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Ovulation and oestrous responses of high and low fecundity ewes to ingestion of isoflavone-rich pasture

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ABSTRACT

Groups of high and low fecundity Booroola x Romney (B x R) and Romney (R) two-tooth ewes grazed a pasture containing 85% 'Grasslands Pawera' red clover for 10 days; similar groups of ewes grazed ryegrass-white clover control pastures. Oestrus and ovulation were synchronised in all ewes to occur during the last 5 days of grazing. Pawera leaf material contained 0.7% formononetin and the pasture was highly oestrogenic to sheep ($>40 \mu\text{g}$ oestradiol — 17β). Mean ovulation rates in the B x R-High, B x R-Low and R ewes were consistently but not significantly, lower in the flocks grazing Pawera than control pastures ($2.08 \nu 2.69$, $1.08 \nu 1.30$, $1.09 \nu 1.40$, respectively). The mean ovulation rate of the ewes grazing Pawera was lower ($P < 0.05$) at the time of grazing the pastures than 1 month later at joining. There were no significant effects of pasture treatment on proportion of ewes ovulating or marked by vasectomised rams. There was no evidence that fecundity status influenced the responsiveness of ewes to isoflavone-rich pasture.

INTRODUCTION

The recent availability to farmers of the highly productive and persistent tetraploid red clover *Trifolium pratense* L. c.v. 'Grasslands Pawera' has led to a renewal of studies on the influence of isoflavone-rich pastures on the reproductive performance of ewes. Leaf material of Pawera may contain up to 1.4% of the isoflavone formononetin and pure swards are highly oestrogenic to ovariectomized ewes (Kelly *et al.*, 1979). The reproductive performance of Romney ewes is markedly impaired when pure swards of Pawera are grazed during joining (Kelly *et al.*, 1980).

The fecundity status of ewes may modify the response of the animal to exogenous oestrogens. Land (1976) reported that Finn ewes were less sensitive to oestradiol benzoate, as measured by the ability to ovulate, than lower fecundity Blackface ewes. Ch'ang (1963) found that ewes born as twins had higher lambing percentages than ewes born as singles when red clover pastures were grazed about joining, largely due to differences in barrenness levels.

This study reports the oestrous and ovulation responses of high and low fecundity Booroola x Romney (B x R) cross and Romney (R) two-tooth ewes to ingestion of isoflavone-rich Pawera red clover in March 1980.

EXPERIMENTAL

Pastures

The control pasture was predominantly ryegrass—white clover; the red clover pasture contained 85% Pawera, 15% grasses and dead

matter. Leaf samples from the clover in both pastures were assayed for coumestans and formononetin on 27 March using a semi-quantitative technique modified from that of Francis and Millington (1965).

Animals and Design

One hundred and seventy-two B x R cross (F_1 and F_2) and 161 R two-tooth ewes were randomly divided into 4 groups of about 80 containing approximately 40 ewes of each breed. Two groups grazed the Pawera and 2 groups the control pastures. Recent reports (Piper and Bindon, 1980; Davis *et al.*, 1981) have indicated that the fecundity of the Booroola is controlled by a major gene; consequently on the basis of the subsequent ovulation rates at two- and four-tooth joining, using a triple ovulation as the segregation criterion, the B x R ewes were classified as those carrying the high fecundity gene (H) and those without it (L). The experimental design was therefore 2 pasture treatments (control, Pawera) by 3 genotypes of ewe (B x R-H, B x R-L, R), replicated twice.

To measure oestrogenicity of the Pawera sward 70 ovariectomized ewes were divided at random into 7 groups of 10 and pretreated for 10 days with intravaginal progestagen sponges. Two groups grazed Pawera from 3 days prior to sponge withdrawal. The remaining 5 groups grazed control pastures and 3 groups received a single intramuscular injection of either 10, 20 or $40 \mu\text{g}$ of oestradiol— 17β 24 h after removal of sponges. The production of cervical mucus (Lindsay and Francis, 1968) in 1 h was measured 24 h after sponge withdrawal or injection of oestradiol— 17β .

Management

All two-tooth ewes received a subcutaneous implant containing 375 mg progesterone on 9 March. Implants were removed on 24 March and harnessed vasectomised rams joined with each flock. Flocks were inspected twice daily and marked ewes removed and run in other Pawera or control paddocks. Experimental pastures were grazed from 21 to 30 March. Thereafter the ewes were run as one flock on ryegrass—white clover pastures. Mating records were taken daily for the second oestrus following synchronisation. Ovulation rates (number of corpora lutea per ovulating ewe) were observed by laparoscopy in all ewes on 31 March, 17 April and 13 May (end of first period of joining). Live weights were recorded following the grazing of experimental pastures.

RESULTS

The formononetin content of Pawera leaf material was 0.7% (dry matter basis). No isoflavones were detected in the control pastures and no coumestans in either pasture. The Pawera pasture was highly oestrogenic to the ovariectomised ewes, producing cervical mucus responses exceeding those for 40 µg of oestradiol—17β.

The means of the 2 replicates for live weights, ovulation rates, proportion of ewes ovulating, and proportion of ewes marked by a ram for each genotype within pasture treatments during the grazing of the experimental pastures are presented in Table 1. All ewes marked were recorded during the period 25 to 27 March. Mean ovulation rates were consistently but not significantly lower (17–23%) in the ewes grazing Pawera than control pastures. There was large variation in means between replicates for the B x R-H flocks. When the replicated flocks were used as their own 'controls' by calculating the difference between mean ovulation rates recorded on the experimental pastures and those following the

first period of joining (i.e., after at least 1 month recovery period), there was a significant effect ($P < 0.05$) of pasture treatment. For ewes grazing Pawera the mean ovulation rates were lower by 1.09 (B x R-H), 0.46 (B x R-L) and 0.30 (R) when grazing the experimental pastures. In comparison the respective differences for ewes grazing control pastures were 0.32, 0.08 and -0.14. Live weights, proportion of ewes ovulating and proportion of ewes marked did not differ significantly between pasture treatments.

Ewe genotype had significant effects on ovulation rate ($P < 0.01$), proportion of ewes ovulating ($P < 0.05$) and proportion of ewes marked ($P < 0.05$). There were no significant interactions.

At the second oestrus following synchronisation (12 to 14 April), about 2 weeks after the ewes were removed from the experimental pastures, there were no significant effects of pasture treatment on ovulation rate, proportion of ewes ovulating or marked. Ewe genotype had a significant effect on ovulation rate ($P < 0.001$), being 2.78, 1.22 and 1.39 for the B x R-H, B x R-L and R ewes respectively (s.e.d. = 0.10). At joining, commencing 1 month after exposure to the experimental pastures, there were no significant effects of the prior pasture treatment on mating performances recorded over the 3 17-day periods of joining, or ovulation rates recorded at the end of the first period of joining.

DISCUSSION

Clearly, there is no evidence of any moderation in the response of ewes to short term grazing of isoflavone-rich pastures by their fecundity status. The magnitude of the depression in ovulation rate was of the order of 20%. In contrast to previous work (Kelly *et al.*, 1980), there were no significant effects of Pawera on the proportion of ewes ovulating or marked, probably due to its lower formononetin content and/or the shorter period of exposure in this

TABLE 1 Mean live weight, ovulation rate, proportion of ewes ovulating and proportion of ewes marked while grazing experimental pastures

Genotype of ewe	Pasture treatment	Live weight (kg)	Ovulation rate	Proportion of ewes ovulating	Proportion of ewes marked
B x R-H	Control	44	2.69	1.00	0.96
	Pawera	42	2.08	1.00	0.91
B x R-L	Control	44	1.30	0.92	0.90
	Pawera	43	1.08	0.96	0.88
Romney	Control	51	1.40	0.90	0.67
	Pawera	49	1.09	0.76	0.52
s.e.d. ¹		0.9	0.24	0.05	0.12
s.e.d. ²		0.6	0.25	0.06	0.09

1. of means except for same pasture treatment.

2. of means within pasture treatment.

study. Ovulation rates quickly recovered to normal levels of fecundity following such short exposure. Prolonged exposure to oestrogenic clover pasture has also failed to reduce ovulation rates in ewes when they are removed to non-oestrogenic pastures, and there is some evidence that it may even be increased (Adams *et al.*, 1979). In contrast, recovery of ewe fertility if any, is slower.

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