FORMONONETIN CONTENT IN SELECTED RED CLOVER STRAINS AND ITS EFFECTS ON REPRODUCTION IN EWES

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MUHAMMAD ANWAR

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ABSTRACT

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A series of trials were conducted to investigate the oestrogenicity of a low formononetin selection of red clover, 'G27', as compared to the original Pawera red clover. Formononetin concentration was measured in the plants at various stages of their growth. In ewes which grazed Pawera, G27, or Ryegrass-white clover (Control) pastures, comparisons were made of the incidence of ovulation, ovulation rate, and fertility.

As the individual isoflavone level for any one strain may vary with growth stage, a study was conducted to characterize formononetin concentration in various components of G27 red clover and Pawera red clover during different stages of plant growth under field conditions. Mean formononetin concentration (percent dry weight) of leaflets and petioles was lower for G27 than for Pawera ($P<0.05$) at various stages of vegetative leaf development. G27 leaflet concentrations (0.29 ± 0.02) changed little during development compared to Pawera leaflets which declined from 2.16 ± 0.10 in the youngest leaflets to 0.75 ± 0.08 by the end of vegetative leaf development. Formononetin concentration in G27 leaves (leaflet + petiole) at the pre-flowering stage was 0.35% compared to 0.97% in Pawera leaves ($P<0.05$). At early and late-flowering stages, the formononetin concentration in G27 red clover, on a whole plant basis, was 50% of that in Pawera red clover because the formononetin concentration in petioles and stem of G27 did not decline to the same extent as that in the leaflets. When calculated only for the upper parts of the plant, which are usually ingested by sheep, G27 and Pawera red clover contained 0.27% and 0.99% formononetin, respectively, at the early-flowering stage, and 0.19% and 0.53% formononetin, respectively, at the late-flowering stage.

In ewes grazed on various red clover and Control pastures, the main effects studied were ovulation rate and fertility. An investigation was made of the development and the number of follicles in the ovaries and plasma FSH concentrations in ewes grazing

either G27 red clover, Pawera red clover, or Control pasture, close to oestrus. A prostaglandin F2α (PGF) injection was used on day 13 of a synchronized cycle to enhance the synchrony of oestrus in ewes. Mean level of blood equol, which is the main oestrogenic metabolite of formononetin in ewes, was significantly lower on G27 red clover (1.81 ± 0.28 μg/ml) than on Pawera red clover (7.25 ± 1.70 μg/ml) ($P<0.01$). Total number of ovarian surface follicles in Pawera ewes (9.40 ± 1.13) was lower than that in G27 (15.36 ± 1.87) or Control ewes (16.18 ± 2.32) 24 h after PGF injection ($P<0.05$). Histological examination of the left ovaries conducted 72 h after PGF injection showed that the number of healthy follicles with diameter (D) $1mm<D\leq2mm$ was marginally lower in Pawera ewes (2.80 ± 0.66) than that in G27 (5.50 ± 1.04), or that in Control animals (5.18 ± 0.64) ($P<0.06$). Cellular atresia was observed in some of the large follicles ($D>4mm$) in Pawera ewes but not in any of the ewes in the other two treatments. No differences were observed in the mean plasma FSH concentrations between ewes from the three treatments at various sampling times.

Two trials were conducted to compare sperm transport in ewes mated after grazing on Pawera red clover, G27 red clover, or Control pastures. In the first experiment 84 ewes were inseminated each with 500 million spermatozoa at oestrus, after grazing for two oestrous cycles. Mean numbers of spermatozoa in the cranial part of the cervix were not different between various treatments 2 h after insemination. No spermatozoa were recovered from the Fallopian tubes and uteri of many ewes, but this was considered to be due to technical problems. In a second experiment 30 Romney ewes (10 per treatment) were mated to rams after 28 days of grazing either on Pawera red clover, G27 red clover, or Control pastures. The ewes were killed 24 h after service and sperm were recovered from the tract and counted using an improved technique. The number of spermatozoa recovered from different parts of the tract did not differ significantly between treatments, although there was a trend for the low formononetin (G27) ewes to have higher mean sperm numbers than Pawera and Control ewes.

In another two trials, ewes ($n = 16$ per group), that were potential recipients for embryo transfer, grazed on the high oestrogenic red clover (Pawera), low oestrogenic red clover (G27), and Ryegrass-white clover (Control) pastures for 5 weeks around oestrus. In both the trials, the number of ovular ewes and ovulation rate were lower ($P<0.05$) in Pawera ewes. The ovulation rate in Pawera, G27, and Control ewes in trial 1 was 0.62 ± 0.15, 1.62 ± 0.18 and 1.93 ± 0.27; in trial 2 it was 0.31 ± 0.18, 1.17 ± 0.27 and 1.54 ± 0.14 for the three groups respectively. Following the transfer into

suitable recipients of two embryos per ewe, post-mortem examination at 35 days showed a survival rate in Pawera, G27 and Control groups of 50%, 90% and 85% in trial 1, and 50%, 50% and 69% in trial 2.

Fertility, and litter size in ewes when fed on the two types of clovers close to the time of mating were studied in another experiment. 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. Ewes were mated on non-oestrogenic pasture. Ovulation rates in ewes after the first service were not different for all treatment groups ($P>0.05$). The incidence of returns 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. The litter size was not significantly different between various treatment groups after 3 cycles of matings.

It is concluded that G27 red clover has significantly lower formononetin concentrations than Pawera red clover at different stages of plant growth and development. Follicle growth and ovulation rate in ewes on G27 red clover were not different from those in ewes on non-oestrogenic pasture, and were better than those in ewes on Pawera red clover. The performance of ewes after grazing 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. Sperm transport in the reproductive tract, and embryo survival in ewes after transfer of fertilized eggs were also not different in G27 and Control ewes. The study showed that the oestrogenicity of G27 red clover was significantly reduced compared to that of Pawera red clover from which the selection was made.

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TABLE OF CONTENTS

CHAPTER III: Comparison of formononetin concentration and yield between 'Grasslands' Pawera red clover and G27 red clover, at different stages of plant development

CHAPTER IV: Follicular development, plasma progesterone, FSH, and equol concentration in ewes grazed on Pawera and G27 red clovers

CHAPTER V: Sperm transport in ewes grazed on red clover

CHAPTER VI: Ovarian activity and embryo survival in ewes grazing high and low oestrogenic red clovers

CHAPTER VII: Reproductive performance of ewes after grazing on G27 or Pawera red clovers

LIST OF TABLES

Appendix Tables

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

The following abbreviations have been used in the text without prior definition:

Units:

°C	degree Celcius
cm	centimetre(s)
g	gram(s) or acceleration due to gravity
h	hour(s)
l.u.	international units
kg	kilogram(s)
mg	milligram(s)
ml	millilitre(s)
mm	millimetre(s)
ng	nanogram(s)
ppm	parts per million
µg	microgram(s)
µl	microlitre(s)

Hormones

FSH	follicle stimulating hormone
GnRH	gonadotrophin releasing hormone
oGH	ovine growth hormone
oLH	ovine luteinizing hormone
oPRL	ovine prolactin
PMSG	pregnant mares' serum gonadotrophin

Others

SEM	standard error of the mean
vs	versus
v/v	volume/volume
w/v	weight/volume
NaCl	sodium chloride