The effect of long-day photoperiod treatments on plasma prolactin and wool follicle activity in New Zealand Wiltshire sheep

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ABSTRACT

Exposure of NZ Wiltshire sheep to a long-day photoperiod during late winter/spring has previously been shown to alter wool follicle growth patterns. The present experiment was undertaken to evaluate the plasma prolactin and wool follicle response to treatment with either 1, 2 or 3 months of long days.

Four groups of six, NZ Wiltshire ewes were subjected to long-day photoperiod (16L:8D) for 1 month (LD1), 2 months (LD2), 3 months (LD3) or natural photoperiod (control), commencing on the 3 August, after which they were released from light treatment into natural (indoor) spring daylength. Weekly jugular blood samples were assayed for prolactin (PRL) and skin biopsies were histologically assessed for wool follicle activity.

Circulating PRL concentrations were low in all treated and control sheep at the start of the experiment (19 ± 2 ng/ml). In treated sheep mean PRL concentration increased ($P<0.05$) above control levels (8 ± 1 ng/ml) within 1 week after exposure to long days (52 ± 4, 51 ± 5 and 55 ± 2 ng/ml for LD1, LD2 and LD3, respectively). PRL concentrations remained significantly higher than controls ($P<0.05$) for the duration of each treatment reaching maxima of 148 ± 27 ng/ml, 180 ± 43 ng/ml, and 199 ± 33 ng/ml for LD1, LD2 and LD3 respectively. In LD2 and LD3 treatments, PRL concentrations declined to control levels by 3 and 2 weeks after treatment had ceased, respectively, but in the LD1 treatment PRL remained significantly above ($P<0.05$) control levels until between 2 and 3 months after treatment. Primary follicle activity (PFA) decreased in response to long-day treatment ($P < 0.01$) reaching minima of 47 ± 9%, 36 ± 6%, and 33 ± 11% in LD1, LD2, LD3 sheep, respectively. Secondary follicle activity (SFA) responses were most marked in the LD2 and LD3 treatments where they fell below ($P < 0.05$) control levels reaching minima at 2 months into treatment (66 ± 15% and 58 ± 13% for LD2 and LD3, respectively). Both PFA and SFA increased to over 90%, within 1 month after release into natural light, in all treatments groups (except PFA for the LD1 group which took 2 months after release to reach over 90%).

In support of previously reported trials an increasing level of plasma PRL was followed by a decline in follicle activity. The optimal time frame to achieve an effective synchronised follicle regression was between 2 and 3 months of long-day treatment. The long-day treatment system produced a complete follicle growth cycle in temporal synchrony and is therefore a potential model for the investigation of structural and biochemical processes surrounding transitions between active and resting stages of fibre growth.

Keywords: long-day photoperiod; Wiltshire sheep; prolactin; wool follicles.

INTRODUCTION

The photoperiodic signal that modulates the seasonal pelage cycles in many mammals (Morris, 1961; Ryder, 1978) is transduced via the endocrine system (Johnson, 1981). In particular, seasonally varying levels of prolactin (PRL) are closely associated with changes in follicle growth patterns in a number of species including sheep (Smale *et al.*, 1990; Lincoln 1990). Of the sheep breeds available in New Zealand, the Wiltshire, which originated from the Wiltshire Horn of Great Britian, exhibits a highly seasonal fleece growth pattern (Slee, 1965; Parry *et al.* 1991). The winter decline in wool growth in this breed results from a high proportion of follicles entering a resting stage followed by a recrudescence in fibre growth which culminates in a late spring moult (Ryder, 1969; Parry *et al.*, 1991). The New Zealand Wiltshire genotype has recently been characterised as a model for the study of follicle growth processes (Parry *et al.*, 1991).

In a previous trial NZ Wiltshire sheep were exposed to a 6 month short-day (8L:16D) treatment period from mid-winter followed by exposure to natural midsummer long-days (16L:8D). Within 4 weeks of the short-day - long-day transition a synchronised decline in wool follicle activity occurred (Pearson *et al.*, 1992). A second treatment regime involving exposure to 3 months of long-days (16L:8D) from late winter was subsequently demonstrated to induce the same effect (Parry *et al.*, 1993). Both treatments caused growing wool follicles to enter into the regressive catagen phase of the growth cycle. The long-day treatment however was shorter, easier to apply and more cost effective than a 6 month period of short-day preconditioning. The present study aimed to further develop the use of long-days applied in late winter to initiate a wool follicle growth cycle. The effectiveness of three different long-day treatment durations in altering both circulating PRL and wool follicle activity in NZ Wiltshire sheep were examined.

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METHODS

Sampling and Measurements

Twenty four, one year old non-pregnant New Zealand Wiltshire ewes were randomly allocated to 4 groups (n=6). Each group was housed indoors from the 3 August, 1992 under either natural photoperiod (control) or an artificially extended long-day photoperiod (16L:8D) with the use of incandescent electric lighting. The treatment groups were exposed to either 1 month (LD1); 2 months (LD2); or 3 months (LD3) of the long-day photoperiod. Following treatment, the sheep were released into the natural photoperiod, but kept indoors for 10 (LD1), 6 (LD2) and 6 (LD3) weeks after which they grazed pasture outdoors. Indoors the sheep were fed a diet of lucerne pellets (480g/hd/day) and meadow hay *ad libitum*.

Skin samples were taken as snip biopsies (Parry *et al.*, 1992) on the right midside of all sheep at 3-weekly intervals, with more intensive skin sampling occurring at strategic times during and after long-day treatments. The skin was fixed in 10% phosphate buffered formalin, wax embedded, sectioned and stained by the 'Sacpic' method (modified from Auber, 1952). Each skin sample was examined and assessed for the proportion of active primary follicles (PFA) and active secondary follicles (SFA) in transverse section (Nixon, 1993).

Blood was collected weekly during the light treatments and then subsequently for 4 weeks (LD1) or 6 weeks (LD2, LD3, Controls) post-treatment, after which monthly samples were taken until the 14 December (LD1, LD2) or until the following July (LD3, Controls). Blood samples (10 ml) were collected at 11 am by jugular venipuncture into EDTA-Vacutainers (Becton Dickinson, New Jersey, USA.). The blood was centrifuged and the plasma stored at -20° C until PRL assay was performed in duplicate by radioimmunoassay.

Statistical Analysis

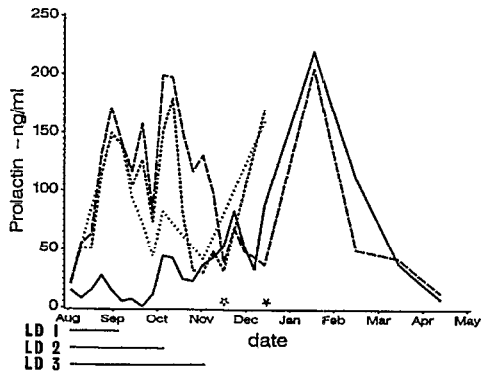
The treatment effects on PFA, SFA and PRL concentrations (log10 transformed) were examined using general linear models with repeated measures (GLM procedure, SAS Institute, USA).

RESULTS

Prolactin

Circulating PRL concentrations were low in all treated and control groups at the start of the experiment (19 ± 2 ng/ml). In control animals PRL increased in early October and reached a summer peak in January (220 ± 31 ng/ml) after which PRL declined to levels similar to the previous August (Figure 1). In all treated sheep (Figure 1), PRL increased ($P<0.05$) above control levels (8 ± 1 ng/ml) within 1 week after exposure to long days (52 ± 4, 51 ± 5 and 55 ± 2 ng/ml for LD1, LD2 and LD3, respectively). PRL concentrations remained significantly higher than controls for the duration of treatment, reaching maxima of 148 ± 27 ng/ml, 180 ± 43 ng/ml, and 199 ± 33 ng/ml for LD1, LD2 and LD3, respectively. In LD1 sheep, PRL concentration declined after treatment but remained significantly above ($P<0.05$) control levels until between 2 and 3 months after treatment had ceased. In LD2 sheep, PRL concentration declined to control levels within 3 weeks after termination of light treatment (Figure 1). PRL concentrations in both the LD1 and LD2 groups increased significantly above control levels ($P<0.05$) in mid-December when both groups were transferred from an indoor to an outdoor environment, after which sampling ceased for these groups (Figure 1). In the LD3 group, PRL concentrations declined to control levels within 2 weeks after treatment and remained similar to control levels thereafter, so that both controls and LD3 reached summer maxima in January before declining rapidly in late summer and autumn (shortening days) (Figure 1).

FIGURE 1: Mean plasma prolactin levels (ng/ml) in long day (LD) treated (LD1 · · ·, LD2 - - - -, LD3 — — —) and control (_____) sheep. Treatment duration is shown by horizontal bars at the base of the graph. Stars on the graph show release dates from an indoor to an outdoor environment, for LD1 and LD2 (open) and LD3 and Controls (solid).



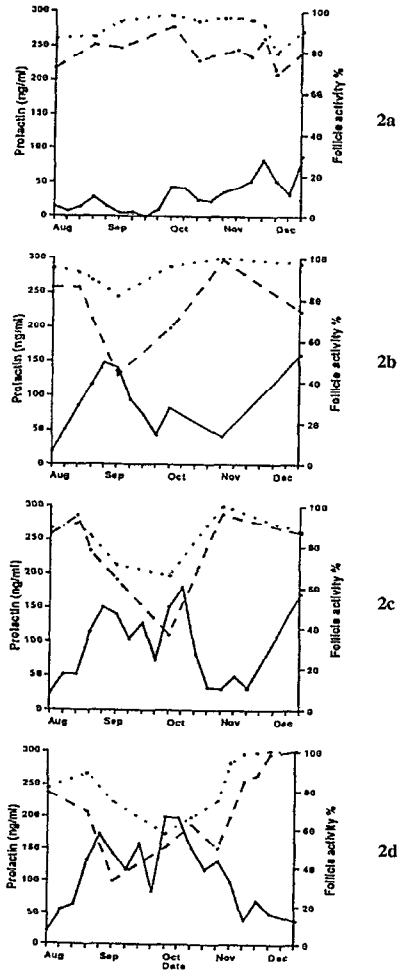
Follicle Activity

Mean follicle activity in the control group was generally high during the 3 month trial period (Figure 2a) and ranged between 73 ± 10 % and 93 ± 3 % for PFA and 87 ± 5 % and 97 ± 1 % for SFA. Both PFA and SFA had increased to near 100% by February and remained at that level until the end of the trial.

During the LD1 treatment, PFA declined ($P<0.05$) below control levels, reaching a minimum of 47 ± 9 % at the end of treatment in September (Figure 2b). One month after treatment (in October), PFA increased to 66 ± 7 % remaining lower than controls ($P<0.05$) then rose above ($P<0.05$) control levels one month later (99 ± 4% vs 80 ± 4% for LD1 and control). Mean SFA in LD1 sheep, did not fall significantly below control levels at any time during or after treatment.

PFA in both LD2 and LD3 groups declined below control PFA ($P<0.05$) to less than half of pre-treatment levels. Mean minimum values occured at 2 months (37 ± 6%, LD2) and 1 month (33 ± 11%, LD3) into treatment (Figures 2c, 2d). SFA also declined below control SFA in both groups ($P<0.05$) but was consistently above PFA. Mean minimum SFA occured in October in both groups and was 66 ± 15% and 58 ± 13% for LD2 and LD3, respectively. Mean PFA and SFA in the LD2 group reached over 90% by 1 month after treatment then declined to 86 ± 4 and 87 ± 6, respectively, which was not significantly different from control levels. In the LD3 group, both PFA and SFA began to increase between 2 and 3 months into treatment, reaching control levels by 2 weeks after the end of treatment.

FIGURE 2: Mean plasma prolactin (——), primary follicle activity (— — —) and secondary follicle activity (· · · ·) for Control (2a), LD1 (2b), LD2 (2c) and LD3 (2d) sheep.



DISCUSSION

The effectiveness of different photoperiod treatments in the present study was evaluated on two criteria. The first was based on the ability to induce a change in follicle activity and the second on the degree of synchronisation within and between follicle types and between animals. An experimentally induced, synchronised follicle growth cycle has application in the study of the biochemical processes underlying the control of wool growth.

The long-day treatments were applied in August after the mid-winter period of follicle inactivity (Parry *et al.* 1991). All long-day treatments were effective in altering PRL and follicle activity. In all treatment groups, a higher proportion of primary than secondary follicles entered the regressive stage, as reflected in the low percentage of active primary follicles. Both the LD2 and the LD3 treatments produced a synchronised regression in both primary and secondary follicles, whereas in LD1 animals, only primary follicles showed a significant regression. During LD1 treatment, SFA declined in only 2 of the 6 animals to a minimum of 82±10%. Therefore, 1 month of long-day treatment appears to be too short to cause a marked effect on the secondary follicle population. The increase in follicle activity before the end of the LD3 treatment, suggests that the optimal length for long-day treatment is between 2 and 3 months.

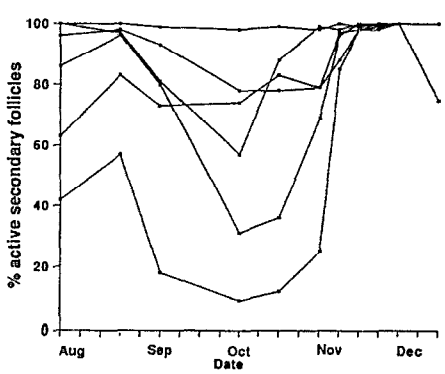
The control animals showed the characteristic seasonal PRL secretion pattern with low concentrations in winter, increasing in October to a summer peak in January. Circulating PRL increased rapidly in response to long day treatment, as found in previous studies with the NZ Wiltshire (Parry *et al.*, 1993) and Soay sheep (Ryder & Lincoln, 1979). Maximal PRL concentrations during treatment in long-day treated animals were similar to those found in control animals later in mid-summer.

Plasma prolactin appeared to increase following the transfer from an indoor to an outdoor environment (in LD1 and LD2 groups on November 16 and in the LD3 and Control groups on December 14). Since the photoperiod was not altered, the changed diet (from pellets to pasture) or an increased light intensity or ambient temperature could have been factors contributing to this increase. It is known, for example, that increasing ambient temperature stimulates ovine prolactin secretion (Bell *et al.*, 1989). The change to outdoor grazing may have also caused a shift in the seasonal prolactin maximum, from December (Pearson *et al.*, 1992) to late January, in the Control and LD3 groups. In a variety of sheep breeds grazing pasture, the seasonal peak of prolactin has been reported in the month preceeding the summer solstice (Lincoln, 1990) which is in accordance with our earlier trials involving NZ Wiltshires housed indoors under natural photoperiod (Pearson *et al.*, 1992; Pearson *et al.*, 1993a).

Parry *et al.* (1993) and Pearson *et al.* (1993a) have suggested that a decline in follicle activity is associated with an increase in levels of plasma PRL. In addition, Pearson *et al.* (1993b) have also shown that active fibre growth was maintained, and follicle regression delayed, by the use of bromocryptine to suppress the PRL surge normally associated with the release of short-day treated sheep into long-days. A similar association between follicle regression and an increase in PRL was seen in the present study, in all 3 treatments.

Considerable variation was found in the follicle activity response between animals within the same treatment group. The differences in SFA between individuals in the LD3 groups were evident in the amplitude but not the timing of the follicle regression (Figure 3). Individual variation in the amplitude of response is likely to reflect genetic variation associated with an infusion of Poll Dorset genes in the Wiltshires used in this study (Parry *et al.*, 1991). The Dorset influence is likely to reduce the high degree of seasonality of wool growth which is characteristic of the Wiltshire and, by implication, also the sensitivity of the wool follicles to changes in seasonally responsive hormones.

FIGURE 3: Percentage of active secondary follicles for individual sheep, treated with 3 months long days from 3 August to 2 November.



CONCLUSIONS

This study has demonstrated that a late winter long-day photoperiod is a suitable technique for the study of an induced wool follicle growth cycle. The treatment is easily applied, inexpensive and avoids the use of pharmaceuticals with possible side effects. The optimal time to achieve an effective synchronised follicle regression is between 2 and 3 months of long-day treatment. The long-day treatment produces a complete follicle growth cycle within a short time frame and is therefore suitable for studies of both catagen and proanagen phases of the follicle growth cycle and the role of PRL in seasonal fleece growth processes.

There was a clear association between raised PRL concentrations and follicle regression, though it is yet to be shown whether or not this is a direct cause and effect relationship. Individual variation in the amplitude of response, indicates that Wiltshires may also provide a valuable model for studies of the genetic basis of variation in seasonal follicle growth.

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