six years. The structure of these lines has been described by Blair *et al.*, (1984, 1985). Both flocks are grazed together at all times except during mating. Thirteen rams from each line were available after selection of breeding stock, aged between fourteen and fifteen months at the start of the trial and reared on naturally infected pasture.

All sheep were drenched with an anthelmintic eight days prior to infection to remove any existing parasite burden ('Leviben', 20g/l ricobendazole and 37.5g/l levamisole hydrochloride, dose rate 2ml/5kg liveweight).

### 2. Housing and feeding

Sheep were housed in raised pens, with two sheep per pen. Allocation to pens was random across lines. A maintenance diet was fed consisting of lucerne chaff and formulated pellets, each supplying 50% of daily energy requirement. Water was available *ad libitum*. A period of 8 days elapsed before infection (day 0) to allow adjustment to the diet.

### 3. Parasites

Each sheep was infected with a mix of three species of infective (third stage) nematode larvae: *Haemonchus contortus* (n=4000), *Ostertagia circumcincta* (n=22750), and *Trichostrongylus colubriformis* (n=25000). These larvae were sheep-derived strains originating from Massey University (*Haemonchus*), AgResearch Palmerston North (*Ostertagia*) and Wallaceville (*Ostertagia* and *Trichostrongylus*). The number of larvae in the infective dose was expected to achieve a low to moderate level of parasitism consistent with ethical considerations. Larvae were administered directly into the rumen by tube, in a water medium.

### 4. Sampling techniques

Faecal egg counts (FEC) were assessed at days 21 and 27 using a Modified McMaster Technique where each egg counted represented 50 eggs per gram of wet faeces. FEC was also assessed on day 0 to determine the efficacy of the anthelmintic drench (no eggs were found).

All animals were slaughtered on day 28 using a captive bolt gun. The abomasum and small intestine were removed, ligated separately, and frozen for later parasite counts. Mucosal mast cells (including globule leucocytes) were counted as described by Douch *et al.*, (1986). Sections for cell counts were taken from the fundic region of the abomasum, and one metre below the pylorus. Numbers of parasites were estimated by flushing the abomasal and small intestine contents and washing the mucosa of each into 2 litres of water. Aliquots were randomly drawn to make up 100 mls in which the total number of male and female parasites of each species were counted including 4th stage larvae.

The 'potential pathogenicity' of the total adult parasite burden was estimated using a 'points system' described by

McKenna (1981) in which 500 *Haemonchus*, 4000 *Ostertagia* and 6000 *Trichostrongylus* are each equal to one point. This enabled a 'Total Pathogenic Index' to be calculated for each sheep. Actual pathogenicity of a parasite burden can only be measured in terms of effects on the host, which may differ between host genotypes. However a 'Total Pathogenic Index' is still a more useful measure than total burden when comparing the potential seriousness of a mixed infection between lines.

### 5. Statistical Analysis

Differences between flock means for the variables measured were examined using analysis of variance techniques. Between-line differences in the relationship between numbers of parasites and faecal egg count were examined using a general linear regression homogeneity of slopes model ( $H_0: \beta_{FW} = \beta_C$ ).

The error distribution of parasite counts, FECs and small intestine mucosal mast cell counts tended to be negatively skewed, particularly in the C line, and variances tended to be larger in the FW line. These variables were therefore square-root transformed to normalise distributions and improve homoscedasticity. Where values were below 10 the transformation  $\sqrt{x+0.5}$  was applied (Steel and Torrie, 1980).

## RESULTS

During the infection period there were no clinical effects of parasitism such as inappetance, diarrhoea, or listlessness. Numbers of *Haemonchus* at slaughter ( $\sqrt{x}$  transformed) were higher in FW sheep than C sheep for both males ( $p < 0.0001$ ) and females ( $p < 0.005$ ), and for *Ostertagia* ( $p < 0.05$ , both sexes) (Table 1). There was no significant difference in numbers of *Trichostrongylus* or in total worm count (TWC), between lines.

Seven sheep from the FW line had early 4th stage larvae present at post-mortem, whereas early 4th stage larvae were present in only one C sheep. However the numbers of these larvae were very low and unlikely to be of biological significance.

The female to male ratio of the three species did not differ significantly between lines.

**TABLE 1:** Worm burdens at post-mortem in FW selected and Control sheep artificially infected with three nematode species (untransformed means and S.E's)

Species	Flock	No. of Worms	% Est. <sup>1</sup>	Prop.(%) <sup>2</sup>
Haem.	FW	1151 ± 207	28.8 ± 5.2	12.4
	C	249 ± 102	6.20 ± 2.5	4.1
Ost.	FW	2268 ± 690	10.0 ± 3.0	24.5
	C	600 ± 169	2.60 ± 0.7	9.8
Trich.	FW	5838 ± 1213	23.4 ± 4.9	63.1
	C	5266 ± 1619	21.1 ± 6.5	86.1
TWC	FW	9257 ± 1748	17.9 ± 3.4	100
	C	6115 ± 1618	11.8 ± 3.1	100

<sup>1</sup> Number of parasites which established as a percentage of larvae in the infective dose.

<sup>2</sup> Number of adult parasites of each species at slaughter expressed as a percentage of the total burden.

The calculated 'Total Pathogenic Index' (TPI), was higher in FW sheep than C sheep (3.8 vs 1.5;  $p < 0.005$ ). Faecal

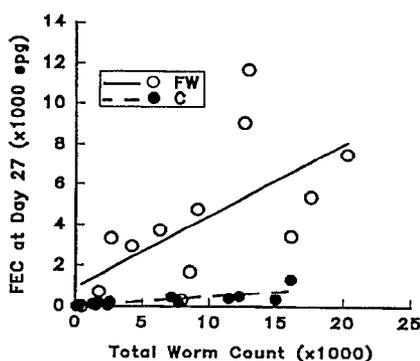
egg counts (sqrt transformed) were higher in FW sheep at day 21 ( $p < 0.05$ ) and at day 27 ( $p < 0.0001$ ) (Table 2).

**TABLE 2:** Faecal Egg Count at day 21 and day 27 in FW selected and Control sheep artificially infected with larvae (eggs/g faeces, untransformed means and S.E.'s)

	C	FW
Day 21	262 ± 107	1300 ± 386
Day 27	300 ± 99	4204 ± 970

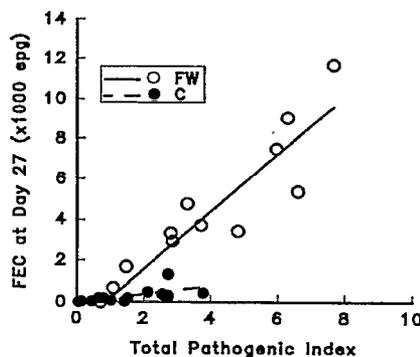
The regression coefficient relating FEC at day 27 to total parasite burden was significantly larger ( $p < 0.01$ ) in FW sheep than in C sheep ( $0.35 \pm 0.13$ ,  $p < 0.05$  vs  $0.05 \pm 0.01$ ,  $p < 0.005$  eggs per gram faeces/worm) (Fig.1).

**FIGURE 1:** The relationship between faecal egg count at day 27 and Total Worm Count in FW selected and C sheep artificially infected with larvae.



The regression coefficient relating FEC at day 27 to TPI was significantly larger ( $p < 0.001$ ) in FW sheep than in C sheep ( $1410 \pm 200$ ,  $p < 0.0001$  vs  $202 \pm 71$ ,  $p < 0.05$  eggs per gram faeces/index unit) (Fig.2).

**FIGURE 2:** The relationship between faecal egg count at day 27 and Total Pathogenic Index in FW selected and C sheep artificially infected with larvae.



Abomasum and small intestine mucosal mast cell counts did not differ significantly between lines (Table 3).

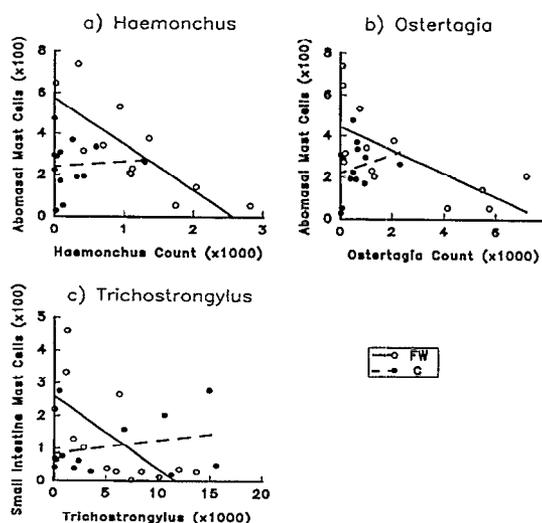
In FW sheep there was a strong inverse relationship between numbers of *Haemonchus* and numbers of mucosal mast cells in the abomasum ( $-2.78 \pm 0.66$  worms/cell  $p < 0.005$ ) and a similar relationship for *Ostertagia* ( $-7.80 \pm 2.65$  worms/

**TABLE 3:** Mucosal mast cell counts in the abomasum and small intestine of FW selected and C sheep artificially infected with larvae (arithmetic means and S.E.'s)

	Abomasum	Small Intestine
FW	317 ± 59	130 ± 40
C	246 ± 34	106 ± 26

cell  $p < 0.05$ ). This relationship was also evident for numbers of *Trichostrongylus* and numbers of mucosal mast cells in the small intestine ( $-20.16 \pm 6.72$  worms/cell  $p < 0.05$ ) in FW sheep (Fig. 3). There were no such relationships between parasite counts and cell counts in C sheep.

**FIGURE 3:** The relationship between mucosal mast cell count and worm count in FW selected and C sheep artificially infected with larvae.



## DISCUSSION

The rams from the FW selection line used in this trial were those which remained after selection of breeding sires and therefore did not constitute a random sample of the total flock. If there is an unfavourable genetic correlation between FEC and wool weight as suggested by Garrick *et al.*, (1992), the rams used in this trial would be expected to have lower FECs than the flock average. This difference would be small and would tend to underestimate any between-line differences in the parasitological parameters measured. The C rams used were those which remained after random selection of breeding sires and can therefore be considered a random sample themselves.

### 1. Worm Counts

The generally low level of establishment of parasites in both lines indicates that most sheep had acquired a degree of immunocompetence, making them largely refractory to infection. However there were clear differences between sheep selection lines in the level of establishment of *Haemonchus* and *Ostertagia*, with the numbers of adult parasites at slaughter being higher in the FW line than in the C line.

The lack of a significant difference in total parasite burden between lines reflects the fact that *Trichostrongylus*,

whose level of establishment did not differ between lines, made up the greatest proportion of worms recovered at post-mortem in both lines (Table 1). Between-line differences in worm burden were species-specific i.e. FW sheep were more susceptible to the abomasal parasites *Haemonchus* and *Ostertagia*, but not to the small intestine parasite *Trichostrongylus*. This result is in contrast to studies (Dineen *et al.*, 1977; Douch, 1989; Stewart, 1953, 1955) which have found that although a specific antigenic trigger is required for resistance to be expressed, the actual effector mechanism is non-specific. A greater worm burden in FW sheep would have been expected for all three species of parasite if this effector mechanism is non-specific. Work on the high-producing Woodlands lines suggests that worm burdens in these sheep have increased non-specifically (McEwan *et al.*, 1992).

The worm burden in FW sheep was made up of a higher proportion of species normally considered to be highly pathogenic, in particular *Haemonchus*, and therefore the mean 'Total Pathogenic Index' (TPI) of the FW line was greater than that of the C line. Although the total parasite burden was not significantly larger in the FW line, on a theoretical basis the potential pathogenicity of the burden in FW sheep is considerably greater. There is some evidence to suggest that the 'realised pathogenicity' of the burden acquired by FW lambs grazing pasture is indeed greater than that of C lambs, with FW lambs tending to suffer more production depression when facing the same challenge (Williamson *et al.*, in press).

## 2. Faecal Egg Counts.

The higher mean faecal egg count in the FW line is consistent with observations made by Howse *et al.*, (1992) under field conditions. FEC increased rapidly between days 21 and 27 in the FW line, but increased only slightly in the C line. Little is known about possible differences in the kinetics of parasitic infection between these two lines, but the more rapidly increasing FEC in FW sheep may reflect greater parasite fecundity in this line. In-utero egg counts of female worms were not performed in this trial so it was not possible to determine whether parasitic fecundity differed between lines.

## 3. Relationship between faecal egg count and total parasite burden.

The relationship between FEC and parasite burden appears to differ between lines as a consequence of selection for fleece weight. Even though both lines carried a similar total burden, the FW line had a larger FEC at day 21 and a considerably larger FEC at day 27. This difference is partly due to the fact that *Haemonchus* is more fecund than the other species (McKenna, 1981) and therefore contributes more to a sheep's total FEC. Thus FW sheep, having a greater proportion of this species, had higher FECs even though the total burden was not significantly different.

In terms of helping to account for the large difference in FEC between lines, greater parasite fecundity may also be a contributing factor, over and above the effect of different proportions of each species in the burden. A higher female to male ratio would also result in a higher FEC from a similar total burden. However results from this trial do not indicate that the female to male ratio has been changed by selection for fleece weight.

The relationship between FEC and total parasite burden has been changed by selection in the sense that the same burden no longer results in the same FEC. However within both lines the relationship between FEC and total burden was still reasonably strong. This relationship tended to be stronger in the C line than the FW line, reflecting the larger FEC and total worm burden (and therefore larger variance) in the FW line.

## 4. Relationship between mucosal mast cells and parasites.

A large number of trials using a range of host-parasite systems have shown a close association between inflammatory cells of the gut mucosa (including mast cells and globule leucocytes) and resistance to internal parasites (Rothwell, 1989). An inverse relationship between numbers of globule leucocytes, and numbers of parasites has been demonstrated in sheep in several trials (eg. O'Sullivan and Donald, 1973; Dineen *et al.*, 1978; Dineen and Windon, 1980; Douch *et al.*, 1986). There has been some controversy over the relationship between mast cells and globule leucocytes. However it is now generally accepted that globule leucocytes are partially degranulated mast cells and that these two cell types can be included in one count as was done in this trial.

The expected inverse relationship between mucosal mast cell numbers and parasite numbers was observed only in the FW line. It is unclear why there was no such relationship in the C line. This result provides further evidence for a difference between lines in the response to parasitism.

## CONCLUSIONS

This trial confirms that resistance to internal parasites (of some species) has decreased as a result of long term selection for high fleece weight. This lends support to the view of Howse *et al.*, (1992) that low FEC may not be a suitable selection criteria where the aim is to increase sheep productivity. Fleeceweight-selected sheep have become less resistant than control sheep yet are more productive, suggesting an unfavourable genetic association between resistance and fleece weight.

These two lines provide a useful resource for examining physiological mechanisms involved in resistance to parasites. Determining which mechanisms differ between resistant and susceptible genotypes may lead to the identification of new criteria for the selection of genetically resistant sheep.

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