Effect of *Fusarium* culture and zearalenone on the reproductive performance of ewes

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ABSTRACT

Groups of 33 Coopworth ewes were drenched with either 25mg zearalenone, *Fusarium* culture (containing 25mg zearalenone) or water (controls) daily for 10 days pre-mating commencing on day 7 of the cycle following oestrus synchronisation with CIDRs.

Reproductive performance was markedly reduced in the ewes treated with zearalenone or the *Fusarium* culture, with only 9.1% and 15.6% of ewes yielding fertilised eggs compared to 57.6% of the control ewes.

Treatment reduced the number of ewes ovulating although displaying oestrus. Treated groups had 46% of ewes anovular compared to 12% in the controls. The ovulation rate for those ewes ovulating was less in the treated ewes (1.55) than the controls (1.86). Treatment with zearalenone or *Fusarium* culture reduced the proportion of egg-yielding ewes that had fertilised eggs (27.3% and 55.5%) compared to the controls (100%).

Treated ewes had a markedly longer duration of oestrus and it was evident from corpus luteum size that the oestrus-ovulation relationship had been disturbed.

Keywords Zearalenone; Fusarium; ovulations; fertilisation; oestrus duration; ewes; reproduction; pre-mating; egg recovery

INTRODUCTION

Large-spored Fusaria are a common component of the mycoflora of leaves of pastures in the North Island of New Zealand (di Menna and Parle, 1970). They are present in greatest numbers from February to April and counts of 10^4 to 10^5 *Fusarium* macroconidia/g wet weight of leaves have been recorded. Various *Fusarium* strains isolated from New Zealand pastures have been shown to produce zearalenone when cultured (Gallagher, 1985; di Menna *et al.*, 1985) and recently zearalenone has been isolated from pasture leaf samples collected in February and March (di Menna *et al.*, 1985).

Zearalenone has oestrogenic properties (Blankenship *et al.*, 1982; Mirocha *et al.*, 1978) and detrimental effects on the reproductive performance of sows and cows have been reported (Chang *et al.*, 1979; Kurtz *et al.*, 1969; Mirocha *et al.*, 1968; Diekman and Long, 1984; Long *et al.*, 1983). Reports of the effects of zearalenone on reproduction of sheep are sparse and tend to be associated with its effect on the pregnant animal (Mitton *et al.*, 1975). However because of its oestrognic actions it is likely to influence oestrus, ovulation and fertilisation if administred pre-mating in the ewe.

This experiment was performed to determine the effects of zearalenone and a *Fusarium* culture containing zearalenone, administered pre-mating to ewes, on their reproductive performance.

MATERIALS AND METHODS

Ninety-nine Coopworth ewes (4 and 5yrs) of known reproductive performance were divided into 3 groups on the basis of age, live weight at weaning and number of lambs weaned. One group was drenched with 25mg/d of purified zearalenone in 10ml of a 30% v:v soln of ethanol. A second group was dosed with 40ml of a slurry of a culture of a *Fusarium* isolate (strain 9FA14) grown on rice. This amount of slurry contained 25mg zearalenone and 6.8g milled rice. The third group was dosed with 10ml of tap water (controls).

Ewes were treated with CIDRs (Ainsworth and Downey, 1984) for 14 days to synchronise oestrus and were dosed with their respective treatments for a period of 10 days commencing on day 7 of the cycle following CIDR removal. After 7 days of treatment, ewes were joined with a group of 3 rams fitted with harnesses and crayons. Ewes were inspected twice daily (0800h and 1600h) for mating marks. Marked ewes were shifted to groups of 3 rams fitted with a different coloured raddle. This procedure was continued until ewes were not marked for 3 consecutive inspections. Five groups of rams were used and, where necessary, ewes were crutched to remove raddle marks and rejoined to the first groups of rams.

Ewes were grazed as one group on ryegrass white clover pasture at an allowance of 4.0kg DM/ewe/d for the period between CIDR removal and the end of drenching. Pasture yields were assessed visually. Paddocks were sprayed with benlate for fungal control and were monitored for numbers of *Fusarium* macroconidia and *Pithomyces chartarum* spores thrice weekly. Leaf samples, collected from the paddock being grazed on the collection date and from the paddock next in rotation, were shaken with 10 times

their weight of water and the spores in 2 cu mm of wash water were counted in haemocytometer slides. Plasma GGT levels were measured at the beginning and end of the trial.

Seven days after the final dosing the ewes were slaughtered and pre-slaughter live weight, carcass weight and liver weight were recorded. The reproductive tract was removed and the ovaries examined for number of corpora lutea. The fallopian tubes and uterus were flushed and eggs collected and examined for fertilisation and stage of development.

Data were analysed using analyses of variance and chi square tests.

RESULTS

The reproductive performance of ewes treated with zearalenone or the *Fusarium* culture was markedly altered compared to the control ewes (Table 1).

TABLE 1 Mean values for reproductive traits of groups of 33 ewes.

	Zearalenone	Fusarium	Control	Significance[4]
Incidence of oestrus (%)	87.8	72.7	92.9	*
Duration of oestrus (h)	39.7	25.5	16.0	***
Incidence of ovulation (%)	54.5	53.1	87.9	***
Ovulation rate[1]	1.55	1.50	1.86	*
Ewes yielding eggs/ewes ovulating (%)	61.1	52.9	65.5	ns
Egg recovery rate (%)[2]	57.1	53.8	48.1	ns
Ewes with fertilised eggs/ewes yielding eggs (%)	27.3	55.5	100.0	***
Fertilisation rate (%)[3]	28.8	42.9	100.0	***
Ewes with fertilised eggs/ewes treated (%)	9.1	15.6	57.6	***

[1] Number of ovulations/number of ewes ovulating

[2] Number of eggs recovered/number of ovulations

[3] Number of fertilised eggs/number of eggs recovered

[4] Significance of difference between treated (zearalenone and Fusarium) and control groups

Incidence and duration of oestrus

The *Fusarium* treated group showed a lower incidence of oestrus than the controls ($P<0.05$). Both treated groups had a longer mean duration of oestrus than the controls ($P<0.001$) and there were 49% and 25% of the ewes in the zearalenone and *Fusarium* treated groups respectively with an oestrus duration longer than the maximum of 36h recorded for the control ewes.

Incidence and rate of ovulation

The proportion of ewes ovulating was significantly reduced by treatment as was the ovulation rate of those ewes ovulating. There was no difference between the zearalenone and the *Fusarium* treatments.

Embryo recovery and fertilisation

There were no significant differences between groups on the proportion of ewes ovulating which yielded eggs, nor on the egg recovery rate (proportion of ova shed that were recovered). However both treated groups showed reduced levels of fertilisation. Treatment with zearalenone or *Fusarium* significantly reduced the proportion of ewes yielding eggs which yielded fertilised eggs and reduced the proportion of eggs recovered that were fertilised.

Live weight, carcass and organ weights

There were no significant effects of treatment on carcass weight, dressing percentage, liver, ovary or uterine weights (Table 2).

TABLE 2 Mean values for ewe live weight, organ weights and plasma GGT levels (n = 33).

	Zearalenone	Fusarium	Control
Live weight (kg)			
Start[1]	57.8	55.9	53.9
Final[2]	59.6	58.4	56.4
Live-weight gain (g/d)	95.0	130.0	132.0
Carcass weight (kg)	33.6	29.8	28.3
Dressing %	51.0	50.8	49.5
Live weight (kg)	1.14	1.18	1.10
Ovary weight (g)	3.28	3.88	4.16
Uterine weight (g)	87.1	88.9	83.5
Plasma GGT ($\log_e$)			
Start[1]	4.394	4.320	4.503
Final[2]	4.168	4.132	4.313

[1] Start = time of laparoscopy and start of daily drenching

[2] Final = time of slaughter

Pasture intake and spore counts

The 99 ewes were grazed as one mob on a daily shift of 0.17ha of pasture with average yield of 2400kg dry matter (DM)/ha and 80% green material. The average residual was 1500kg DM/ha giving an estimated intake of 1.5kg DM/d/ewe.

The highest count of *Fusarium* macroconidia was 60 000/g of leaves, wet weight, and of *Pithomyces chartarum* spores 70 000 but counts were usually low and macroconidia were undetectable or at their lowest detectable level (5000/g) in 11 of the 17 samples. *P.chartarum* spores were below or at the limit of detection in 12 of the 17 samples.

There were no differences between groups in plasma GGT levels at the start or end of the trial nor was there any difference in level between the 2 sampling times (Table 2).

DISCUSSION

Treatment of ewes pre-mating with zearalenone or a *Fusarium* culture markedly affected their reproductive performance. The major reduction in ewes ovulating and in ovulation rate, coupled with the markedly lower fertilisation rates, produced an almost complete failure of the reproductive process. Treatments resulted in a 70% reduction in reproductive performance. The number of fertilised eggs produced to one cycle of mating as a percentage of ewes treated was reduced from 79% in the controls to values of 9% and 19% for the zearalenone and *Fusarium* culture treatments respectively.

The similar response of the 2 treated groups indicates that the effect of the *Fusarium* culture can be attributed to its zearalenone content.

The oestrogenic properties of zearalenone are manifest in this trial with

(i) the prolongation of oestrous behaviour (Fletcher and Lindsay, 1971),

(ii) the failure of ewes to ovulate, most probably due to an interference with LH release from the pituitary (Scaramuzzi *et al.*, 1971),

(iii) reduced fertilisation rate through a possible oestrogenic effect on sperm transport (Croker *et al.*, 1975).

There was also a major disturbance of the oestrus-ovulation time relationship in the 2 treated groups as evidenced by different sized corpora lutea on the day of slaughter and by the incidence of 2, 4 and 8 cell embryos recovered from ewes in this group. The overall egg recovery rate was low and could be due to stage of cycle of recovery (Killeen, 1982) with unfertilised eggs that had hatched being difficult to detect.

Thus treatment of ewes with zearalenone pre-mating could lead to an increase in the number of dry ewes through both anovulation and fertilisation failure while the incidence of ewes twinning would be lowered by a reduction in the ovulation rate.

The amount of zearalenone administered to each ewe in the present experiment (25mg/d) represents approximately 16ppm of the dietary DM intake. This is considerably lower than the zearalenone intakes reported in pigs for a similar degree of depression of reproductive performance (Chang *et al.*, 1979; Diekman and Long, 1984) and thus the ewe would appear to be more sensitive than the sow to zearalenone.

This higher sensitivity could be due to the metabolism of zearalenone to zearalenol by the ruminal protozoa (Kiessling *et al.*, 1984). Zearalenol has a greater absorbtion rate and is more oestrogenic than zearalenone (Mirocha *et al.*, 1978). The effects of zearalenone treatment in the present trial exceed those recorded following daily injections of 128ng stilboestrol to ewes before mating (Morley *et al.*, 1963).

The levels of zearalenone administered in the present trial are some 5 times the levels expected to be consumed by ewes in the field based on levels subsequently found in pasture (0.2 to 2.6 ppm). However while it is most likely that these lower levels will adversely affect reproduction, further research is needed to quantitate these effects.

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