

# SEASONAL INCIDENCE OF OESTROGENIC ACTIVITY IN IRRIGATED PASTURE AS ASSESSED BY WETHER TEAT LENGTH INCREASE

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## Summary

In three groups of wether sheep, the increase in teat lengths, caused by the ingestion of legume-dominant pastures was determined over a 12-month period commencing March 1964.

The dominant species in each of the three pastures were *Trifolium subterraneum* L. variety Bacchus Marsh, *T. subterraneum* variety Yarloop, and *T. repens* L. variety Ladino, respectively. Measurements were also made on a companion flock continuously grazing a clover-free native pasture.

Oestrogenic activity as assessed by teat length increase was maximal in early spring. This coincided with dominance of the pasture by the legume component.

Milk secretion was recorded during autumn-winter in Merino wethers continuously grazing a near-pure stand of Yarloop subterranean clover.

## I. INTRODUCTION

The use of teat length bioassay for investigation of seasonal changes in effective oestrogen intake of sheep at pasture has been suggested by Braden, Southcott and Moule (1964). Using this technique, Millington, Francis and McKeown (1964) and Francis and Millington (1965) have assayed nine strains of subterranean clover (*Trifolium subterraneum* L.) in both the dry and green state when grown under natural rainfall conditions in Western Australia. The oestrogenic potency of some strains was high in the green state and almost negligible in the dry state. The importance of this potency in relation to time of mating has been demonstrated by Morley, Axelsen and Bennett (1963) and Clark (1965).

This paper reports on the seasonal incidence of oestrogenic activity, as determined by wether teat length increase, in three irrigated pastures having different legume components.

## II. EXPERIMENTAL

### (a) Pastures

Three irrigated pastures and a non-irrigated native pasture, which was free of clover, were used in this study. The pasture designated Yarloop consisted in spring of 20 cwt/ac of 95% *T. subterraneum* variety Yarloop and 5% *Hordeum* spp. The pasture designated Bacchus Marsh consisted in spring of 16 cwt/ac of 50% subterranean clover variety Bacchus Marsh, 25% *Echium plantagineum* L., 20% *Lolium rigidum* Gaud. and 5% annual *Hordeum* spp. The third pasture, designated Ladino clover, consisted in spring of 30 cwt/ac of 60% *T. repens* L. variety Ladino, 18% of *Lolium perenne* L., 12.5% *Paspalum dilatatum* Poir., 6% *Juncus* spp. and 3% *Phalaris minor* Retz.

No attempt was made to determine selectivity of diet.

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### **(b) Sheep**

Four flocks of medium-woolled Merino wethers were used and they grazed on the four pastures as follows:—

Group 1. Clover-free native pasture.

Group 2. Yarloop pasture.

Group 3. Bacchus Marsh pasture.

Group 4. Ladino clover pasture.

### **(c) Procedure**

Wether hoggets for Groups 1 and 2 were drawn from a flock that had been reared on a clover-free native pasture. The control flock (Group 1) of ten wethers remained on a clover-free pasture while the remaining ten (Group 2) were continuously grazed at 8 sheep/ac on the Yarloop pasture. Groups 3 and 4 **were four** year old wethers that had previously been confined to the pasture under test for two years. Ten sheep were measured from each flock.

The increase in teat length was determined over a 12-month period commencing March 1964. Measurements were made on each teat at monthly intervals by means of vernier calipers read to the nearest 0.1 mm. All measurements were made by one observer.

## III. RESULTS

Due to the disparity in age and previous grazing experience there were differences in the initial mean teat length between groups. For this reason, the changes in mean teat length for each of the four groups is shown in Figure 1 in **terms** of absolute teat length.

### **(a) Yarloop pasture**

The teat length response of wethers on this pasture after 30 days was positive ( $+ 0.90 \pm 0.05$  cm) in contrast to a negative response ( $- 0.04 \pm 0.01$  cm) by the companion flock on native pasture. The peak teat length increase of  $+ 1.30 \pm 0.09$  cm in August coincided with obvious mammary development and milk secretion. No attempt was made to measure milk production **but secretion was** recorded for the months of May, June and August 1964 and February and March 1965. Figure 2 shows mammary development typical of the wethers in February 1965.

Partial recession of teat length occurred in spring and **continued through** summer until the opening autumn irrigation in mid-February.

### **(b) Bacchus Marsh pasture**

Initial teat length increase was slight ( $+ 0.20 \pm 0.01$  cm) in comparison with the Yarloop group. The pasture, as grazed, showed two major peaks of oestrogenic activity: the first in September-October 1964 (mean teat length increase  $+ 0.60$  cm) when the subterranean clover was the dominant species and the second in February ( $+ 0.43 \pm 0.09$  cm) after the opening autumn irrigation.

### **(c) Ladino clover pasture**

The changes in teat length were similar to those of the Bacchus Marsh group with a peak increase of  $+ 0.49 \pm 0.07$  cm in September. Teat length recession in spring and summer was rapid and almost complete.

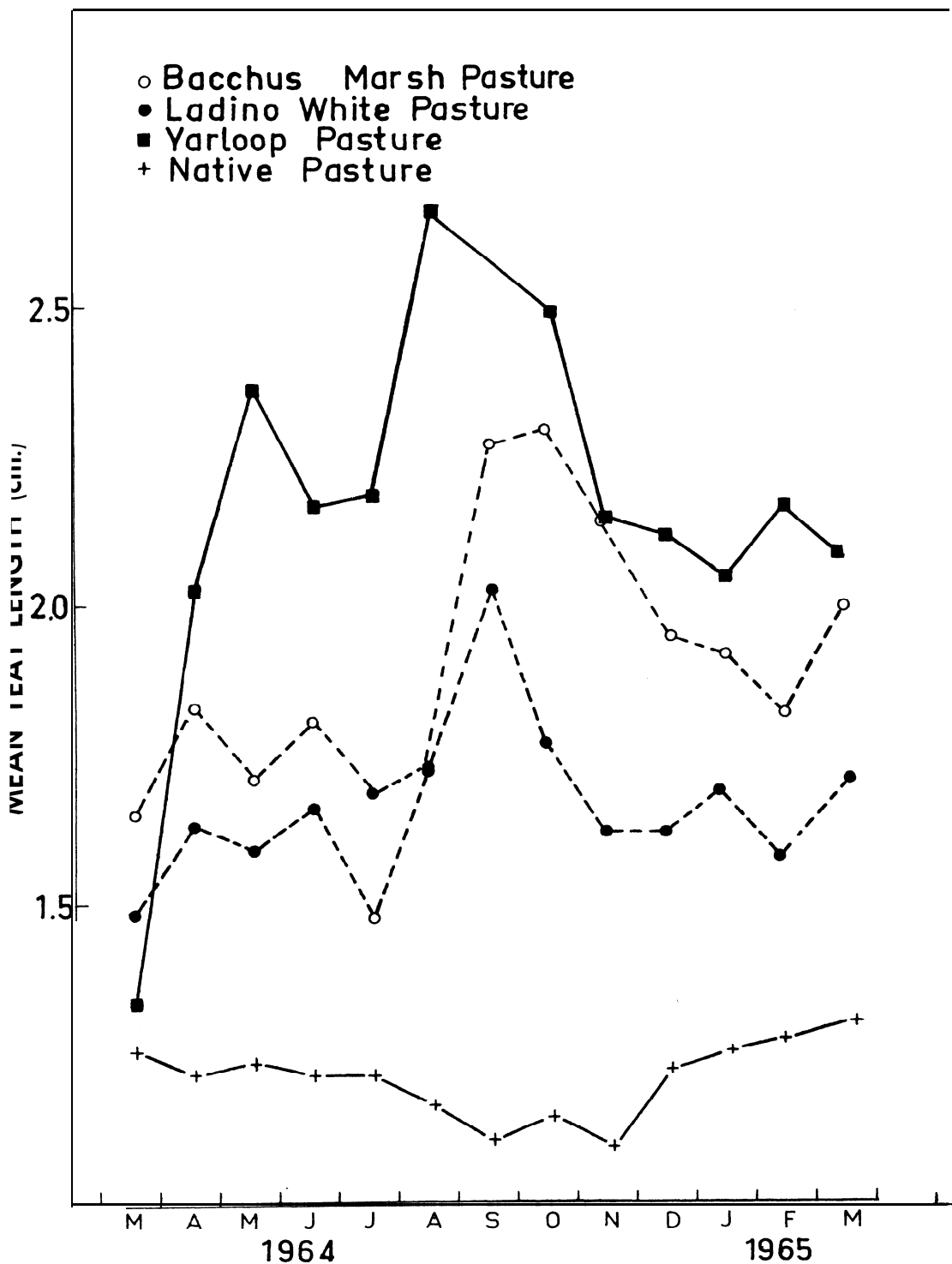


Fig. 1.—The influence of some legume-dominant pastures on the teat length of Merino wethers.

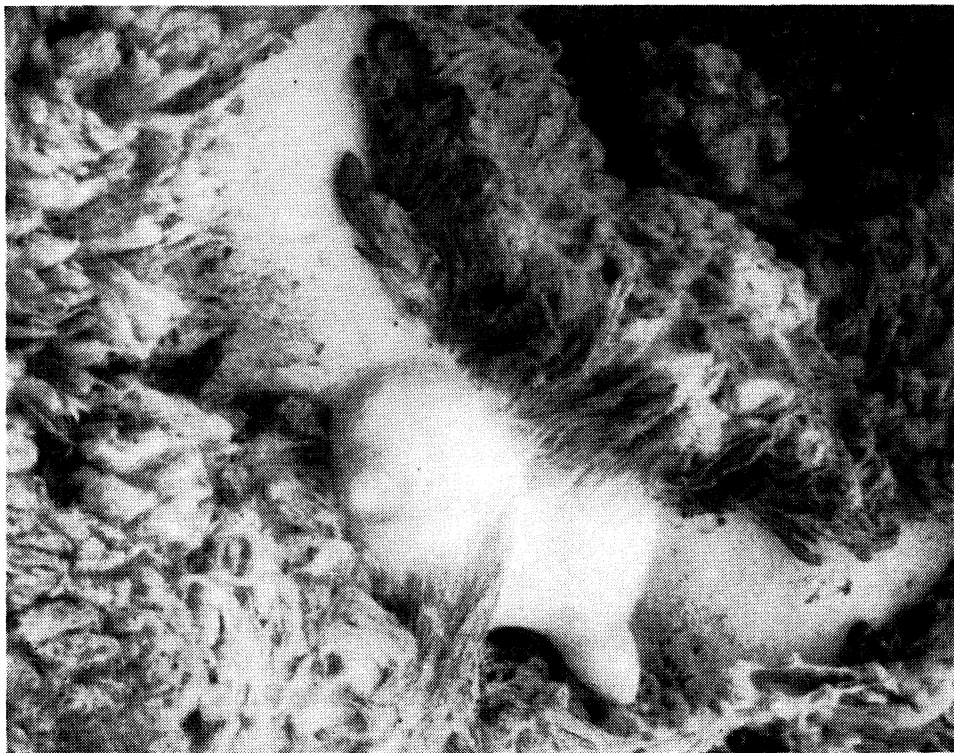


Fig. 2.—Mammary development of Merino wether on February 18, 1965 due to intake of oestrogen from Yarloop subterranean clover pasture.

#### IV. DISCUSSION

Although the dominant legume in each of the pasture mixtures was the sole legume present, the extent of the contamination by the associated species precludes the possibility of ranking them in terms of their oestrogenic potency.

However these observations are in agreement with rankings made by other workers. Millington, Francis and McKeown (1964) in a wether bioassay of pure stands of nine strains of subterranean clover have graded them on the basis of diethylstilboestrol (D.E.S.) equivalents. Yarloop strain induced a response exceeding  $16\text{ }\mu\text{g}$  D.E.S. per day and Bacchus Marsh strain a response approximating  $4\text{ }\mu\text{g}$  D.E.S. per day. Clark (1965) using a vaginal smear bioassay found that the oestrogenic potency of a Bacchus Marsh pasture was equivalent to a daily intake of  $7\text{ }\mu\text{g}$  stilboestrol dipropionate.

There have been variable results in assays on white clover and these differences may be related to variety, season and age of the stand (Moule, Braden and Lamond 1963). Braden, Southcott and Moule (1964) found no activity in white clover at either Armidale or Prospect, but Morley, Bennett and Axelsen (unpublished data) have found moderate activity in winter at Canberra, and Sanger and Bell (1961) found some oestrogenic effects on sheep grazing Ladino white clover in North America.

Morley, Axelsen and Bennett (1963) have found that a daily dose of 8  $\mu\text{g}$  stilboestrol dipropionate administered to ewes at mating reduced conception rates and induced other reproductive disturbances. While no stilboestrol injections were made in the present experiment to determine the dose response curve, the teat length increases of wethers grazing the three irrigated pastures *suggest* an oestrogen intake above the level necessary to adversely affect reproduction.

It is desirable to restrict mating to periods of low oestrogenic activity (Clark 1965) but this is more difficult under irrigated conditions at Deniliquin where sheep are grazing subterranean clover pasture in the green state for more than nine months each year. Furthermore, one of the alternative irrigated pastures, Ladino white clover, also has a low level of oestrogenic activity.

The oestrogenic activity of the common strains of subterranean clover, in the dry state, is negligible (Francis and Millington 1965). Similarly Morley, Bennett and Axelsen (1964) report that at Canberra the Yarloop strain was inactive after wilting. In the present experiment where wethers were continuously depastured on a near-pure stand of Yarloop subterranean clover for nine months before senescence of the pasture, teat length recession was far from complete during the period of negligible oestrogenic activity. It might be concluded that continuous ingestion of Yarloop subterranean clover may have led to some permanent changes in the teat (Figure 2).

With continuous grazing, the teat length at any time depends: on past as well as current intake. However, Braden, Southcott and Moule (1964) in a wether bioassay at Armidale have demonstrated that changes in mean teat length of wethers depastured continuously on red clover showed similar patterns to the mean teat lengths of wethers on the same pasture for two week intervals interspersed with periods of rest on inactive pastures.

## V. ACKNOWLEDGMENTS

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