

## Relationship of Ambient Temperature to Serum Prolactin in Heifers<sup>1,2</sup> (38217)

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Prolactin is generally considered to be necessary for milk production in laboratory animals, and serum prolactin is correlated with average monthly milk production in cows (1). Stimuli associated with lactation can alter blood serum prolactin in cattle. For example, milking or washing the udder increase serum prolactin concentration (2-3). Furthermore, Koprowski and Tucker (1) demonstrated that serum prolactin concentration in lactating cows was greater during the warmer months of the year than during the winter months. However, the environmental factors which cause these changes in serum prolactin are not known. Knowledge of these factors will be essential before the role of prolactin during lactation will be clearly understood. Thus, the objective of this study was to examine the relationship of ambient temperature to serum prolactin in cattle.

*Materials and Methods.* Four Holstein heifers, 3 mo of age, were paired randomly, and the 2 pairs were confined in 2 environmental chambers (I and II). A pair of heifers was stanchioned beside each other, but physical contact between calves was

restricted. Relative humidity was maintained at  $50 \pm 10\%$ , and animals were exposed to 12 hr of light daily starting at 8 am. Water and a complete ground ration (grain concentrate plus roughage) were supplied *ad libitum*, and chambers were cleaned at 8:30 am and 5:00 pm.

After an initial 2-week adjustment period to the chambers (maintained at  $21 \pm 0.5^\circ$ ) and the day before blood sampling started, a cannula (Silastic, 95 cm length; 2.16 mm o.d., Dow Corning Corp.) was inserted into one jugular vein of each heifer. About 20 cm of cannula were placed in the vein and attached to the neck with adhesive tape. The remaining 75 cm of cannula were taped to the dorsal midline of the heifer to permit blood samples to be taken while standing at the rear of the animal. If a cannula became nonfunctional, it was replaced. The blood sample scheduled to be taken at the time a cannula became nonfunctional was omitted.

The pair of heifers in chamber I was maintained at  $21 \pm 0.5^\circ$  for 5 days, then the temperature was elevated to  $27^\circ$  at the rate of approximately  $3^\circ$  per hr beginning at 10:00 am. Exposure to  $27 \pm 0.5^\circ$  was continued for 5 days. On the 5th day at 10:00 am the heifers were injected via the jugular cannula with 10  $\mu\text{g}$  of synthetic thyrotropin-releasing hormone (pyro-glutamyl-histidyl-proline amide, TRH).<sup>3</sup> The TRH injection was used to evaluate the

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<sup>3</sup> TRH kindly supplied by Dr. R. Rippel, Abbott Laboratories, North Chicago.

ability of the anterior pituitary to release prolactin (4). The next day the temperature was returned to 21° for 5 days. After 5 days at 21° the temperature was reduced to 10° at the rate of about 3° per hr beginning at 10:00 am. Exposure to 10 ± 0.5° was maintained for 5 days and on the 5th day the heifers were infused with 10 µg of TRH via jugular cannula. The pair of heifers in chamber II were treated similarly to and concurrently with those in chamber I, except the order of the periods of exposure to 27° and 10° was reversed.

Blood serum samples were collected via cannulae twice daily at 8 am and 8 pm. On those days when temperature was altered from 21°, 12 samples were taken at hourly intervals, starting 1 hr before the start of the temperature change. Prior to treatment of heifers with TRH, blood samples were taken and discarded at 15-min intervals for 1.5 hr in an effort to achieve stable baseline values (3). Thereafter, blood samples were collected 30, 20, 10 min and immediately before (0) TRH injection. After TRH infusion, blood samples were taken at 5, 10, 15, 20, 25, 30, 45 and 60 min. Sera were stored at -20° until prolactin was quantified by a double antibody radioimmunoassay (5). The acute responses to temperature changes were subjected to regression analysis. Prolactin values for the 12-hr sampling period were regressed on temperature on a within animal basis. Chronic temperature effects were analyzed using a *t*-test. The differences between means for each heifer at either 10° and 21° or 21° and 27° were tested to determine if they were different from zero.

**Results.** During the 4-hr interval when ambient temperature was reduced from 21° to 10°, serum prolactin concentration decreased linearly ( $P < 0.001$ ; Fig. 1). Prolactin averaged 13 ng/ml before the start of the temperature decline, and decreased at the rate of  $0.88 \pm 0.11$  ng/ml per degree C, to approximately 4 ng/ml. As ambient temperature was increased from 21° to 27°, serum prolactin increased linearly ( $P < 0.01$ ; Fig. 2) from approximately 8 ng/ml, at the rate of  $1.73 \pm 0.62$  ng/ml per degree C, to a maximum of 22 ng/ml.

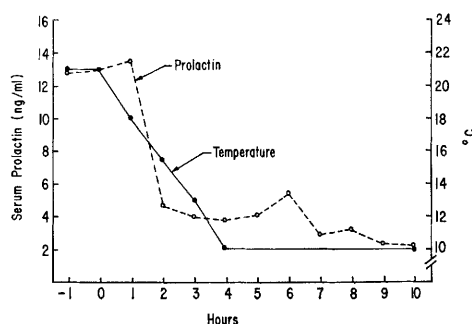


FIG. 1. Serum prolactin and ambient temperature during the change from 21° to 10°. Each point for serum prolactin is the mean of four heifers. Standard error of the overall mean for prolactin was 1.6 ng/ml serum.

Chronic exposure for 5 days of the heifers to either 10°, 21° or 27° altered serum prolactin. Since average serum prolactin concentrations were not significantly different between daily samples taken at 8 am and 8 pm or between days within a given temperature, the values for all samples taken for each heifer were averaged (Table I). When heifers were exposed to 10° after the adjustment period at 21°, serum prolactin was significantly reduced (6.7 vs 10.8 ng/ml, respectively;  $P < 0.05$ ). Serum prolactin was twice as concentrated in heifers maintained at 27° as when they were at 21°. However, serum prolactin concentrations in heifers at 27° were much more variable than those in heifers at lower environmental temperatures, and this difference between 27°

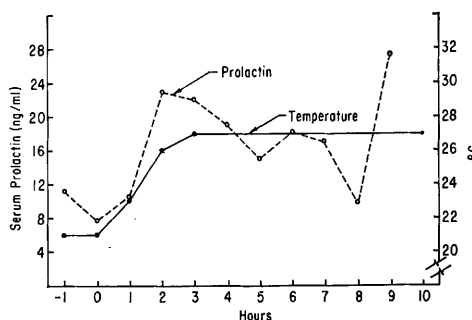


FIG. 2. Serum prolactin and ambient temperature during the change from 21° to 27°. Each point for serum prolactin is the mean of four heifers. Standard error of the overall mean for prolactin was 4.4 ng/ml serum.

TABLE I. Serum Prolactin (ng/ml) in Heifers During Exposure to 10°, 21° or 27° for 5 Days.

Heifer number	Treatment		
	10°	21°	27°
1	8.0 <sup>a</sup>	12.0	21.4
2	3.4	9.0	16.6
3	5.7	9.9	26.5
4	9.6	12.3	26.7
$\bar{X}$	6.7	10.8	22.8

<sup>a</sup> Mean for 7-10 samples per heifer taken during a 5-day period.

and 21° only approached statistical significance ( $P < 0.10$ ).

Within 5 min of TRH administration serum prolactin increased 9.7 fold in heifers at 10° and 7.4 fold in heifers at 27° (Fig. 3). In comparison with heifers at 10°, absolute concentrations of serum prolactin were significantly greater ( $P < 0.01$ ) in the heifers at 27° before and after TRH. When the prolactin concentration during the first 20 min after TRH was expressed as a percentage of the pretreatment concentration, heifers at both temperatures responded similarly to TRH. But during 25-60 min after TRH, heifers at 27° had a greater ( $P < 0.05$ ) serum concentration of prolactin percentage wise than heifers at 10°. In fact within 1 hr after TRH infusion, prolactin

concentration had decreased to pretreatment values in heifers at 10°, but in heifers at 27° serum prolactin concentration was still elevated about 2.5 times above pretreatment values.

*Discussion.* Alteration of blood serum prolactin by changes in environmental temperature has been suggested previously. Koprowski and Tucker (1) observed in lactating cows that serum prolactin concentration was greatest in summer and lowest in winter, but the temperature effect was confounded with changes in day length and diet. The rapid and consistent changes in serum prolactin which occurred within 3-4 hr during which time temperature was altered suggests the need to control temperature when studying relationships of prolactin to physiological functions. The diurnal changes in prolactin previously described in cattle (6) were not observed in our study, possibly because temperature was controlled or the number of observations we made throughout the day were insufficient to detect significant differences.

Injection of TRH markedly increased serum prolactin similar to previous reports in cattle (5, 7) and sheep (8). When the data were expressed as a percentage of pretreatment values, ambient temperature had no influence on the initial prolactin concentrations after TRH. However, the absolute concentrations of serum prolactin after TRH in heifers at 27° were at least twice as great as those for heifers at 10°. In contrast the prolactin response to the milking stimulus in lactating cows is greatest in the cool months of the year (1). Whether this reflects differences in anterior pituitary responsiveness between calves and cows or between TRH and the milking stimulus must await further study. In any event the data clearly showed that environmental temperature markedly influenced the ability of the anterior pituitary to release prolactin within 5 min after TRH injection.

Not only was the initial serum prolactin response to TRH greater at higher temperatures but it remained above pretreatment concentrations for a longer period of time. The disappearance rate of prolactin in cattle is estimated to be 22-25 min (9, 10).

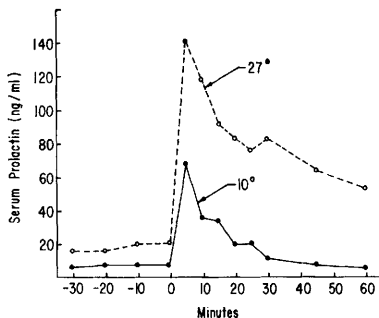


FIG. 3. Serum prolactin response to infusion of 10  $\mu$ g of TRH (at 0 min) into each of four heifers at 10° and 27°. Standard errors of mean ( $n = 4$  at each point) for heifers at 10° ranged from 1.6 to 27.7 ng/ml serum and for heifers at 27° ranged from 2.9 to 38.9 ng/ml serum. Standard errors were proportional to the means.

But whether elevated temperatures decreased clearance rates or sustained an increased release rate for prolactin over a prolonged period cannot be answered from these data.

*Summary.* Four heifers were exposed to 10° and 27° for 5 days after preconditioning to 21° in a controlled environment chamber. Serum prolactin decreased from 13 to 4 ng/ml during the 4-hr interval when ambient temperature was reduced from 21° to 10°. Prolactin increased from 8 to 22 ng/ml during the 3-hr period when ambient temperature was increased from 21° to 27°. During 5 days of chronic exposure to 10°, serum prolactin was 38% lower ( $P < 0.05$ ) than during a control period at 21°; and while at 27°, heifers had twice the concentration of serum prolactin ( $P < 0.10$ ) as those at 21°. Injection of 10 µg of thyrotropin releasing hormone (TRH) caused serum prolactin to increase within 5 min from 20 to 140 ng/ml in heifers at 27° and from 8 to 70 ng/ml in heifers at 10°. We conclude that ambient temperature influences basal and TRH-

stimulated concentrations of serum prolactin in heifers.

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