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## Zearalenone challenge in sheep: variation in ovulation rate

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### ABSTRACT

Zearalenone is a fungal toxin produced by *Fusarium* species commonly found on autumn pastures in New Zealand, and it may interfere with reproductive functions in sheep. We have measured both ovulation rate after challenge with zearalenone, and also a metabolic indicator, Zen/Cr (the concentration of zearalenone and its breakdown products (Zen) in urine, after adjustment for urinary volume using creatinine concentration (Cr)). A small self-replacing flock of Coopworth ewes was used for the study. This paper reports a preliminary assessment of whether Zen/Cr might be an equivalent but cheaper method than laparoscopic ovulation rate for measuring an animal's resistance to zearalenone. Ovulation rates were determined in ewes in autumn before dosing (Lap1; Years 1 and 2), and after dosing (Lap2; Years 1 to 4) with 10 mg zearalenone per animal per day for 6 days. The average reduction in ovulation rate following dosing (Years 1 and 2: Lap1 minus Lap2) was 0.60 ovulations or 31% of the mean. Lap2 was used for the present correlation analyses. The heritability of Lap2 was estimated ( $0.11 \pm 0.09$ ), and breeding values (BVs) for Lap2 were determined. Subsequent to the laparoscopy, the ewes were mated at pasture, and the resulting natural litter sizes were monitored in relation to BVs for ovulation rate after challenge. Urine samples were taken during and after dosing in Years 3 and 4, to measure Zen/Cr, and a heritability for Zen/Cr ( $0.32 \pm 0.10$ ) was estimated from these and other Zen/Cr data. Preliminary analyses of BVs for Lap2 and Zen/Cr revealed a negative correlation between them ( $r = -0.55$ ). Ewes with higher BVs for ovulation rate also had higher mean litter sizes following natural ovulation. In conclusion, zearalenone affects ovulation rate at moderate challenge levels, and this ovulation rate may be negatively related to the concentration of measurable urinary zearalenone metabolites.

**Keywords:** sheep; zearalenone; dose rate; ovulation; heritability.

### INTRODUCTION

Zearalenone is a fungal toxin produced by *Fusarium* species commonly found on autumn pastures in New Zealand. The toxin may interfere with reproductive functions in sheep because its chemical structure is similar to that of an oestrogen. Examples of the potential reproductive cost of zearalenone in the diet have been given for sheep by Jagusch *et al.* (1986), Smith *et al.* (1988, 1990) and Towers & Sprosen (1993). We have recorded ovulation rates following zearalenone dosing in breeding ewes, in a series of studies over four years at Ruakura. The animals came from a self-replacing flock with recorded pedigree. The purpose of the present study in sheep was to determine animal variation in ovulation rates and in zearalenone breakdown products in urine, following zearalenone challenge, along with preliminary genetic parameters for these traits.

### MATERIALS AND METHODS

#### Animals

Animals in this study consisted of Coopworth ewes and lambs from a self-replacing pedigree-recorded 'Zearalenone' flock at Ruakura. The flock was established in the autumn of 2000, and was derived from animals used in studies at Ruakura by J. F. Smith in 1997 into the effects of zearalenone and Androvax™ on reproductive function. All the ewes were derived from the 'Zearalenone' treatment of that study, and they

included high responders for ovulation rate and/or lambing rate (3 eggs and/or 3 lambs), and low (single) responders for ovulation rate. Breeding ewe numbers ranged from 31 in 2000 to 56 in 2003 (Years 1 to 4), and replacement females and some service sires were also bred within this flock. Following oral administration of zearalenone, ovulation rates of ewes were recorded (primary objective, Years 1 to 4), and urinary responses of lambs or ewes to zearalenone were measured (secondary objective, Years 3 to 4). Details are described below.

#### Dosing

The zearalenone dosing procedure for ewes, applied in Years 1 to 4, was similar to that given by Smith *et al.* (1998). Briefly, ewes were synchronised each autumn for 12 days using CIDR-G devices (InterAg NZ Ltd, Hamilton, New Zealand), beginning at Day -8 relative to the first day of dosing (Day 0). CIDRs were withdrawn on Day 3, and oestrous dates were recorded after introducing vasectomised/harnessed rams (Days 3 to 9). The ewes (average liveweight 58 kg) were dosed for 6 days with zearalenone as a fine suspension in aqueous ethanol (10 mg zearalenone per animal per day), using a drench gun. Ewes were laparoscoped on Day 12 (i.e. centred on about 6 days after oestrus) in order to record ovulation data. These ovulation data are referred to as 'Lap2'. In Years 1 and 2, the synchronisation procedure began earlier in the autumn, in order that 'Lap1' data could be recorded from ewes before dosing. Ewes were then re-

synchronised, and dosing began (Day 0) as described above. The recording of 'Lap1' was abandoned after the first two years because it appeared better to use Lap2 as an indicator of ovulation rate after zearalenone dosing, than to use ovulation rate depression (Lap1 minus Lap2; details are given later). Lap2 was used for all estimates of breeding values (BVs) for ovulation rate.

### Urine samples

In Years 3 and 4, urine samples from individual ewes were obtained after the third dosing (Day 2), and again two days after dosing had terminated (Day 7). Urine sample collection was by the respiratory occlusion method (Divers, 1992). The concentration of zearalenone and its breakdown products (Zen) in urine was determined at Ruakura by ELISA (Garthwaite *et al.*, 1994). Urine samples were also analysed in a commercial laboratory to determine the concentration of creatinine (Cr), so that an approximate adjustment for urinary volume could be made by using the ratio, Zen/Cr. These urine-sample data were merged with the corresponding Zen/Cr data collected after dosing lambs in 2004 (Morris *et al.*, 2005).

### Other management and recording

No genetic selection was carried out during the 4-year period; records were collected so that future selection might be possible, after genetic parameters had been determined. Ewes were mated in individual ram-mating groups for two cycles (approx. 5 weeks), directly after the dosing/ovulation studies described above. Individual litter size data were recorded in all years, and litter size records were also available on the same foundation ewes for the previous two years (1998 and 1999).

Zearalenone challenge on pasture at Ruakura was encountered during the natural mating periods in Years 3 and 4 (high zearalenone levels on pasture especially in Year 3, with less opportunity among ewes for diet selection than in Year 4). The consequences of this challenge were monitored, both in terms of further urine collections for Zen/Cr during the mating period, and also in terms of subsequent litter size data.

### Data analyses

Analyses of variance models were tested with SAS (1995). Fixed effects for Lap2 were year of record, and age of ewe. The heritability of Lap2 was estimated using restricted maximum likelihood (REML) procedures (Gilmour, 1997), with an animal model and a relationship matrix. The Zen/Cr data from ewes, combined with the corresponding data from lambs (Morris *et al.*, 2005), were processed by REML after standardisation for all combinations of dose rate by collection time (and, for lambs, adjustment for genetic flock). A 2-trait animal REML model was applied to Lap2 and Zen/Cr (standardised for each group sampled), to determine the genetic correlation between the two traits.

## RESULTS

### Initial laparoscopy results

In Years 1 and 2, the overall means of Lap1 and Lap2 were 1.94 and 1.34 ovulations/ewe, respectively, giving a zearalenone effect of 0.60 ovulations, a 31% reduction in ovulation rate.

### Theoretical selection criteria: Lap2 versus (Lap1 – Lap2)

Hypothetical selection of ewes for a breeding programme to minimise (Lap1 – Lap2) would lead to animals with either (a) high Lap2, or (b) low Lap1, or both. The residual correlation between (Lap1 – Lap2) data recorded in 2000 and 2001 and natural litter size (lambings in 1998-2001) was negative (-0.06) and not significant, but we had a concern over the longer term that including Lap1 as part of a selection criterion might be undesirable. The correlation between Lap1 and Lap2 was of moderate size ( $0.36 \pm 0.11$ ). Although not conclusive at this stage, it was decided to abandon recording Lap1 and to use Lap2 alone (ovulation rate under zearalenone challenge), instead of ovulation rate depression (Lap1 – Lap2).

### Heritability estimates

The heritability estimate for Lap2 (based on 75 ewes and 167 records) was  $0.11 \pm 0.09$ , with a repeatability of  $0.19 \pm 0.08$  and a phenotypic standard deviation of 0.65. Using these parameters, BVs for Lap2 were calculated for all ewes with ovulation records (up to 4 years of data), and the ewes were then divided into two similar-sized groups on BV (Lap2): positives or negatives. A heritability for Lap1 was not estimated from the limited data available, but it was noted that the standard deviation for Lap1 was lower (as a % of the mean) at 37% than for Lap2 at 52%. The heritability for Zen/Cr was  $0.32 \pm 0.10$ , using data combined from 243 lambs and ewes (Morris *et al.*, 2005).

### Effect on natural litter size

The natural litter sizes over six years (1998-2004, but excluding 2002 when there was natural zearalenone challenge at mating) for those with positive vs negative BVs for Lap2 averaged 1.68 and 1.60 lambs born, respectively, a difference of  $0.08 \pm 0.09$ . This was not significant, but consistent with a trend for ewes that expressed higher ovulation rates under zearalenone challenge to produce higher natural litter sizes. For Year 3 (2002) alone, the difference in lamb numbers born between ewes with positive and negative BVs for Lap2 was larger, but also with larger standard error ( $0.21 \pm 0.21$  lambs born).

### Correlations between Lap2 and Zen/Cr

The phenotypic and genetic correlations between ovulation rate under challenge (Lap2) and Zen/Cr were  $-0.10 \pm 0.08$  and  $-0.55 \pm 0.47$ , respectively. The Zen/Cr data above included all urine sample data after artificial (dosing) challenges. Another opportunity to

test the relationship of reproduction with Zen/Cr came from the natural zearalenone challenge of ewes during mating in Year 3. From Zen/Cr samples collected during mating under natural challenge, the subsequent litter sizes of animals with a high versus low phenotype for Zen/Cr were 1.57 for the 50% of animals below average for Zen/Cr and 1.39 for the animals above average for Zen/Cr, a difference of  $0.18 \pm 0.18$  lambs, which again was not significant but in the expected direction. The genetic correlation between Zen/Cr measures taken after natural versus artificial challenge conditions was positive, and not significantly different from 1.0; the corresponding phenotypic correlation was also positive ( $0.23 \pm 0.06$ ;  $P < 0.01$ ).

## DISCUSSION

### Initial laparoscopy results

The dose rate of 10 mg per ewe per day which was used in this trial was intermediate within the rates tested by Smith *et al.* (1990), and it generated about the same depression in ovulation rate (31%) as their 6 mg treatment, administered daily over a longer period (10 days, compared with our 6 days).

We did not have a control, namely an undosed group recorded by laparoscopy at the same time as the dosed group. Therefore (Lap1 – Lap2) was originally taken as the dosing effect, although this may have been unreliable because there is the possibility of both time or season effects on ovulation rate through autumn (Smith *et al.*, 1987) and an effect due to changes in nutritive value of the pasture grazed over time (Rattray *et al.*, 1980).

### Heritability of Lap2

The heritability estimate for Lap2 under zearalenone challenge ( $0.11 \pm 0.09$ ) and the repeatability estimate ( $0.19 \pm 0.08$ ) were both slightly lower than those reported by Davis *et al.* (1998) for three breeds under natural grazing conditions in New Zealand (pooled within-breed estimates being  $0.14 \pm 0.03$  and  $0.26 \pm 0.02$ , respectively). The difference could be associated with (a) the sample of animals involved, (b) greater variability of ovulation rate when animals were under zearalenone challenge, giving phenotypic standard deviations of 0.65 ovulations for this trial, and 0.57 in the Davis *et al.* (1998) study where there was no challenge, (c) lower heritabilities when the mean ovulation rate is lower (1.34 ovulations for Lap2) compared with 2.32 in the Davis *et al.* (1998) study. Nevertheless, the heritability under the present challenge conditions was high enough for a Lap2 selection response to be achievable, if selection was applied, probably using BV estimates and half-sib selection of young males.

### Correlations between Lap2 and Zen/Cr

This was the primary objective of the present study. Given the small animal resource available, the genetic correlation between Lap2 under challenge and standardised Zen/Cr was negative ( $-0.55 \pm 0.47$ ), but not

statistically significant. However, the relationship between Lap2 and Zen/Cr was found to be negative both for artificial challenge conditions and also when the ewe flock experienced natural zearalenone challenge at mating time in Year 3 (2002, the most serious natural-challenge year encountered). As might be expected, the genetic correlation between Zen/Cr measures taken after natural versus artificial challenge conditions was positive, and not significantly different from 1.0, but the phenotypic correlation in this case ( $0.23 \pm 0.06$ ) was not close to 1.0, probably indicating the variability introduced under grazing conditions by diet selection when zearalenone is present in the pasture (Cosgrove *et al.*, 2002), and also by variable food intake.

### Implications for genetic selection

Since Zen/Cr is heritable and appears to be genetically correlated negatively with ovulation rate ( $-0.55$ ), an implication is that, where zearalenone is commonly found on autumn pastures and cannot be avoided, breeders may wish to select for resistance (ovulation rate after zearalenone challenge). Recording Zen/Cr from urine could be a more convenient and cheaper method for ranking and selecting animals for higher reproduction after zearalenone challenge, than using laparoscopy. The advantages are that (a) it can be carried out in lambs (i.e., generation intervals can be reduced), (b) it is not sex-limited, unlike an ovulation record, so that ram lambs can be phenotyped, (c) it is considerably less labour-intensive than laparoscopy, and (d) less specialised staff can be utilised to dose orally and to urine sample than to perform laparoscopic examinations. The relative genetic progress (per unit selection differential and per generation) from using indirect selection would probably be similar to the direct progress for ovulation rate, because the product of the genetic correlation and the square root of the heritability ratios is close to unity (i.e.,  $0.55 \times (0.32/0.11)^{1/2}$ , or 0.94). However, with the addition of selection among males, with likely higher selection differentials, and shorter generation intervals, the balance would favour indirect selection, perhaps by a factor of up to two.

Zearalenone is much more widespread across New Zealand (Towers & Sprosen, 1993) than sporidesmin (facial eczema), and it is of interest that ewes from a Ruakura line selected for facial eczema resistance were more susceptible to the effects of zearalenone (lower ovulation rates under zearalenone challenge) than those from a line selected for facial eczema susceptibility (Smith *et al.*, 1988). A possible reason is that a major break-down product of zearalenone, alpha-zearalenol, is more oestrogenic than zearalenone itself (Hagler *et al.*, 1979), so that animals which can break down zearalenone more quickly may be more susceptible to its effects.

## CONCLUSIONS

It is concluded that zearalenone affects ovulation rate at moderate challenge levels, and that the ovulation rate

after zearalenone dosing may be negatively related to the concentration of measurable urinary zearalenone metabolites (Zen/Cr).

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#### REFERENCES

- Cosgrove, G.P.; Anderson, C.B.; Phillot, M.; Nyfeler, D.; Hume, D.E.; Parsons, A.J.; Lane, G.A. 2002: The effect of endophyte alkaloids on diet selection by sheep. *Proceedings of the New Zealand Society of Animal Production* 62: 167-170
- Davis, G.H.; Morris, C.A.; Dodds, K.G. 1998: Genetic studies of prolificacy in New Zealand sheep. *Animal science* 67: 289-297
- Divers, T.J. 1992: Assessment of the urinary system. In: Wilson, J.H. ed. *The Veterinary Clinics of North America; food animal practice, physical examination vol. 8*. pp 372-382
- Garthwaite, I.; Sprosen, J.M.; Briggs, L.; Collin, R.; Towers, N.R. 1994: Food quality on the farm: immunological detection of mycotoxins in New Zealand pastoral agriculture. *Food and agricultural immunology* 6: 123-129
- Gilmour, A.R. 1997: ASREML for testing fixed effects and estimating multiple trait variance components. *Proceedings of the Association for the Advancement of Animal Breeding and Genetics* 12: 386-390
- Hagler, W.M.; Mirocha, C.J.; Pathre, S.V.; Behrens, J.C. 1979: Identification of the naturally occurring isomer of zearalenol produced by *Fusarium roseum* 'Gibbosum' in rice culture. *Applied and environmental microbiology* 37: 849-853
- Jagus, K.T.; Gray, M.H.; Maclean, K.S.; Towers, N.R.; di Menna, M.E.; McMillan, W.H. 1986: The cause of reproductive loss in Gisborne-East Coast ewe flocks. *Proceedings of the New Zealand Society of Animal Production* 46: 251-253
- Morris, C.A.; Amyes, N.C.; Sprosen, J.M.; Hawkes, A.D.; Finch, S.M.; Miles, C.O. 2005: Zearalenone challenge in sheep: urine sampling to measure response. *Proceedings of the New Zealand Society of Animal Production* 65: 316-319
- Rattray, P.V.; Jagusch, K.T.; Smith, J.F.; Winn, G.W.; MacLean, K.S. 1980: Flushing responses from heavy and light ewes. *Proceedings of the New Zealand Society of Animal Production* 40: 34-37
- SAS. 1995: JMP Version 3. SAS Institute, Cary, NC, USA.
- Smith, J.F.; McGowan, L.T.; MacLean, K.S.; Farquhar, P.A. 1987: The effect of steroid immunisation on the seasonal pattern of oestrus, ovulation and ovulation rate in Coopworth ewes. *Proceedings of the New Zealand Society of Animal Production* 47: 143-146
- Smith, J.F.; Campbell, A.G.; Towers, N.R.; Cox, N.R.; McGowan, L.T.; Wesselink, C. 1988: Ovulation rates in ewes selected for resistance to facial eczema and the effect of exposure to zearalenone. *Proceedings of the New Zealand Society of Animal Production* 48: 135-138
- Smith, J.F.; di Menna, M.E.; McGowan, L.T. 1990: Reproductive performance of Coopworth ewes following the oral administration of various doses of zearalenone pre- and post-mating. *Journal of reproduction and fertility* 89: 99-106
- Towers, N.R.; Sprosen, J.M. 1993: Zearalenone-induced infertility in sheep and cattle in New Zealand. *New Zealand veterinary journal* 41: 223-224 (Abstr.)