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Selection for or against facial eczema susceptibility in sheep

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ABSTRACT

Facial eczema (FE) in sheep is associated with liver damage and considerable production losses. Romney flocks, resistant (R) and susceptible (S) to FE were established at Ruakura in 1975; a control (C) and a second resistant (R2) flock were added after 1981, the R and R2 flocks were merged in 1988, and three flocks (R, C and S) still exist. Progeny testing was carried out in early years for ram selection, and performance testing was used after 1981. The R flock was dosed with sporidesmin in most years since 1981 at 0.15 mg/kg live weight, with the dose rate for the S flock initially 0.066 mg/kg and now reduced to 0.04 mg/kg. The C flock was divided into two groups, with one group dosed at 0.15 and one at 0.04 mg/kg, to provide an indirect comparison of the R and S flocks. Blood samples were taken after dosing to monitor liver damage via plasma gamma-glutamyltransferase (GGT) concentration. Log_e GGT levels increase in proportion to increasing severity of liver damage. Thus, changes in mean log_e GGT value reflect changes in relative susceptibility or resistance, with negative changes indicating increasing resistance.

Two distinct peaks appear in the log_e GGT distribution, centred around values of about 40 and 500 international units (i.u./l) for both the R and S flocks. The selection flocks differ in the proportions of animals at each peak, suggesting that the R and S flocks may be indirectly compared via the C flock although different dose rates were used. Annual divergence in log_e GGT in the R flock relative to the C flock was -0.081±0.047 i.u./l, and the R-flock difference from control in the most recent years averaged -1.24±0.14 i.u./l. Divergence of the S flock from the C flock was more variable from year to year and the S-flock difference from control for the last 6 years averaged 0.48±0.11 i.u./l.

A total of 101 industry flocks have used the commercial FE testing service, Ramguard, with 9 of them testing for at least 10 years. A comparison with the Ruakura R-flock response was obtained from one such flock selecting for production traits *and* FE resistance since 1979. Their GGT trend was 34% of the R-flock trend, indicating that less but worthwhile genetic progress has been obtained from using a selection programme with a multiple objective.

Keywords: sheep; facial eczema; breeding; selection; resistance; susceptibility.

INTRODUCTION

Facial eczema (FE) is a costly animal health problem in the lower lying areas of the North Island. The disease is caused by sporidesmin, a toxin found in spores from the saprophytic fungus *Pithomyces chartarum*, growing on the dead litter at the base of most pastures. In the warm, humid conditions common in autumn, fungal growth and spore formation can be rapid, resulting in high numbers of toxic spores in the pasture. Sporidesmin is a potent toxin that injures many body tissues including the liver. It can cause extensive cell damage and cell death, particularly in the bile ducts which may become completely blocked in some animals. Damage to the bile ducts prevents excretion of waste products which then spill over into the bloodstream. In turn this causes the photosensitivity and tissue damage to the exposed areas of the face, udder etc that are responsible for the common name of the disease. However, in all except the most severe outbreaks, the majority of affected animals show no outward sign of the disease.

Post-mortem studies of liver damage have shown a wide range of susceptibilities from animal to animal (Campbell *et al.*, 1975), and these animal differences have subsequently been shown to be highly inherited, with a heritability of 0.42±0.09 (Campbell *et al.*, 1981).

The two objectives of this paper are to describe the selection responses achieved at Ruakura in breeding for changes in FE susceptibility, and to discuss experiences with the commercial service, Ramguard, which provides breeders with tests of rams for FE susceptibility.

MATERIALS AND METHODS

Selection flocks

Flock descriptions

A selection experiment was set up at Ruakura in 1975 to breed two Romney flocks, one for resistance (R) and one for susceptibility (S) to FE (Campbell *et al.*, 1975, 1981). In the years until 1981, the susceptibility of potential sires was assessed by progeny testing, followed by scoring liver damage in slaughtered progeny. The FE challenge was provided by dosing with sporidesmin. From 1981 onwards, a performance test has been used to select sires, monitoring elevated gamma-glutamyltransferase (GGT) concentrations in the blood of animals 3 weeks after dosing with sporidesmin (Towers and Stratton, 1978). The experiment was expanded after 1981 by establishing two further Romney flocks, both originating from a common group of Romneys drawn widely from industry sources, to form the control (C) and second resistant (R2) flocks at Ruakura. These last two flocks were used for demonstration purposes, to show the responses achievable from performance testing. By the 1988 matings, they had fulfilled this role, and the R and R2 flocks were subsequently merged, leaving three flocks (R, C and S) which still exist at Ruakura.

Ewe numbers mated annually in each flock have generally ranged from 100 to 150.

Dosing methods

Performance testing of lambs by the sporidesmin dosing procedure began with the lamb crop born in 1981 for the R2 flock, the 1982 crop for R and S, and the 1984 crop for C (with C rams being selected at random within sire group before this). Some aspects of the experimental protocol have changed with time. Animals were dosed with sporidesmin in summer as lambs in early years, but in April/May in later years. Generally only males were dosed, but females from some flocks were dosed in some years. Sporidesmin was administered orally, in standard quantity per unit fasted live weight. In earlier years the total dose was split over 2 or 3 days, but in later years it was administered as a single dose. It has not been possible to use the same dose rates for R and S flock animals, for both practical and welfare reasons. Most animals in the R flock would be unaffected by the low doses of toxin used in the S flock, and conversely most animals in the S flock would die following the higher doses of the R flock. This would compromise our objectives to minimise clinical FE and avoid production/reproduction losses in all flocks wherever possible (Morris *et al.*, 1991). The dose rates have varied across years, but the most common has been 0.15 mg/kg live weight in the R flock, whilst in the S flock the dose has been gradually reduced from 0.066 to 0.05 and then 0.04 mg/kg. To obtain direct flock comparisons, balanced samples of C flock males have been dosed at 0.15 mg/kg alongside R males, whilst other C flock males have been dosed at 0.04 mg/kg alongside S males. In some years, some C and R flock females were also dosed at 0.10 mg/kg.

For R flock males, a second higher dose was administered in some early years, to assist in ranking the most resistant R flock outliers. This has been discontinued, and round-2 dosing results are not shown here.

Line crosses

In 1990 and 1991, RxS and SxR crosses were generated in addition to the R and S pure line animals. FE resistance levels of the crosses as lambs are included in the present summary.

Data analyses

Because GGT values following sporidesmin dosing showed a skewed distribution, they were transformed to a $\log_e$ basis. This did not completely normalise the distribution. Genetic trends for $\log_e$ GGT were estimated by least squares analyses for the R (and R2) and C flocks, and then the S and C flocks, i.e. separately for animals receiving the high and low dose rates respectively. Distributions of $\log_e$ GGT phenotypic results, 21 days after dosing, were calculated for combinations of years, flocks and dosing rates.

Ramguard

Some progressive breeders initially established a performance testing procedure based on field testing with toxic pasture in the late 1970s and early 1980s. This was used successfully but suffered from the need to do daily spore counting to control toxin intakes, with no guarantee of developing toxic conditions to complete the test. A private commercial performance testing service based on sporidesmin dosing was established in 1984. Since 1987 this service has been run from Ruakura as Ramguard.

Ramguard produces and provides toxin, calculates the required doses, arranges for analysis of blood samples and ranks the rams for FE resistance. Dose rates are calculated for each breeder individually on the basis of past results for that flock. When a second round of dosing is required the results of the first sporidesmin challenge are taken into account in selecting the dose rate required. The field work for most performance tests is carried out by veterinary practitioners.

RESULTS AND DISCUSSION

Selection flocks

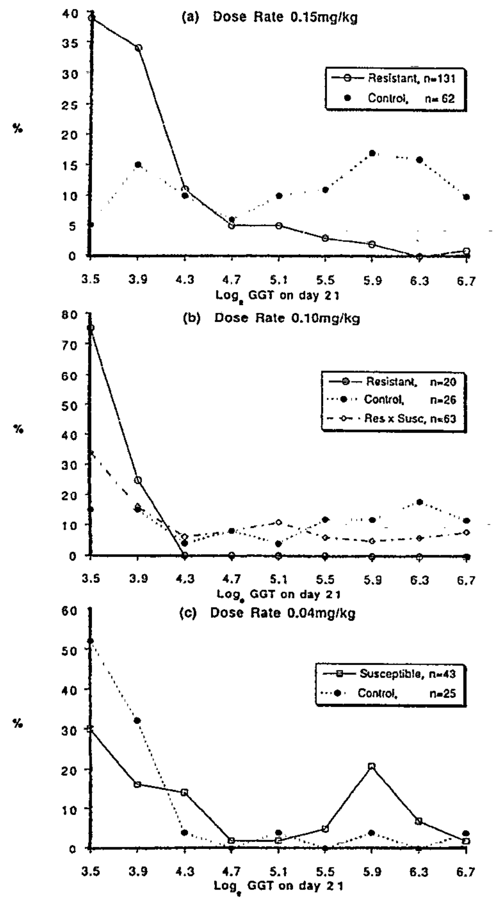
GGT distributions

Figure 1a shows the distribution of actual $\log_e$ GGT values for the 1990-92 lamb crops of the R and C flocks at the high dose rate. There were peaks at about 3.6 for the R flock and at 3.8 and 6.2 $\log_e$ units for the C flock (corresponding to actual GGT values of about 40 and 500 international units/l respectively). Figure 1b shows the same peaks for females in the R and C flocks dosed at a lower rate of 0.10 mg/kg. The RxS and SxR animals had slightly lower $\log_e$ GGTs than the C animals, indicating that they were slightly more resistant than C animals. Figure 1c compares the $\log_e$ GGT distributions for S and C animals dosed at the lowest rate. There was a marked bimodal distribution for S animals. The R flock animals dosed at this rate had 100% of animals resistant (i.e. all records fell into the lowest $\log_e$GGT class). These data suggest that flock effects reflect different percentages of animals in the two peaks.

Since variation in susceptibility to FE is a heritable trait (Campbell *et al.*, 1981; Morris *et al.*, 1989), gene effects must contribute to the two peaks described above. If a gene with large effect was segregating, this could also lead to a bimodal distribution of $\log_e$ GGT values. However, the rise in GGT concentrations with sporidesmin-induced liver damage is a secondary response to injury to the epithelial cells forming the biliary tree. It is not a direct reflection of the toxic action of sporidesmin, and is non-specific to FE. At present we have not resolved the mode of inheritance, as phenotypes or phenotypic boundaries distinguishing $\log_e$ GGT values of animals with possible different genotypes have not been determined.

The percentages of animals recorded as resistant (GGT <50 i.u./l and $\log_e$GGT <3.91) within dose rates led to flocks being ranked in order R, RxS/SxR and C (Figure 1b). In the absence of heterosis, the RxS/SxR flock mean is expected to be midway between the R and the S values. It is uncertain whether the R flock has advanced in one direction from the C flock further than the S flock has advanced in the other direction, because of the different dose rates applied. Further statistical analyses are presently being tested to answer this question, and to find a way of combining first and second rounds of dosing (where the second dose is restricted to a biased sample, i.e. the most resistant animals). However, our preliminary studies of this kind suggest that it should be possible to make direct flock comparisons of R and S flocks via the C flock using $\log_e$ GGT in a single combined analysis, in spite of the different dose rates (to be reported elsewhere).

FIGURE 1: Frequency distributions of $\log_e$ GGT for male and female lambs born in 1990-92, and dosed at (a) 0.15, (b) 0.10 or (c) 0.04 mg/kg.



Response to selection : $\log_e$ GGT

The rates of change in $\log_e$ GGT were similar in the R and R2 flocks from 1981 to 1987, and subsequent to merging the two flocks the rate of change has remained about the same. Overall, least squares analysis showed that the average annual rate of divergence in $\log_e$ GGT between the R (and R2) and C flocks from 1984-92 was -0.081±0.047 i.u./l (P<0.10), about two-thirds of the theoretical rate of response achievable. This may be the result of difficulty in discriminating amongst resistant rams which have not reacted to the sporidesmin challenge, and which have similar phenotypic GGTs. Nevertheless, the average difference between the R and C flocks (1990-92 birth years) was -1.24±0.14 i.u./l (P<0.001).

In the C flock, no sporidesmin testing was carried out before the 1984 lamb crop. As a result, although new rams were selected at random, there were no GGT records to assist in minimising any drift in flock mean GGT. In later years, the selection of C flock rams has been carried out with the restriction that their average breeding value (BV) is the same as that of all contemporaries. Applying best linear unbiased prediction (BLUP) techniques to the C flock showed that there was minimal drift in $\log_e$ GGT for the C flock each year after 1985 (overall, a value of 0.014±0.006 i.u./l).

In the S and C flock comparisons, numbers tested were smaller and there was not a significant selection response over time, using least squares analysis. A BLUP analysis, assuming a heritability of 0.42, did show a significant annual trend. However, the more important estimate was perhaps the flock difference by least squares (0.48±0.11 i.u./l, P<0.001); this estimate was obtained from the 1987-92 years of birth (more years than in the comparison of R and C flocks, because there were fewer S and C animals tested per year).

Having found the bimodal distributions in Figures 1a-1c, showing peaks at similar positions for each flock, it has already been noted that the R and S flocks may be directly comparable via the C flock. Therefore, assuming that the recent responses of the R and S flocks may be compared with the controls on the same scale, change in the S flock (0.48 i.u./l) thus appeared to be less than half of that in the R flock (-1.24 i.u./l). This finding was consistent with the expectation that S flock responses would be less than in the R flock because of major difficulties experienced with reproductive rate in both sexes of the S flock (Morris *et al.*, 1991).

Response to selection : percent resistant

By 1992, the difference in $\log_e$ GGT between the R and C flocks was 1.34 i.u./l. From the relationship obtained between $\log_e$ GGT and the percent of animals resistant (Morris *et al.*, 1989), i.e. 38% more animals resistant per unit change in $\log_e$ GGT, there is expected to be a 51% difference in the percentage of R and C flock animals resistant to the high sporidesmin dose. The actual differences in per cent resistant between males in the two flocks were 41% and 67% for the 1991 and 1992 lamb crops respectively, averaging 54%. Similarly, the 1992 data for the S and C flocks were equivalent to an expected 26% difference in susceptible numbers per flock, although the actual value was much higher at 67% (based on 9/9 C flock animals resistant and 11/33 S flock animals resistant).

In a recent year where a field challenge was applied (involving a maximum Brook spore trap count of >2.88 x 10^6 spores m^{-3} of air, i.e. 81 600 spores per cubic foot), 53% of Controls were susceptible, compared with only 7% of R flock animals grazed together with them. This indicates a large flock difference in susceptibility to a natural challenge, under circumstances likely to occur under commercial conditions in the field.

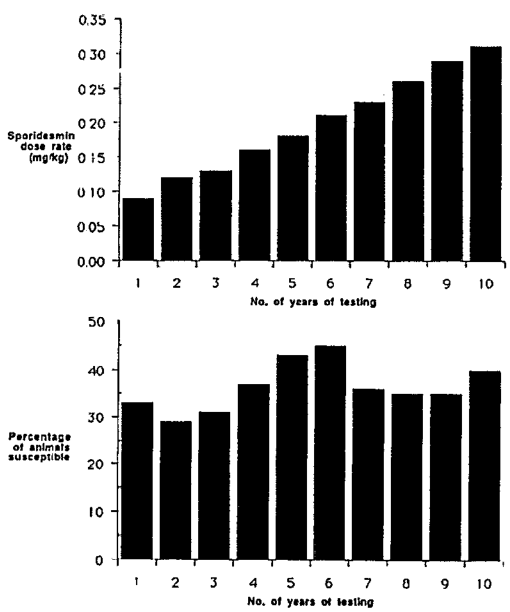
Ramguard

Over the period 1984-93, the Ramguard service has been used by 101 breeders, each testing at least one crop of rams. While some have withdrawn from the scheme, over 70 breeders now use GGT levels as one of their multiple selection criteria along with production traits, and 9 breeders have tested for at least 10 years. Initially ram lambs were tested, to take advantage of their lower live weights and thus lower testing costs, but over the last five years there has been a trend to delay testing until the animals are two-tooths and BVs for wool production are available to aid selection of rams for testing. Most breeders test from 10 to 30 rams (mean = 18), although the number tested has ranged from 3 to 99. Those testing for at least 8 years have averaged 23 rams per year.

As might be expected, most breeders selecting for resistance are located in those areas most prone to FE, such as Northland, South Auckland, Waikato and the East Coast of the North Island. However, there are increasing numbers of breeders from other areas now selecting for FE resistance, probably as a result of increasing demand from clients. All major breeds in New Zealand are represented amongst Ramguard clients.

An assessment of the progress achieved by ram breeders overall can be made by examining changes in the relationship between the amount of toxin administered and the proportion of susceptible animals, i.e. animals with elevated plasma GGT concentrations. If genetic progress is being made, one would expect that the dose response curve should shift so that more toxin was needed to maintain the same proportion of affected animals with each succeeding crop of rams tested. Figure 2 shows plots of the percent of animals affected and the mean rate applied at the first dose, for those Ramguard clients who have tested for one to 10 years.

FIGURE 2: Relationships between a farmer's number of years of testing and sporidesmin dose rate or percentage of susceptible animals, in the facial eczema testing scheme Ramguard.



The trend for increasing dose rates is indicative of genetic progress for FE resistance, although the absence of adequate genetic links across years (i.e. very small numbers tested per flock per year) means that the rate of progress is uncertain. However, a 3-fold increase in dose rate in the flocks testing for longest now produces similar percentages of affected animals to the original low dose rates in first-year tests. Similar but slightly smaller changes have occurred for the second-round dose rates, but the interpretation of these data are complicated, as the numbers of flocks using second doses has fallen from about 65 to 35 percent of all testing flocks over the last 6 years.

One flock testing for FE resistance using GGT since the 1979 lamb crop, first by field testing on toxic pasture and in later years by sporidesmin dosing, has subjected data to a BLUP analysis. This has shown a significant trend of increasing resistance. Relative to the single-trait objective for FE resistance at Ruakura, their rate of genetic progress is 34% of the Ruakura R flock trend. This indicates lower but still useful genetic progress, where FE resistance is part of a multiple objective.

CONCLUSIONS

The Ruakura experiment is providing a pool of genetically resistant and susceptible animals for use in biochemical and genetic studies on the control of FE resistance. A liver protein difference has been identified between R and S flocks (Lu *et al.*, 1994), and at least one genetic marker for FE has been found with a log odds ratio of >3 (i.e. probability of better than 1:1000), using DNA from the progeny of an SxR ram (Hermans, I.F., Jordan, T.W., Morris, C.A. and Towers, N.R., unpublished data). Sporidesmin testing is being used commercially in ram breeding flocks, and evidence of genetic progress in FE resistance has been obtained.

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