Residual effect of a four weeks grazing of oestrogenic red clover on reproductive performance of ewes in the following six weeks

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ABSTRACT

The trial was conducted to study if the temporary infertility in ewes induced by oestrogenic clover, would recover accompanied by a concomitant increase in ovulation rate and/or litter size following their removal to a 'safe' pasture. Romney ewes (N = 155) either grazed on Pawera red clover or Ryegrass-white clover (control) pasture for four weeks. Afterwards all ewes grazed ryegrass-white clover pasture and were mated in 3 groups either at the first, third or sixth week post-treatment. Mean ovulation rate was significantly lower ($P<0.05$) in the Pawera ewes (1.22 ± 0.06) than in the controls (1.49 ± 0.07) on respective treatments after two weeks of grazing. Ovulation rates in ewes after the 1st, 2nd and 3rd mating cycle were 1.60 ± 0.12, 1.41 ± 0.10, 1.27 ± 0.09 (red clover), and 1.64 ± 0.10, 1.67 ± 0.12, 1.30 ± 0.09 (control) respectively. Ovulation rate and litter size were similar for the two treatment groups after joining in week 1, 3 or 6 post-treatment ($P>0.05$). No increase in ovulation rate/litter size in Pawera group ewes was observed over and above those observed in the control ewes within the six week post-treatment period.

Keywords: Ewe; phytoestrogen; formononetin; red clover; reproductive performance; ovulation rate.

INTRODUCTION

Short term grazing on an oestrogenic clover results in a reduced ovulation rate/ovulation failure in ewes (Lightfoot and Wroth 1974; Kelly *et al.*, 1980; Anwar *et al.*, 1993). An increased return to service has been observed in ewes joined on or shortly after grazing on such pasture (Morley *et al.*, 1966; Kelly *et al.*, 1980). This reproductive disturbance is due to the presence of phytoestrogens, specifically formononetin, in the clover (Millington *et al.*, 1964), and recovers within a few weeks after the ewes are removed to non-oestrogenic pasture. Ovulation rates recover quickly, while return to service takes a few weeks to become normal (Morley *et al.*, 1966; Kelly *et al.*, 1980). Ewes grazed for several seasons on oestrogenic clover may suffer cumulative and therefore permanent infertility characterized by reduced fertilization but relatively normal ovulation rate (Adams 1990).

In a previous study conducted in the 1990 breeding season where the ewes were mated on non-oestrogenic pasture immediately after six weeks of grazing an oestrogenic red clover (Pawera) or control (Ryegrass-white clover) pastures, a significantly higher percentage of the Pawera ewes returned to service (McDonald *et al.*, 1994). Fertility in the Pawera ewes recovered after one cycle. The litter size after 34 days of mating, although not significantly different, was found to be higher in the Pawera compared to the control ewes (1.64 v 1.38). It was speculated that the higher litter size might be a part of the phenomenon of recovery from phytoestrogen induced infertility and might be associated with an increase in ovulation rate. In that study 90.5% ewes from the control group conceived during the first cycle compared with only 27.3% ewes from the Pawera group. It is also possible that variation in the conception time might have contributed to the higher ovulation and lambing rate in Pawera group ewes (McDonald and Ch'ang, 1966).

In permanent phytoestrogenic infertility, where ovulation rate remains relatively normal, there is some evidence of an increased ovulation rate in Merino ewes which had grazed oestrogenic pasture for three years (Adams *et al.*, 1979). The difference was reported to be statistically significant at the peak of the breeding season.

The present study was conducted to determine whether the ewes recover with a concomitant increase in ovulation and lambing rate after short term grazing on oestrogenic pasture. The residual effect on reproductive performance of ewes following grazing highly oestrogenic Pawera red clover (for four weeks) was studied in the subsequent 3 cycles and compared with ewes that had grazed non-oestrogenic pasture.

MATERIALS AND METHODS

Animals and grazing

Non-pregnant Romney ewes (N = 155) drawn from the same flock and with a history of normal fertility were used during the 1992 mating season (March - June). The ewes were 6-7 years old and had no previous exposure to oestrogenic clover. They were divided into two groups with similar average weight during the third week of March and then grazed either Pawera red clover (R) or ryegrass-white clover (Control-C) pastures. After 28 days of this grazing treatment, the ewes on red clover were transferred to the ryegrass-white clover pasture. At this stage, the ewes in each group were divided into three subgroups, identified as R1, R2 and R3, or

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C1, C2 and C3 in the red clover and control treatments respectively. The subgroups were paired between treatments and grazed on non-oestrogenic pasture in three mobs. Ewes were weighed at start of the trial, at the end of 28 days grazing and at three weeks after treatment. The Pawera red clover was a fresh regrowth containing more than 70% red clover. Herbage was sampled at the start and middle of the grazing period and formononetin concentration was measured by a fluorimetric method (Gosden and Jones 1978).

Ovulation rate and mating of ewes

Intravaginal progestagen sponges (each containing 40 mg medroxy progesterone acetate) were placed in the ewes at the start of the grazing treatments for a period of 12 days. Ovulation rate in all the ewes was recorded by laparoscopy 6 days after induced heat. The subgroups within each treatment were balanced for ovulation rate as determined at laparoscopy. The first post-treatment oestrus in ewes occurred within one week after removal from treatment. Mobs 1, 2 and 3 were joined with three entire rams in weeks 1, 3 and 6 post-treatment respectively. Two-tooth Romney rams were used for the first mating period but they were replaced by three mature Border Leicester rams for the second and third mating periods. Ovulation rate in each mob was checked 6 days after mating. Mean ovulation rates were compared between the two treatment groups after induced heat and then between the treatment subgroups in the same mating mob.

Litter size

Ewes in each subgroup were slaughtered 27 to 36 days after mating. Reproductive organs were collected, tagged and taken to the laboratory where pregnancy status of each ewe and number of developing embryos (expressed as litter size) were determined. Conception rate and litter size were compared between the treatment subgroups that were mated as one mob.

Statistical analysis

Ovulation rate, conception rate and litter size between treatment groups were compared by the Chi square method. Differences in live weight gain between treatments were compared by t-test. The data are presented as mean ± SEM. All statistical analyses were performed using the statistical analysis system computer package (SAS 1985).

RESULTS

The mean formononetin concentration present in Pawera clover was 1.00 ± 0.11 percent on a dry weight basis.

Live weights

Mean live weights of ewes in the two treatment groups were similar at the start of the trial (Table 1). Both the groups gained weight during 28 days of grazing treatment. Ewes that grazed Pawera red clover gained significantly more weight than the ewes that grazed control pasture (P<0.01). Both the groups continued to gain weight until three weeks after treatment.

TABLE 1: Live weight change (kg) in ewes grazing Pawera red clover or Control pasture.

Observation time relevant to treatment	Red clover ewes (n–78)	Control ewes (n–77)	Significance
Start	53.3 ± 0.5	53.1 ± 0.6	NS
At end	59.9 ± 0.6	55.2 ± 0.6	P<0.01
3 weeks post-treatment	61.1 ± 0.6	56.5 ± 0.7	P<0.01

Ovulation rate

Ovulation rate in the ewes is shown in Table 2. Mean ovulation rate was significantly higher in control ewes (due to a higher percentage of multiple ovulating ewes) than that in Pawera ewes after the induced heat (P<0.05). Ewes at this time were grazing the two different pastures. Mean ovulation rates were similar in the two treatment subgroups at the first post-treatment heat. The differences in mean ovulation rate remained non significant between the two treatments at week three (second cycle) and week six (third cycle) post-treatment (P>0.05).

Conception rate

The conception rate was lower in Pawera ewes (48% - subgroup R1) than that in control ewes (64% - subgroup C1) after joining in week one, but the difference was not significant (P>0.05) (Table 3). Conception rates were similar in the two treatment sub-groups after joining in week three and week six post-treatment.

TABLE 2: Effect of grazing Pawera red clover or Control (ryegrass-white clover) pastures, on ovulation rate (OR) in ewes.

Time of observation	Treatment group					
	Red clover			Control		
	n	OR mean ± SEM	Ewes with ≥2 CLs (%)	n	OR mean ± SEM	Ewes with ≥2 CLs (%)
3rd week of treatment	73[1]	1.22 ± 0.06[a]	19 (26)	74[2]	1.49 ± 0.07[b]	37 (50)
1st week post-treatment	25	1.60 ± 0.12[a]	14 (56)	25	1.64 ± 0.10[a]	16 (64)
3rd week post-treatment	27	1.41 ± 0.10[a]	11 (41)	24[3]	1.67 ± 0.12[a]	15 (63)
6th week post-treatment	26	1.27 ± 0.09[a]	7 (27)	27	1.30 ± 0.09[a]	8 (30)

Means in same row with different superscript letters are significantly different (P<0.05).

[1] 5 ewes lost sponges.

[2] 3 ewes lost sponges.

[3] 1 ewe died.

TABLE 3: Residual effect of grazing Pawera red clover or Control pastures on conception rate (CR) and litter size in ewes mated on non-oestrogenic pasture.

	Treatment group*							
	Red clover				Control			
Joining time after treatment	n	CR (%)	Litter Size mean ± SEM	Ewes with ≥2 lambs(%)	n	CR (%)	Litter Size mean ± SEM	Ewes with ≥2 lambs(%)
1st week	25	48	0.68 ± 0.16	5 (20)	25	64	0.92 ± 0.16	7 (28)
3rd week	26	88	1.15 ± 0.12	7 (27)	24	88	1.38 ± 0.16	11 (46)
6th week	26	85	0.92 ± 0.09	2 (8)	27	89	1.11 ± 0.11	6 (22)

* All between treatment differences are not significant.

Litter size

Mean litter sizes after joining in weeks 1, 3, and 6 post-treatment were consistently lower in Pawera than in control ewes but the differences were not significant (P>0.05) (Table 3). Lower litter sizes in Pawera ewes were due to a lower percentage of ewes with multiple (≥2) lambs. A similar rise and fall in mean litter size was observed in ewes from two treatments during the second and third mating periods.

DISCUSSION

Mean ovulation rate in the ewes grazing oestrogenic Pawera red clover was significantly lower than in those grazing non-oestrogenic control pasture. Ovulation rates became similar in the two treatments within one week of red clover ewes being moved to non-oestrogenic pasture, and were not different at 3 weeks and 6 weeks post-treatment. Ewes that had grazed on red clover did not have an ovulation rate higher than that in the control ewes at any stage of the trial, despite a higher live weight gain during treatment. Rather the mean ovulation rate in the clover ewes was consistently lower than that in the control ewes during the three mating cycles. Similarly, mean litter size was consistently lower in the red clover ewes than that in the control ewes although the differences were not statistically significant. A rise in the mean litter size was observed on both treatments in ewes mated during the second cycle post-treatment. This may be due to different rams (possibly with better fertility) used in the second mating period.

A low ovulation rate in ewes grazing red clover for a short term, followed by a quick recovery once they were moved to a 'safe' pasture, is consistent with earlier studies (Kelly *et al.*, 1980; Kelly and Shackell 1982). But no increased ovulation rate or litter size occurred in red clover ewes over and above those observed in the control ewes within the six week post-treatment period of this trial. It was contrary to our hypothesis that recovery from phytoestrogenic infertility might accompany a rise in ovulation rate and/or litter size. In a normal oestrous cycle at luteolysis, the secretion of oestradiol begins to increase in response to an increasing frequency of LH pulses, culminating in a peak of oestradiol secretion that triggers the preovulatory surge of GnRH and the gonadotrophins (Scaramuzzi *et al.*, (1993). It was assumed that a continuous presence of high levels of phytoestrogenic substance in the blood might interfere with the control mechanism of oestradiol over the hypothalamus and in turn give rise to a higher ovulation rate. There is evidence of a higher ovulation rate in ewes suffering from permanent infertility due to a long term grazing (for several seasons) on oestrogenic clover (Adams *et al.*, 1979). Ewes suffering from permanent infertility are less able to elicit an increase in LH after treatment with oestrogen due to long term interference with the hypothalamic control mechanism (Findlay *et al.*, 1973; Adams and Martin 1983) and it may also account for a higher number of ovarian follicles developing to meet the increased threshold for oestrogen in the hypothalamus (Adams *et al.*, 1979). But Hearnshaw *et al.*, (1977) observed that ingestion of oestrogenic clover for 5 days did not cause any saturation or refractoriness of hypothalamic receptor sites as the ewes exhibited a normal release of LH following oestradiol administration. The hypothalamic response of ewes that grazed oestrogenic red clover for 28 days in the present trial, may fall into the same category as described in the latter study. Thus any residual oestrogenic effect in terms of ovulation rate may be lacking and the hypothalamus may maintain a normal threshold for oestrogen after such a short exposure (28 days) to the clover.

However other possibilities to explain the higher lambing rate observed in Pawera red clover ewes in our previous study (McDonald et al., 1994) could include the later time when mating and conception occurred. Ovulation rate and lambing rate can vary with successive heat periods (McDonald and Ch'ang, 1966; Quinlivan and Martin, 1971). It is concluded that recovery of fertility after a short term grazing on oestrogenic clover might not result in an above normal ovulation/litter size in ewes.

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