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*J Anim Sci* 2003. 81:279-284.

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# Effects of prolactin administered to a perfused area of the skin of Angora goats<sup>1,2</sup>

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**ABSTRACT:** It is suspected that prolactin may affect mohair growth; therefore, effects of infusing prolactin on mohair growth were investigated using a skin perfusion technique. Seven Angora wethers (average body weight,  $30 \pm 3$  kg) were implanted bilaterally with silicon catheters into the superficial branches of the deep circumflex iliac artery and vein. For the first 14 d of the experiment, animals were infused (2.4 mL/h) with prolactin (one side) or control (other side) into the deep circumflex iliac arteries. The infusion rate of prolactin was 2.21 mg/d and was calculated to triple prolactin blood concentration in the perfused region. The area of skin supplied by the deep circumflex iliac artery was approximately 240 cm<sup>2</sup>. Two weeks after the cessation of infusions, 100-cm<sup>2</sup> areas within the perfused regions were shorn to determine mohair growth. Greasy and

clean mohair production was decreased ( $P < 0.05$ ) by prolactin compared with control (3.79 vs 4.62 and 3.02 vs 3.67 g/[100 cm<sup>2</sup> × 28 d], respectively). Oxygen saturation in blood hemoglobin from the deep circumflex iliac veins was greater ( $P < 0.02$ ) on the side infused with prolactin than on the control side (75.1 vs 68.2%). Higher concentrations of methionine, lysine, valine, isoleucine, and leucine were observed in blood of the deep circumflex iliac vein on the side infused with prolactin vs that infused with control ( $P < 0.05$ ). In conclusion, direct skin infusion with prolactin decreased mohair fiber synthesis by the skin and may have concomitantly lessened oxygen consumption. Thus, effects of increasing prolactin concentration approximately two-fold in the skin on mohair fiber growth may not be limited to simple competition for nutrients between skin and other tissues such as the mammary gland.

Key Words: Angora, Goats, Mohair, Prolactin

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J. Anim. Sci. 2003. 81:279–284

## Introduction

Decreased fiber growth by some breeds of sheep and Angora goats in early lactation has been attributed to a regulatory role of prolactin on increased nutrient use by the mammary gland (Russel, 1992). In addition to indirect influence, direct effects of prolactin on follicles are possible (Choy et al., 1995). A recent study indicated that higher plasma prolactin concentrations occur in the skin venous circulation of goats and sheep than in the systemic venous supply (Litherland, 1996). It is widely accepted that the pituitary is the major site of prolactin synthesis, which is largely regulated by hypothalamic dopamine and the pineal hormone, melatonin.

However, molecules with a structure similar to prolactin are synthesized in the placenta (Yamakawa et al., 1990), lymphocytes (Sabharwal et al., 1992; Swarlow-Santo, 1992; Arkins et al., 1993), and possibly in the skin (Walker, 1989; Nixon et al., 2002).

Published reports on the effect of prolactin on fiber growth are contradictory. Nixon et al. (1993) observed that in cashmere-producing goats, an increase in fiber growth was associated with suppression of the normal rise in plasma prolactin concentration by previous treatment with melatonin. McCloghry et al. (1992) reported no differences in wool growth among pinealectomized, sham-pinealectomized, or untreated Merino wethers. Isolated hair follicles from cashmere-bearing goats (Ibraheem et al., 1993) showed an increase in cashmere fiber growth when exposed to prolactin. The relatively low mohair fiber growth by Angora goats during short photoperiod observed by Litherland et al. (2000) may be related to high blood prolactin concentrations; however, effects of prolactin on mohair fiber growth in Angora goats have not been extensively studied. Objectives of this experiment were to study direct effects of prolactin on mohair fiber growth and metabolite concentrations in the venous blood of skin in Angora goats.

<sup>1</sup>This research was supported by USDA Grant No. 97-38814-4149.

<sup>2</sup>The authors wish to thank the farm crew at the E (Kika) de la Garza Institute for Goat Research for animal care.

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Received May 3, 2002.

Accepted September 11, 2002.

## Materials and Methods

### *Animals*

The experimental protocol was approved by the Langston University Animal Care Committee and experiment occurred during late fall as one of many experiments investigating the effects of hormones and amino acids on mohair growth. Seven Angora wethers ( $30 \pm 3$  kg BW, approximately 15 mo of age) were used. Animals were implanted bilaterally with Silastic catheters (Dow Corning, Midland, MI) in the superficial branch of the deep circumflex iliac artery and vein, as described by Pierzynowski et al. (1994). At the same time, a Silastic catheter was placed in the carotid artery. Areas supplied by the arteries were identified by infusing methylene blue (Sigma, St. Louis, MO), and areas ( $10 \times 10$  cm) in the middle of perfused regions were marked by tattoo. Goats were placed in metabolic crates 1 d after surgery. Animals were fed a mixed concentrate-forage diet (37.8% bermudagrass hay, 7% alfalfa, 25% oats, 19% ground corn, 9% solvent-extracted soybean meal, 1% trace mineralized salt, 1% calcium carbonate, and 0.2% vitamin premix; DM basis) prepared according to NRC (1981) recommendations. Feed was provided once daily at 0800 at approximately 110% of consumption on the preceding few days, and goats had free access to water.

### *Treatments and Sampling*

Goats were shorn before the 28-d experimental period, and tattooed regions were also shorn using a small Oster (Milwaukee, WI) clipper with a number 40 blade. For the first 14 d, goats were infused with a prolactin/control solution into the deep circumflex iliac artery on one side and control alone on the other. Sides for infusion treatments were chosen randomly. For prolactin infusion, 38.4 mg of prolactin (from sheep pituitary, 40 IU/mg; Sigma, St. Louis, MO) and 0.5 g of BSA were dissolved in 1 L of saline, and pH was adjusted to 7.4 with NaOH. For control infusions, 0.5 g of BSA was dissolved in 1 L of saline, and pH was adjusted to 7.4 using NaOH. Solutions were infused with 60-mL syringe pumps (Harvard Apparatus, South Natick, MA). The infusion rate was 2.4 mL/h for control and prolactin. The infusion provided 92.16  $\mu$ g/h of prolactin and was estimated to triple plasma prolactin concentration on the prolactin-infused side. A relatively small amount of prolactin (1.2% of the whole-body flow, calculated using blood prolactin concentration and blood volume) was infused to limit effects to a defined area of skin, and so that the infusate of one side would not influence metabolism on the other side (Pierzynowski et al., 1994; Puchala et al., 1995).

On d 7 and 14 of infusions, blood was collected at 0800 from both deep circumflex iliac veins. Blood was collected into three 7-mL tubes containing  $K_3$  EDTA for hormone assays, sodium heparin for amino acid

analysis, or potassium oxalate-sodium fluoride for other metabolites (Becton Dickinson, Rutherford, NJ). Blood samples were immediately chilled in an ice bath, transported to the laboratory, and centrifuged at  $1,500 \times g$  at 4°C for 20 min. Plasma aliquots were stored at -20°C until analyzed. Also, on d 14, blood flow to perfused regions was measured through a primary dose of 10 mL of 0.5% (wt/vol) para-aminohippuric acid into iliac arteries followed by continuous infusion of the same solution with added prolactin at a rate of 12 mL/h. The amount of prolactin added to the para-aminohippuric acid solution was designed so that prolactin and control delivery rates were the same as described previously. After a 30-min equilibration period, six samples were taken at 20-min intervals from the carotid artery and iliac veins for analysis of para-aminohippuric acid, hemoglobin, oxygen saturation of hemoglobin, and packed cell volume. Two weeks after infusions were stopped, tattooed regions were shorn and mohair fiber was collected for analysis of yield and diameter.

### *Analyses*

Plasma hormones were analyzed using commercially available kits from ICN Biomedicals, Inc. (Costa Mesa, CA; insulin, kit No. NK9910; total triiodothyronine, kit No. LN1305; and total thyroxine, kit No. LN1301). Analyses for the specific hormones were carried out in a single assay. Intraassay coefficients of variation were 5.9% for insulin, 4.8% for triiodothyronine, and 6.2% for thyroxine. Plasma prolactin was analyzed using an ELISA kit (DRG Int., Mountainside, NJ). Amino acid analyses were performed with an AminoQuant 1090 system (Hewlett-Packard, San Fernando, CA), utilizing precolumn derivatization with *o*-phthalaldehyde and 9-fluorenylmethylchloroformate and UV detection. Plasma (0.45 mL) was deproteinized with 0.05 mL of 50% (wt/vol) sulfosalicylic acid with internal standards (norvaline and sarcosine). Plasma glucose concentration was analyzed colorimetrically using a Sigma Diagnostic kit (catalog No. 315). Plasma urea N was determined as described by Chaney and Marbach (1962). Plasma was analyzed for para-aminohippuric acid by an automated procedure (Technicon Industrial Systems, 1972; No. 216-72T). Samples for oxygen and hemoglobin were drawn anaerobically. Samples were immediately analyzed for hemoglobin percentage and oxygen saturation of hemoglobin with a OSM 3 hemoximeter (Radiometer, Westlake, OH). Remaining sample was then used to determine packed cell volume with heparinized tubes (Clay Adams, Parsippany, NJ).

Staple length and greasy and clean mohair yields were determined according to standards of ASTM (1988). Fiber diameter was determined using the Optical Fibre Diameter Analyzer (BSC Electronics, Myaree, Australia). To determine mohair amino acid profile, mohair samples were allowed to react with 3,3'-dithiodipropionic acid to convert Cys to stable Cys-mercaptodipropionic acid and were hydrolyzed with 6 N HCl using

**Table 1.** Mohair fiber measurements in Angora goats at 14 d after 14 d (d 28) of infusion of the skin with prolactin or control<sup>a</sup>

Items	Infusion		SE	P
	Prolactin	Control		
Greasy mohair, g/100 cm <sup>2</sup>	3.79	4.62	0.24	0.05
Clean mohair, g/100 cm <sup>2</sup>	3.02	3.67	0.19	0.05
Staple length, mm	17.1	18.5	0.11	0.05
Diameter, μm	30.4	30.0	0.19	0.33

<sup>a</sup>n = seven goats.

a MDSB2000 microwave system (CEM, Matthews, NC). The amino acid profile of digested mohair samples was determined using an AminoQuant system (Hewlett Packard) as noted earlier.

### Statistical Analyses

To compare effects of prolactin and control treatments, data were analyzed as a randomized block design with a model consisting of treatment and animal (block) by GLM procedures of SAS (SAS Inst., Inc., Cary, NC). For blood measures taken on both d 7 and 14 of infusions, data were analyzed as a split-plot with animal within treatment as the error term to test the main plot of treatment. Residual error was used to test the subplot of sampling time and the interaction of time and treatment. To determine whether there was no effect of infusion on feed intake, data from the infusion (first 14 d) and the rest periods (last 14 d) were analyzed as split-plots.

### Results

Feed intake was not affected by infusions and was similar during the entire experiment (966 g/d in the infusion period vs 974 g/d in the rest period;  $P = 0.84$ ). Prolactin infusion decreased greasy and clean mohair fiber harvested at the end of the 28-d experimental period ( $P < 0.05$ ; Table 1). Mohair staple length was lower for infusion of prolactin vs control ( $P < 0.05$ ), although diameter did not differ ( $P = 0.33$ ).

For measures at d 7 and 14 of infusion, the effect of sampling time and the interaction between time and treatment were not significant ( $P > 0.05$ ). Blood flow in the perfused region, packed cell volume, and concentrations of insulin, triiodothyronine, and thyroxine in blood from the superficial branch of the deep circumflex iliac vein did not differ between treatments (Table 2). The absence of effects of prolactin on concentrations of these hormones suggests that prolactin had only a local effect in the perfused area. Although it was estimated that infusion would triple blood prolactin concentration from the superficial branch of the deep circumflex iliac vein (calculated using average blood prolactin concentrations in deep circumflex iliac circulation and average blood flow in the perfused area), only a 100% increase

was observed. The amount of infused prolactin was low and only sufficient to change prolactin concentration in the perfused area, with the concentration in arterial blood similar to that in venous samples (34.6 ng/mL). Oxygen saturation of hemoglobin was lower and prolactin concentration greater with infusion of prolactin than control ( $P < 0.05$ ).

Concentrations of methionine ( $P < 0.01$ ), cysteine ( $P < 0.01$ ), lysine ( $P < 0.05$ ), valine ( $P < 0.01$ ), isoleucine ( $P < 0.02$ ), leucine ( $P < 0.01$ ), threonine ( $P < 0.03$ ), alanine ( $P < 0.01$ ), and tyrosine ( $P < 0.01$ ) in blood from the superficial branch of the deep circumflex iliac vein were greater for prolactin than for control infusion (Table 3). There were no treatment differences in the amino acid profile of mohair fiber (data not shown), with values similar to those reported previously (Puchala et al., 2002).

### Discussion

In vitro studies of the influence of prolactin on fiber growth generally have not agreed well with in vivo experiments, including the depression of mohair fiber growth noted in the present experiment. In vitro exposure of isolated hair follicles from cashmere-bearing goats (Ibraheem et al., 1993) and red deer (Thomas et al., 1993) to prolactin for 5 d increased fiber growth, but isolated hair follicles from cashmere-bearing goats (Ibraheem et al., 1994) also had increased fiber growth during 5 d of melatonin exposure. It is not clear why both treatments similarly increased cashmere fiber growth in studies conducted by Ibraheem et al. (1993; 1994) because melatonin suppresses prolactin concentration in goats (Nixon et al., 1993). Furthermore, melatonin and prolactin have had contrasting effects on fiber growth in several experiments (Parry and Craven, 1992; Pearson et al., 1993; 1996; Litherland, 1996). Conversely, with in vitro maintenance of follicles from Drysdale, English Leicester, or Wiltshire sheep, supraphysiological concentrations of prolactin and melatonin did not affect growth rate or viability of wool follicles (Winder et al. 1995). It is also possible that the media used and the accumulation of metabolites in the media increased fiber growth in the study by Ibraheem et al. (1993) and prevented a response in the Winder et al. (1995) study. Differences between results of the present experiment and these reports could indicate that the duration of in vitro incubations used by Ibraheem et al. (1993; 1994) might have been inadequate for full expression of prolactin or melatonin effects. In support of this suggestion, Pearson et al. (1993) observed that prolactin effects on wool growth in vivo occurred after about 21 d of exposure. In the present experiment, prolactin was infused for 14 d and fiber growth was assessed 14 d after infusions ended, thereby allowing enough time for prolactin to affect fiber growth.

Results of this experiment indicate a direct and substantial negative effect of prolactin on mohair fiber growth in Angora goats. Sahlu et al. (1999) observed

**Table 2.** Blood parameters in the superficial branches of the deep circumflex iliac vein in Angora goats during infusion of the skin with prolactin or control<sup>a</sup>

Items	Infusion		SE	P
	Prolactin	Control		
Blood flow, mL/min	24.3	23.9	4.1	0.98
Oxygen saturation, %	75.1	68.2	1.2	0.05
Hemoglobin, g/100 g	10.1	10.1	0.3	0.98
Packed cell volume, %	22.2	22.3	0.3	0.98
Glucose, mg/dL	62.3	60.4	1.6	0.44
Insulin, $\mu$ IU/mL	21.3	21.2	0.5	0.75
Triiodothyronine, ng/dL	222.4	229.9	14.2	0.89
Thyroxine, $\mu$ g/dL	6.8	6.7	0.3	0.93
Prolactin, ng/mL	77.1	32.6	3.3	0.01

<sup>a</sup>n = seven goats.

that mohair fiber growth rate decreased during lactation, which may be linked to prolactin. In late pregnancy and early lactation, there may be partitioning of nutrients away from the fiber-producing follicles to the mammary gland for milk production because of increased blood flow and/or extraction of nutrients by mammary (Russel, 1992). The increased concentration of amino acids and higher oxygen saturation of hemoglobin in venous blood draining the region infused with prolactin in the present experiment indicate decreased nutrient use by skin, which contributed to increased nutrient availability to other tissues. Therefore, decreased mohair fiber growth during lactation is probably not just a function of increased nutrient uptake by the mammary gland, which is stimulated by prolactin, but also of direct effects of prolactin on skin metabolism (i.e., decreased nutrient uptake). Glucose concentration was not influenced by infusion treatment, probably because glucose is not a major energy source for fiber follicles (Harris et al., 1989).

Prolactin receptors have been found on fiber follicles of Wiltshire sheep (Choy et al., 1995) and might likewise be present in Angora goats. In this regard, Pearson et al. (1996) suggested that natural and experimental increases in daylength had short-term inhibitory effects on wool follicle growth in New Zealand Wiltshire ewes that could be mediated by increasing the concentration of plasma prolactin because increasing photoperiod resulted in higher prolactin and lower melatonin concentrations (Nixon et al., 1993). Increased plasma prolactin concentrations have been associated with deactivation of follicles and fleece shedding in Wiltshire sheep (Parry and Craven, 1992; Pearson et al., 1993; 1996) and cashmere-producing goats (Litherland, 1996). Although follicle activity was not measured in the present experiment, the lack of shedding and decrease in mohair fiber length on the side infused with prolactin imply that all or a substantial portion of change in fiber growth was due to reduced fiber production by active follicles, per-

**Table 3.** Blood amino acid levels in the superficial branches of the deep circumflex iliac vein in Angora goats during infusion of the skin with prolactin and control<sup>a</sup>

Items	Infusion		SE	P
	Prolactin	Control		
	$\mu$ M			
Essential AA				
Methionine	22.0	16.6	1.0	0.01
Cystine	65.1	52.1	4.6	0.01
Lysine	115.2	106.2	3.4	0.05
Phenylalanine	58.9	53.3	2.0	0.06
Valine	294.7	252.8	10.4	0.01
Isoleucine	109.9	93.4	3.3	0.02
Leucine	160.8	139.4	4.0	0.01
Threonine	101.9	84.0	5.5	0.03
Arginine	197.3	200.6	6.4	0.71
Non-essential AA				
Glutamic acid	276.9	295.3	13.8	0.36
Serine	71.6	69.3	4.1	0.94
Glycine	1,209.3	1,091.8	50.0	0.11
Alanine	398.4	339.8	14.9	0.01
Tyrosine	80.2	72.2	2.0	0.01

<sup>a</sup>n = seven goats.

haps because of the limited supply of nutrients to follicles.

Because the experiment was performed during late fall, which is characterized by short photoperiod resulting in relatively low prolactin and high melatonin concentrations, skin sensitivity to the sudden increase in prolactin concentration may have been high. Melatonin, produced by the pineal gland, regulates seasonal secretion of prolactin produced by the pituitary of goats (Maeda et al., 1984; 1988; Mori and Okamaru, 1986). Plasma prolactin concentration increases as photoperiod increases and decreases with decreasing daylength (Buttle, 1974; Kloren, 1991). Continuous melatonin treatment provides an extremely short photoperiod endocrine signal (O'Callaghan et al., 1991) that suppresses plasma prolactin concentration. Foldes and Maxwell (1993) observed that in young pinealectomized Merino wethers, a decline in wool growth in mid-side patches associated with changing season was more rapid than in pineal-intact animals, presumably because in pineal-intact animals melatonin concentration was higher. It was suggested that melatonin might be involved in partitioning nutrients to wool follicles. In cashmere goats, Nixon et al. (1993) observed that previous treatment with melatonin suppressed a natural rise in plasma prolactin concentration and thereby allowed for continued fiber growth. However, McCloghry et al. (1992) did not observe differences in wool growth, total follicle density, or BW among pinealectomized, sham-pinealectomized, and control Merino wethers. In the present experiment, the sudden reverse in the melatonin:prolactin ratio due to prolactin infusion during the short photoperiod simulated an increasing photoperiod, and this may have contributed to decreased mohair fiber growth. This observation is in contradiction to other experiments investigating prolactin effects on fiber growth using either pinealectomized animals or *in vitro* measurements (McCloghry et al., 1992; Ibraheem et al., 1993; Winder et al., 1995).

Lincoln (1990) suggested that in highly selected Romney and Merino sheep breeds, wool continuously grows throughout the year, is not affected by changes in prolactin concentrations, and there is no clear seasonal shedding. However, despite the fact that Angora goats are only half the size of mature Merino sheep, fiber growth rates of Angoras are comparable to those of Merinos (4.2 kg/year; Gallagher and Shelton, 1972; Bunge et al., 1996). Therefore, when mohair fiber production is expressed per unit of BW, Angora goats are more efficient than Merino sheep as fiber producers. Nonetheless, in terms of seasonality and prolactin effects on fiber growth, based on findings of this experiment and other studies such as Litherland et al. (2000), Angoras may not respond in the same way as highly selected Merino sheep. Compared with other goat breeds, Angoras are known to have lower blood amino acid concentrations, with methionine blood concentration being approximately half that of Spanish or Alpine goats (Puchala et al., 1995) and Merino sheep (Reis et

al., 1973). Lower blood amino acid concentrations in Angora goats suggest more efficient utilization of amino acids for mohair fiber/protein production than in Merino sheep. Therefore, it is possible that the high sensitivity of Angoras fiber follicles to prolactin ensures that amino acids can be partitioned away from the skin and to reproductive tissues and the mammary gland during late gestation and lactation when demands are elevated.

## Implications

Prolactin decreased mohair fiber growth in a perfused area of skin of Angora goats, implying a direct effect on skin metabolism and fiber growth. The decrease in mohair fiber growth was accompanied by a decrease in mohair staple length, indicating that all or a substantial portion of change in fiber growth was because of actions on active follicles rather than an increased number of inactive follicles. Decreased amino acid use by follicles of Angora goats when prolactin is elevated, such as in late pregnancy and early lactation, may contribute to partitioning of nutrients to other tissues.

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