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Ultrasonic measurement of backfat and muscling in sheep selected for parasite-related traits

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ABSTRACT

This paper reports on backfat depth and muscling comparisons of sheep selected for nematode parasite-related traits. Results are from two AgResearch projects, the first located at Kaitoke Farm (Upper Hutt), in which experimental Romney lines were selectively bred for susceptibility (S) or resistance (R) to nematode infection (high or low faecal egg count), or for resilience to nematode challenge whilst maintaining acceptable growth with minimal anthelmintic treatment (RL line); these lines grazed alongside an unselected control line (C). The second project was located at Ballantrae (Northern Wairarapa), in which Romney breeding lines were selected for resilience, with two replicate farmlets under 'Conventional' parasite management (with anthelmintic treatment), and with two further replicate farmlets under 'Low-Chemical' management. The four replicate lines at Ballantrae were serviced by rams of equivalent genetics, from elite RL-line sires bred at Kaitoke or Ballantrae. Ultrasonic backfat depth and eye muscle dimensions (length, depth and area) of ewe lambs from each project were assessed at 8-9 months of age (234 C-, S-, R- and RL-line lambs born in 2003 at Kaitoke, and 154 resilient lambs born in 2004 at Ballantrae). Adjustments were made for significant fixed effects including covariate adjustment for live weight. No significant differences were found among the R, C and RL lines for either trait, but the S line had 5-10% smaller eye-muscle dimensions ($P < 0.05$) than the other lines following an extended period of natural nematode challenge with minimal drench treatment. The two replicate lines under Low-Chemical management were 3.8 ± 0.6 kg (11.8%) lighter in May than the replicates under Conventional management ($P < 0.001$). After adjustment for live weight, lambs in the Conventional treatments had 15% larger eye-muscle areas ($P < 0.001$) but not backfat depths than those in the Low-Chemical treatments. Results suggest that muscle dimensions of the S-line lambs were more affected than those of their R-line counterparts by parasite infection, whilst the C-, R- and RL-line lambs did not differ significantly. At Ballantrae, even at equivalent live weights, the two Conventional replicates had more muscling but not fat depth than the two Low-Chemical replicates.

Keywords: sheep; nematodes; genetics; selection; ultrasound; composition.

INTRODUCTION

Breeding sheep with a greater ability to cope with roundworm challenge is one of several options that may assist farmers in New Zealand to manage the growing anthelmintic resistance problem and to meet consumer demands to minimise drug usage in livestock. Two different types of host response, "resistance" and "resilience", were originally described by Albers *et al.* (1987). Resistant lambs can maintain low worm burdens while grazing pasture contaminated with worm larvae, but these lambs may not have good growth or fleece weights (see below). However, resilient lambs can maintain good health and growth rates without worm treatment while grazing heavily contaminated pasture, although worm burdens in the resilient animals may not decline over generations.

A common question from ram breeders interested in the host genetics of resistance or resilience in sheep is whether there are any favourable responses or negative side effects to selection. In the case of selection for resistance as monitored through low faecal egg count (FEC) in coarse-woolled breeds, significant reductions in live weight have been documented in Romneys (Morris *et al.,* 2000) and in Perendales (Morris *et al.,* 2005), along with reduced fleece weights (Morris *et al.,* 1997; 2005), but many other aspects of productivity in these lines, such as body composition have not been investigated. In the case of selection for resilience, faster post-weaning liveweight gains have been achieved (Morris *et al.*, 2004), which leads to the question of what is the cost to the animal of faster gains, in terms of body composition? This paper addresses the question of genetic changes in body composition, as assessed by ultrasound, in the AgResearch parasite-related selection lines. The study involved ultrasonic assessment of ewe lambs in the 2003-born selection lines at Kaitoke (Upper Hutt), and in the 2004-born selection lines at Ballantrae (Northern Wairarapa). Additionally, we were interested to test for any effect of two different treatments at Ballantrae (Conventional *vs*. Low Chemical

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farmlets) on fat depth and muscling, *i.e.* whilst the lambs experienced different worm burdens.

MATERIALS AND METHODS

Ethics

This experiment was carried out with trial designs approved by the AgResearch Wallaceville Animal Ethics Committee (#7793), and the AgResearch Grasslands Animal Ethics Committee (29/03).

Faecal egg count (FEC) selection lines

The FEC selection experiment at Kaitoke consists of Resistant (R), Susceptible (S) and Control (C) lines of Romney sheep. Establishment of the lines in 1979 and early experimental procedures are described in detail by Morris *et al.* (2000). An Elite Resilient line grazes with the FEC selection lines, as described below. The selection objective in the R and S lines is to reduce/increase mean FEC, respectively, in lambs post-weaning. The FEC data are from two sampling times, 'FEC1' and 'FEC2', generally in January/February and March/April, respectively. These two sampling times are separated by administration of an anthelmintic drench, which is effective according to faecal egg count reduction tests on the flock.

Management and selection procedures applied are essentially the same from year to year. In 2003, the year in which the Kaitoke ewe lambs in this study were born, the details were as follows: After mating in single-sire groups, pregnant ewes from all four selection lines grazed together. Lambs were identified to dam and tagged within 24 hours of birth. At weaning on 11 December 2003, at an average of approximately 12 weeks of age, the lambs received an effective anthelmintic drench and were split into separate ewe and ram lamb mobs. All lines of ewe lambs (total = 234) grazed together from weaning onwards, on nematode-infested pasture. Fresh faecal samples were collected routinely from a small random group of lambs, across all lines, and monitored for FEC. When the mean FEC in the monitor group reached approximately 1500 eggs per g, all lambs were faecal-sampled for FEC1 on 23 February 2004. They then received an effective anthelmintic drench (referred to as the FEC1 drench). This cycle was repeated, with a further nematode challenge, followed by a faecal sampling (FEC2) and a drench on 4 May 2004. An autumn live weight was taken on 7 May 2004, followed by ultrasonic measurements in June, as described below.

Resilient selection lines

The establishment of the Resilient selection line of Romney sheep at Kaitoke in 1994, and its subsequent management, are described by Bisset & Morris (1996) and Morris *et al.* (2001, 2004). In brief, Resilient-line rams were individually tested each year for resilience traits (age at first drench and number of drenches) alongside rams from the unselected FEC Control line described above. In 1997, additional ewes were screened in using an index of high growth and low dag score, forming an expanded flock of four 90-ewe replicate sublines which was managed at Ballantrae from that year onwards. At Ballantrae, the four replicate sublines of ewes graze year-round on separate self-contained farmlets. These consist of two farmlets under 'Conventional' parasite management (with regular chemical use, including anthelmintic), referred to as Cv1 and Cv2, and two farmlets under 'Low-Chemical' management, referred to as LC1 and LC2 (Mackay *et al*., 1998). An Elite Resilient line of ewes, generated from the best of the animals in each subline in 1999, was returned to Wallaceville. This Elite line has grazed alongside the FEC Resistant, FEC Susceptible selection lines and the Control line at Kaitoke since 1999, and is evaluated for resilience each year alongside the FEC Control, R and S lines.

The rams used for mating at Ballantrae are balanced across the sublines in terms of breeding values for resilience each year, with repeated reference sires being used across years and farmlets. This ensures that the four farmlets are as close to being genetically equivalent as possible. The Elite Resilient line at Kaitoke and the Ballantrae flock itself supply the eight rams used each year in the Resilient sublines to breed female replacement stock (two rams per farmlet). Since 2000, another four Romney rams from industry sources have also been used annually on representative samples of Ballantrae ewes from the replicate lines (one ram per farmlet). The resulting lambs by industry sires are evaluated for resilience but then culled before 12 months of age, thus being excluded as breeding replacement stock, and their ultrasonic measures are excluded from this paper.

Ewe lambs born at Ballantrae remain in their farmlets, whilst ram lambs are transferred to Kaitoke after weaning each year, for evaluation of age at first drench, alongside the Kaitoke-based FEC lines. Since 1997, management and selection procedures applied at Ballantrae have been essentially the same from year to year. In 2004, the year in which the Ballantrae ewe lambs in this study were born, the details were as follows: The ewes from the two Cv replicates received anthelmintic treatment in bolus form just before lambing. Lambs were identified to dam and tagged within 24 hours of birth. Lambs from all four replicates were weaned on 14 December 2004, at an average of approximately 13 weeks of age, and those from the two Cv replicates received an effective anthelmintic drench. Sexes were separated at weaning. From weaning onwards, the replicate lines of ewe lambs (total = 154 lambs sired by AgResearch rams) grazed on their separate farmlets, on nematode-infested pasture. Fresh faecal samples were collected routinely from a small random group of ewe lambs in each replicate, and monitored for FEC. Lambs from the two Cv replicates received an effective anthelmintic drench, approximately 4-weekly. Live weights of lambs in all replicates were recorded regularly. This provided the opportunity to monitor the two LC replicates and determine the resilience of their lambs post-weaning, namely their individual ability to maintain growth (or not) under nematode challenge but without the routine drenches received by the two Cv replicates. The control against which the LC weight gain was compared was the average of lambs in the Cv farmlets. No chemicals of any description were used on animals or land in the two Low-Chemical farmlets, except for ethical reasons (salvage drenching applied to poor-gaining individual lambs, after which any treated animals were quarantined in a designated quarantine area for twice the withholding period, before being returned to the mob). Ultrasonic measurements and a live weight were taken on all ewe lambs in May, described below.

Ultrasonic recording procedures

The ewe lambs born at Kaitoke in 2003 (R and S FEC selection line lambs and C-line lambs, and the Elite Resilient lambs) were scanned on 3 June 2004, using an Aloka SSD500 ultrasonic scanner with a 5 MHz linear transducer. Backfat depth (FD), eye muscle length (EML) and eye muscle depth (EMD) were measured. Eye muscle area (EMA) was calculated as the product of the length and depth measurements, making the assumption in all that follows that the eye muscle was a rectangular shape. These procedures were repeated on 25 May 2005 in the replicate Resilient lines at Ballantrae (ewe lambs born in 2004).

Statistical methods

Traits reported here include least-squares means (SAS, 1995) for FD and EMA, and live weight in May. EML and EMD were also analysed. In the Kaitoke data, the fixed effects in the least-squares models were selection line and sire within line. The effects of birth rank (single *vs.* multiple), age of dam and birth-date deviation were also tested for significance, and included in the model where significant. In the Ballantrae data, the fixed effects were Cv *vs.* LC management, farmlet replicate (within Cv or LC management), and sire within farmlet. Effects of birth rank, age of dam and birth-date deviation were included in the model where significant. Both sets of analyses were carried out with and without covariate adjustment for live weight in May. Levels of significance for the effects of selection lines were tested against the residual, and then additionally against the 'sire-within-line' mean squares (Kaitoke data) or the 'replicate-within-treatment' mean squares (Ballantrae data).

Least-squares means for live weights recorded throughout the post-weaning period from weaning to May were included to compare lines, with fixed effects tested as described above, but without May liveweight adjustment by covariate.

RESULTS

Live weights

Figure 1 shows the mean live weights from weaning to the following May, by trial and by line. At Kaitoke, the Elite Resilient line was consistently heavier than the three FEC lines. Relative to the S and C lines, the R line gained less weight after weaning (both $P < 0.001$). At Ballantrae, the Cv lines showed less of a weight check in March/April than the LC lines, and lambs in the Cv2 farmlet were heavier in April and May than those in the other three farmlets ($P < 0.001$). All lambs in the LC1 farmlet were salvage-drenched on 31 March 2005, and 94% of lambs in the LC2 farmlet had been salvage-drenched by 29 April 2005. Lambs in the LC farmlets were 3.8 ± 0.6 kg (11.8%) lighter in May, on average, than those in the Cv farmlets ($P < 0.001$).

Kaitoke ewe-lamb muscling and fat

Least-squares means for FD and EMA for the Kaitoke R, S, C and Elite Resilient lines are presented in Table 1. Selection line least-squares means for EML (mean 44.2 mm) and EMD (mean 22.1 mm) followed the same pattern as shown for EMA, and are not tabulated. There were no significant line differences in FD, with or without adjustment for live weight, but the regression of FD on live weight was significant and positive ($P < 0.01$). The Elite Resilient line had significantly greater EMA than both the C line ($P < 0.05$) and the other FEC lines ($P < 0.001$), when no adjustments were made for live weight, but it lost this advantage after adjustment. The R line had significantly greater EMA than the S line after adjustment for live weight ($P < 0.01$).

Figure 1: Least-squares means of live weight by selection line from weaning to May for Kaitoke lambs born in 2003 (Panel 1) and Ballantrae lambs born in 2004 (Panel 2); standard error bars of line means are shown in one direction.

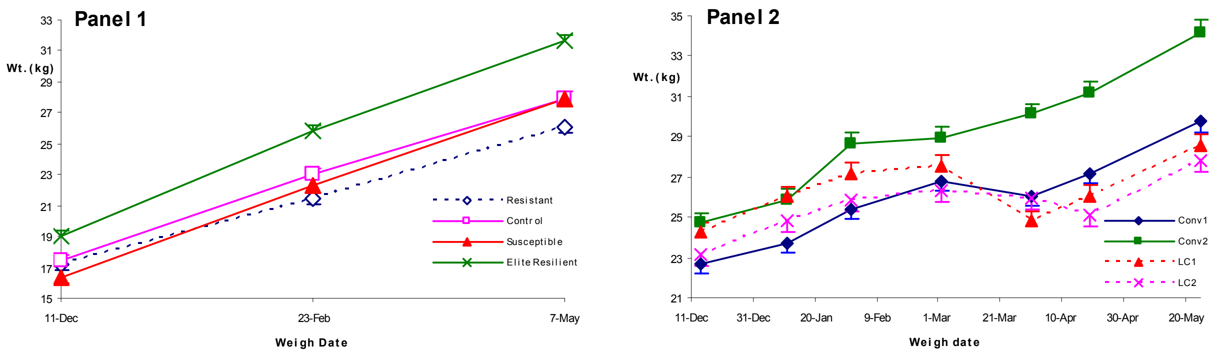

Table 1: Least-squares means of fat depth and eye muscle area in ewe lambs at Kaitoke, showing effects of selection line (Resistant (R), Control (C), Susceptible (S), and Resilient (Re)), sire pooled within line, birth rank, May live weight covariate, and date of birth covariate. The adjusted R^2 for each model is also shown.

Measurement	May weight covariate	Effect	Signif. level	Birth line	Least square mean	Diff from R line	Adj. R^2
Fat depth, mm (mean = 2.37, n = 233)	No	Line	n.s.	R	2.32 ± 0.09		0.02
		Sire / line	n.s.	C	2.39 ± 0.08	n.s.	
		Birth rank	*	S	2.34 ± 0.10	n.s.	
				Re	2.45 ± 0.08	n.s.	
	Yes, slope = 0.045 (s.e. 0.014) mm/kg	Line	n.s.	R	2.41 ± 0.09		0.05
		Sire / line	n.s.	C	2.39 ± 0.08	n.s.	
		May weight	**	S	2.39 ± 0.10	n.s.	
				Re	2.31 ± 0.09	n.s.	
Eye muscle area, mm^2 (mean = 988, n = 234)	No	Line[1]	***	R	941 ± 27		0.14
		Sire / line	n.s.	C	1006 ± 25	+	
		Birth rank	*	S	907 ± 31	n.s.	
		Date of birth	*	Re	1092 ± 26	***	
	Yes, slope = 35.4 (s.e. 3.7) mm^2/kg	Line[1]	*	R	1022 ± 24		0.37
		Sire / line	n.s.	C	1011 ± 21	n.s.	
		May weight	***	S	924 ± 26	**	
				Re	988 ± 25	n.s.	

[1]Additionally for EMA, testing the Line effect against the sire-within-Line mean square showed that it was significant ($P < 0.05$ and $P < 0.01$) in analyses with and without adjustment for live weight, respectively.

Ballantrae ewe lamb muscling and fat

Least-squares means for FD and EMA in the Ballantrae sublines (farmlets) are presented in Table 2. Least-squares means for EML (mean 41.1 mm) and EMD (mean 20.0 mm) followed the same pattern as shown for EMA, and are not tabulated. The Cv *versus* LC contrast showed no significant differences in FD, with or without adjustment for live weight, but the regression of FD on live weight was significant and positive ($P < 0.001$). The Cv2 replicate had significantly greater FD than Cv1 ($P < 0.05$) and LC2 ($P < 0.01$), but this effect disappeared when an adjustment was made for live weight. For EMA, the Cv *vs.* LC contrast was highly significant ($P < 0.001$), with or without adjustment for live weight. However, the regression of EMA on May weight was highly significant ($P < 0.001$), and the heavier Cv lambs lost some of their advantage over the LC lambs after adjustment for live weight, falling from 33% greater EMA pre-adjustment to 15% greater EMA post-adjustment.

DISCUSSION

Kaitoke ewe lambs

Differences in FD among the lines were small and non-significant, although the power of the line comparisons was small given the high coefficient of variation for FD (27%, from a residual standard error of 0.64 mm). In contrast, line differences in EMA were significant ($P < 0.001$), with a

Table 2: Least-squares means of fat depth and eye muscle area in ewe lambs at Ballantrae, showing effects of replicate line (farmlet: Conventional (Cv) versus Low-chemical (C)), sire pooled within farmlet, birth type, May live weight covariate, and date of birth covariate. The adjusted R^2 for each model is also shown.

Measurement	May weight covariate	Effect	Signif. level	Farmlet	Least square mean	Cv vs. LC	Adj. R^2
Fat depth, mm (mean = 2.23, n =154)	No	Cv *versus* LC	n.s.	Cv1	2.12 ± 0.10	2.31 ± 0.07	0.09
		Farmlet	*	Cv2	2.50 ± 0.11		
		Sire / farmlet	n.s.	LC1	2.29 ± 0.11	2.17 ± 0.08	
		Date of birth	*	LC2	2.04 ± 0.12		
	Yes, slope = 0.096 (s.e. 0.012) mm/kg	Cv *versus* LC	+	Cv1	2.14 ± 0.08	2.13 ± 0.07	0.34
		Farmlet	n.s.	Cv2	2.11 ± 0.11		
		Sire / farmlet	n.s.	LC1	2.40 ± 0.09	2.31 ± 0.07	
		May Weight	***	LC2	2.22 ± 0.10		
EMA, mm^2 (mean = 838, n = 153)	No	Cv *versus* LC[2]	***	Cv1	928 ± 30	976 ± 23	0.31
		Farmlet	*	Cv2	1024 ± 33		
		Sire / farmlet	n.s.	LC1	700 ± 31	733 ± 23	
		Birth type	**	LC2	765 ± 35		
	Yes, slope = 34.2 (s.e. 3.3) mm^2/kg	Cv *versus* LC[2]	***	Cv1	914 ± 22	889 ± 18	0.58
		Farmlet	*	Cv2	863 ± 29		
		Sire / farmlet	*	LC1	730 ± 25	775 ± 19	
		Date of birth	**	LC2	820 ± 28		
		May Weight	***				

[1]Farmlet effects fitted as replicates within the main treatment (Cv or LC).
[2]Additionally for EMA, testing the Line effect against the replicate-within-Line mean square showed that it was not significant in analyses with adjustment for live weight, and it was P = 0.055 without adjustment for live weight.

maximum difference of 185 mm^2 (or 20%) found between the Resilient and S lines (coefficient of variation also 20%). Much of this was associated with the EMA-live weight relationship and, after covariate adjustment for live weight, there remained a significant difference only between the R and S lines, of 11%, and between the C and S lines, of 10%. The R line was the lightest of the four lines in May, as was also found in earlier lamb crops in this breeding experiment (Morris *et al*., 2000), but this appeared not to have affected R-line muscling, compared with the C line. In contrast, the S and C lines were about equal in live weight, but the S line tended to be lower in muscling. It is important to note that these measurements were taken on lambs following an extended period of natural nematode challenge, with minimal drench treatment (10.4 weeks from weaning to drenching at FEC1 and an additional 10.1 weeks to drenching at FEC2), which contributed to the low FD levels.

Ballantrae ewe lambs

The most striking feature of the Ballantrae results was the clear advantage in mean live weight of over 4 kg in the Cv2 line compared with the weights of all others. Results from earlier lamb crops (Mackay *et al*., 2006) also showed a consistent advantage in live weight of the Cv over the LC farmlets, but no significant interaction between treatment and replicate (although the statistical unit analysed by Mackay *et al*. (2006) was the treatment x replicate mean for each year, rather than individual animal live weights).

Differences in FD were small at Ballantrae, as also found in the Kaitoke lambs. Although it is not possible to be specific about the consequences of the management effects, over half of the difference in EMA between treatments was associated with live weight. A significant (15%) difference in EMA persisted after correction for liveweight differences, and the LC lines had a lower mean EMA than the Cv lines (the latter lines being only minimally challenged with parasites). Nutrient loss in parasitised LC animals was probably the result of reduced protein availability (as reviewed, for example, by Sykes & Greer (2003), who described losses in serum proteins, sloughing of gut epithelial cells, and mucus secretion into the alimentary tract, all of which potentially could affect muscling in the lines). Faecal egg counts in lambs from the four replicates at weaning and at 32 weeks of age showed significantly higher (P < 0.001) levels of infection in the LC replicates, and there were also significant differences in the same direction in breeding ewes from those lines (Mackay *et al*., 2006). These differences were found in spite of higher Cv-line grazing pressure during lambing and in spring, due to ewes in the Cv lines being heavier, which was associated with higher Cv-line ovulation rates and greater lamb numbers weaned (122% lambs weaned in the Cv line compared with 104% in the LC line, per ewe lambing, P < 0.001).

CONCLUSIONS

It is concluded that muscle dimensions of the S-line lambs were more affected than those of their R-line counterparts by parasite infection, whilst the C-, R- and RL-line lambs did not differ significantly. At Ballantrae, after liveweight adjustment, lambs in the Cv lines had 15% larger eye-muscle areas (P < 0.001), but not FD, than those in the LC treatments. Equivalent genetics in the four Ballantrae replicates led us to the conclusion that these were management treatment effects rather than genetic differences.

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