Testing glutathione-S-transferase for an association with facial eczema resistance in cattle

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ABSTRACT

Resistance to facial eczema (FE) in cattle is known to be a heritable trait. The commonly used indicators of susceptibility in affected groups of animals are gamma glutamyl-transferase (GGT) and glutamate dehydrogenase (GDH), which indicate the severity of the liver injury associated with FE. Published research using mouse models suggests that glutathione-S-transferase (GST) may be a downstream indicator of variation in FE susceptibility, and a study was carried out in calves to test this. Friesian-sired and Jersey-sired bull calves were purchased from dairy herds in spring 2004, artificially reared from about four days of age, weaned at 75-80 kg, monitored for base-line GGT activity in blood, and then dosed orally with sporidesmin. At 14 and 21 days after dosing, blood samples were taken to measure the activities of both GDH and GGT. A sample of 45 Friesian calves and 45 Jersey calves from the high and low outliers (using GDH activity within breed, 14 days after dosing) were blood-sampled 15 or 16 days after dosing and measured for GST activity, with 20-25 animals sampled each day, in balanced groups. There were significant differences in GST activity between high and low GDH groups ($P < 0.01$), and the pooled within-analysis-day regression of GST activity on logGGT activity (day 21) was negative ($P < 0.05$). Results confirmed that low erythrocytic GST activity is an indicator of FE susceptibility, although the differences seen between resistant and susceptible animals are insufficient for use as a predictive tool.

Keywords: cattle; facial eczema; resistance; glutathione-S-transferase.

INTRODUCTION

Facial eczema (FE) can be a serious animal health problem to ruminants in New Zealand. It is caused by sporidesmin, a toxin produced by spores of the fungus, *Pithomyces chartarum*, which are found on many pastures in summer and autumn in the North Island of New Zealand. In susceptible cows, sporidesmin causes liver injury, with deleterious effects on milk production (Towers & Smith, 1978) and on survival in the herd (Steffert, 1970; Morris *et al*., 2002). The cost of FE to the dairy industry can amount to tens of millions of dollars. For example, multiplying up to current dairy cow numbers for the North Island from data monitored in the Manawatu and Taranaki regions (Faull, 1991), for the 1985/86 to 1988/89 seasons which had 'minimal' to 'serious' outbreaks, it was found that the North Island costs were between $3.6M and $66.2M *per annum.* Then converting these results to 2007 milk values (assuming $4.15 per kg milksolids), the range was from $6.8M to $84.2M *per annum*.

Resistance to FE in cattle is known to be a heritable trait (Cullen *et al*., 2006). The commonly used indicators of susceptibility in affected groups of animals are gamma glutamyl-transferase (GGT) (Towers & Stratton, 1978) and glutamate dehydrogenase (GDH). These enzymes do not cause differences in FE susceptibility, but are merely associated with differences in FE susceptibility; they were chosen for use because they are robust measures of susceptibility under commonly encountered field conditions. The most recent heritability estimates are 0.40±0.04 for log GGT, and 0.35±0.06 for log GDH (Cullen *et al*., 2006), and the genetic correlation between them is close to unity (0.93±0.03). These estimates followed a series of papers progressively increasing the amount of data available (Morris *et al*., 1990; 1991a; 1991b;, 1998). Other enzymes and metabolites have been studied in the past to provide a greater understanding of the response to sporidesmin (the FE toxin), for example serum arginase, bilirubin, glutamate oxaloacetate transaminase, sorbital dehydrogenase and urea in sheep (Ford, 1974), and serum aspartate transaminase, bile acids, bilirubin, ferroxidase and 5'nucleotidase in cattle (Morris *et al*., 1998). Published research using mouse models suggests that glutathione-S-transferase (GST; EC 2.5.1.18) may be another downstream indicator of variation in susceptibility (Abel *et al.*, 2004), and an AgResearch study was carried out in calves to test this, as an adjunct to a genetic project (Cullen *et al*., 2006). The present paper reports results from this study, evaluating circulating GST activity as another indicator of liver injury in cattle after pasture challenge with facial eczema spores. Activities of the GGT and GDH enzymes are used as the criteria for comparison.

MATERIALS AND METHODS

Ethics

This experiment was carried out using a trial design approved by the Ruakura Animal Ethics Committee (RAEC 4663).

Animals

In 2004, as part of a project to rank dairy industry sires for genetic merit for FE susceptibility, Friesian-sired (F) and Jersey-sired (J) bull calves (nominated by AmBreed New Zealand Ltd, and of known sire pedigree) were purchased from dairy farmers at about 4 days of age. The calves were transferred to AgResearch's Tokanui Station or to a private local rearing farm, and reared on milk replacers until weaning at about 80 kg live weight (F) and 75 kg (J). They were then transferred in groups to Ruakura Research Centre, where a pre-dose live weight and a blood sample were taken on Day -1 (relative to dosing day, Day 0). The blood sample was to check for base-line GGT activity, because GGT is also present in very high quantities in colostrum (Wesselink *et al*., 1999). Calves were then challenged with sporidesmin by oral dose at 0.14 mg sporidesmin (first dose group: 20 October, 2004) or 0.16 mg sporidesmin (third dose group: 24 November, 2004) per kg live weight. Blood samples were taken at Days 14 and 21 after dosing, to measure activities of GGT and GDH, in order to determine the response to sporidesmin challenge. A further live weight was taken on Day 21 for each dose group. The procedures described so far were also applied to all other calves over three years, as reported by Cullen *et al*. (2006); calves from the two dose groups above were then also included in the GST study, as follows:

A total of 25 F-sired and 25 J-sired calves from dose group 1 were selected as high or low within breed on GDH activity on Day 14 post-dosing. They were split into two balanced sub-groups, and were blood-sampled in these sub-groups on Days 15 or 16 (12 or 13 animals per breed per day), providing samples to be assayed for GST enzyme activity. Similarly, a total of 20 F-sired and 20 J-sired calves from dose group 3 were selected as high or low within sire breed on GDH activity on Day 14 post-dosing. They were split into two balanced sub-groups, and were blood-sampled in these sub-groups on Days 15 or 16 (10 animals per breed per day), providing samples to be assayed for GST enzyme activity. Dose group 2 animals were not included in this GST study.

Glutathione-S-transferase assay

Blood packed cell volume (PCV) was determined by the microhaematocrit method, using an IEC Micro-MB centrifuge. Concentrations of haemoglobin (Hb) were assayed by the method of Evelyn & Malloy (1938). Erythrocytic GST activity was determined by the method of Habig *et al*., (1974), using 1-chloro-2,4-dinitrobenzene as substrate. Estimates of GST activity per 100 g Hb (GSThb) and GST activity per unit PCV (GSTpcv) were then derived.

Data analysis

The GGT and GDH variates were transformed to natural logarithms before statistical analysis, to account for their non-normal distributions with tails to the right. Linear models (SAS, 1995) were applied to each trait, $\log_e$GGT, $\log_e$GDH, PCV, Hb, GSThb and GSTpcv, with fixed effects to account for breed (covariate on breed composition (fractional 16ths of F): 0 for a pure J through to 16 for a pure F), dose group (1 or 3), sample analysis day (Day 15 or 16), and GDH response group (high or low).

RESULTS

Live weights

Mean live weight gains of calves, between Day -1 and Day 21, were 12.1 kg (F) and 10.1 kg (J), indicating that gains of almost 0.5 kg/day were achieved whilst the calves were recovering from sporidesmin challenge.

Correlations of GST with GDH and GGT activities

The most appropriate sample times post-dosing for measuring GDH and GGT activities are approximately Days 14 and 21, respectively (Morris *et al*., 1998). For GSThb activity, the pooled within-analysis-day regression on $\log_e$GDH at Day 14 was -0.07±0.03 units per $\log_e$ unit ($P < 0.05$), and on $\log_e$GGT at Day 21 it was -0.07±0.03 units per $\log_e$ unit ($P < 0.05$). Table 1 shows the pooled within-analysis-day correlations between the activities of GST and either $\log_e$GDH or $\log_e$GGT, ranging from -0.17 to -0.28. These correlations may, however, be biased because the choice of animals to sample for GST analysis was made on Day 14 GDH activity, so another way of assessing the results is to compare groups classified by the Day 14 GDH criterion (see below).

Group difference in GST activity

Table 2 shows the comparison of 'high' and 'low' animals classified according to Day 14 GDH activity. Those in the 'high' GDH activity response group were significantly lower in GST activity

Table 1: Correlations of measures of the activity of glutathione-S-transferase (GST), per unit packed cell volume (pcv) or per unit haemoglobin (hb), with the activities of glutamate dehydrogenase (GDH) and gamma-glutamyltransferase (GGT) sampled 14 and 21 days after dosing, from cattle selected at day 14 for low GDH (n = 45) or for high GDH (n = 45); correlations adjusted for analysis age.

	PCV	Hb	GSTpcv	GSThb
$\log_e$GDH day 14	0.17	0.20	-0.20	-0.21
$\log_e$GGT day 14	0.08	0.08	-0.18	-0.17
$\log_e$GDH day 21	0.15	0.18	-0.27	-0.28
$\log_e$GGT day 21	0.08	0.09	-0.22	-0.22

than those in the 'low' GDH activity response group ($P < 0.01$). GST activity (by both measures, *i.e*., per unit of PCV, or per unit of Hb) was 14% lower in Day 14 high GDH animals than in low GDH animals, which differed by a factor of 7 in GDH activity. There were no significant differences in GST activity between dose groups, indicating that there was no unintentional bias in the sub-groups studied.

Table 2: Differences between high and low response groups (as determined by glutamate dehydrogenase (GDH) activity, 14 days after dosing) in the activity of glutathione-S-transferase (GST), per unit of packed cell volume (pcv) or of haemoglobin (hb).

Response group	GSTpcv	GSThb
High GDH	4.17	1.28
Low GDH	4.84	1.50
Difference	-0.67	-0.21
Standard error of difference	0.24	0.07

DISCUSSION

GDH and GGT responses to dosing

The responses of GDH at Day 14 and GGT at Day 21 in dose groups 1 and 3 were lower than observed in calves a year earlier, although the reason is not clear. The dose rate in dose group 3 was increased accordingly to 0.16 instead of 0.14 mg/kg live weight, but this adjustment was also insufficient to make an appreciable difference to the responses.

GST differences

GSTpcv and GSThb were both found to be associated significantly with the activities of GGT and GDH, in a negative direction, so that high GST activity tended to be a characteristic of FE-resistant animals. Significant differences in GST were found between 'high' and 'low' response groups classified on GDH activity at Day 14. Other significant associations between enzyme or metabolite and GGT activity have been reported in cattle (Morris *et al*., 1998) for aspartate transaminase, bile acids, bilirubin and 5'nucleotidase, and in sheep (Hohenboken *et al*., 2004) for catalase, glutathione peroxidase, and cytoplasmic CuZn-superoxide dismutase.

Our findings suggest that GST is also in the downstream cascade of metabolic events involved in the host's attempts to detoxify cells affected by sporidesmin, but that differences in GST were relatively small compared with those shown by the GDH and GGT enzymes. In the future, this information about GST may assist in understanding more about the process of detoxification after sporidesmin challenge, but the field test for sub-clinical symptoms will continue to be the GGT and GDH enzymes, because they are robust under typical field conditions, and because the differences in GGT and in GDH activities, between high and low responders, were much greater than those found for GST activity.

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REFERENCES

Abel, E.L.; Bammler, T.K.; Eaton, D.L. 2004: Biotransformation of methyl parathion by glutathione S-transferases. *Toxicological Sciences* **79**: 224-232.

Cullen, N.G.; Morris, C.A.; Hickey, S.M. 2006: Genetic parameters for resistance to facial eczema in dairy cattle. *Proceedings of the New Zealand Society of Animal Production* **66**: 310-314, 319-324.

Evelyn, K.A.; Malloy, H.T. 1938: Microdetermination of oxyhemoglobin, methemoglobin, and sulfhemoglobin in a single sample of blood. *Journal of Biological Chemistry* **126**: 655-662.

Faull, B.W. 1991: Prevalence and cost of facial eczema on dairy farms. *Surveillance* **18**(2): 14-16.

Ford, E.J.H. 1974: Activity of gamma-glutamyl

transpeptidase and other enzymes in the serum of sheep with liver or kidney damage. *Journal of Comparative Pathology* **84**: 231-243.

Habig, W.H.; Pabst, M.J.; Jakoby, W.B. 1974: Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. *Journal of Biological Chemistry* **249**: 7130-7139.

Hohenboken, W.D.; Morris, C.A.; Munday, R.; de Nicolo, G.; Amyes, N.C.; Towers, N.R.; Phua, S.H. 2004: Antioxidants in blood from sheep lines divergently selected for facial eczema resistance. *New Zealand Journal of Agricultural Research* **47**: 119-127.

Morris, C.A.; Burton, L.J.; Towers, N.R.; Cullen, N.G.; Rendel, J.M.; Johnson, D.L. 1998: Genetics of susceptibility to facial eczema in Friesian and Jersey cattle. *New Zealand Journal of Agricultural Research* **41**: 347-357.

Morris, C.A.; Hickey, S.M.; de Nicolo, G.; Tempero, H.J. 2002: Lifetime survival of Jersey-sired cows following natural challenge with facial eczema during first lactation. *New Zealand Journal of Agricultural Research* **45**: 165-170.

Morris, C.A.; Towers, N.R.; Smith, B.L.; Southey, B.R. 1991a: Progeny testing bulls for susceptibility to facial eczema. *New Zealand Journal of Agricultural Research* **34**: 413-417.

Morris, C.A.; Towers, N.R.; Tempero, H.J. 1991b: Breeding for resistance to facial eczema in dairy cattle. *Proceedings of the New Zealand Grassland Association* **53**: 221-224.

Morris, C.A.; Towers, N.R.; Tempero, H.J.; Cox, N.R.; Henderson, H.V. 1990: Facial eczema in Jersey cattle: heritability and correlation with production. *Proceedings of the New Zealand Society of Animal Production* **50**: 255-259.

SAS. 1995: JMP version 3. SAS Institute, Cary, NC.

Steffert, I.J. 1970: Facial eczema: the extent of damage in dairy cows. *Dairyfarming Annual* **22**: 95-101.

Towers, N.R.; Smith, B.L. 1978: The protective effect of zinc sulphate in experimental sporidesmin intoxication of lactating dairy cows. *New Zealand Veterinary Journal* **26**: 199-202.

Towers, N.R.; Stratton, G.C. 1978: Serum gamma-glutamyltransferase as a measure of sporidesmin-induced liver damage in sheep. *New Zealand Veterinary Journal* **26**: 109-112.

Wesselink, R.; Stafford, K.J.; Mellor, D.J.; Todd, S.; Gregory, N.G. 1999: Colostrum intake by dairy calves. *New Zealand Veterinary Journal* **47**: 31-34.