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The effect of exogenous melatonin, administered in summer, on wool growth and testis diameter of Romneys

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ABSTRACT

To study the influence of melatonin on seasonal changes in wool production of crossbred sheep, a pharmacological dose of sustained release melatonin was administered over a month in early summer to both fleeceweight-selected and control rams from the Massey University Progeny Test lines. Wool growth and testis diameter were compared with those of untreated animals during the period of melatonin treatment and for a further 2 month period.

Melatonin treatment did not significantly change either greasy or clean fleeceweight production. However, the testis diameter of the melatonin-treated animals was significantly greater than in untreated animals during the period of melatonin treatment and significantly smaller during the recovery period. Hence, although the melatonin treatment was sufficient to produce a change in reproductive characteristics, it was not sufficient to induce a measurable change in the pattern of wool production.

Keywords Melatonin; wool; sheep; testis size

INTRODUCTION

The photoperiodicity, or seasonality, of wool production has two significant commercial implications for the grower. First, elimination of the decline in production over winter may increase total yearly wool production by 10%; second, the quality of the wool produced may be increased because of the more consistent fibre diameter and associated fibre strength. The magnitude of seasonality varies greatly in different breeds of sheep; very little in breeds such as Merinos through to a complete cessation of wool growth, and even shedding, in more 'primitive' breeds such as the Soay, Moufflon and Limousine. The predominant breeds farmed in New Zealand show very significant reductions in production during winter.

The causes of the winter decline in wool production are not yet completely understood. Usually there is a nutritional component as a result of poorer feed quality and the additional stress of pregnancy and lactation. However, the major influence is not nutritional. There is an obvious associative relationship between the seasonality of wool growth and the seasonal cycle of melatonin secretion. Pinealectomy (removal of the pineal gland, the source of melatonin) has been shown to prevent the winter cessation of primary hair follicle activity in Limousine rams (Rouget *et al*., 1984). Although there is no direct evidence of a causal relationship between melatonin and seasonality of wool production, Lincoln and Ebling (1985) did show that implantation of constant release implants of melatonin into Soay rams during periods of long day lighting caused a premature moult. In contrast, there is substantial evidence that melatonin plays a direct and key role in mediating the effects of changing photoperiod on reproductive activities of adult sheep (Arendt, 1986), and a less direct, but still significant, effect on reproductive responses of ram lambs (Kennaway and Gilmore, 1985).

The Massey University Progeny Test flock now shows a 25% difference between the fleeceweight-selected (FW) and unselected control (C) lines in their hogget fleeceweights.

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This increased fleeceweight is a consequence of a greater wool production throughout the year and also a proportionately greater production during winter (McClelland *et al.*, 1987).

In undertaking the present study it was anticipated that the continuous release of melatonin would simulate the pattern produced through continuous darkness and would thus induce a winter pattern of physiological responses. The aim was to induce a change in summer wool production towards that of winter while monitoring testis diameter as a reflection of any change in reproductive status. Because of the difference between the FW and C lines in their winter wool production, both lines were treated with melatonin to test for a difference between the lines in sensitivity to the hormone.

EXPERIMENTAL

Twenty four ram hoggets (aged 15 months) from each of the FW and C lines were weighed and divided into two groups of 12 animals on 17 November 1987. A preliminary blood sample was taken from all animals into vacutainers containing 15 mg (K_3) EDTA and placed on ice. Samples were centrifuged for 20 minutes at 2,500 G and the plasma removed and frozen at -20°C until analysis. On the same day midside patches were clipped on each animal and the testis diameter measured with calipers.

On 18 November 1987 half of the animals from each line (FW/M and C/M) were injected subcutaneously in the back of the neck with micro-encapsulated melatonin in adjuvant (at 1.9 mg melatonin/kg body weight), while the two remaining groups from each line (FW/C and C/C) were given adjuvant only.

Further blood samples were taken from all animals on days 2, 5, 9, 16, 23, 35, 58, 89 and 104 after treatment and treated and stored as for the preliminary sample. Midside patches were recut and weighed on 15 January 1980 and 1 March 1988 (i.e. 59 and 105 days after treatment). The wool samples were washed and then weighed at 16% moisture regain to give clean wool growth. Testes diameters were measured at 16, 35, 58, 78, 104, 124 and 145 days after treatment.

Plasma samples were pooled within each treatment group for each time of sampling and the pooled samples assayed for concentration of melatonin using the radioimmunoassay of Fraser *et al.* (1983).

Values presented for midside patch and testes diameter are least squares means of each factor fitting the following model to data at each time point and adjusting the bodyweight at all points to 55.6 kg (the overall mean during the experimental period):

Diameter (or midside patch weight) = Birth rank + line + treatment + all first order interactions + bodyweight

RESULTS AND DISCUSSION

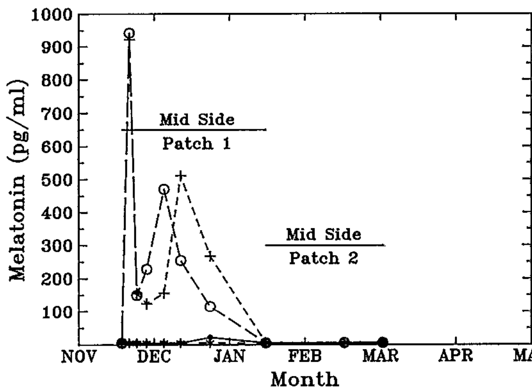

FIG. 1 Levels of melatonin (pg/ml) throughout the trial in pooled plasma from untreated control animals (•), melatonin treated control animals (+), untreated fleece weight-selected animals (*) and melatonin-treated fleeceweight-selected animals (o). Horizontal bars represent the 2 periods of sampling a midside patch for wool growth.

There was no significant difference in bodyweight between the FW or C animals or between groups FW/M, FW/C, C/M, C/C at either the start of the trial or two months into the trial (15 January 1988) when the animals were reweighed. The mean (±S.E.) body weight was 55.6 ± 2.0 kg.

Levels of melatonin in the pooled plasma samples (Fig. 1) show a biphasic pattern of release from the microcapsules in treatment groups FW/M and C/M. Both the treatment groups had greatly enhanced levels of plasma melatonin for a

TABLE 1 The effect of exogenous melatonin on wool growth (g/d) of Romney rams, measured on a midside patch of 100mm x 100mm.

Flock	Treatment	Greasy wool Period 1	Greasy wool Period 2	Clean wool Period 1	Clean wool Period 2
FW	Control	0.18 ± 0.007	0.14 ± 0.006	0.15 ± 0.006	0.11 ± 0.005
	Melatonin	0.20 ± 0.007	0.14 ± 0.006	0.15 ± 0.006	0.10 ± 0.005
Control	Control	0.15 ± 0.007	0.13 ± 0.006	0.12 ± 0.006	0.10 ± 0.005
	Melatonin	0.16 ± 0.007	0.12 ± 0.006	0.12 ± 0.005	0.09 ± 0.005
Significance					
	FW *v* Control	<0.001	0.002	<0.001	0.02
	Control *v* Treatment	0.12	0.53	0.34	0.24
	Interaction	0.72	0.74	0.77	0.93

month, compared with untreated groups FW/C and C/C.

The timing of midside patch sample 1 (Fig. 1) coincided with the period of enhanced plasma melatonin in the treated animals while period 2 coincided with the recovery period.. The FW animals produced ($P<0.001$) more wool (greasy or clean) than control animals in both periods 1 and 2 of sampling (Table 1). There was a trend ($P<0.12$) for the melatonin-treated animals to produce more greasy wool in period 1. However this trend was not apparent in the clean wool produced during period 1, suggesting that the treatment effect was on suint production, not on production of the fibre itself. During period 2, this trend had disappeared leaving no differences between treatment groups in either greasy or clean wool production.

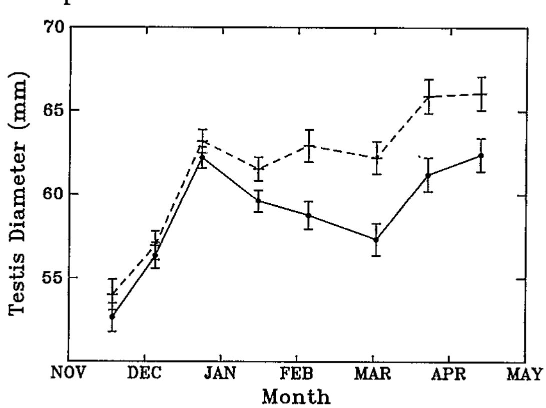

FIG. 2 Changes in testis diameter (means with s.e. bars) in animals from the control flock (●) and the fleece-weight selected flock (+) throughout the period of the trial.

After the seasonal increase in testis diameter throughout November and December the FW group maintained significantly larger testes than the Control animals (Fig. 2). Fig. 3 shows the effect of melatonin treatment on testis diameter. Because both FW and C groups responded similarly to treatment only the combined data (corrected for line effects as described in the Methods) are shown. Melatonin treatment caused an increase in testis diameter throughout the period of treatment. However on removal of the enhanced levels of melatonin from the plasma there was a depression in testis diameter in the treated animals so that the testes of untreated animals were significantly larger. By the end of the measurement period in April, the treated animals had recovered from the depression in testis size and there was no longer any significant difference

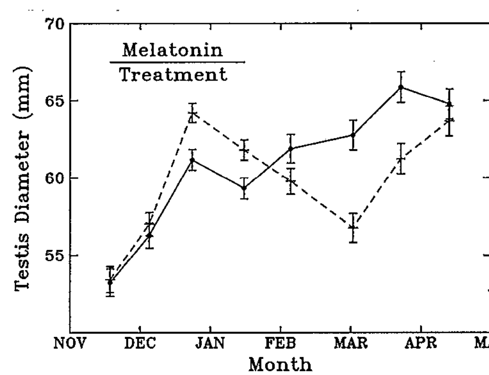

FIG. 3 Changes in testis diameter (means with s.e. bars) in combined data from the untreated animals (●), and melatonin treated animals (+) with flock effects removed. The horizontal bar represents the period of melatonin treatment.

The changes found in testis diameter in response to melatonin treatment are very similar to those observed by Lincoln and Ebling (1985) after implanting melatonin into Soay rams. When melatonin implants were given to adult rams during artificially manipulated 'long days' there was a significant increase in testis diameter. When further implants were given to ram lambs during natural light 'long days' the treated lambs had normal or slightly enlarged testes during treatment but a decline in testis diameter after removal of the implant. It therefore appears that, in both the present work and that of Lincoln and Ebling (1985), increased levels of circulating melatonin during 'long days' induced the changes in testis size normally associated with 'short days'.

Although the present work did achieve a change in testis diameter, it did not alter wool production to mimic 'short day' production (in the case of Soay sheep an alteration in the timing of moult (Lincoln and Ebling, 1985). This could be due to either the timing or the level of melatonin given in the present work; enhanced levels of circulating melatonin were maintained for only a month in the present work compared with more than 5 months in the work of Lincoln and Ebling (1985). The melatonin administered in the present work (3.5 mg/day) was only about half that given by Lincoln and Ebling (1985) (8.4 mg/day) assuming an average release pattern. However, when Kennaway and Gilmore (1985) implanted sustained release melatonin sachets in 4 week-old ram lambs, they did not change the fleeceweight production by the treated animals at either 20 or 72 weeks of age, despite achieving enhanced levels of melatonin for 52 weeks.

Rouget *et al.* (1984) suggest that prolactin may be the main pituitary hormone controlling coat renewal whereas the effects of day length on ram fertility appear to be mediated through gonadotrophic hormones (Lincoln and Short, 1980). Thus, the failure of the present study to demonstrate a significant change in wool growth despite significant changes in testis size may not mean that seasonality of wool growth is not mediated by melatonin. The system controlling wool growth may be less sensitive to exogenous melatonin treatment compared to the system controlling seasonal reproduction. Furthermore, the time of melatonin treatment may also explain the discrepancy in the responses obtained. In the present study, melatonin was given during the transitional period from the non-breeding to the breeding season in rams of this breed. The exogenous melatonin administered may simply hasten the process of testicular recrudescence normally occurring prior to the onset of the breeding season.

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