Milk Yields and Hormone Concentrations of Holstein Cows in Response to Sometribove (Somatotropin) Treatment During the Dry Period


Dairy Science Department
University of Florida
Gainesville 32611

ABSTRACT

Holstein cows (n = 135) under commercial management were used to determine whether sometribove (recombinant methionyl bST, 25 mg/d) administered during the dry period affected milk yield during the ensuing lactation. Cows scheduled to begin lactations (≥22) during January to March were assigned randomly to treatments of sodium bicarbonate excipient (n = 67) or bST (25 mg/d, n = 68). Subcutaneous injections were given for 14 d, corresponding to d -21 to -7 relative to expected calving date. Days dry prior to first injection (64.0, 60.2) and number of injections received (13.9, 13.8) were similar for control and treatment groups, but days from last injection to calving (8.8, 7.1) differed. No differences in incidence of dystocia or udder edema were detected. Previous lactation yields were 8251 and 7952 kg, and yields for lactations following treatment were 8328 and 7852 kg, based on complete lactation data. Mean test date 3.5% FCM yields for control and treated groups during experimental lactation differed before (30.3 vs. 28.1 kg) but not after (29.5 vs. 28.4 kg) covariance adjustment for previous total lactation milk yield. Test of heterogeneity of regression provided no evidence that respective curves for FCM yield during lactation were not parallel or of different magnitude. Elevation of serum bST during 2 wk of the dry period resulted in no apparent increase in extent of mammmogenesis or lactogenesis that was translated into an increase in milk yield.

(Key words: somatotropin, dry period, milk yield, hormones)

INTRODUCTION

Efficacy of recombinant bST when administered to lactating dairy cows to enhance milk yield has been well documented (6, 7, 32). Rapidity of onset and cessation of the increased milk yield response suggested that activity rather than number of secretory cells was affected by exogenous bST (17). Increased metabolic activity of mammary tissue, which likely is effected via bST-mediated insulin-like growth factor-I (IGF-I) (8, 15, 16, 17, 29, 36), could promote local production of vasodilators, which, in turn, would result in an increased percentage of cardiac output perfusing the mammary gland (10). This increase in mammary blood flow would contribute to a partitioning of nutrients to the mammary gland (4, 27) and to an increase in milk component synthesis and secretion, because many key enzymes, notably lactose synthetase, inherently operate below their respective maximum velocity (20).

Because mammary cell activity can be increased by exogenous bST, further increases in...
milk yield require increase and retention of cell numbers (34). Involvement of bST, directly or indirectly via growth factors, in regulation of mammary secretory cell proliferation and maintenance is likely (13, 25, 28). The periparturient period is characterized by a large increase in mammary DNA, which reflects an increase in number of secretory cells (1, 34). One objective of this study was to determine whether administration of bST during the dry periods of multiparous cows increases milk yield during the ensuing lactations. A second objective was to evaluate concentrations of hormones in plasma during the dry period and in early lactation.

MATERIALS AND METHODS

Experiment 1: Field Study

A large commercial dairy enrolled in the Florida DHI testing program provided the Holstein cows used in this study. Dry cows (n = 156) that had previous on-site lactation records and were due to calve within a 12-wk interval received injections of bST or excipient during January through March 1988. Each cow was scheduled to receive 14 daily injections, starting 21 d before the expected calving date obtained from DHI records. Cows in the control group (C; n = 76) received sodium bicarbonate excipient and cows in the treatment group (T; n = 80) received bST (sometrilo, 25 mg/d; Monsanto Co., St. Louis, MO). Both preparations were maintained at -20°C until the day of injection. Injection volumes were 2.5 ml, following daily solubilization of the lyophilized bST preparation in pyrogen-free water and daily thawing of excipient solution.

Daily, at 1200 h, cows scheduled for injection were moved from the dry cow pasture to a covered corral with an adjacent cattle chute that accommodated 4 cows. At the time of first injection, cows within the chute were assigned alternately to either C or T. Cows were marked on the rump with colored crayons to avoid misinjection and to indicate the treatment and date of last injection. Injections were subcutaneous over the dorsal rib cage. After a cow received her last scheduled injection, she was moved to the freshening lot.

Monthly DHI records were the primary source of data used to characterize lactation and reproductive parameters. All cows were milked three times daily during the lactations that preceded and followed the dry period when treatments were administered. Milk yields were monitored on a monthly basis for the entire lactation. Twice daily mature equivalent 305-d milk (305-d-2X-ME) yield was based on full lactation information and not on projections of partial lactations.

The data set contained the following variables: treatment, parity, total days dry, days dry before first injection, number of injections received, days from last injection to calving, age at calving, month of calving, lactation length, calving interval, days open, test month, milk yield, fat percentage, 3.5% FCM, SCC, DIM, 305-d-2X-ME of lactations preceding and following the injections, BW at calving, body condition at calving, and sex of calf.

The general linear models procedure of SAS (14) was used for least squares analysis of variance. The model included parity, month of freshening, treatment, their interactions, cow nested in parity, month of freshening and treatment, and DIM. Heterogeneity of regression was used to determine whether lactation curves differed between treatments. Milk yields of cows during lactations preceding treatment were used as covariates to adjust experimental lactation milk yields.

Experiment 2: Blood Sampling, Hormones, and IGF-I

In the second study, designed to evaluate plasma hormone and IGF-I concentrations, dry cows (n = 10) and primiparous cows (n = 10) from the University herd were treated using the same injection protocol and were distributed equally within C and T. Blood samples (5 to 8 ml) collected from the coccygeal vein into heparinized vacutainer tubes were centrifuged at 1000 × g for 30 min at 5°C, plasma was decanted, and samples were stored at -20°C until analyzed. Sampling was scheduled for d -42, -28, -14, -7, -5, -3, -1, 0, +1, +3, +5, +7, +14, and +21 relative to expected or actual calving date. When both blood sample collection and injection were scheduled on the same day, the blood sample was collected prior to administering the injection.

Double antibody radioimmunoassay procedures were used to measure insulin (3), trio-
dothyronine (3), thyroxine (3), somatotropin (3), cortisol (11), prolactin (22), and IGF-I (12). All samples were assayed in duplicate in a single assay, except for assays for cortisol and IGF-I. For the latter two determinations, all samples from individual cows were in the same assay. The intrasample coefficients of variation were less than 10%.

The model for statistical analyses included treatment, parity, calving period, cow nested in treatment by parity by calving period, and blood sampling interval. Calving period categorized cows and samples relative to days between last injection and calving (≤3 d, >3 d). Blood sampling interval considered injection phase and parturition (before, during, or after) to convert time within a phase from a continuous to a discrete variable for estimation of least squares means.

Because cows within a calving period by parity by treatment varied in terms of hormone or IGF-I concentration and also differed from each other, covariance analysis was performed to adjust treatment least squares means during injection for concentrations before injection and to adjust least squares means after injection for concentrations before and during injections.

RESULTS

Experiment 1: Field Study

Retention rates (88 vs. 85%) were similar for cows in C and T; 9 cows in C and 12 in T were removed primarily because actual calving date differed appreciably from the expected calving date. Cows that calved 21 d beyond expected calving date or that had received fewer than 10 injections prior to calving were removed from the data set. Six cows from each group were eliminated as a result of ailments that typically occur in a commercial dairy, e.g., milk fever, displaced abomasum, and udder edema. Records from 135 cows (67 C, 68 T) were analyzed.

Days dry prior to first injection for C and T (mean ± SEM: 64.0 ± 4.2, 60.2 ± 4.2) did not differ. Number of infections received by cows was similar for C and T (13.9 ± 1.1, 13.8 ± 1.1). However, interval from last injection to calving differed (P < .03); the average cow in T calved 40.8 h sooner relative to date of last injection than for the average cow in C (7.1 ± .5 vs. 8.8 ± .5 d). A physiological basis for this difference was not apparent from calving data collected. Notably, distribution of male and female calves was similar between groups.

In the lactation prior to the experimental lactation, cows assigned to C averaged 8251 ± 149 kg (305-d-2X-ME), and cows assigned to T averaged 7952 ± 166 kg or 299 ± 225 kg greater per cow for C. During lactations that followed dry period treatments, average lactation yields were 8328 ± 160 and 7852 ± 195 kg for C and T; the difference was significant (476 ± 115 kg per cow; P < .03). A greater difference between groups for the experimental lactation relative to that for the preceding lactation (476 vs. 299 kg per cow) was the result of opposite trends. Namely, cows in C showed an increase in 305-d-2X-ME milk yield with advancing lactation number (parity), whereas yield for cows in T decreased slightly.

Least squares analysis of variance was performed to determine whether the mean test date FCM yields of the two groups differed. The least squares means, unadjusted for previous total lactation milk yield, differed (P < .03, Table 1) for test date 3.5% FCM yield. On average, a cow in C yielded 2.2 kg/d more milk than a cow in T (30.3 ± .68 vs. 28.1 ± .67 kg/d). After covariance analysis, in which experimental milk yield of a cow was adjusted for her previous total lactation milk yield, no difference (P > .3) was detected between means for test date FCM yield (29.5 ± .65 vs. 28.4 ± .67 kg/d).

Although the interval from last injection to calving differed between C and T (8.8 vs. 7.1

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Control</th>
<th>bST</th>
<th>Treatment comparison</th>
</tr>
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<tbody>
<tr>
<td>LSM</td>
<td>SEM</td>
<td>LSM</td>
<td>SEM</td>
</tr>
<tr>
<td>Unadjusted</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30.3</td>
<td>.68</td>
<td>28.1</td>
<td>.67</td>
</tr>
<tr>
<td>Adjusted</td>
<td>29.5</td>
<td>.65</td>
<td>28.4</td>
</tr>
</tbody>
</table>

1Observations were 638 and 636 for control and bST groups.
Figure 1. Relationship of FCM yield to DIM for control cows (C) and for cows treated with bST (T) during the preceding dry period. Regression equations for curves:

Control cows: \[ y = 36.15 + 20.5075 \times 10^{-2}x - 27.4380 \times 10^{-4}x^2 + 9.3265 \times 10^{-6}x^3 - 1.04608 \times 10^{-8}x^4 \]

Cows treated with bST: \[ y = 32.37 + 27.5240 \times 10^{-2}x - 34.9902 \times 10^{-4}x^2 + 12.6626 \times 10^{-6}x^3 - 1.55234 \times 10^{-8}x^4 \]

where \( x = \text{DIM} \).

Regression curves that described the relationship between 3.5% FCM yield and DIM for C and T were similar (Figure 1). A test of heterogeneity of regression provided no evidence that the curves were not parallel or that heights of the curves differed. Mathematical model for the pooled regression analysis (curve not shown) included parity, month of freshening, two- and three-way treatment interactions, cow in parity by month of freshening by treatment, and DIM to quintic power.

Experiment 2: Blood Sampling, Hormones, and IGF-I

Cow groups for the second experiment were similar except that cows in C varied more in days dry prior to first injection (Table 2). Within groups, cows calving \( \leq 3 \) d after last injection consisted of two primiparous and two multiparous cows, whereas three cows of each parity calved after 3 d. Thus, groups had equal distributions of cows in both parity and calving period.

Initial analysis to evaluate concentrations of hormones and IGF-I in plasma utilized the overall model described and revealed that, in the phase before injection, concentrations of IGF-I were greater \( (P < .05) \) for T (64.4 vs. 51.5 ng/ml; Table 3). During the injection phase, IGF-I \( (P < .08) \), prolactin \( (P < .05) \), and bST \( (P < .004) \) differed by treatment (values not shown). No differences from treatment were detected for concentration of any hormone or IGF-I during the posttreatment phase. Because differences in concentrations that existed before bST or excipient was injected could have affected the concentration in subsequent periods, the least squares means for hormones and IGF-I within the treatment and posttreatment phases were adjusted for concentrations in the preceding phases. The least squares means obtained by this analysis are in Table 3.

Adjusted concentrations of bST were 183\% \( (P < .0001) \) greater in T cows, and those of cortisol were 38\% \( (P < .04) \) greater in C cows during the injection phase, but adjusted concentrations of other hormones and IGF-I did not differ \( (P > .1) \). To assess the effect of actual time of calving on concentrations of hormones, some of which may show a periparturient surge, calving period \( (\leq 3 \) or >3 d) was included in the model to remove variation because of the interval from last injection to calving. Cows calving \( \leq 3 \) d after receiving their last injection had greater concentrations of prolactin than those calving >3 d \( (P < .05; 22.1 \text{ vs. } 8.6 \text{ ng/ml}) \). Concentrations of prolactin during the injection phase were greater in multiparous than in primiparous cows \( (P < \)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Experimental group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (kg/d)</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>545 118 550 159</td>
</tr>
<tr>
<td>Dry prior to first injection, d</td>
<td>52 8.9 40 2.5</td>
</tr>
<tr>
<td>Injections received</td>
<td>13.5 .4 14 0</td>
</tr>
<tr>
<td>Last injection to calving, d</td>
<td>5.3 1.3 5.4 1.3</td>
</tr>
</tbody>
</table>

1Each group consisted of 5 primiparous and 5 multiparous dry cows.
TABLE 3. Concentrations of hormones in plasma of Holsteins injected prepartum with excipient or bST.

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Group</th>
<th>Injection phase</th>
<th>Before</th>
<th>During</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>LSM</td>
<td>SEM</td>
<td>LSM</td>
</tr>
<tr>
<td>Insulin</td>
<td>Control 4</td>
<td>1.38</td>
<td>.04</td>
<td>1.35</td>
<td>.04</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>1.35</td>
<td>.04</td>
<td>1.30</td>
<td>.04</td>
</tr>
<tr>
<td>Triiodothyronine</td>
<td>Control</td>
<td>.46</td>
<td>.09</td>
<td>.47</td>
<td>.04</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>.51</td>
<td>.09</td>
<td>.50</td>
<td>.04</td>
</tr>
<tr>
<td>Thyroxine</td>
<td>Control</td>
<td>29.0</td>
<td>3.0</td>
<td>24.9</td>
<td>2.2</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>33.2</td>
<td>2.9</td>
<td>25.9</td>
<td>2.2</td>
</tr>
<tr>
<td>Cortisol</td>
<td>Control</td>
<td>15.17</td>
<td>2.0</td>
<td>13.36</td>
<td>1.06*</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>18.36</td>
<td>2.49</td>
<td>9.64</td>
<td>1.18</td>
</tr>
<tr>
<td>Prolactin</td>
<td>Control</td>
<td>6.03</td>
<td>.47</td>
<td>9.00</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>8.75</td>
<td>.47</td>
<td>8.27</td>
<td>1.5</td>
</tr>
<tr>
<td>Somatotropin</td>
<td>Control</td>
<td>3.46</td>
<td>.63</td>
<td>4.71</td>
<td>1.01**</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>3.28</td>
<td>.71</td>
<td>13.35</td>
<td>1.13</td>
</tr>
<tr>
<td>Insulin-like growth factor-I</td>
<td>Control</td>
<td>51.46</td>
<td>3.64*</td>
<td>48.83</td>
<td>6.35</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>64.35</td>
<td>4.07</td>
<td>55.96</td>
<td>7.2</td>
</tr>
</tbody>
</table>

1Control heifers and dry cows received sodium bicarbonate excipient, and treated group received bST (25 mg/d) for 14 consecutive d.
2Adjusted for before treatment values.
3Adjusted for both before and during treatment values.
4Samples analyzed for control and treated groups before, during, and after injection were 21 and 22, 23 and 26, and 80 and 79 within each hormone analyzed. Means for control and treatment groups within phase were compared.

DISCUSSION

No evidence was obtained to indicate that treatment of Holstein cows with bST during the dry period increased milk yield during the ensuing lactation. Upon calving, all injected cows were integrated into the lactating herd of over 1500 cows, fed corn silage-based diets, and managed to permit full expression of their milk yielding ability. Thus, nutrition and management were not limiting milk yield response to treatment.

Capacity of the mammary gland to synthesize milk is a function of number and activity of mammary epithelial cells (34). Analysis of shapes of lactation curves (Figure 1) and comparison of total lactation yields and of mean daily yields of milk (Table 1), adjusted for previous lactation milk yields, showed no differences that were due to treatment. If mamogenesis had been increased by dry period injection of bST, an increase in milk yield would have been expected in the absence of nutritional or managerial limitations. Although repeated records are not perfectly correlated \( r = .5; (38) \), differences in milk yields for either C or T from one lactation to another were expected to be relatively small because the yields were adjusted for maturity, milkings per day, and length of record. Because all cows had full lactation records, no long-term projections of lactation yield were made. Also, distributions of age and lactation number were similar between groups. Approximately 46, 34, and 20% of the cows in each group had completed their first, second or third, and fourth through seventh lactation prior to treatment. Conse-
frequently, the milk yield results indicate that exogenous bST during late gestation did not affect extent of mammary tissue development.

Lactogenesis occurs concurrently with mammary development during late pregnancy. A surge in prolactin, and perhaps other hormones, seems to be needed for maximal stimulation of lactogenesis (1, 33, 34). Analysis of lactation curves for the two groups showed that dry period treatment with bST did not affect extent of lactogenesis. Intercepts of the lactation curves for the two groups did not differ, and similar times were required to reach peak yields (Figure 1). Timing of lactogenesis is limited to a short period around calving and could have been affected, but not detected, because monthly DHI milk weight records were used to generate the lactation curves. Indeed, results of several studies suggest that timing of lactogenesis may have been affected by treatment of cows (2) or goats (21) with bST during the dry period or treatment of cows during the last trimester of first pregnancy (31). Akers and Cleale (2) reported that Holstein cows showed positive milk yield response when 25 mg/d of bST were injected for about 40 days beginning at 20 d after dry-off and continuing through calving. Average cumulative 30-d milk yield for the bST-injected cows was greater ($P < .05$), but 100- and 305-d yields did not differ. This pattern of yields would result if lactogenesis, but not mammosgenesis, were affected, in that the initial increase in milk yield was not maintained throughout lactation. Although peak, weekly, and cumulative milk yields of goats treated or untreated with bST during late pregnancy did not differ, control goats showed an increase in udder volume during the first 2 wk postpartum (21). Again, this implied that bST treatment caused a more rapid onset of lactogenesis. Primiparous cows injected daily with 20 or 40 mg of bST for 84 d during the last trimester of gestation showed a curvilinear response in milk yields during the first 100 d of lactation (31). The low dose of bST increased milk yield, but the high dose caused a decrease in milk yield relative to control (24.9; 20.9 vs. 23.8 kg/d). However, when expressed as FCM, yields decreased linearly as dose of bST increased (22.4, 22.0, 19.1; $P < .05$). Although colostrum yields did not differ, prepartum milk accumulation reportedly was stimulated by bST injections when administered until the time of parturition. Our analysis of 3.5% FCM yields using monthly DHI records likely would not have detected these very early effects on lactogenesis had they occurred.

The dry period is hormonally dynamic (33); resultant dramatic changes occur in the involution and remodeling of mammary tissue in preparation for the next lactation (5, 18, 19, 28, 35). Although large-scale sloughing of mammary epithelial cells does not occur during the normal 45- to 60-d dry period (18), some replacement of cells prior to the next lactation does occur (1, 5, 19, 34). Starting the injection of bST shortly after drying cows off (2) or during the very rapid and extensive growth phase characteristic of the last third of first pregnancy (31) may have altered cellular growth and the timing of lactogenesis differently than in our protocol. Our daily injections, which totaled 14, were started later in the dry period and were scheduled to stop 7 d before calving. This injection protocol was chosen to allow a longer period of involution and yet to provide exogenous bST during a time frame coinciding with the rapid acquisition of new cellular DNA that occurs around the time of parturition (1, 34). We hypothesized that injection of bST at that time would not interfere with lactogenesis.

Timing of bST injections within the dry period might be very important if effects of bST on plasmin concentration in mammary secretions and on mammary tissue involution were similar to those hypothesized to occur during lactation (28). Plasmin is a serine protease implicated in the gradual remodeling of mammary tissue that occurs during the declining phase of lactation. Injection of bST during lactation causes the cow to maintain milk plasmin at low concentrations, thereby potentially increasing lactational persistency by retarding the gradual involution of the mammary tissue. When injection of bST ceased immediately prior to dry-off, a 300% increase in milk plasmin concentrations occurred (28). This increase could reflect the removal of a suppressive effect that IGF-I has displayed upon secretion of plasminogen activator by mammary epithelial cells in vitro (35). Furthermore, cows withdrawn from bST immediately prior to final milk removal dried off at a faster rate. Consequently, starting bST injections too early...
in the dry period might inhibit involution and, thereby, prevent epithelial cells from regressing to a fully nonsecretory state. Inherent length of the active involution period might be regulated by the concentration of IGF-I in serum and mammary secretions during the first part of the dry period. The observed elevation in IGF-I at this time (23, 26) might suppress production of plasmin and, thus, decrease the rate of tissue remodeling.

Continuation of injections of bST too close to parturition might allow IGF-I, which normally is increased by injections of bST (8), to enhance cellular differentiation and, thus, effect an earlier than desired onset of lactogenesis. Clearly, additional information is needed to understand factors responsible for active involution and to determine the appropriate time to attempt to enhance mammary epithelial cell proliferation during the dry period.

There were no major differences in the concentrations of hormones measured during the injection and postinjection phases between cows in C and T. Unexpectedly, concentrations of IGF-I did not increase when bST was injected during the dry period. Injections of bST given during lactation or dry period increased IGF-I concentrations severalfold (8, 37). Concentration of IGF-I in bovine serum decreases during the last 2 to 4 wk of pregnancy to the concentrations that we observed (36). Perhaps the response of IGF-I to bST was attenuated because of metabolic changes associated with impending parturition.

In addition to similar and adequate involution intervals prior to first injection, cows used in the present study were in excellent body condition. To obtain full benefit of bST use during lactation, cows must have adequate reserves of body tissue, particularly adipose tissue (6), which can be mobilized to support milk synthesis. The relationship between body condition score and epithelial cell proliferation within the mammary fat pad of multiparous cows has not been studied. In contrast, overconditioning of prepubertal heifers interferes with subsequent lactational performance by altering mammary gland development (30). Both quantity and composition of mammary fat pad apparently are important in determining extent of mammogenesis and lactogenesis (9, 24, 25).

CONCLUSIONS

Exogenous bST administered during the late dry period did not have a positive or negative effect on milk yield during the ensuing lactation. Therefore, inherent concentrations of bST apparently are not limiting mammary epithelial cell proliferation that occurs during the periparturient period. An unanticipated attenuated response of serum IGF-I concentration to bST administered during the late dry period was observed.

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