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# Effect of melatonin immunisation on liveweight gain of red deer

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## ABSTRACT

Red deer calves (10 of each sex) were immunised at birth against a melatonin conjugate or injected with adjuvant only (10 of each sex). Booster injections were given on 5 occasions over the next two years and effects of immunisation on live-weight gain, pelage and reproductive changes were monitored throughout this period. Immunised stags were 7-10% heavier than their controls between 9 and 11 months of age and at 16 and 20 months of age and a similar, but not significant, effect was recorded for immunised hinds. Immunisation did not affect calving date of the hinds, or casting date and time of stripping of the antlers in the stags or pelage changes in deer of either sex. These results indicate that immunisation of red deer against melatonin can modify the seasonal pattern of liveweight changes during their first two years of growth.

**Keywords** Red deer; melatonin; immunisation; liveweight gain; growth

## INTRODUCTION

In red deer, seasonal changes in breeding activity, antler development, pelage, feed intake and growth are under photoperiodic control (Pollock, 1974; Kay and Staines, 1981; Webster and Barrell, 1985; Suttie and Simpson, 1985). It has been shown in sheep that the length of darkness determines the duration of the nocturnal secretion of melatonin from the pineal gland (Lincoln *et al.* 1981; Bittman *et al.* 1983; Kennaway *et al.* 1983) and thus changes in daily photoperiod entrain the timing of seasonal events via changes in the daily pattern of melatonin secretion (Arendt, 1985). Removal of the pineal gland, or disruption of its innervation (by removal of the superior cervical ganglia), abolishes the nocturnal rise of melatonin in sheep (Arendt *et al.* 1980; Lincoln *et al.* 1981; Bittman *et al.* 1983) and, although pinealectomised and ganglionectomised sheep remain seasonal, they tend to lose synchrony with entire animals and do not respond appropriately to artificially manipulated photoperiods (Lincoln, 1979; Barrell and Lapwood, 1979). Abolition of the daily pattern of melatonin secretion by active immunisation against melatonin has been attempted in mature rams (Lincoln and Almeida, 1981) and ewes (Arendt, 1986) but it did not have any effect on reproductive activity. However, the

consequences of such immunisation at an early age, possibly before the endogenous annual rhythms have become established, are not known.

This study investigated the effect of active immunisation of young red deer against melatonin on liveweight gain and seasonal antler, pelage and reproductive changes.

## MATERIALS AND METHODS

### Animals

The trial was conducted at the Research Farm, Lincoln College using 40 red deer calves (20 of each sex) born on the property during November and December 1985 (mean date of birth  $\pm$  SEM, 26 November 1985  $\pm$  9.9 d). Throughout the trial, animals were grazed on ryegrass/white clover pasture. They were weaned from their dams on 5 May 1986 and separated by sex into two mobs in February 1987. In March 1986 an immunised hind calf died after an accident and a control hind died from malignant catarrhal fever in January 1987.

### Immunisation Procedure

Within the first two days of birth calves were injected (s.c. in the frontal axilla) either with

melatonin conjugate (melatonin conjugated to rabbit gamma globulin, prepared by T. Stelmasiak and S. van Mourik) plus adjuvant mixture (10 of each sex, immunised) or with adjuvant mixture only (10 of each sex, controls). For immunised animals the melatonin conjugate was suspended in sterile 0.9% saline solution before being mixed with adjuvant (See Table 1).

### Measurements

Plasma samples were collected from all calves on 27 February and 23 May 1986 for estimation of melatonin binding activity in the plasma. Duplicate 100  $\mu$ l aliquots of plasma were incubated (either undiluted or diluted 1:100 in tricine buffer) at 10°C with 60 pg (2500 cpm) of tritiated melatonin ( $[^3\text{H}]$  melatonin, specific activity = 38.6 Ci/mmol, New England Nuclear, Boston). After 24 h, bound and unbound melatonin were separated by centrifugation with dextran coated charcoal and the bound melatonin tracer was counted. Non-specific binding (NSB) of the tracer was estimated using 100  $\mu$ l of sheep plasma and this varied from 1.6 to 2.9% of the total radioactivity.

Live weights were recorded on 21 February, 20 March, 5 and 28 April, 23 May, 19 August, 22 September, 6 November, 2 December, 1986; 5 March, 29 July, 1987 and 24 February, 1988. On 2 December 1986 the degree of moulting of the winter coat was recorded. Calves were scored from 1 to 5 with 1 representing an animal in full winter coat and 5 signifying a complete change to summer pelage. For stags, pedicle and antler development, the date of stripping of velvet in 1986 and of antler casting in 1988 were recorded. Hinds were run in a single mob with a red deer stag in March and April 1987 and subsequent calving dates were recorded.

### Statistical Analysis

Effects of treatment were examined using analysis of covariance and unpaired t-tests. Prior to analysis, live weights were transformed to their logarithms and date of birth was used as the covariate.

**TABLE 1** Melatonin immunisation schedule for red deer calves.

Date	Total volume (ml/dose)	Melatonin conjugate (mg/dose)	Immunisation mixture
At birth November/ December 1985	2.0	1	25 mg conjugate <sup>1</sup> 22 ml saline solution 22 ml sorbitan triolate <sup>2</sup> 6 ml Sontex <sup>3</sup>
20 February 1986	1.0	1	25 mg conjugate <sup>1</sup> 11 ml saline 11 ml sorbitan triolate <sup>2</sup> 3 ml Sontex <sup>3</sup>
16 June 1986	0.5	1	25 mg conjugate <sup>1</sup> 12 ml saline 12 ml Freund's incomplete adjuvant <sup>4</sup> 500 $\mu$ g muramyl dipeptide <sup>5</sup>
5 March 1987 29 July 1987 23 February 1988	2.0	1	25 mg conjugate <sup>1</sup> 25 ml saline solution 25 ml Freund's incomplete adjuvant <sup>4</sup>

<sup>1</sup> Immunised animals only.

<sup>2</sup> Polyoxythene 20 sorbitan triolate : sorbitan triolate (ratio 1:1); Sigma Chemical Co., St Louis, Missouri.

<sup>3</sup> Sontex, Marathon Marco Co., Texas.

<sup>4</sup> Freund's incomplete adjuvant, Gibco Laboratories, Grand Island, New York.

<sup>5</sup> N-acetyl muramyl-L-alanyl-D-isoglutamine-6-O-steroyl, Behring Diagnostics, La Jolla, California.

## RESULTS

### Melatonin Binding Activity of Plasma

Plasma from 11 of the 19 immunised calves sampled on 27 February bound significant amounts of the melatonin tracer (> twice NSB). Binding ranged between 5 and 26%. On 23 May 1986 plasma from 15 of the 19 immunised calves bound

between 7 and 33% of the tracer added. In all cases a 1:100 dilution of the sample reduced binding to within the range of the NSB. Plasma from four of the immunised animals (2 male and 2 female) did not bind melatonin on either occasion. There was no detectable binding of melatonin in plasma samples from control animals.

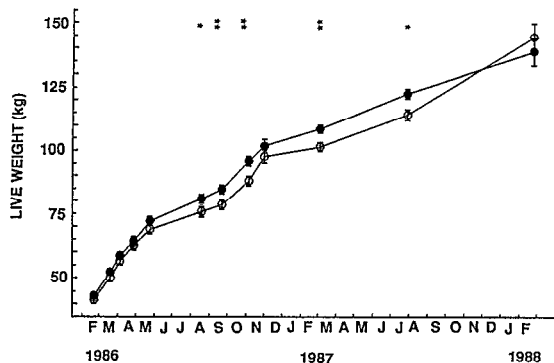


FIG. 1 Adjusted mean live weight of red deer stags immunised against melatonin (●) and non-immunised controls (○) recorded from 3 to 26 months of age. Vertical bars represent SEM.

\* significant difference for log transformed data.

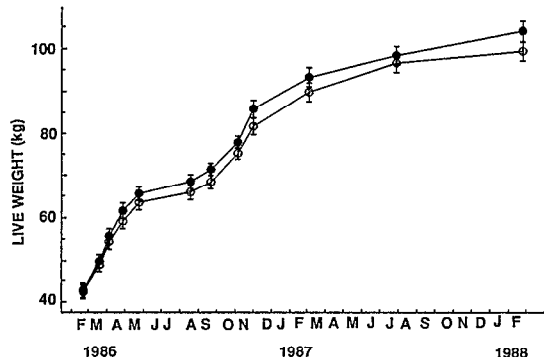


FIG. 2 Adjusted mean live weight of red deer hinds immunised against melatonin (●) and non-immunised controls (○) recorded from 3 to 26 months of age. Vertical bars represent SEM.

### Live Weight

Initially treatment and sex of the calf had no effect on live weight. However, by 20 March 1986 stag calves weighed significantly more than hinds (mean  $\pm$  SEM; males  $50.7 \pm 1.1$  v females  $48.7 \pm 1.0$  kg,  $P < 0.05$ ) and remained heavier throughout the trial. From 19 August until 6 November 1986 and on 5 March and 29 July 1987, immunised stags were significantly heavier ( $P < 0.05$ ) than the male controls (Fig. 1). The greatest between-treatment difference in mean live weight was recorded in November 1986 when immunised and control stags weighed  $95.5 \pm 1.01$  and  $87.4 \pm 1.02$  kg, mean  $\pm$  SEM respectively. Immunised hinds tended to be heavier than female controls (Fig. 2) but the difference in live weight was not

significant. Immunised animals which did not produce significant melatonin binding activity in response to immunisation (non-responders; 2 male and 2 female) had similar live weights to controls. Consequently, elimination of non responders from the immunised groups enhanced treatment differences so that immunised animals with significant binding activity in plasma were heavier ( $P < 0.05$ ) than their controls at most weighing dates for stags and on three occasions in the case of hinds.

### Pelage

On 2 December 1986, shedding of the winter coat was more advanced in stags than in hinds (mean pelage score  $\pm$  SEM,  $4.7 \pm 0.2$  and  $4.00 \pm 0.3$  for stags and hinds respectively,  $P < 0.01$ ). There was no effect of treatment on the time of shedding of the winter coat.

### Antler Development

In 1986, immunised and control stags initiated velvet stripping at similar times (17 November  $\pm$  12.7 d and 9 November  $\pm$  13.5 d; mean  $\pm$  SEM respectively) and in 1988 there was no difference in the date of antler casting (2 October  $\pm$  3.7 d v 30 September  $\pm$  2.4 d).

### Calving Date

All hinds conceived in 1987 although 2 of the calves arising from these conceptions (one from each treatment group) died as a result of calving difficulties. There was no effect of treatment on the mean date of calving (9 December  $\pm$  2.0 d v 7 December  $\pm$  3.4 d for immunised and control hinds respectively)

### DISCUSSION

In this study, active immunisation of red deer calves against a melatonin conjugate has altered the pattern of liveweight gain in the animals that responded to the treatment. Growth in red deer varies with season, feed availability and the age and sex of the animal (Mitchell *et al.*, 1976;

Fennessy, 1981; Moore *et al.*, 1988). As a consequence weight gain of farmed red deer is usually rapid during spring and summer but slows down in winter (Fennessy *et al.*, 1981; Adam and Asher, 1986; Moore *et al.*, 1988) when voluntary intake is decreased, probably in response to the shorter daily photoperiod (Pollock, 1974; Simpson *et al.*, 1984). It is of note that in the present study the effect of immunisation did not appear until the first winter. This is when the duration of melatonin secretion at night is long and any perturbation of the melatonin secretion profile might be expected to have its greatest effect on 'short day' photoperiod-dependent effects.

The fact that elimination of the non-responders (deer without appreciable melatonin binding activity in plasma) from the immunised group of deer has enhanced the differences in live weight between immunised and control deer provides support for the view that the effect was due to the production of anti-melatonin antibodies in the responders. In spite of this it was not possible to demonstrate the presence of high antibody titres against melatonin in plasma even at a 1:100 dilution. The present results are in contrast with those of Lincoln and Almeida (1981) where active immunisation of mature Soay rams against melatonin-bovine serum albumin conjugate resulted in high antibody titres (1:1600 to 1:32000 at 50% binding of 10 pg [<sup>3</sup>H] melatonin) in all animals. Presumably the procedure used in the present trial to estimate binding activity in plasma was unable to quantify antibody titre adequately or samples were taken at inappropriate times, so it is possible that substantial antibody production did occur at some occasions during the study.

In sheep (Kennaway *et al.*, 1982; Nett and Niswender, 1982; Arendt *et al.*, 1983; Lincoln and Ebling, 1985) and deer (Bubenik and Smith, 1985; Adam and Atkinson, 1984; Webster and Barrell, 1985; Fisher *et al.*, 1988) administration of melatonin or provision of 'short days' advances the onset of autumnal events such as loss of appetite, decline in growth rate and testicular development or oestrous activity, shedding of antler velvet and moulting of the summer coat. If immunisation against melatonin prevented the deer from perceiving the natural autumnal decrease in

photoperiod then the associated decline in appetite and growth rate may have been delayed which would provide the major explanation for the heavier live weight of the immunised animals recorded here. This is similar to what could be expected following surgical modifications such as pinealectomy or superior cervical ganglionectomy. Immunisation against melatonin did not affect the reproductive seasonality of Suffolk-cross ewes (Arendt, 1986) or Soay rams (Lincoln and Almeida, 1981) maintained under natural photoperiods. Furthermore pinealectomised or ganglionectomised sheep retained their annual rhythms (Lincoln, 1979; Kennaway *et al.*, 1981), although the first seasonal cycle was delayed in sheep modified prepubertally (Lincoln and Almeida, 1981; Kennaway, 1984; Ebling *et al.*, 1988). Similarly, when adult white-tailed deer were pinealectomised, changes in their seasonality were minor (Brown *et al.*, 1978) yet when the pineal gland was removed from young white-tailed deer, antler, testicular and pelage changes were delayed by several months (Brown *et al.*, 1978; Snyder *et al.*, 1983). Ganglionectomy of immature red deer stags also delayed the cycle of antler development during the following season although its timing returned to normal in subsequent years (Lincoln, 1985). The liveweight patterns of animals in these studies were not reported. On balance these results suggest that disruption of the melatonin pattern, whether by immunisation against melatonin or surgical modification, would be expected to have little effect on the seasonality of adult animals but that it may alter the annual cycles of immature animals possibly by interfering with the entrainment the endogenous rhythms to the natural photoperiod. The results of the present study indicate that this view may hold for immature red deer immunised against melatonin; at least for live-weight patterns.

In this study disruption of the melatonin signal, possibly from an early age, may have prevented the entrainment of endogenous annual rhythms in feed intake and growth to the appropriate photoperiod without affecting the seasonal antler, pelage and reproductive changes rhythms in the same way. This apparent dichotomy is difficult to explain but it is possible to conjecture that

melatonin must influence growth and reproduction by separate neurohormonal pathways or that different thresholds of sensitivity to melatonin secretion are involved.

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