Reproductive performance of ewes after grazing on G27 red clover, a low formononetin selection in cultivar Pawera

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ABSTRACT

One hundred and fifty ewes grazed either on a high oestrogenic red clover (Pawera), or a new, low oestrogenic selection of red clover (G27), or control pastures before mating. The treatment groups (n = 25) and grazing lengths prior to mating were (1) Pawera, 6 weeks; (2) G27, 6 weeks; (3) G27, 12 weeks; (4) G27 / Ryegrass-white clover (rg-wc), 6 weeks/6 weeks; (5) Rg-wc (control 1), 6 weeks, and (6) White clover (control 2), 6 weeks. The ewes were mated as one group on rg-wc pasture immediately after the grazing treatments. Formononetin concentration was significantly lower in G27 (0.26%) than in Pawera red clover (0.55%) ($P<0.05$). Ovulation rates in ewes after the first service were similar for all treatment groups ($P>0.05$). The incidence of return to service was significantly higher in Pawera ewes (72.7%) than in any of the other groups ($P<0.01$). The return rates for the other groups were 33.3% (G27/6 weeks), 25.0% (G27/12 weeks), 4.8% (G27/ rg-wc), 9.5% (rg-wc) and 14.3% (white clover). Most ewes which were mated at the next two cycles became pregnant. It is concluded that the fertility in the ewes grazing G27 red clover was better than that in ewes grazing Pawera red clover, however there may have been some impairment in contrast to animals grazing control pasture.

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

INTRODUCTION

Oestrogenic clover frequently causes reproductive problems in sheep (Collins and Cox, 1985). Plant constituents that have been identified as oestrogenic are mainly isoflavones and include formononetin, daidzein, genistein and biochanin A (Cox and Braden, 1974). Formononetin is generally recognized as the most important of these with regard to long term oestrogenic effects in grazing sheep (Millington *et al.*, 1964; Morley *et al.*, 1968; Davies *et al.*, 1970).

Plant oestrogens can cause a temporary or permanent infertility in sheep (Lightfoot, 1974). Ewes mated on, or mated after a short term grazing on an oestrogenic clover suffer from an impairment to fertility which resolves within a few weeks following removal to non oestrogenic pasture (Morley *et al.*, 1966; Kelly *et al.*, 1980). Prolonged exposure (≥2 breeding seasons) to oestrogenic clover can cause infertility which persists for years after removal from oestrogenic pasture (Adams *et al.*, 1988). According to Familton (1990), permanent sterility is uncertain to occur under New Zealand conditions but a reduction in performance has been recorded in ewes when grazing legume dominant pastures. A recent study has shown that Pawera red clover may cause permanent infertility in ewes when grazed for more than two years (Shackell *et al.*, 1993). Temporary infertility is characterized by a lower ovulation rate, an increase in return to service, and a lower lambing rate (Morley *et al.*, 1966; Lightfoot and Wroth 1974; Kelly *et al.*, 1980; Shackell and Kelly, 1984). After removal from oestrogenic clover, ovulation rate appears to recover earlier whereas return to service takes longer to return to normal (Morley *et al.*, 1966; Kelly *et al.*, 1980). An important method to control infertility due to oestrogenic cultivars is the use/breeding of clovers low in formononetin (Lightfoot, 1974).

'Grasslands Pawera', a highly productive and persistent cultivar of red clover (Anderson, 1973), became available in New Zealand in 1970s, but it was found to have a high formononetin concentration (Kelly *et al.*, 1979) which impaired the fertility of ewes mated on this cultivar (Kelly *et al.*, 1980). G27 red clover is a sixth generation selection within cultivar Pawera based on decreasing the formononetin level of the leaflets. Ovulation rate in ewes on G27 red clover was found to be higher than that on Pawera red clover after two weeks of grazing (Anwar *et al.*, 1993). The objective of the present trial was to study the mating performance of ewes after grazing on G27 red clover for a period of 6 or 12 weeks compared to a 6 week grazing on Pawera red clover, or on control pastures.

MATERIALS AND METHODS

Animals

Mixed age Romney ewes, with a history of normal fertility, from a flock at Massey University were allocated to one of six treatment groups (n = 25) balanced for age and live weight. One half of the ewes were 2.5 years old while the remainder ranged from 4-6 years in age. Average live weight of the ewes at the start of the trial was 57.4 ± 0.6 kg. The ewes had no previous exposure to oestrogenic pasture.

Treatments

The trial was conducted in the breeding season of 1990. Treatment pastures and grazing periods before mating for

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various groups were (1) Pawera red clover, 6 weeks; (2) G27 red clover, 6 weeks; (3) G27 red clover, 12 weeks; (4) G27 red clover / Ryegrass-white clover (rg-wc), 6 weeks / 6 weeks; (5) Ryegrass-white clover (control 1), 6 weeks, and (6) White clover (control 2), 6 weeks.

Ewes in groups 3 and 4 were put on G27 red clover in the first week of January. All the other ewes were kept on ryegrass-white clover pasture during this time as one mob. After 6 weeks of grazing on G27, group 4 ewes were moved to ryegrass-white clover pasture. In the second week of February, the remainder of the ewes were also put on the respective grazing treatments and all ewes were drenched for parasite control. All the animals were shorn on 21st of February. Grazing finished on 23rd of March.

Live weight was monitored at weekly intervals during grazing treatments. An effort was made to keep the weight gain similar in all groups by restricting feed available to the heavier group/s, to minimise the effects of the weight change on ovulation rate.

Red clover samples were collected at regular intervals during the trial and were chemically assayed (Gosden and Jones, 1978) to determine formononetin concentration. Pawera and G27 plots grazed in the trial contained more than 70% red clover. The plants were at the flowering stage at the time of grazing.

Matings

All the ewes received intravaginal sponges impregnated with 40 mg medroxy progesterone acetate for a period of two weeks from February 21, to synchronise oestrus. Harnessed teaser rams were used for heat detection which occurred about 9th March. Two days before the following natural heat, the ewes were run as one mob on ryegrass-white clover pasture along with four harnessed, entire rams of proven fertility. Ovulation rate was recorded at laparoscopy 5 days after the first mating which occurred during the last week of March and the first week of April. Return to service was checked by regular change of crayons fixed to entire rams in the following two cycles. Pregnancy and the number of lambs per ewe (litter size) were determined by using real-time ultrasound seven weeks after the third time of mating.

Due to the likelihood of facial eczema outbreak during early autumn which is common in the area, the ewes were drenched with zinc salts at biweekly interval as a prophylactic measure (Smith *et al.*, 1977). The ewes were blood sampled at the start of the trial, at mating time and at the end of the trial. Serum gamma-glutamyltransferase (GGT) was measured to estimate any impairment of liver function (Towers and Stratton, 1978). There was an attack of the disease during the first mating cycle and affected ewes were excluded from the trial.

Ovulation rate, return to service and litter size data were analysed by the Chi square method. Formononetin concentration in the two types of red clover was compared by t-test. Data are presented as mean ± SEM. All statistical analyses were performed using the statistical analysis system computer package (SAS 1985).

RESULTS

Mean formononetin concentration in Pawera (4 samples) and G27 red clover (10 samples) was 0.55 ± 0.08 and 0.26 ± 0.01% (of dry matter) respectively. The concentration was significantly higher in Pawera than that in G27 red clover ($P<0.05$).

Details of ovulation rate in the ewes during the first cycle of mating are given in Table 1. All the ewes included in the trial ovulated. Ewes in the white clover group had a higher mean ovulation rate due to slightly more ewes with multiple ovulations than those in the other groups, but the mean ovulation rates were not significantly different between the various treatment groups ($P>0.05$).

TABLE 1: Ovulation rate in ewes after grazing different red clover and control pastures.

Treatment Group	Grazing period (weeks)	n	Ovulation rate[1] (mean ± SEM)	No. (%) of ewes with ≥2 ovulations
Pawera	6	22	1.50 ± 0.13	10 (45.5)
G27	6	24	1.46 ± 0.10	11 (45.8)
G27	12	16	1.50 ± 0.16	7 (43.8)
G27/rg-wc	6 / 6	21	1.48 ± 0.13	9 (42.9)
Rg-wc (C1)[2]	6	21	1.43 ± 0.11	9 (42.9)
Wc (C2)[3]	6	21	1.62 ± 0.11	13 (61.9)

[1] Group means not significantly different ($P>0.05$).
[2] Ryegrass-white clover (control 1)
[3] White clover (control 2)

Return to service and conception pattern of ewes are presented in Table 2. A significantly higher percentage of ewes in the Pawera red clover group returned to first service than did ewes in other groups ($P<0.01$). There was no difference in the fertility due to grazing G27 for 6 or 12 weeks. Return rates were greater in the ewes which grazed G27 until the end of treatment than in the animals which grazed rg-wc or white clover (Gr. 2 and 3, 12 of 40 ewes vs controls, 5 of 42 ewes; $P<0.05$). A majority of the ewes that returned to service after the first cycle, conceived in the following mating cycle (i.e. 14 out of 16 Pawera ewes, and 26 out of 34 ewes over all treatments). Three ewes were not pregnant after a third cycle (one each from the Pawera, G27/12 weeks, and rg-wc group).

Litter size (Table 3) was similar for all treatment groups after the third service ($P>0.05$). The ewes which grazed with G27 and then ryegrass-white clover did not have many multiple births, somewhat different to that found in the control groups or to those grazing G27 alone. The Pawera group had the highest mean litter size due to a higher percentage of multiple lamb bearing ewes compared to that in the other groups.

DISCUSSION

An important way to minimise phytoestrogen-related fertility problems in clovers is through the selection and breeding of cultivars low in formononetin. Monitoring the reproductive performance of ewes on them is necessary to prove their safety before they are released for commercial use. Pawera red clover has been shown to contain high concentrations of formononetin throughout the year, and this drastically reduced ovulation and conception rate in ewes joined while grazing it (Kelly *et al.*, 1979; Kelly *et al.*, 1980). G27 red clover contains less than half the amount of formononetin in Pawera. In the present trial the ewes that

TABLE 2: Returns to service and conception pattern in the ewes after grazing different red clover and control pastures.

Treatment Group	Grazing period (weeks)	n	No. (and %) of ewes returning to 1st service	Number (and %) of ewes conceiving in cycle 1	cycle 2	cycle 3
Pawera	6	22	16 (72.7)	6 (27.3)	14 (63.6)	1 (4.5)
G27	6	24	8 (33.3)	16 (66.7)	6 (25.0)	2 (8.3)
G27	12	16	4 (25.0)	11 (68.8)	3 (18.8)	1 (6.2)
G27 / rg-wc	6 / 6	21	1 (4.8)	20 (95.2)	0 (0.0)	1 (4.8)
Rg-wc (C1)[1]	6	21	2 (9.5)	18 (85.7)	1 (4.8)	1 (4.8)
Wc (C2)[2]	6	21	3 (14.3)	18 (85.7)	2 (9.5)	1 (4.8)

[1] Ryegrass-white clover (control 1).
[2] White clover (control 2).

TABLE 3: Litter size in ewes after completion of three mating cycles.

Treatment Group	Grazing period (weeks)	n	Litter Size[1] (mean ± SEM)	Number (and %) of ewes with lambs 0	1	≥2
Pawera	6	22	1.64 ± 0.15	1 (4.5)	8 (36.4)	13 (59.1)
G27	6	24	1.54 ± 0.10	0 (0.0)	11 (45.8)	13 (54.2)
G27	12	16	1.31 ± 0.15	1 (6.2)	9 (56.3)	6 (37.5)
G27 / rg-wc	6 / 6	21	1.19 ± 0.09	0 (0.0)	17 (80.9)	4 (19.1)
Rg-wc (C1)[2]	6	21	1.38 ± 0.13	1 (4.8)	11 (52.4)	9 (42.8)
Wc (C2)[3]	6	21	1.57 ± 0.11	0 (0.0)	9 (42.9)	12 (57.1)

[1] Between treatment differences in litter size not significant (P>0.05).
[2] Ryegrass-white clover (control 1)
[3] White clover (control 2)

grazed G27 red clover showed a significantly lower rate of return to service than the Pawera group ewes. There were more returns to service in the G27 groups compared to those in the controls but the size of the difference was less than that after grazing on Pawera clover. Although the effects of six weeks of grazing on Pawera red clover apparently disappeared by the second cycle in this trial, as most of the returning ewes conceived in the very next cycle, a significantly higher percentage of Pawera group ewes potentially had later lambing dates and consequently the group had a greater spread of lambing compared to the ewes that had grazed G27 red clover for 6 or even 12 weeks. The litter size was higher for Pawera group ewes, as a high percentage of returning ewes delivered multiple lambs, but the differences were not significant among different groups. The higher litter size in Pawera group ewes might be due to seasonal variation as a majority of the Pawera group ewes conceived in a different cycle. Within season variation in ovulation rate has been shown in several studies (e.g. McDonald and Ch'ang, 1966). Another possibility is that it was an after effect of the short term grazing on oestrogenic pasture. An elevated ovulation rate has been reported in ewes after removal from oestrogenic pasture which they had grazed for three years, and were permanently infertile (Adams *et al.*, 1979).

The mean ovulation rates (checked after the grazing treatments finished) were similar in different red clover and control groups. Although ovulation rates in the present trial were not checked while the ewes were grazing on different treatment pastures, a previous study showed a significantly higher ovulation rate in the ewes grazing on G27 red clover than those grazing Pawera red clover (Anwar *et al.*, 1993). It has been noted that ovulation rates in ewes recover shortly after removal from oestrogenic clover (Morley *et al.*, 1966; Kelly *et al.*, 1980).

The major control programme for clover infertility has been to produce and disseminate cultivars of clover with a low content of formononetin. Selection of G27 red clover based on lowering the formononetin content of plant laminae, in New Zealand, is a step in this direction. In the present study the reproductive performance of ewes that had grazed the low formononetin G27 red clover, was better than that of the ewes that grazed the high formononetin Pawera red clover because of fewer returns to service and thus earlier mean lambing date.

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REFERENCES

Adams, N.R.; Oldham, C. M.; Heydon, R. A., 1979. Ovulation rate and oocyte numbers in ewes after prolonged exposure to oestrogenic pasture. *Journal of Reproduction and Fertility* **55:** 87-89.

Adams, N.R.; Sanders, M.R.; Ritar, A.J. 1988. Oestrogenic damage and reduced fertility in ewe flocks in South Western Australia. *Australian Journal of Agricultural Research* **39:** 71-77.

Anderson, L.B. 1973. Relative performance of the late-flowering tetraploid red clover 'Grasslands 4706', five diploid red clovers, and white clover. *New Zealand Journal of Experimental Agriculture* **1:** 233-237.

Anwar, M.; Keogh, R.G.; McDonald, M.F. 1993. Oestrus, ovarian activity and embryo survival in ewes grazing high and low oestrogenic red clover. *Proceedings of the New Zealand Society of Animal Production* **53:** 303-305.

Collins, W.J.; Cox, R.I. 1985. Oestrogenic activity in forage legumes. *In:* Forage for Energy-Efficient Animal Production. Eds. R. F. Barnes *et al.*, Agricultural Research Service, US Department of Agriculture, Springfield, VA. pp 268-276.

Cox, R.I.; Braden, A.W.H. 1974. The metabolism and physiological effects of phyto-oestrogens in livestock. *Proceedings of the Australian Society of Animal Production* **10:** 122-129.

Davies, H.L.; Rossiter, R.C.; Maller, R. 1970: The effect of different cultivars of subterranean clover (T. Subterraneum L) on sheep reproduction in the south-west of Western Australia. *Australian Journal of Agricultural Research* **21:** 359-369.

Familton, A.S. 1990. Animal disorders arising from consumption of pasture. *In:* Pastures - their ecology and management. Ed. Langer, R.H.M. Oxford University Press, Auckland. pp 284-298.

Gosden, A.F.; Jones, R. 1978. A routine method for predicting the formononetin content of red clover. *Journal of the Science of Food and Agriculture* **29:** 925-929.

Kelly, R.W.; Hay, R.J.M.; Shackell, G.H. 1979. Formononetin content of 'Grasslands Pawera' red clover and its oestrogenic activity to sheep. *New Zealand Journal of Experimental Agriculture* **7:** 131-134.

Kelly, R.W.; Shackell, G.H.; Allison, A.J. 1980. Reproductive performance of ewes grazing red clover (Grasslands Pawera) or white clover-grass pasture at mating. *New Zealand Journal of Experimental Agriculture* **8:** 87-91.

Lightfoot, R.J. 1974. A look at recommendations for the control of infertility due to clover disease in sheep. *Proceedings of the Australian Society of Animal Production* **10:** 113-121.

Lightfoot, R.J; Wroth, R.H. 1974. The mechanism of temporary infertility in ewes grazing oestrogenic subterranean clover prior to and during joining. *Proceedings of the Australian Society of Animal Production* **10:** 130-134.

McDonald, M.F.; Ch'ang T.S. 1966. Variation in ovarian activity of Romney Marsh ewes. *Proceedings of the New Zealand Society of Animal Production* **26:** 98-106.

Millington, A.J.; Francis, C.M.; McKeown, N.R. 1964. Wether bioassay of annual pasture legumes. II. The oestrogenic activity of nine strains of Trifolium subterraneum L. *Australian Journal of Agricultural Research* **15:** 527-536.

Morley, F.H.W.; Axelsen, A.; Bennett, D. 1966. Recovery of normal fertility after grazing on oestrogenic red clover. *Australian Veterinary Journal* **42:** 204-206.

Morley, F.H.W.; Bennett, D.; Braden, A.W.H.; Turnbull, K.E.; Axelsen, A. 1968. Comparison of mice, guinea-pigs and sheep as test animals for bioassay of oestrogenic pasture legumes. *Proceedings of the New Zealand Society of Animal Production* **28:** 11-21.

SAS 1985. SAS User's Guide, Statistics, Version 5 Edition. SAS Institute Inc. Box 800, Cary, North Carolina, 27511-8000.

Shackell, G.H.; Kelly, R.W. 1984. Residual effects of short term grazing of red clover pastures on the reproductive performance of ewes. *New Zealand Journal of Experimental Agriculture* **12:** 113-117.

Shackell, G.H.; Kelly, R.W.; Johnstone, P.D. 1993. Effect of prolonged exposure of ewes to oestrogenic pasture. 1. Permanent flock infertility following long-term grazing of red clover ('Grasslands Pawera') - dominant pasture. *New Zealand Journal of Agricultural Research.* **36:** 451-457.

Smith, B.L.; Embling, P.P.; Towers, N.R.; Wright, D.E.; Payne, E. 1977. The protective effect of zinc sulphate in experimental sporidesmin poisoning in sheep. *New Zealand Veterinary Journal* **25:** 124-127.

Towers, N.R.; Stratton, G.C. 1978. Serum gamma-glutamyltransferase as a measure of sporidesmin induced liver damage in sheep. *New Zealand Veterinary Journal* **26:** 109-112.