Responses to selection in ryegrass staggers lines of sheep

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ABSTRACT

Ryegrass Staggers (RGS) is predominantly a summer/autumn metabolic disorder in ruminants, caused by ingestion of the lolitrem-B toxin from endophyte-infected perennial ryegrass, and it is common in New Zealand. It causes lack of neuromuscular co-ordination in susceptible animals under stress, *e.g*. when mustered by sheep dogs. It is of welfare concern, and it is costly to farmers because it severely compromises grazing management. A flock was established at AgResearch in 1993, with two lines bred for resistance (RGS-R) or susceptibility (RGS-S) to RGS. Lambs are scored for susceptibility each year, when grazing on high endophyte pastures, and mustered by sheep dogs. In 2005 and 2006, 94% and 91% of lambs respectively in the RGS-S line showed clinical staggers, when grazing together with the RGS-R line which had 2% and 6.5% of clinical cases, respectively ($P < 0.001$). Using the latest standardised RGS scoring system, the heritability estimate of resistance/susceptibility to RGS was 0.36 ± 0.04. To understand more about why and how the RGS lines differ, blood samples were taken from the breeding-ewe flock (n = 126) in June 2005, outside the usual RGS and facial eczema season. Compared with the RGS-R line, the RGS-S line ewes had a 23% higher activity ($P < 0.001$) of aspartate transaminase (AST), an enzyme indicating hepatic or muscular damage; there were similar findings in ewes sampled four weeks apart (between-animal repeatability for AST = 0.85). In plasma samples from selection-line yearling females (two birth years; n = 95), the RGS-S line had 27% and 37% higher AST and alanine transaminase activities, respectively (both $P < 0.001$), and a 38% higher creatine kinase activity ($P < 0.05$) than the RGS-R line. One explanation of these results is that, compared with the RGS-R line, selection in the RGS-S line may have changed two functions, a reduced ability to detoxify lolitrem-B, and an increased sensitivity to stress expressed as a tendency to show increased muscle tetany and damage.

Keywords: sheep; ryegrass staggers; breeding; selection; aspartate transaminase.

INTRODUCTION

Ryegrass Staggers (RGS) is predominantly a summer/autumn metabolic disorder in ruminants, caused by ingestion of the toxin lolitrem-B (Fletcher, 2005). The toxin is produced by an endophyte (*Neotyphodium lolii*) found in perennial ryegrass (*Lolium perenne*). In clinical cases of RGS, animals experience lack of neuromuscular co-ordination when under stress, *e.g.* when mustered by sheep dogs. RGS is of welfare concern, and it is costly to farmers because it severely compromises grazing management. The disease is common in New Zealand.

Much is understood about the perennial ryegrass/endophyte association, and methods have been developed for reducing or preventing lolitrem-B production whilst still providing the host plant with the means to continue producing toxins against insects (Easton & Tapper, 2005). However, much less is known about effects of the toxin on livestock, and on animal variation in resisting the toxin. A paper presented to the New Zealand Grassland Association by Hewett (1983) showed sire differences in RGS susceptibility on his North Canterbury sheep farm. Follow-up breeding work by Ruakura staff (Campbell, 1986; Morris *et al*., 1995a), culminated with the establishment by AgResearch in 1993 of two breeding lines of sheep selected for resistance (R) or susceptibility (S) to RGS. Over the last two years, blood samples have been taken from breeding ewes and the young stock, outside the usual RGS and facial eczema season. Our objective was to begin studying why and how these RGS lines differ in enzymes selected to indicate hepatic or muscular injury. The present paper describes recent RGS score results from the breeding lines, and preliminary comparisons of the lines for the enzymes tested.

MATERIALS AND METHODS

Ethics

This experiment was carried out using trial designs approved by the Ruakura Animal Ethics Committee (RAEC 10737).

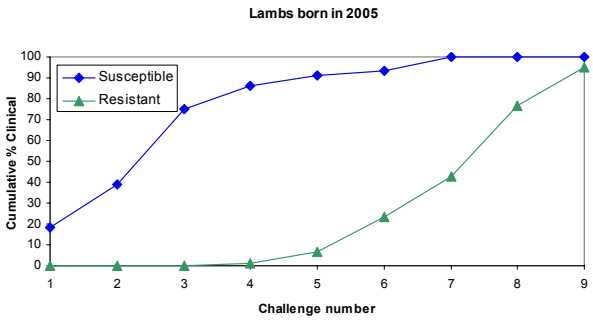
Animals

Following a 5-year period of testing sires for susceptibility to RGS, based on records of their progeny as lambs, yearlings or ewes (mainly Romney x Coopworth crosses), R and S breeding lines were established at Ruakura from 1993 onwards, using elite-ranked rams and ewes. Matings among selected parents have continued each year up to 2007. Ewes have grazed at two other sites in the 2000s, but stock management has remained similar. Lambs were born mainly in September, and were scored for RGS on high-endophyte perennial ryegrass pastures, generally in January and February (as described below). Yearlings and ewes were also scored where possible, in separate contemporary groups. Lambs from the 1993-1995 and 1998-2000 birth years were scored at Ruakura, and the 1996-1997 birth years were scored at Poukawa, Hawke's Bay. From the 2001 lamb crop onwards, they have been scored on AgResearch's Lincoln Farm, using control high-endophyte perennial ryegrass pastures of the Forage Improvement Section's modified endophyte programme.

Scoring for susceptibility

With large numbers of lambs to score regularly, and limited labour availability, we initially modified Keogh's (1973) 0-5 system in 1988/89 to a less labour-intensive RGS 0/1 scoring procedure. Score '1' was clinical RGS (inability to walk: scores of 4 or 5 on the Keogh scale), as observed at least once for an animal by the end of the season, when under the stress of being mustered by sheep dogs. Since 2001, the scoring system was improved to take account of increasing severity of challenge over the grazing season, as follows: tag-numbers and a date were recorded for any animal showing clinical RGS on the first scoring occasion; clinically affected animals from both lines were removed from the grazing group, as required for welfare reasons. (A high between-animal repeatability over time would be expected if clinical cases were not removed from the group, because of toxin accumulation in the body over time.) The challenge cycle was repeated on the remainder of animals, a few days later. An example of the scores allocated to lambs experiencing clinical RGS over time is given in Figure 1 by line. There were 9 scoring days in that particular year (and up to 14 days in other years). Because of variable toxin challenge over time, the intervals between adjacent dates where new clinical cases occurred were not uniform, so we avoided using date (or number of grazing days) as part of the RGS score.

Figure 1: Example showing the time course of recorded cases of clinical ryegrass staggers in lambs from the genetically resistant and susceptible lines. Clinical cases on Day 1 (challenge number 1) were scored 9, those on Day 2 were scored 8.…those on Day 9 were scored 1, and those from the Resistant line remaining non-clinical on Day 9 were scored 0.



Blood samples

Blood samples from sheep were drawn from the jugular into 10 ml vacutainer tubes, to test for enzymes whose activity may differ between the two lines. Samples were collected outside the summer/autumn season of natural challenge with RGS or facial eczema; within these dates (generally January to May), elevated enzyme activity would have been expected from liver injury caused by facial eczema. Animals from the two lines were compared as follows: breeding ewes (two samplings in June 2005; n = 126 ewes); 2004 lamb crop (in August at 11 months of age; n = 46 females), 2005 lamb crop (3, 9 and 11 months of age; n = 121 males and females, with only females sampled after 3 months of age), 2006 lamb crop (3 months of age; 100 males and females). The 3-month samples were taken within 24 h of weaning in 2005, and 15 days after weaning in 2006. Samples were assayed on each occasion for aspartate transaminase (AST), indicating hepatic (Ford, 1974) or muscular injury. On some of the above dates, the same blood samples were also analysed for alanine transaminase (ALT), another indicator of hepatic or muscular injury, gamma-glutamyltransferase (GGT) and glutamate dehydrogenase (GDH), both indicators of liver injury (Towers & Stratton, 1978), and creatine kinase (CK), an indicator of animals experiencing physical stress (muscle damage).

Data analysis - RGS scores

A contemporary group was defined as the year-of-scoring x stock-class of animal (ram lamb, ewe lamb, breeding ewe, *etc*.). All coded data for RGS score were standardised within contemporary group each year, to reflect the difference in ranges

of scores collected in different years (with varying numbers and severities of challenge). Accumulated standardised data (named as 'standardised RGS score') were analysed each year to rank all the new season's lambs, and to update the scores on parents (both sires and dams). The analysis used a restricted maximum likelihood procedure (Gilmour, 1997), including a full pedigree relationship matrix, with a fixed effect for contemporary group. Effectively, all animals were compared with contemporaries from the same grazing group, with results then pooled over contemporary groups. Analysis of the standardised RGS scores provided a Breeding Value (BV) for RGS resistance for each animal, where the BV is defined as the value of an animal as a parent. All the back-cross and out-cross lambs, derived in a related study and scored alongside lambs from the selection lines (Amyes *et al*., 2002), were also included in the BV analyses.

Data analysis - blood samples

All enzyme activities were analysed by least squares (SAS, 1995), fitting fixed effects for selection line, and for sex and year where necessary. Two-way interactions were tested, where two sexes or two years of data were available on the lambs. The enzymes analysed were: AST, ALT, GGT, GDH and CK. Two commercial laboratories were used for enzyme analyses, and different equipment and sample-analysis temperatures were used within those laboratories over time, and in some cases there were sampling days with different means and reference ranges, *e.g*. AST on 31 Aug 2005 (reference range 64-225 i.u./L) in Lab#1, on 2 Dec 2005 (reference range 0-55 i.u./L) in Lab#2 and on 12 Dec 2006 (reference range 0-90 i.u./L) in Lab #2. Enzyme activity levels of lambs were thus analysed after transformation to a $\log_e$ scale, so that the fixed effect for sample day (confounded with laboratory) and the transformation accounted for the differences in mean and variance, respectively. Using standard methods, an estimate of the between-animal repeatability of AST over time was made, for repeated records from ewes (June 2005 data), and for serially sampled ewe lambs born in 2005 (9 and 11 months of age).

RESULTS

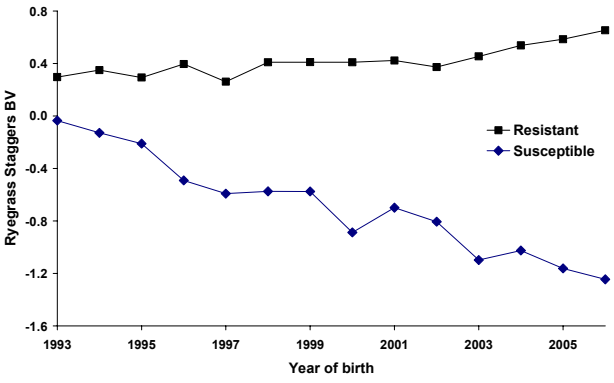
Parameters

Analysis of standardised RGS score yielded a heritability estimate of 0.36 ± 0.04, and the phenotypic standard deviation (σ_P) was 0.78 score units.

Long-term responses in susceptibility

Figure 2 shows the response to the original screening achieved in the 1993 lamb crop, and indicates that most of the subsequent within-line responses until 2002 were in the S line. This was because the ryegrass-dominant pastures to which we had access at Ruakura did not provide sufficiently severe natural challenges to rank R-line rams accurately for susceptibility. Since the 2001 birth year, lambs scored for susceptibility to RGS at Lincoln have experienced more severe RGS challenges at pasture. The divergence achieved in 13 years between the lines, through to the 2006 lamb crop, was about 2.4 σ_P in RGS score (Figure 2). About 28% of the selection response to 2006 was in the R line, and 72% in the S line. Larger annual responses have been recorded in the R line since 2002, as a result of the greater RGS challenge now being experienced. Over the four years 2002-2006, annual genetic response in the R line was 76% of that in the S line. In the lamb crops born in 2004 and 2005, 2% and 6.5% of R-line lambs, respectively, showed clinical staggers, compared with 94% and 91% of S-line lambs, respectively ($P < 0.001$).

Figure 2: Response to genetic selection for resistance or susceptibility to ryegrass staggers in two lines of sheep, by year of birth, 1993-2006. (The Breeding Value (BV) for resistance to ryegrass staggers (standardised RGS score) is defined as the 'value of an animal as a parent'; signs are reversed in the graph so that resistance is shown with a positive sign and susceptibility with a negative sign).



Enzyme results

There were no animals with GGT activity elevated above the reference range (range 15 to 140 i.u./L), confirming that blood samples were collected outside the usual facial eczema season. Sampling was also outside the RGS season. The GGT results also indicated that liver injury from facial eczema did not contribute to the other

enzyme differences between lines, described below. It was common, however, to find GDH activity levels marginally elevated, and the GDH difference between lines approached significance in one sampling (immediately after the natural facial eczema season, June 2006), where there appeared to be a greater percentage of RGS-S animals (96%) than RGS-R animals (78%) with elevated GDH activity ($P = 0.055$).

Compared with the RGS-R line breeding ewes, those in the RGS-S line (Table 1) had a 23% higher AST activity ($P < 0.001$), and there were similar findings in ewes sampled four weeks apart, with a high between-animal repeatability of 0.85. In 3-month-old lambs (Table 1), there was already a 9% higher AST activity in RGS-S line than in RGS-R line animals ($P < 0.05$), and lambs from the RGS-S selection line in August at 11 months of age (two birth years, $n = 95$) had 27% and 37% higher AST and ALT activities, respectively (both $P < 0.001$), than those from the RGS-R line. CK activity levels were 38% higher in RGS-S line than RGS-R line animals ($P < 0.05$). On average, the AST activity from 2005-born lambs sampled in both June and August was 24% higher in the RGS-S line than in the RGS-R line ($P < 0.01$). The between-animal repeatability of $\log_e$AST over time (June to August) was high, at 0.76 within line, 0.78 overall; corresponding repeatability estimates for $\log_e$ALT were 0.68 and 0.73, and for $\log_e$CK 0.14 and 0.18.

From the 11-month lambs measured in two lamb crops, there were positive correlations among the logarithms of ALT, AST and CK activities: ALT x AST 0.59, ALT x CK 0.62, AST x CK 0.55. For the log-transformed data, standard deviations of ALT, AST and CK, adjusted for line and year, were 0.36, 0.31 and 0.74, respectively, indicating that CK was the most variable of the three enzymes. Correlations of these three log-transformed traits with BVs for RGS susceptibility were 0.39, 0.36, and 0.22 overall, and 0.02, 0.05, and 0.06, on a within-line basis.

DISCUSSION

Genetic parameters

The heritability for the standardised RGS score, at 0.36 ± 0.04, was moderate/high, and it is thus a trait which should respond to selection. Realised rates of response to selection in the S line were broadly consistent with what would be expected for a multi-genic trait, but the response could also be explained by a major gene segregating. Therefore, all recent selection-line animals, and the back-cross and out-cross lambs derived in a related study (Amyes *et al*., 2002), have had DNA collected and stored, for future molecular analysis.

Resistance/susceptibility to facial eczema is also an inherited trait in sheep (Morris *et al*., 1995b: heritability 0.45 ± 0.05), and earlier work at Ruakura scoring both RGS and facial eczema resistances on the same animals has shown a genetic correlation of 0.31 between resistance to RGS and to facial eczema (Morris *et al*., 1995a). Therefore at least some of the genes for detoxification are in common for these two metabolic conditions, in spite of the toxins not being closely related chemically.

Enzyme results

The AST enzyme activity was consistently higher in RGS-S than RGS-R line animals, of either sex, or any age group measured, and it was a repeatable measure over time. In addition to AST, activities of ALT and CK were also significantly higher in the RGS-S than RGS-R line. The ALT result confirmed the AST result, but an explanation must be sought for the CK findings: it is possible that, compared with the RGS-R line, selection in the RGS-S line may have changed two functions, not only a reduced ability to detoxify lolitrem-B, but also an increased sensitivity to stress expressed as a tendency to show increased muscle tetany and damage. It is possible to see how such an unfavourable stress response might be getting incorporated into the RGS scoring (and selection)

Table 1: Effects of ryegrass staggers selection line (Susceptible (S) or Resistant (R)), on activities of aspartate transaminase (AST) in ewes (sampled twice) and in lambs (two birth years). Alanine transaminase (ALT) and creatine kinase (CK) comparisons are also shown for the two lamb crops. All enzyme activities were analysed after natural logarithm transformation.

Trait	S line	R line	Difference	s.e.d.	Enzyme activity in S line, as % of R line activity [1]
Ewes – $\log_e$AST	4.18	3.97	0.210	0.035	123
Lambs – 3 months $\log_e$AST	4.54	4.45	0.085	0.039	109
Lambs – 11 months $\log_e$AST	4.84	4.60	0.240	0.065	127
$\log_e$ALT	2.62	2.30	0.317	0.077	137
$\log_e$CK	5.95	5.63	0.320	0.156	138

[1] After back-transformation.

criterion for the RGS-S line, because RGS scores are only recorded after mustering. It would probably be necessary to obtain blood samples remotely from catheterised R and S animals in order to test for a CK enzyme difference without the associated animal stress experienced during the mustering and blood-sampling process.

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