be antagonistic. FE Resistant-line sheep may have higher activities of Phase I liver detoxification enzymes than FE Susceptible-line sheep (Smith *et al*., 1980; Parkinson, 2001). If these same enzymes are also necessary for the conversion of zearalenone to its breakdown product, α-zearalenol (Liehr *et al*., 1998), then this could be an asset for FE resistance but a penalty for zearalenone resistance, because α-zearalenol is more oestrogenic than zearalenone (Hagler *et al*., 1979; Galtier, 1999), at least in monogastrics. This would contribute to a positive genetic correlation between the breakdown or metabolism of zearalenone and sporidesmin, but a negative genetic correlation between resistance to zearalenone and FE.

Genetic correlation with ovulation rate

There was a genetic correlation of -0.55 between urinary Zen/Cr after dosing, and ovulation rate response (Morris *et al*., 2005b) (i.e. the ewes with lower levels of zearalenone breakdown-products in urine had higher ovulation rates). The genetic correlations between resistance to zearalenone and other production traits need to be worked out, and this should be possible from an industry trial underway in 2005/06, involving eight flocks.

GENERAL CONCLUSIONS

- *Fusarium* and the zearalenone toxin are found distributed on New Zealand pastures nationwide, in some years.
- Zearalenone is a potential cause of severely reduced reproduction in ewes, through interference with levels of both fertility and litter size.
- Its effect on production levels in lambs is not known.
- Control of zearalenone production or of *Fusarium* growth on pasture is not possible on a large scale.
- *Fusarium* and zearalenone are most commonly found between March and May, precisely the sheep mating season,
- The annual economic cost to the nation is unknown, but probably amounts to tens of millions of dollars.
- Resistance to zearalenone is inherited in sheep.
- A simple challenge-test has been developed and could be offered to breeders, using the infrastructure already built up by Ramguard.

Genetic parameters for resistance to facial eczema in dairy cattle

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ABSTRACT

A project was designed to progeny-test dairy industry sires for their resistance to facial eczema (FE), as a preliminary step towards identifying DNA markers or genes for FE resistance. The FE disease is caused by the toxin, sporidesmin, produced by spores of a fungus, *Pithomyces chartarum*, found on many pastures in summer and autumn in the North Island of New Zealand. In susceptible animals, sporidesmin causes liver injury, and the cost of FE to the dairy industry is measured in tens of millions of dollars in years with serious outbreaks ($3.6 to 66.2M *per annum*). Earlier studies in New Zealand have established that resistance to FE in cattle is a heritable trait, with resistance measured by variation in activity in blood of liver-derived enzymes, gamma-glutamyltransferase (GGT) and the associated glutamate dehydrogenase (GDH). Widely-used Friesian and Jersey sires were progeny-tested via 572 specially-reared sons (born in 2002-04 and dosed with sporidesmin), and also via 3761 daughters in autumns 2004 and 2005 in 17 herds with 3-22% clinical cases of FE per herd. The data were combined from all sources, including four earlier years of GGT records (1173 animals born in the 1986, 1989, 1990 and 1992 birth years), and standardisation was applied to each contemporary group. Heritabilities were estimated for Friesians (0.47 ± 0.07 for log GGT; 0.32 ± 0.08 for log GDH) and for Jerseys (0.37 ± 0.06 and 0.39 ± 0.09, respectively), and there was a very high genetic correlation between activities of the two enzymes (0.93 ± 0.03). Sixty-eight sires had reliabilities of >0.70 for log GGT Breeding Value and 71 others had reliabilities between 0.60 and 0.70. The sires progeny-tested

in this way generally were already proven for dairy traits and had been widely used, so that FE selection among these sires would be an additional trait when they were already old. Alternative approaches would be to performance-test young bulls for FE resistance, or to use sire and progeny data and their DNA to identify DNA markers or genes for FE resistance.

Keywords: facial eczema; cattle; heritability; resistance.

INTRODUCTION

Facial eczema (FE) is caused by the toxin, sporidesmin, produced by spores of a fungus, *Pithomyces chartarum*, 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 and 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 (e.g., scaling up to current dairy cow numbers for the North Island, from four years of monitoring, 1985/6 to 1988/9 (minimal to serious outbreaks) in the Manawatu and Taranaki regions (Faull, 1991): between $3.6M and $66.2M *per annum*).

In sheep the resistance of animals to FE has been shown to be inherited (heritability estimate = 0.45 ± 0.05: Morris *et al*., 1995a), and three dairy industry studies have established that resistance to FE is also inherited in cattle (Morris *et al*., 1990, 1991a, 1998); for example, a heritability of 0.29 ± 0.15 in Friesians, from the 1998 publication. The present study was set up to continue the progeny testing of sires for FE resistance, but using a different method, whereby large numbers of progeny and large numbers of sires could be evaluated. Our ultimate goal is to rank enough sires so that the extreme resistant and the extreme susceptible sires may be used for DNA marker studies on FE resistance/susceptibility.

MATERIALS AND METHODS

Animals

Beginning in the autumn of 1989, a series of trial designs with dairy cattle has been used to progeny test Friesian and Jersey sires for resistance/susceptibility to FE. Data from all the trials have been combined for the present analyses, because many male ancestors were in common across the trials. Table 1 provides a general summary of the number of animals measured. Three previous trial designs (Trials 1-3) are described briefly here, whilst the two recent designs (Trials 4-5), are described in greater detail.

Trial 1

Daughters in 60 herds recorded in the Livestock Improvement Corporation's (LIC) Jersey Sire Proving Scheme were blood-sampled during their first lactation, following unintentional natural exposure to FE, in a joint study by LIC and AgResearch Ruakura (Morris *et al*., 1990). Blood samples were analysed for the activity of gamma-glutamyltransferase (GGT), a liver-derived enzyme indicating the degree of liver injury from FE (Towers & Stratton, 1978). By using a threshold to

TABLE 1: Summary of animals tested for resistance/susceptibility to facial eczema (FE).

Trial	Sire breed[1]	Number of sires	Progeny year of birth	Sex[2] of progeny	Age at testing (yr)	Number of progeny in analysis	Number of herds	Type[3] of FE challenge
1	J	63	1986	F	2.6	571	24	N
2a	F, J	21	1989	M	0.6	132	1	N
2b	F, J	16	1990	M	0.6	61	1	A
2c	F, J	36	1992	M	0.6	335	2	A
3	J	10	1990	F, M	0.3	74	1	A
4	F, J	-[4]	2002-04	M	0.3	572	20[5]	A
5	F, J	-[4]	mixed[6]	F	2 to 12	3761	17	N
Total		139[4]				5506		

[1] F = Friesian; J = Jersey.
[2] F = female; M = male.
[3] N= natural challenge; A = artificial challenge by oral dosing.
[4] Within year and herd, numbers of progeny per sire were often insufficient for accurate proofs. However, by the use of reference sires, all data from Trials 1-5 were pooled. Minimal reliability for the bulls included in the overall analysis = 0.60.
[5] Up to 8 contemporary groups per year.
[6] Mixed-age cows, sampled in autumn, in the production years 2003/04 and 2004/05.

screen which herds had received sufficient challenge to rank animals (minimum criterion for a herd: 30% samples with GGT activity above 30 i.u./l), data were retained in the present analysis from 24 herds with 571 daughters (63 sire groups). This was a more stringent threshold than used in earlier analyses of the data (Morris *et al*., 1998).

Trial 2

Also in collaborative work between LIC and AgResearch Ruakura (Morris *et al*., 1998), bull calves born in 1989 were purchased by LIC to be progeny tested for milk and other traits in the LIC's Sire Proving Scheme. Following exposure at 7 months of age to toxic pasture containing FE spores, 132 young Friesian and Jersey bulls (by 10 and 11 sires per breed, respectively) were blood sampled for GGT assay. For the next (1990) crop of Sire Proving Scheme bull calves (61 young Friesians and Jerseys, by 8 sires in each breed), blood samples were taken following an oral dose with sporidesmin at 0.2 mg/kg live weight, using a split-dose regime where a third of the dose was administered on three consecutive days. In a third group of bulls (335 LIC-owned progeny born in 1992, sired by 18 bulls per breed, not for testing in the Sire Proving Scheme), a single oral dose of sporidesmin was administered at a dose rate of 0.14 mg/kg live weight. Serial blood samples were then taken from bulls in each group for GGT monitoring. The activity of a second enzyme (glutamate dehydrogenase (GDH), an earlier indicator of liver injury than GGT (Ford, 1974)), was also assayed from the same blood samples. Overall, the animals from Trial 2 consisted of progeny by 34 separate Friesian sires and 28 separate Jersey sires (i.e., avoiding double-counting sires where they were represented in more than one year).

Trial 3

At Ruakura, 74 male and female experimental calves were born in 1990; they were sired by one of five high GGT Jersey sires or five low GGT Jersey sires (previously identified in Trial 1 above). The calves received an oral dose of sporidesmin over a 4-day period (25% per day, with a total of 0.3 mg/kg live weight), and they were blood-sampled for GGT at about weekly intervals from 9 days after dosing (Morris *et al*., 1991b).

Trial 4

In the spring seasons of 2002-04, bull progeny by Ambreed-nominated Friesian and Jersey sires were purchased, transferred to one of two central rearing sites in the Waikato, weaned at 75-80 kg, and then challenged with sporidesmin by oral dose (Day 0) at 0.10 to 0.18 mg of sporidesmin /kg in each mob. The predominant sire breeds were Friesian and Jersey, but there were also small numbers of Hereford sires. Bull calves were born in July and August, and they were challenged with sporidesmin at 3 to 4 months of age. There were up to four dose groups each year, and blood samples for GGT were taken at Day -1, and again at Days 14 and 21 after dosing. GDH activity was also measured at Days 14 and 21. The pre-dose GGT enzyme level is usually used as a pre-dose negative control. However, GGT is also present in very high quantities in colostrum (Wesselink *et al*., 1999), so the pre-dose GGT provided a check against any animals carrying residual GGT from colostrum consumed early in life.

Trial 5

This Trial was used to generate data on much larger numbers of sires than earlier Trials, using their female progeny. Herds were first identified in the autumn containing at least 3% clinical cases of FE (actual range in the study, 3-22% clinical cases per herd). Blood samples for GGT analysis were then taken from all cows in each grazing mob in these herds, regardless of clinical FE status of individual cows. Pedigrees of cows were obtained from the herd-owners' records. The sampled cows generally had at least sire-pedigree known, so that the procedure led to the progeny testing of sires via daughters within and across herds. Information from the herd-owner was used in order to define each cow's grazing group over the last 3-4 months during unintentional exposure to FE-challenge.

The definition of a contemporary group is critical to the integrity of the FE analyses: if low-challenge mobs (or low-'reactor' mobs) are included in the data, there is a problem in ranking sires whose progeny would mainly score low on the GGT scale. Seventeen herds were sampled in Trial 5 in the autumns of 2004 and 2005. Using a qualifying criterion for a herd as having at least 30% animals with GGT activity levels above the 'reference range' (Morris *et al*., 1998), these herds were found to have 49 to 93% cows with elevated GGT levels. Samples were analysed in one of two commercial laboratories; the relevant GGT threshold (upper reference range) was 32 i.u./l at Gribbles Veterinary Pathology (Hamilton) where the analytical temperature for the assay was 30 ºC, and 36 i.u./l at NZ Veterinary Pathology Ltd (Hamilton) where the analytical temperature was 37 ºC. Four other herds, where preliminary monitoring of GGT was carried out, failed to reach

the 30% threshold, and these herds were not followed any further.

Data analyses

As much pedigree information as possible was obtained on all animals from Trials 1-5. Each animal's sire and paternal grandsire were known in all cases and, where possible, dam and maternal grandsire information were also included in the analysis. Initial analyses were carried out using the 'JMP' option in SAS (1995) to test for fixed effects, with GGT values (and, where available, GDH values) transformed to natural logarithms. Animal-model restricted maximum likelihood (REML) analyses were then used (Gilmour, 1997), to estimate heritabilities (log GGT, log GDH) and genetic correlations, with a repeated-record model. In Trials 2, 3 and 4, involving repeated records of GGT and/or GDH, all sampling days where at least 30% of animals had GGT values above the threshold (defined above) were included in the analysis.

The full statistical model included a contemporary group as follows: year, herd, grazing group within herd, rearer and dose rate within herd and sampling day x grazing group where relevant. Breeding values (BVs) were obtained from the solution files. In the case of cow data from Trial 5, the mix of breeds and breed crosses within herds provided the opportunity to compare breeds and also to run the analyses separately for each breed. An additional refinement of the data was to standardise every contemporary group before inclusion in the REML analyses. This was expected to reduce any effects of varying levels of FE challenge, and thus reduce some of the between-herd variation in GGT levels.

RESULTS

Heritabilities and breed effects

Table 2 shows the population parameters overall, and separately for Friesian-sired and Jersey-sired animals from affected contemporary groups. From the unstandardised data, there were significantly higher heritability estimates for $\log_e$GGT in Friesians than in Jerseys. From the standardised data, the corresponding heritability estimates for $\log_e$GGT were closer together and not significantly different by breed. Repeatabilities for log GGT were significantly different in Friesians compared with Jerseys, and estimates were slightly reduced by the standardisation process, but generally the repeatabilities were very high, indicating that repeated sampling of the same animals over time gave consistent GGT or GDH results. Overall, heritability estimates for the standardised traits were 0.40 ± 0.04 for $\log_e$GGT and 0.35 ± 0.06 for $\log_e$GDH, with a genetic correlation of 0.93 ± 0.03 between them. The repeatabilities overall were 0.86 ± 0.00 for standardised $\log_e$GGT and 0.71 ± 0.01 for standardised $\log_e$GDH. Sixty-eight sires had reliabilities >0.70 for BVs for standardised $\log_e$GGT, and 71 others had reliabilities between 0.60 and 0.70. Phenotypic standard deviations from the unstandardised data for $\log_e$GGT were 1.48 and 1.42 $\log_e$ i.u./l for Friesians and Jerseys, respectively, and for $\log_e$GDH they were 1.47 and 1.35 units, respectively.

TABLE 2: Parameter estimates (± standard errors) for the enzymes gamma-glutamyltransferase and glutamate dehydrogenase in blood ($\log_e$GGT and $\log_e$GDH), as indicators of resistance to facial eczema in Friesian-sired, Jersey-sired and pooled ('All') data, from animals in affected contemporary groups. The parameters were: heritability (h^2), genetic correlation (r_g), phenotypic correlation (r_p), and repeatability ('rep').

Parameter/Enzyme	All	Friesian	Jersey
Unstandardised			
h^2 $\log_e$GGT	0.41±0.04	0.52±0.06	0.34±0.06
h^2 $\log_e$GDH	0.36±0.05	0.34±0.07	0.43±0.09
r_g	0.95±0.02	0.96±0.02	0.97±0.03
r_p	0.88±0.00	0.88±0.01	0.88±0.01
rep $\log_e$GGT	0.90±0.00	0.92±0.00	0.88±0.01
rep $\log_e$GDH	0.78±0.01	0.78±0.01	0.77±0.01
Standardised			
h^2 $\log_e$GGT	0.40±0.04	0.47±0.07	0.37±0.06
h^2 $\log_e$GDH	0.35±0.06	0.32±0.08	0.39±0.09
r_g	0.93±0.03	0.93±0.03	0.95±0.03
r_p	0.84±0.01	0.85±0.01	0.82±0.01
rep $\log_e$GGT	0.86±0.00	0.89±0.01	0.84±0.01
rep $\log_e$GDH	0.71±0.01	0.71±0.01	0.70±0.02

DISCUSSION

Heritabilities and breed effects

Heritability estimates for the two breeds were medium-to-high and broadly similar to each other, and this applied to both enzyme measures. Most estimates were generally consistent with earlier reports (Morris *et al.*, 1998), the main exception being the heritability estimate for $\log_e$GGT in Jerseys, where the 1998 value was 0.77 ± 0.13, compared with half of that value now (0.37 ± 0.06) with a much larger Jersey data set now added and tighter qualifying criteria applied than in the 1998 analyses. The heritabilities were sufficiently high that genetic progress would be made if selection was applied to reduce FE susceptibility, as has also been reported in studies with experimental sheep (Morris *et al.*, 1995a). There were large differences between the BVs of some of the proven sires whose semen is, or has been, widely available. For example, the range of

BVs for standardised BV (log_eGGT) is illustrated by taking the means of the highest 10% and the lowest 10% of the Friesian sires with accurate proofs: their difference was 1.47 standard deviations. The corresponding value was greater for Jerseys with accurate proofs, at 1.90 standard deviations, mainly because there were four outlier resistant Jersey sires (two of these being sired by one imported bull). The overall ranges of standardised BV (log_eGGT) for Friesian and Jersey sires with accurate proofs were 1.94 and 2.71 standard deviations, respectively, again showing a breed difference accounted for by the same outlier Jersey sires.

Both enzyme activity levels were highly repeatable over time and, genetically, GGT and GDH were good indicators of each other, with a high genetic correlation estimate of 0.93 ± 0.03.

Thresholds

The choice of threshold for inclusion of data was tested by Morris *et al*. (1998), where it was concluded that "changing the threshold from 30% to 20 or 40% for defining qualifying groups made minimal difference to the heritability estimates for log GGT". However, the threshold was a potential problem in the 2002-2004 bull-calf data (Trial 4), where the %reactors averaged only 35% overall. In this case, including both log GGT and log GDH in the 2-trait REML assisted, because the peak for GDH was higher and earlier than the peak for GGT (Morris *et al.,* 1998), and the genetic correlation estimate between log GGT and log GDH was very high at 0.93 ± 0.03.

Herd incidence

In an earlier (1976) FE survey of dairy industry herds (Towers, 1978), four of ten selected herds had at least 5 clinically-affected cows (percentages affected being 5.1, 8.1, 33.1 and 36.3, per herd). Moving from the clinical to the subclinical cases, those four herds contained 34.3, 42.1, 96.5 and 97.6% animals with elevated GGT levels, respectively. The reported incidences of % clinical cases and elevated GGT in Towers' study had a similar range to the current study, where the range of clinical cases was 3-22% per herd, with 49 to 93% elevated GGT samples per herd.

Choice of testing procedures

In performance-testing bulls (Trials 2a, 2b) or progeny-testing them (Trials 1, 2c, 3-5), there appear to be practical constraints to testing, whichever protocol is chosen. There are difficulties in making use of the BV results for FE in practice, with sires already 'proven' for dairy traits (Trial 5), in that the industry sires are all old. For a progeny-testing approach with many young sires (Trials 1, 4), the numbers of progeny per sire are low, unless most of the Sire Proving Scheme herds can be included, and this would be an expensive target for FE-testing. The design in Trials 2a and 2b has limitations for future use because of concerns over the dosing of potentially valuable young dairy bulls, and Trials 2c and 3 are expensive because all the overheads of generating progeny specifically to progeny-test for FE susceptibility are allocated against FE alone. A total of 139 sires have been ranked so far. Two routes for future sire-proving for FE could be (i). to continue further phenotyping each year as in Trial 5 to rank new sires, and/or (ii). to identify DNA markers for FE resistance so that, thereafter, the costs of ranking further animals would be reduced, but the returns (to the whole dairy industry) could be much higher. Preparations are underway to search for DNA markers associated with the genes carried by outlier resistant and susceptible sires, as well as progeny-testing more sires using the Trial 5 protocol.

Conclusions

The present study has followed from earlier work on the genetics of resistance to FE in dairy cattle. The current heritability estimate overall (using standardised log_eGGT) is 0.40 ± 0.04, based on the most records yet collated and with the lowest standard error. Standardised log_eGDH was also heritable (0.35 ± 0.06), with a genetic correlation of 0.93 ± 0.03 between transformed measures of the two enzymes. There were no statistically significant breed differences in heritability for standardised enzyme activity levels. Large genetic differences between the BVs of some widely-used industry sires were identified although, with the techniques used so far, the sires were generally old by the time that accurate proofs for FE resistance became available. A DNA-marker approach to ranking sires for FE resistance may be able to circumvent this problem of timing.

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