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A PRELIMINARY STUDY ON BREED DIFFERENCES IN SUSCEPTIBILITY OF SHEEP TO *MYCOTOXIN SPORIDESMIN*

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SUMMARY

Sporidesmin is the mycotoxin that causes facial eczema disease (FE) in New Zealand (NZ) livestock. In an artificial sporidesmin dosing test, the introduced Finnish Landrace sheep breed was found to be significantly more tolerant to FE than the introduced Texel breed. This finding enables cross-breeding strategies to improve disease tolerance. Combining published data with the current study, a tentative inference is that Finnish Landrace, Merino and East Friesian are more FE resistant than Romney, Texel and Border Leicester.

INTRODUCTION

Genetic adaptation to environment is a key evolutionary feature of living things. Organisms with a small genome size, such as unicellular bacteria, adapt to new environment by acquiring extra-chromosomal genes through plasmids, which enable them to grow, for instance, in the presence of antibiotics and heavy metals (Dib *et al*. 2013; Dhanarani *et al*. 2009). On the other hand, higher organisms with larger genome sizes have a wider spectrum of genes and variants that can form novel biochemical pathways for adaptation. Plants for example, when faced with herbicide glyphosate challenge, acquire resistance to the xenobiotics by changing their glyphosate metabolism and translocation (González-Torralva *et al*. 2012), gene amplification, and by increasing the enzymatic activity of a specific gene product (Salas *et al*. 2012). Such adaptive changes in animals give rise to genetic differences of sheep breeds developed in different countries.

As an island nation, NZ has an indigenous problem which is facial eczema (FE). In a severe outbreak, the disease costs the NZ sheep industry an estimated $60M. The current methods used to reduce the impact of FE are by zinc prophylactic treatment of animals and through breeding of resistant livestock. Through selection and cross breeding over the years, NZ sheep flocks generally become more tolerant to the disease. Exotic sheep breeds, developed under different foreign conditions, face a new FE challenge following importation into NZ. In this report, two introduced sheep breeds, Finnish Landrace and Texel, were tested for their relative susceptibility to FE in an artificial sporidesmin challenge experiment.

MATERIALS AND METHODS

Animals. Information on the experimental animals is summarized in Table 1. The Finnish Landrace (Finn) and Coopworth (Coop) animals were from the AgResearch Woodlands farm, Texel and Finn x Texel animals were purchased from 2 different commercial farms and housed at Woodlands for a month before the experiment. Nine animals were obtained from each source and were progeny from three sires (with 3 progeny per sire); the exception was Finn which had only 2 sires with 3 and 6 progeny (Table 1). All animals were 10-month old lambs. All Finn and Coopworth animals were females, while all Texel and Finn x Texel were males. Morris *et al*. (1995) observed no sex differences in susceptibility to FE.

Sporidesmin challenge regime. Ethics approval to conduct this work was obtained from AgResearch Animal Ethics Committee (application AEC-P516). The 36 experimental animals were allocated into 3 dosing groups of equal size. Each group had at least 1 progeny from each sire (Table 1). The 0.2, 0.3 and 0.4 dosing groups had sporidesmin dose rates of 0.2, 0.3 and 0.4 mg/kg live-weight (LWT), respectively.

Animals were weighed and blood sampled a week before dosing: the weights were used for calculating the dosage for each animal, and the blood samples were used to determine the pre-dose levels of liver-specific enzymes, gamma-glutamyl transferase (GGT) and glutamate dehydrogenase (GDH) (in IU/L). After dosing, GGT and GDH were measured weekly for 5 consecutive weeks: GGT0 and GDH0 refer to pre-dose GGT and GDH respectively, GGT1-5 and GDH1-5 refer to GGT and GDH levels at 1- to 5-week post dosing. Levels of GGT and GDH in the blood reflect the severity of liver damage caused by sporidesmin.

Throughout the 5-week period, animals were generally kept outdoors on good pasture. After sporidesmin dosing, animals were monitored thrice daily for clinical signs of photosensitivity. Animals were housed indoors in well-ventilated shed, supplied with feed and water, if they showed any sign of restlessness, stamping of feet, pruritus, shaking or rubbing of the head, swollen eyes/ears/lips, or avoidance of sunlight.

Table 1. Experimental groups and their constituent animals

Breed	Sire[1]	Progeny (n)	Number of animals in each dosing group[2]		
			0.2	0.3	0.4
Finn	Sire A	3	1	1	1
	Sire B	6	2	2	2
Texel	Sire C	3	1	1	1
	Sire D	3	1	0	0
	Sire E	3	1	1	1
Finn x Texel	Sire F	3	1	1	1
	Sire G	3	1	1	1
	Sire H	3	1	1	1
Coopworth	Sire I	3	1	1	1
	Sire J	3	1	1	1
	Sire K	3	1	1	1

[1]Sires A - K denote different sires.
[2]Two Texel progeny (from sire D) died just before the experiment started and no replacements were available.

Statistical analyses. GGT and GDH data were natural log transformed (logGGT & logGDH) and then analysed in a mixed model which included dose rate and breed as fixed effects, and sire within breed as a random effect. The model was fitted by residual maximum likelihood (REML). The sire within breed variance was used to test for differences between breed. Dose rates by breed interactions were tested in the initial models and were dropped because none was significant. The occurrence of clinical signs was analysed in a logistic mixed model, with the same model terms as above. Correlations were calculated between residuals from the analyses at different time points.

RESULTS AND DISCUSSION

The background ranges of logGGT and logGDH for sheep are 0-4.0 and 0-2.6, respectively. With reference to these ranges, the Finn animals did not react to the sporidesmin challenge (Table 2). The Texel animals were significantly more susceptible to the toxin than Finn, with ~29% developing clinical photosensitivity. As expected the Finn x Texel animals showed an intermediate toxin response, between that of Finn and Texel (Table 2). Nonetheless, some of the breed differences observed could be attributed to other factors associated with the different sources of animals; however these factors would be minor. For example, a major factor like rearing rank was found to have no effect on FE resistance (Morris *et al.* 2001).

The Coopworth result showed this group of animals to be at least as FE sensitive as Texel (Table 2). They were from a South Island FE-free region, where animals tend to be more FE-susceptible than their counterparts in North Island. However, Coopworths are genetically diverse, with grade-up from other breeds allowed (http://coopworthgenetics.co.nz/). Hence the group tested here may not be a representation of the whole NZ Coopworth population.

In a toxicological view that “dosage determines poison”, it is intriguing to observe that increasing sporidesmin dose rates from 0.2 to 0.4 mg/kg LWT did not increase the numbers of reactant animals within breed nor between dosing groups. No explanation could be forwarded to account for this observation.

Table 2. Summary of least square means of logGGT and logGDH, and clinical cases of animals after sporidesmin challenge

Trait[1]	Breed least squares means[2]					Dose least squares means[3]			
	Finn	Texel	Finn x Texel	Coop	Mean SED	0.2	0.3	0.4	Mean SED
LogGGT0	3.3[a]	3.42[a]	3.64[a]	3.55[a]	0.16	3.47	3.48	3.48	0.08
LogGGT1	3.51[a]	3.83[b]	3.82[b]	3.75[b]	0.09	3.68	3.78	3.73	0.08
LogGGT2	3.3[a]	4.48[b]	4.19[a,b]	4.90[b]	0.38	4.51	4.23	3.95	0.32
LogGGT3	3.47[a]	5.02[b,c]	4.66[a,b]	6.03[c]	0.51	4.83	4.96	4.59	0.36
LogGGT4	3.43[a]	5.22[b,c]	4.9[b]	6.18[c]	0.57	4.91	5.02	4.89	0.31
LogGGT5	3.42[a]	5.23[b]	4.93[b]	6.04[b]	0.58	4.89	4.94	4.90	0.32
LogGDH0	0.33[a]	1.62[a,b]	1.35[a,b]	2.48[b]	0.48	2.28	1.73	1.57	0.42
LogGDH1	0.34[a]	2.60[b]	2.27[b]	2.20[b]	0.45	2.06	2.05	1.94	0.30
LogGDH2	0.46[a]	4.25[b]	3.13[b]	4.44[b]	0.60	3.60	3.16	3.26	0.34
LogGDH3	0.37[a]	4.71[b,c]	3.66[b]	5.63[c]	0.54	3.88	4.01	4.03	0.46
LogGDH4	0.34[a]	5.20[b,c]	4.38[b]	5.96[c]	0.50	4.45	4.17	4.43	0.43
LogGDH5	0.36[a]	5.49[b,c]	4.61[b]	5.83[c]	0.52	4.65	4.40	4.44	0.43
% Clinical	0[a]	28.6[a]	22.2[a]	55.6[a]		33.3	27.3	18.2	

[1]LogGGT0/logGDH0 refer to pre-dose logGGT/logGDH, logGGT1-5/logGDH1-5 refer to logGGT/logGDH at 1-5 weeks after dosing. The % Clinical refers to percentage of animals showing signs of photosensitivity over all dosage rates.
[2]Values are breed least squares means (over all dosing groups). Means with the same superscript do not differ significantly at $P<0.05$ (within trait/row).
[3]These values are dose least squares means (over all breeds); they do not differ significantly at $P<0.05$ (within trait/row).

The observation of decreasing clinical percentages with increasing dose rates was not significant (Table 2). There is evidence (authors’ unpublished data) that shows that not all sheep with severe liver damage caused by sporidesmin develop photosensitivity. A current view is that a separate set of genes are involved in other factors required for clinical manifestation.

Correlations between residuals from the models were all positive from week 2 onwards, in which case they ranged between 0.71 and 0.97 for logGGT, and between 0.68 and 0.82 for logGDH. Given this and the fact that the greatest differences observed here were in weeks 3 and 4, there is no reason to revise the industry practice of measurement at 3 weeks post-dose.

Year 1972 saw the first importation into NZ of Finn, East Friesian, German Whiteheaded Mutton and Oxford breeds from the United Kingdom and Ireland (Clarke and Meyer 1977). These animals failed the quarantine due to the suspected occurrence of scrapie in some individuals (Tervit *et al*. 1986). The second importation in 1984 using frozen embryos successfully introduced the Texel and Oxford Down from Denmark, and Finn and Texel from Finland (Tervit *et al*. 1986); the numbers of rams and ewes used to generate the embryos were 11 rams/47 ewes, 14/46, 17/47 and 5/23, respectively. The final numbers of lambs born from the imported embryos were 39 Texel and 19 Oxford Down from Denmark, and 56 Finn and 28 Texel from Finland (Tervit *et al*. 1986). The present day Finn and Texel animals come from these bottlenecks. This report shows that the Finn breed is significantly more FE resistant than the Texel breed.

Based on a sporidesmin dose rate of 2 mg/kg LWT, and the results of liver injury score and clinical cases, Smith *et al*. (1980) determined the Merino breed to be more FE tolerant than Romneys, Border Leicesters and Romney/Border Leicester cross. On a dose rate of 0.15 mg/kg LWT and the resultant logGGT3 data, Morris *et al*. (1995) found Finn to be more resistant to sporidesmin than Romneys, with Finn/Romney cross being intermediate. Using a 0.14 mg/kg LWT dose rate and the logGGT3 results obtained, East Friesians were shown to be more FE tolerant than Romneys (Morris *et al*. 2001). Combining the above results with the current study suggests that the relative FE sensitivity of various sheep breeds is, Finn, Merino and East Friesian are more FE resistant than Romney, Texel and Border Leicester.

CONCLUSION

This report shows that the introduced Finnish Landrace sheep breed is much more tolerant to sporidesmin, hence to FE, than the introduced Texel breed.

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