

## SHEEP SELECTED FOR RESISTANCE TO FACIAL ECZEMA DISEASE ALSO SHOW HIGHER TOLERANCE TO ACETAMINOPHEN CHALLENGE

S. H. Phua<sup>1</sup>, P. Johnstone<sup>1</sup>, H. Henry<sup>1</sup>, A. Findlay<sup>1</sup> and C. A. Morris<sup>2</sup>

<sup>1</sup>AgResearch Invermay, Private Bag 50034, Mosgiel 9053, New Zealand

<sup>2</sup>AgResearch Ruakura, Private Bag 3123, Hamilton 3240, New Zealand

### SUMMARY

Facial eczema disease (FE) is a hepato-mycotoxicosis caused by sporidesmin, leading to secondary photosensitisation in severely affected sheep and cattle. Two genetic lines of Romney are maintained by selection for resistance and susceptibility to FE. Five groups of 8 animals, each group composed of 4 animals from each line, were artificially challenged with different dose rates (25, 50, 75, 150 & 250 mg/kg liveweight) of acetaminophen (or paracetamol). Weekly blood samples were collected from each animal for 5 weeks after dosing and were used for liver function tests (glutamate dehydrogenase (GDH), gamma-glutamyl transferase (GGT), bilirubin and albumin/globulin ratio) to measure their liver injury. The results showed that FE resistant-line animals were more tolerant of acetaminophen than the susceptible-line animals, and that they also recovered faster after the drug challenge.

### INTRODUCTION

There are many genes contributing to FE resistance in sheep (Phua *et al.* 2009). An obvious group of candidate genes would be those encoding the enzymes involved in the liver detoxification pathways. It was therefore not surprising to find that FE-resistant sheep also tended to be resistant to ryegrass staggers disease (Morris *et al.* 1995a); the latter is a neuromuscular in-coordination caused by the neurotoxin lolitrem B which is produced by a ryegrass endophyte. Similar observations were also made in rodents, where genetic lines of mice selected for resistance and susceptibility to tall fescue toxicosis were also found to be associated with greater and lesser resistance to sporidesmin (Hohenboken *et al.* 2000). We extended similar study here to include a potential hepato-toxin in human. Acetaminophen, also commonly known as paracetamol, is a non-prescriptive drug widely used in humans for the relief of fever and pain. Overdose of this drug causes acute liver failure. But the modes of action and target tissues within liver are different between acetaminophen and sporidesmin. In this experiment, we wanted to assess the acetaminophen-induced liver responses of sheep selected for resistance and susceptibility to sporidesmin.

### MATERIALS AND METHODS

**Animals.** The Romney FE resistant (R) and susceptible (S) selection lines were established in 1975. In the early years, selection was made based on liver injury scores, later changed to blood GGT levels and finally to the present log(GGT) breeding values. In general, about 6 rams and 120 breeding ewes are maintained in each line (Morris *et al.* 1995b). The experimental animals used in this study were 2001-born progeny.

**Acetaminophen trial.** This trial received an ethical approval from the AgResearch-Invermay Animal Ethics Committee. Twenty R and 20 S 14-month ewes were obtained from the FE selection lines; their respective weight ranges were 46-58kg (average 51kg) and 43-59kg (average 50kg). The animals were randomly distributed into 5 groups of 8, having 4 R and 4 S animals per group. Each group was dosed with a different rate of acetaminophen purchased from a hospital

pharmacy: the dose rates were 25, 50, 75, 150 and 250 mg/kg liveweight (LWT). Before dosing (i.e. Week-0), blood samples were collected from the animals to determine their background levels of serum glutamate dehydrogenase (GDH, i.u./l), gamma-glutamyl transferase (GGT, i.u./l), bilirubin ( $\mu\text{mol/l}$ ), albumin (g/l) and globulin (g/l). After dosing, blood samples were collected weekly for 5 weeks (i.e. Weeks 1-5) for the same assays above. Two animals in the highest dose-rate group died from unknown causes: 1 R animal died 1 week after dosing and 1 S animal died 3 weeks post-dosing.

**Statistical analyses.** A mixed model was fitted to the natural logarithm of the GGT and GDH data. The fixed effects were the selection lines, dose rates of acetaminophen and the time (i.e. weeks) of observation. The random model included the experimental animals' sires. The errors were assumed to be autoregressive order 1 (AR1). The model was fitted using the REML directive in GenStat 10<sup>th</sup> Edition (Payne *et al.* 2007).

**RESULTS AND DISCUSSION**

Overall the dose rates of acetaminophen used in this study did not cause permanent liver damage in sheep. This was shown by the calculated albumin/globulin ratios which were within normal range for all animals during the 5-week trial period. Corollary was that there was no elevation of bilirubin in the blood throughout the trial. However, the dose rates were sufficient to elicit some definitive responses in terms of GGT and GDH data.

A mechanism of sporidesmin toxicity is believed to be through the production of reactive oxygen species, and the target tissue is the biliary tracts. In histological sections of FE-affected livers, the foci of pathological lesions are in the portal triad regions (Ozmen *et al.* 2008). The levels of serum GGT are used to measure the degrees of biliary tract damage (Towers & Stratton 1978). When the selection-line sheep were challenged with acetaminophen, the S animals showed significantly higher GGT levels than the R animals ( $P < 0.001$ ), indicating that they were more susceptible to the drug (Figure 1).

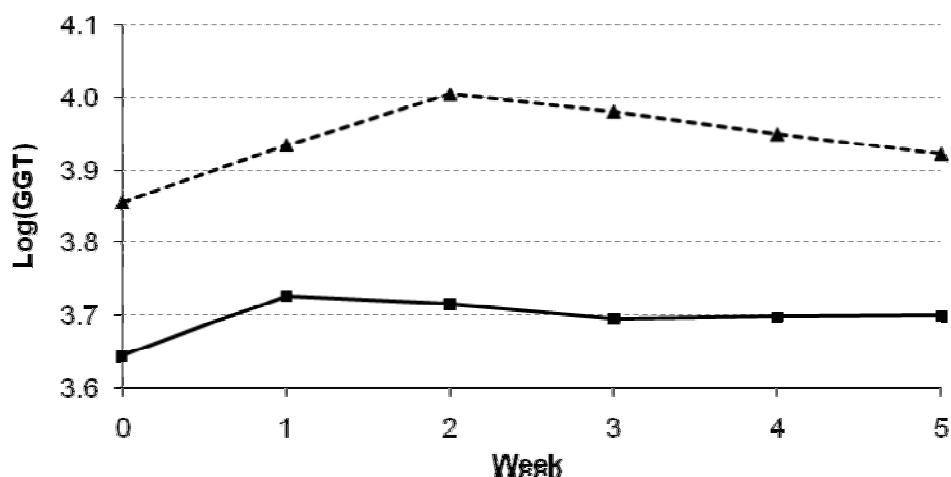
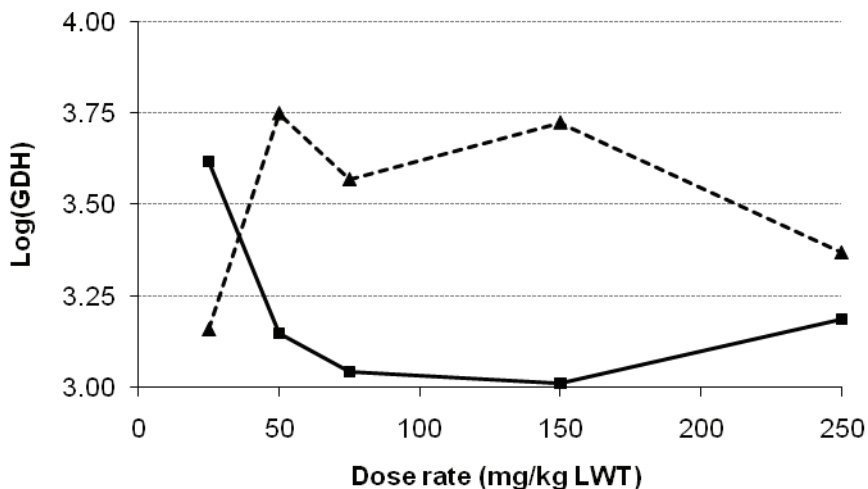
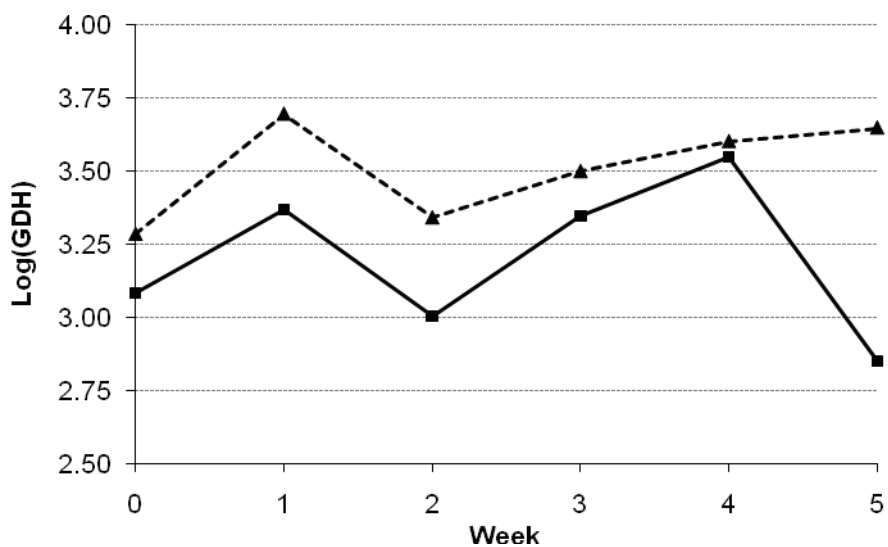


Figure 1. GGT responses of R (squares) and S (triangles) animals over time. Week-0 is the week before acetaminophen dosing, and Weeks 1-5 are 1-5 weeks after dosing. GGT values are averages of all R and S animals from all dose rates. The average standard error of difference (SED) is 0.06.



**Figure 2.** GDH responses of R (squares) and S (triangles) animals under different acetaminophen dose rates. GDH values are averages of R and S animals from all weeks. The average SED is 0.38.



**Figure 3.** GDH responses of R (squares) and S (triangles) animals over time. Week-0 is the week before acetaminophen dosing, and Weeks 1-5 are 1-5 weeks after dosing. GDH values are averages of all R and S animals from all dose rates. The average SED is 0.20.

Acetaminophen is used in humans as an analgesic and antipyretic drug. At therapeutic dose rates, the compound is glucuronidated and sulphated in the liver for elimination from the body. Its toxicity arises when an overdose of the compound is given: under such conditions, the glucuronidation and sulphation pathways are saturated and the compound is diverted into the

glutathione-conjugation pathway for elimination. In the latter pathway an intermediate metabolite is produced which is toxic to hepatocytes and causes lesions in the centrilobular regions of the liver (Prescott 1996). GDH levels in the blood are used to measure such liver damage. It is the GDH data which showed the most interesting differences between the R and S sheep.

Forty percent (8 out of 20) of S animals, compared with 10.5% (2 out of 19) of R, showed at least 3 weeks of elevated serum GDH levels. A chi-square test of this comparison showed that the susceptible selection line had a significantly higher proportion of animals with elevated GDH levels than the resistant line ( $P < 0.05$ ). There was a pronounced difference in GDH profiles between the genetic lines with increasing dose rates of acetaminophen challenge (Figure 2): the S animals had lower GDH than the R at 25 mg/kg, and this was reversed at higher dose rates. As depicted in Figure 3, S animals showed significantly higher GDH than the R animals ( $P < 0.05$ ), indicating that they are more susceptible to acetaminophen. Most interestingly, the graph also showed that the R animals recovered faster than the S after the drug challenge.

### CONCLUSIONS

The acetaminophen dose rates used in this trial were low enough to not cause any permanent liver damage in sheep, but they were high enough to elicit responses which reflected the genetic make-up of the animals. The FE resistant-line sheep consistently showed significantly lower blood GGT and GDH than the susceptible-line animals under acetaminophen challenge. An inference is that sheep selected for resistance to sporidesmin were also resistant to acetaminophen. An extrapolation from this finding is that FE resistant animals could well show tolerance to many other pasture toxins and drugs. Further, it appeared that in the selection for resistance to FE, we also selected resilient animals which could recover quickly after a xenobiotic challenge.

### ACKNOWLEDGMENTS

Funding for this work was provided by the New Zealand Foundation for Research, Science and Technology.

### REFERENCES

- Hohenboken, W.D., Robertson, J.L., Blodgett, D.J., Morris, C.A. and Towers, N.R. (2000) *Journal of Animal Science* **78**:2157.
- Morris, C.A., Towers, N.R., Wheeler, M. and Amyes, N.C. (1995a) *N. Z. Journal of Agricultural Research* **38**:367.
- Morris, C.A., Towers, N.R., Wheeler, M. and Wesselink, C. (1995b) *N. Z. Journal of Agricultural Research* **38**:211.
- Ozmen, O., Sahinduran, S., Haligur, M. and Albay, M.K. (2008) *Trop. Anim. Health Prod.* **40**:545.
- Payne, R.W., Murray, D.A., Harding, S.A., Baird, D.B. and Soutar, D.M. (2007). "GenStat 10<sup>th</sup> Edition for Windows" VSN International, Hemel Hempstead.
- Phua, S.H., Dodds, K.G., Morris, C.A., Henry, H.M., Beattie, A.E., Garmonsway, H.G., Towers, N.R. and Crawford, A.M. (2009) *Animal Genetics* **40**:73.
- Prescott, L.F. (1996) In "Paracetamol (Acetaminophen): A critical bibliographic review", p. 285, Taylor & Francis, Florence, Kentucky, U.S.A.
- Towers, N.R. and Stratton, G.C. (1978) *N. Z. Veterinary Journal* **26**:109.