The effect of manipulation of plasma prolactin concentration on cashmere growth in spring

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ABSTRACT

Domperidone was administered to cashmere goats at 2.5 mg/day either by a daily subcutaneous injection at 1000 hours (INJ, n=6) or by a subcutaneously fitted osmotic minipump (OSP, n=6). There was an untreated control group (C, n=5). The treatments commenced on 13 September 1991 and continued for 14 days.

Daily plasma prolactin concentrations at 1200 hours measured across the 14 days of treatment were 612±32, 73±35, and 60±34 ng/ml in INJ, OSP and C groups respectively (P<0.001), while daily plasma PRL concentrations at 1000 hours, immediately prior to the injection, were 33±6, 72±7 and 60±8 ng/ml in INJ, OSP and C groups respectively (P<0.01).

Mean downlength was lower in INJ goats (34±4 mm) than OSP goats (51±4 mm) and C goats (46±4 mm). A greater percentage of fleece by weight was shed earlier (P<0.05) in INJ compared to the C group. Primary follicle activity also increased earlier (P<0.001) in INJ goats when compared to C and OSP goats. There was no effect of OSP on fibre growth and no overall effect of INJ on guard hair length, shedding score, presence of newly erupted cashmere fibres, and secondary follicle activity.

In conclusion, DOM administered at 2.5 mg/day for 14 days by injection, but not by osmotic pump, perturbed fibre growth in primary and not secondary follicles.

Keywords: Domperidone; subcutaneous injection; osmotic minipump; prolactin; cashmere; follicle activity.

INTRODUCTION

The fleece of the cashmere goat grows and is shed in an circannual growth cycle which is governed by photoperiod (Ryder, 1966; McDonald *et al.*, 1987). During the winter short photoperiod, the fleece of the cashmere goat is composed of long outer-coat guard and down fibres. In summer, the fleece is shorter and the down fibres are no longer detectable by eye (Nixon *et al.*, 1991a). The summer fleece grows and the winter fleece is shed during spring. In animals such as mink and hamsters, the shedding of the winter and the growth of the summer pelage is induced by an increase in plasma prolactin (PRL) concentration (Badura and Goldman, 1992; Martinet *et al.*, 1992). An increase in plasma PRL concentration is also associated with shedding of the winter fleece in cashmere goats (Lynch and Russell, 1990; Kloren *et al.*, 1993).

Release of PRL from the caprine anterior pituitary is primarily controlled by the PRL-inhibiting hypothalamic hormone, dopamine. D2 dopamine receptor antagonists, such as domperidone (DOM), elevate plasma PRL concentrations in ruminants (Milne *et al.*, 1990).

The first objective of this experiment was to develop techniques for the elevation of plasma PRL concentrations, in cashmere goats, using domperidone (DOM). Second, it was to determine whether a 14 day elevation in plasma PRL concentration would advance winter fleece shedding and summer fleece regrowth.

METHODS

Animals

Seventeen, mixed-aged wether goats (mean liveweight 33 kg) were housed indoors, under natural light, in pens of either 3 or 5 individual goats. The goats were fed 250 g per goat of maize between 0800 and 0900 hours daily with meadow hay and water on offer *ad libitum*. The experiment was conducted at the Flock House Agricultural Centre, Bulls, New Zealand and treatments commenced on 13 September 1991 and continued for 14 days.

The goats were randomly allocated to three treatment groups. DOM (Catalogue No. B8910, Sigma Chemical Company, St Louis, Mo, USA) was administered to groups of six goats, at a rate of 2.5 mg/goat/day, either by injection (INJ) or osmotic (OSP) pump (Model Number 2 ML 2, Alzet osmotic minipumps). There was an untreated control group (C) of five goats. DOM, in a 1 ml diluent of dimethylsulfoxide, was injected subcutaneously, daily at 1000 hours, into the anterior neck of INJ goats. Osmotic pumps (5 µl/hr) containing 35 mg of DOM, in 2 ml of dimethlysulfoxide, were placed subcutaneously on the inside of the left back leg of OSP goats and removed after 14 days.

Measurements

Fibre growth was measured at two weekly intervals by a variety of methods commencing on 12 September 1991 and continuing until 07 November 1991. The mean length of

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cashmere and guard hair was measured and calculated according to the procedures of O'Neill et al. 1992). The amount of fibre lost from the fleece at each sampling time (shedding) was assessed by both combing and visual scoring. A hand comb was drawn horizontally and then vertically through the fleece in a single pass over the whole left side of the goat and the shed fibre weighed. Visual scoring of shedding was assessed by plucking the fleece on the right side of the goat. The amount of plucked fibre was scored from 1 (no shedding) up to 5 (large amount of shedding).

Skin snip biopsy samples were taken at two weekly intervals from the left midside region of the goat. The fibre on the each skin sample was trimmed to 5 mm of length. These samples were then viewed under a dissecting microscope and scored for the presence of the intact cashmere fibre tip as follows: 1=newly erupted down fibres (NEDF) (down fibres of less than 3 mm and characterised by an arrow-like tip) and long cashmere (fibre in which the tip exceeds 5 mm and hence was cut); 2=NEDF only; 3=NEDF and short cashmere (fibres 3 to 5 mm with arrow-like tip); 4=short cashmere only; 5=short and long cashmere; 6=long cashmere only.

The skin samples were processed and embedded, epidermal surface uppermost, in paraffin wax. Each wax block was cut into serial 8 μm transverse sections and stained using an adapted Sacpic method (Nixon, 1993). Approximately 10 follicle groups containing both guard hair (primary) and down producing (secondary) follicles were scored according to the characteristics of the outer root sheath (Nixon, 1993). The follicles were scored for two stages of the fibre growth cycle; anagen (follicle actively growing), telogen (no growth).

Catheters were fitted to a jugular of goats on the 12 September and blood samples were drawn at 1000 hours, immediately prior to the DOM injection on treatment days 2, 3, 9, 10, 11 and 14 and at both at 1000 and 1200 hours on days 1, 4, 5 to 9, and 13. After the treatment period, blood samples continued to be collected at 3 weekly intervals by venous jugular puncture at 1100 and 1300 hours until the 10 November 1992. Plasma was separated from blood by centrifugation and plasma samples were frozen at -8°C pending assay for PRL concentration.

The radioimmunoassay of PRL was conducted using ovine PRL (NIDDK-OPRL-1-2) for standards and radioiodination, and ovine PRL antiserum (NIDDK-anti-OPRL-2). PRL was iodinated by the Iodogen technique (Pierce, Rockford, IL) using [^{125}I]-iodide (New England Nuclear NE0033A). Separation of antibody-bound from free labelled PRL was by second antibody precipitation using excess goat antirabbit serum (SAR 265 generated at Ruakura Agricultural Centre). The assay was validated for caprine samples. Sensitivity was 0.6 ng/ml and assay range was up to 100 ng/ml. Intra-assay and inter-assay coefficients of variation at 32 ng/ml PRL concentration were 12.1% and 14.4% respectively.

Statistical Analysis

Because of unequal variances plasma PRL concentrations were $\log_{10}$ transformed prior to statistical analysis. Daily PRL data and fibre growth measurements were statistically analysed using the GLM procedure (SAS, 1987) for a split plot design with repeated measures in time using the Wilks' Lambda test of significance. Pretreatment values were fitted as a covariate. Comparisons of individual treatments were made using orthogonal contrasts. The association between vellus score and mean downlength was determined by accumulating data across sample times and conducting an analysis of variance using GLM procedures of SAS (SAS, 1987) with vellus score fitted as the treatment variable. Data in the text are presented as ls means and ls stderrs.

RESULTS

Mean daily plasma PRL concentrations, in samples collected two hours after the injection of DOM (Figure 1), were greater (P<0.0001) in INJ (612±32 ng/ml) compared to C goats (60±34 ng/ml) and OSP goats (73±35 ng/ml). However 24 hours after the injection of DOM (Figure 2) mean daily plasma PRL concentrations in INJ goats (33±6 ng/ml) had dropped to below that of the C (60±8 ng/ml) and OSP (72±7 ng/ml) goats (P<0.01). Observation of the pumps after removal from the goats showed that the dimethyl sulphoxide had been discharged from the pump at the expected rate. There was no effect of either DOM treatment on plasma PRL concentrations in the post-treatment period.

FIGURE 1: Plasma prolactin concentration at 1200 hours in control goats (C) and goats treated with domperidone at 2.5 mg/goat/day for 14 days by daily injection at 1000 hours (INJ) or by osmotic pump (OSP). Solid bars are LSD values.

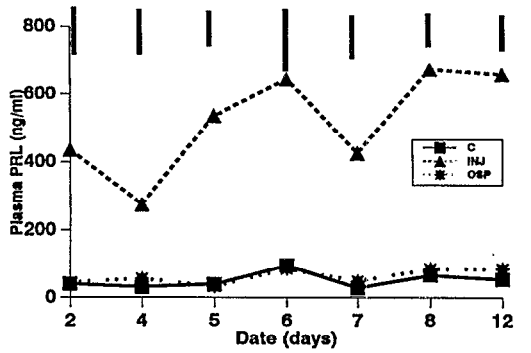

FIGURE 2: Plasma prolcatin concentration at 1000 hours immediately prior to treatment in control goats (C) and goats treated with domperidone at 2.5 mg/goat/day for 14 days by daily injection at 1000 hours (INJ) or by osmotic pump (OSP). Solid bars are LSD values.

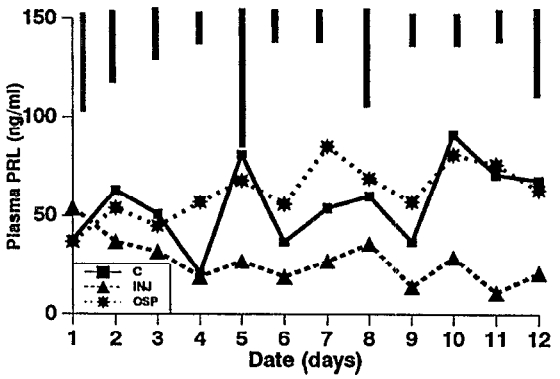

TABLE 1: Fibre Growth (12.09.91 means±se, 26.09.91-07.11.91 ls means±ls se), in control goats (C) and goats treated with 2.5 mg/day of domperidone by injection (INJ) or osmotic pump (OSP) from 13 September until 26 September 1991.

	Fibre Measurement Date				
	12.09.91	26.09.91	10.10.91	24.10.91	07.11.91
Down Length (mm)					
C	67±9	73±8	46±8	31±4^{b}	16±10^{ab}
INJ	68±9	53±7	47±6	27±3^{b}	11± 9^{a}
OSP	71±7	61±7	64±6	54±3^{a}	37± 8^{a}
Percentage of combed shed fibre (%)					
C	49±1	24±8	12±4^{b}	12±5	8±5
INJ	28±3	28±7	29±4^{a}	11±5	4±4
OSP	26±7	23±7	19±4^{ab}	14±5	18±5
Shedding score					
C	1.2±0.4	2.8±0.4^{b}	3.2±0.8	2.1±0.5	1.6±0.5
INJ	0.9±0.2	4.4±0.4^{a}	3.5±0.7	2.5±0.5	1.3±0.4
OSP	0.9±0.4	2.5±0.4^{b}	1.7±0.7	1.9±0.4	1.9±0.4
NEDF score					
C	4±1	4.0±0.9	3.6±0.9	2.6±0.9	2.8±0.9
INJ	6±0	4.5±0.9	2.0±0.9	2.3±0.8	2.2±0.8
OSP	6±0	2.9±0.9	3.2±0.8	3.2±0.8	3.5±0.8
Primary follicle activity (%)					
C	21±10	27±7	27±9^{b}	60±8^{a}	60±12
INJ	11±3	30±7	68±8^{a}	53±8^{a}	68±11
OSP	13±5	22±6	35±8^{b}	33±7^{b}	53±11
Secondary follicle activity (%)					
C	19±10	25±8	21±8	29±8	22±6
INJ	20±9	30±6	17±7	18±8	24±5
OSP	17±6	13±6	29±7	19±8	18±5

Values with different superscripts in each column within each fibre measurement trait are significantly different at the 5% level.

Mean down length, over the entire measurement period, was lower in INJ goats (34±4 mm) than OSP (51±4 mm, P<0.01) and tended to be lower than C goats (46±4 mm, P=0.07). This was consistent trend on all measurement dates (Table 1). There was no effect of treatment on guard hair length.

Overall, treatment with DOM had no effect on the weight of fibre shed from the fleece. However, INJ goats tended to shed their fleeces earlier than C goats (P<0.05) and OSP goats (P=0.07)(Table 1). Shedding scores followed a similar pattern but treatment differences were not significant (Table 1).

Treatment with DOM had no effect on secondary follicle activity or the time at which NEDF's were identified. The appearance of NEDF's was associated with a reduction in mean down length (P<0.001). Primary follicle activity was higher in INJ goats than OSP goats (P<0.05). There was also an treatment interaction with time; primary follicle activity increased more quickly in INJ goats than C goats (P<0.01) and OSP goats (0.05) (Table 1).

DISCUSSION

This study has demonstrated that DOM administered as a daily injection can be used to manipulate plasma prolactin concentration in cashmere goats during spring. The osmotic pump, however, was ineffective at elevating plasma PRL concentration. DOM, administered via the osmotic pump, may either have reached the caprine pituitary in concentrations too small to illicit a response, or the DOM could have come out of solution inside the osmotic pump.

Other studies also have demonstrated that DOM injections can effectively elevate circulating concentrations of PRL in sheep (Thomas *et al.*, 1989; Craven *et al.*, 1993) and in deer (Milne *et al.*, 1990). In deer it was found that the effectiveness of DOM injections had declined at 2 weeks of treatment (Milne *et al.*, 1990). In this study, there was no evidence of a decline in effectiveness of DOM injections after 2 weeks of treatment.

In a previous study involving cashmere goats in summer, a 2.5 mg injection of DOM elevated plasma PRL concentration to 1000 ng/ml 2 hour post-injection in summer (Litherland *et al.*, 1992). In this spring study, the mean elevation in plasma prolactin concentration was only 600 ng/ml. Dopamine receptor sensitivity is affected by season (Devesa *et al.*, 1988; Loudon and Brinklow, 1990) and may be the cause of the differences between these studies.

A single injection of DOM administered in spring, resulted in a complex series of changes in plasma PRL concentration. Immediately following the injection, plasma PRL concentrations were elevated beyond normal goat summer values of 60 to 200 ng/ml (Buttle 1973; Maeda *et al.*, 1986). Subsequently plasma PRL concentration dropped to below that of normal spring concentrations. This fall in plasma PRL concentrations are likely to be due to a feed-back response by the hypothalamus resulting in increased secretion of the prolactin-inhibiting hormones (Greef and van der Schoot, 1985; Lamberts and MacCleod, 1990) and by directly reducing the secretion rate of PRL from the pituitary (Bentley and Wallis, 1987).

This treatment regime, applied in summer to cashmere goats, had no effect on plasma concentrations of growth hormone, insulin and thyroxine (Litherland *et al.*, 1992).

It was expected that an elevation in plasma PRL concentration in spring would advance the shedding of the winter fleece of the goat. By the start of the experiment in September, plasma PRL concentrations had already increased to concentrations ranging between 20 and 80 ng/ml and follicle activity was low. Primary follicles became activated 2 weeks earlier in response to the DOM injections. There was an associated shedding of guard hairs.

In this experiment, the diurnal cycle of plasma PRL concentration was initially increased and then decreased by single DOM injection. In cashmere goats, treated with melatonin in September, plasma PRL concentrations were suppressed and primary follicles were activated by 24 days of treatment (Nixon *et al.*, 1991b). As in this experiment, secondary follicle activity varied considerably during September. The similarity of the response in these two experiments might imply that the DOM injected goats were responding to the reduction in plasma PRL concentration rather than the increase.

Cashmere goats which had been treated with bromocryptine to suppress plasma PRL concentrations from July to December, shedding of secondary follicles was delayed but primary follicle growth was unaffected (Kloren *et*

al., 1993). It is clear that the state of the follicles at the time of treatment is crucial to the treatment response. *In vitro*, actively growing secondary cashmere follicles responded to increasing dose rates of PRL by increasing fibre length growth rate (Ibraheem *et al.*, 1993). The failure of the secondary follicles to respond to the DOM injection in this experiment could be due to the complex changes in plasma PRL concentrations, the lack of synchronisation of the secondary follicles or the short duration of the treatment.

Secondary follicle activity, during the measurement period, was more variable than primary follicle activity between and within individual goats. Consistently, there was the rapid reduction in down length associated with the presence of NEDF's.

In conclusion, the results from this study show that DOM administered at 2.5 mg/day for 14 days by injection, but not by osmotic pump, perturbed fibre growth in only primary follicles.

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