Response of Dairy Cows to High Doses of a Sustained-Release Bovine Somatotropin Administered During Two Lactations.

1. Production Response

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ABSTRACT

This study evaluated the effect of sometribove (zinc methionyl bST) in a sustained-release formulation administered to lactating cows at concentrations up to 3.0 g every 14 d over two lactations. Eighty-two lactating Holstein cows in their first, second, or third lactation were assigned to the study. Cows received .6, 1.8, or 3.0 g of bST in one, three, or five intramuscular injections of a unit dose (.6 g) every 2 wk. Controls received five injections of the vehicle (equivalent volume to the 3.0-g treatment) every 2 wk. Injections were administered from 60 ± 3 d postpartum until dry-off or necropsy. Thirty-eight animals were continued on treatment for a second consecutive lactation.

During the 1st yr of treatment, bST increased mean 3.5% FCM by 7.2, 9.4, and 8.4 kg/d over control production (21.1 kg/d). During the 2nd yr, milk response to .6, 1.8, and 3.0 g of bST averaged 10.6, 3.6, and 4.9 kg/d over controls (24.8 kg/d). The incidence of clinical mastitis increased in the 3.0-g group relative to controls during the 2nd yr. Thus, salable FCM averaged 8.1, 9.1, and 6.2 kg/d above controls (yr 1) and 12.1, 4.7, and -2.8 kg/d (yr 2) for the .6-, 1.8-, and 3.0-g groups. Salable FCM was unaffected by mastitis at a proposed commercial dose (.6 g). Milk fat, protein, lactose, calcium, phosphorus, zinc, magnesium, and ash concentrations were unaffected by bST treatment. Calculated energy, calcium, phosphorus, and protein balances also were unaffected except for early decreases of up to 5 Mcal/d, and 40, 20, and 600 g/d, respectively, until feed intake increased. Milk serum bST concentrations greater than the assay limit of sensitivity (1 ng/ml) were routinely measurable only at doses of 1.8 and 3.0 g. Results confirmed that bST concentrations in milk serum are exceedingly small. Overall, supraphysiological doses of sometribove increased milk production with little effect on composition. No toxic effects of bST were observed.

(Key words: somatotropin, production, health)

INTRODUCTION

With the availability of high purity recombinant DNA-derived bST, in vivo studies have examined the feasibility of bST as a commercial product to increase milk production (6, 20, 21). Various modes of administration (daily injection or multiweek sustained-release vehicles), duration of treatment (18 to 32 wk), and doses (10 to 50 mg/d or equivalent) have been examined (1, 2, 3, 6, 16, 20). Milk response to long-term treatment has varied from approximately 2 to 5.5 kg/d, depending on the dose and frequency of administration (1, 3, 6, 20, 21).

These efficacy studies have also given insight on the long-term health effects of exogenous bST at proposed commercial doses. Health disorders that have been studied include lameness and mastitis, but results to date are...
varied and appear to be affected by management practices and experimental procedures used (23). For example, recent studies have shown bST caused no increased incidence of ketosis, milk fever, or chronic wasting (3, 9, 23)—in contrast to earlier results with pituitary-derived bST, which had indicated that metabolic disorders might occur (14). Body condition scores were decreased by bST doses of 10.3, 20.6, and 41.2 mg/d in the work of Soderholm et al. (26), but no associated health disorders were observed. Some trials have reported no effect (3, 9, 22). Similarly, incidence of mastitis in bST-treated cows (23), but other researchers have reported no effect (3, 9, 22). Similarly, incidence of mastitis has been highly variable across studies (23).

Subtle patterns in health effects are typically examined in two ways: high doses administered to relatively few animals in toxicity studies or lower doses administered to large numbers of cows in a range of environmental and management conditions. In the former strategy, a series of acute (28) and chronic toxicity studies was designed to evaluate potential health concerns for bST. A chronic safety test typically utilizes doses at least one, three, and five times a potential commercial dose administered for two lactations (27). The extremely high doses cumulatively expose the animal to a lifetime equivalent of drug. This scheme also increases the probability that an average animal would have an adverse reaction to the drug in much the same way that a more sensitive animal might react to a commercial dose (27).

Most bST studies have utilized daily injections (20, 21), but the use of a sustained-release system can give more control in the pattern of response achieved for the active molecule. In addition, labor and safety concerns associated with injection are minimized with less frequent administration. Present data suggest that the milk response to sustained-release preparations is within the same range as the response to daily injection, e.g., 4.4 kg/d versus 5.6 kg/d, respectively (6, 20). The objective of this study was to determine the effects of bST administered as one, three, and five times a proposed commercial dose for two lactations. The effect was assessed by response in milk production and composition, body weight, and feed intake.

**MATERIALS AND METHODS**

**General**

Eighty-two first, second, or third parity Holstein cows were assigned to treatments in a randomized block design. The cows had received no previous bST treatment. Treatments were 0, .6, 1.8, and 3.0 g/14 d of zinc methionyl bST [sometribove, (12)] in an oil-based formulation. The single dose (.6 g) is on the linear portion of the sometribove dose-response curve for milk production (10). Somatotropin was produced in *Escherichia coli* K12 W110G via a pbBR322 plasmid, which utilized a tryptophan promoter sequence (5). Sterile bST was purified by standard biochemical techniques; the resulting bST averaged 99.5% purity by reduced SDS-PAGE and passed a series of specifications including dimer-aggregate content and pyrogen content. The biopotency averaged 1.1 U/mg of protein (14% coefficient of variation) as determined in a hypophysectomized rat growth bioassay (15).

Injections were administered every 2 wk into the left or right gluteus, semitendinosus, or semimembranosus muscles. At each interval, treatments were administered as one, three, or five injections of a unit dose of bST (.6 g) or five injections of the vehicle (control, equivalent volume to the 3.0-g bST treatment). Individual syringes and separate, sterile needles were used for each injection. The amount of formulation actually injected was verified by preinjection and postinjection syringe weights. This study was conducted according to FDA Good Laboratory Practice Regulations (11) and Target Animal Safety Guidelines (27).

Guidelines of the National Institutes of Health (18) for the care and use of laboratory animals were followed.

Pregnant nulliparous, primiparous, and multiparous cows were assembled at least 30 d prior to calving at Monsanto's Dardenne, Missouri dairy facility. All animals were screened for bovine paratuberculosis, anaplasmosis, and brucellosis prior to arrival. Treatments were administered from 60 ± 3 d postpartum until dry-off or necropsy. A subset of 38 pregnant cows (14 controls; 8 cows, .6 g of bST; 7 cows, 1.8 g; 9 cows, 3.0 g) continued through a second lactation with the same treatment regimen. Animals were randomly selected to continue into the next lactation prior to the end of the first treatment period (before final repro-

ductive status was known and without consideration of health or production response). Necropsies were conducted on pregnant cows that were not selected to continue on test, any nonpregnant cows (at the end of each lactation), and all moribund or dead animals. Remaining cows were necropsied at the start of the third lactation of study.

Cows were housed in an artificially ventilated and lighted tie-stall barn during both the lactation and dry periods. Stalls were bedded with wood shavings over rubber mats and equipped with feed tubs to allow measurement of individual feed intakes. Ambient temperature and humidity were measured daily at 0800, 1200, and 1600 h. Unless research procedures or weather conditions precluded, all cows were exercised on a dirt and concrete lot at least 2 h daily. Animals were milked twice daily at approximately 0500 and 1700 h in a double-eight herringbone parlor. The DeLaval milking machines included automatic take-off units and a back flush system (Alfa-Laval Agri, Inc., Kansas City, MO). Teats were washed, dried with a paper towel, and forestripped prior to milking. Clinical mastitis was diagnosed by the presence of flakes in the milk or a hot, turgid appearance to the mammary gland. A milk sample from clinically positive animals was collected for microbiologic culture and antibiotic sensitivity testing. Following milking, the infected quarter was treated with a commercial antibiotic, and all teats were dipped with an iodine solution.

Weekly a.m. and p.m. milk samples were analyzed for fat and protein (Multispec Infrared Analyzer, Berwind Instrument Group, York, England) and lactose (Milk-O-Scan 104 A/B, Foss Food Technology, Eden Prairie, MN). Monthly milk samples were analyzed for ash, calcium, phosphorus, and (2nd yr only) magnesium and zinc concentrations by inductively coupled Argon plasma emission spectrometry (Perkin Elmer, Richfield, CT). Subsamples of whole milk were lyophilized, ashed at 500°C, and dissolved in 5% nitric acid prior to mineral analysis. Appropriate analytical standards and blanks were prepared in the same matrix as the ashed samples. A standard reference material (SRM 1573, National Measurement Laboratory, National Bureau of Standards, Washington, DC) was ashed and included in the analyses. Milk bST concentrations were measured on weekly samples from wk -2 through 5 of treatment and monthly thereafter. After centrifugation at 40,000 × g for 30 min, milk serum was incubated with 125I-labeled bST and rabbit anti-bovine antiserum (4°C, 24 h), with goat anti-rabbit antiserum (4°C, 18 h; American Biosystems, Kansas City, KS). After a 25°C, 1-h precipitation with polyethylene glycol, tubes were centrifuged for 30 min at 2000 × g, decanted, blotted, and counted. The sensitivity limit of this assay was 1 ng/ml of milk serum. Intraassay and interassay coefficients of variation and recovery averaged 8.3, 16.6, and 80%, respectively.

Blood was sampled to gain information on endocrine and metabolite concentrations relative to changes in milk composition across lactation. Jugular blood samples were drawn at 1200 to 1600 h during treatment wk -2, -1, 3, 7, and every 8 wk thereafter (during the 1st yr) or every 4 wk thereafter (2nd yr). No blood samples were drawn during the cows' dry periods. Serum was frozen for analysis of calcium, phosphorus, and bST. Blood calcium and phosphorus were analyzed using an ACA IV Clinical Analyzer (DuPont, Wilmington, DE). Intraassay coefficients of variation and recovery averaged <1 and 100%, respectively, for both calcium and phosphorus. Interassay coefficients of variation averaged 4.16 and 9.94% for calcium and phosphorus. Somatotropin was measured by double-antibody radioimmunoasssay using rabbit polyclonal anti-bST (lot 609, Monsanto Company, St. Louis, MO), goat anti-rabbit gamma globulin (Linco Research, Inc., St. Louis, MO), and polyethylene glycol precipitation. The anti-bST antibody cross-reacts less than 1% with bovine prolactin and does not differentiate between bST variants. Methionyl bST (Monsanto Company) was used as the tracer (125I-labeled). Intraassay and interassay coefficients of variation averaged 11.63 and 5.95%, respectively. Recovery of augmented bST in serum averaged 95% over the range from .7 to 67 ng/ml.

Cows were fed for ad libitum intake one of five total mixed diets (Table 1) formulated to meet or exceed NRC requirements (19). Diets contained corn silage, chopped alfalfa hay, beet pulp, whole cottonseeds (2nd yr only), and premixed protein, grain, and mineral supplements. Fresh feed was offered twice daily and orTs measured prior to the a.m. feeding.
TABLE 1. Composition of the complete mixed diets fed during the study.

<table>
<thead>
<tr>
<th>Nutrient content</th>
<th>Lactating cows</th>
<th>Dry cow</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>NE(_L), Mcat/kg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude protein, %</td>
<td>17.6</td>
<td>16.2</td>
</tr>
<tr>
<td>Acid detergent fiber, %</td>
<td>20.1</td>
<td>22.2</td>
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<tr>
<td>Calcium, %</td>
<td>92</td>
<td>88</td>
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<tr>
<td>Phosphorus, %</td>
<td>53</td>
<td>54</td>
</tr>
<tr>
<td>Magnesium, %</td>
<td>.27</td>
<td>.26</td>
</tr>
<tr>
<td>Potassium, %</td>
<td>1.11</td>
<td>1.10</td>
</tr>
<tr>
<td>Sodium, %</td>
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<td>.27</td>
</tr>
<tr>
<td>Sulfur, %</td>
<td>.25</td>
<td>.23</td>
</tr>
<tr>
<td>Iron, ppm</td>
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<td>528</td>
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<tr>
<td>Zinc, ppm</td>
<td>164</td>
<td>166</td>
</tr>
<tr>
<td>Manganese, ppm</td>
<td>139</td>
<td>138</td>
</tr>
<tr>
<td>Copper, ppm</td>
<td>24</td>
<td>23</td>
</tr>
</tbody>
</table>

1Primiparous cows switched from diet 2 to diets 3 or 4 when average milk production decreased to 22 or 25 kg/d, respectively. Multiparous cows switched from diet 1 to diets 2, 3, or 4 when average milk production decreased to 34, 25, or 16 kg/d, respectively. Body condition of the cow also was considered before switching diets in order to limit overconditioning.

2All values presented on DM basis.

Daily feed samples were composited weekly and analyzed by Livestock Nutrition Lab Services, Columbia, MO. All animals were fed the high energy density diets (diet 1, mature cows; diet 2, heifers) through the first 30 d of each year's treatment period. Thereafter, cows were switched to lower energy diets according to milk production (Table 1). Body condition of the cows was also considered before switching diets in order to minimize overconditioning.

Except during the cows' dry periods, body weights were measured once weekly after the a.m. milking. Body condition was scored (1 to 5) on a monthly basis beginning at the end of the 1st yr (29). For NE\(_L\) balance calculations, maintenance requirements and feed component energy were according to NRC (19). The energy values were calculated from the individual NE\(_L\) values for the ingredients and the proportion of that ingredient in the diet. Protein, calcium, and phosphorus balances were calculated similarly. Energy expenditures associated with milk production were calculated according to NRC (19). Salable 3.5% FCM was calculated by subtracting FCM that would have been discarded because of medication from total FCM (no milk was actually marketed from this study). Discarded FCM was calculated by summing milk yield at each milking throughout the labeled period of milk withdrawal for all medications.

Statistics

Data for each year were analyzed separately. Cows in their first lactation during the 1st yr of study formed a primiparous group; cows in their second or third lactation during the 1st yr of study formed a multiparous group. These groupings were maintained for analysis of 2nd-yr data. Data were excluded for any cow removed for health reasons (7) prior to completion of at least two-thirds of the treatment period (approximately 11 injection cycles). Data were truncated at the end of complete injection cycles for all variables.

The primary model for 1st-yr data included treatment, block (based on location in barn), parity group, and interaction between treatment and parity group. Two-week pretreatment means for each cow were included as a covariate, expressed as a deviation from pretreatment mean for the cow's parity group. The model for the 2nd yr excluded block because of reduced number of animals. All analyses used SAS software (24). Pairwise treatment comparisons (least significant difference) of production data were conducted at \(P < .05\) when the main effect was significant \((P < .05)\).
Milk bST concentrations were evaluated by chi-square analysis, because most values were less than the sensitivity limit of the assay (1 ng/ml).

RESULTS AND DISCUSSION

Except where specifically noted, animal health was good throughout the study and did not affect average response during the 1st or 2nd yr. Depending on timing of dry-off, cows generally received 16 to 18 2-wk injection cycles during each of the two lactations of treatment. Data through 16 treatment cycles (224 d) are presented for consistency.

Milk Response

Within each 14-d treatment period, milk production increased steadily through the middle of each cycle and diminished toward basal production thereafter. This peak and valley pattern of galactopoietic response (3) was repeatedly observed throughout the study (Figure 1), and milk production of treated cows never returned to concurrent control levels. The sustained milk response achieved during each lactation of study (Figure 1) could not have been maintained if the cows' overall health was poor.

Milk response to bST treatment during the first lactation of study was similar to the results previously reported for long-term bST treatment (1, 2, 3, 26). Production of 3.5% FCM during the 2 wk prior to treatment did not differ among treatment groups and averaged 24.8 and 30.0 kg/d for the primiparous and multiparous cows, respectively. Initiation of bST injections dramatically increased FCM production and maintained a response throughout the treatment period (Figure 1). When averaged over 16 injection cycles, .6, 1.8, and 3.0 g/14 d of bST increased FCM by 7.2, 9.4, and 8.4 kg/d over controls (21.1 kg/d) during the first lactation. These data agree with those of the dose determination study of Franson et al. (10), who reported 3.0, 3.7, and 5.5 kg/d milk responses to doses of .25, .50, and .75 g/14 d of sometribove, respectively. However, during the second lactation, FCM was increased 10.6, 3.6, and 4.9 kg/d over control production (24.8 kg/d) by the .6, 1.8, and 3.0 of bST, respectively.

A similar pattern was noted for standardized salable 3.5% FCM, which excludes milk that would not have been marketable because of medication withdrawal times (Table 2). In the present test, those medications were primarily to treat clinical mastitis (7). Prior to treatment during the 1st yr, clinical mastitis was observed in 4, 7, 5, and 8 cows randomly assigned to the control, .6-, 1.8-, and 3.0-g groups, respectively. Salable FCM was increased 8.1, 9.1, and 6.2 kg/d over control production by .6, 1.8, and 3.0 of bST during the first lactation (Table 2). The number of days that cows were mastitic during the 1st-yr pretreatment period was statistically significant covariate (P = .01), if included in the model for salable FCM, although the resulting means were similar to those presented without covariate (Table 2). During the 2nd yr, the control and 3.0-g groups had more mastitis than other treatments: 6 out of 14, 3 of 8, 1 of 7, and 7 of 9 cows in the control, .6-, 1.8-, and 3.0-g groups had clinical infections sometime during lactation. During the second lactation, salable FCM was increased 12.1 and 4.7 kg/d over control (21.9 kg/d) by the .6- and 1.8-g treatments, respectively. An increase in mastitis in the 3.0-g group during the 2nd yr (7) obscured the bST response, and salable FCM averaged 19.1 kg/d (P > .10). Cows infected with chronic mastitis during the 1st yr, which would normally have been culled from commercial herds, had a major impact on salable and discarded milk of the 3.0-g group in the 2nd yr. Cows that were allowed to continue into the second lactation were selected randomly prior to the end of the first treatment period (before final reproductive status was known and without consideration of health or production response). Moreover, no animals were culled from study because this was a toxicity evaluation (27). At the .6- and 1.8-g doses, production was unaffected by response to natural health disorders such as mastitis. Interestingly, because medications were administered to cows during illnesses such as mastitis or displaced abomasum that often decrease milk production, days of lower milk production should be excluded. The resulting least squares means correctly reflect control and bST cows' production after subtraction of that "discarded milk", but the .6-g group during the 2nd yr appears artificially
Year 1

Year 2

Control  .6 g bST  1.8 g bST  3.0 g bST

Day of Treatment

Figure 1. Effect of 0, .6, 1.8, or 3.0 g of bST injected every 2 wk on average daily 3.5% FCM during each year of study. Overall pooled SEM = .93 and 1.38 for yr 1 and 2.

inflated relative to total FCM for that group. Regardless, the higher incidence of pretreatment clinical mastitis among cows in bST groups compared with controls was not changed by subsequent bST treatment. During the 1st or 2nd yr of study, 4 cows were necropsied due to mastitis: 1 cow treated with .6 g of bST; 2 cows, 1.8 g; 1 cow, 3.0 g. Although the ability of bST to increase milk production has been well documented (1, 6, 21), the present study demonstrates that mastitis, if not controlled, may negatively affect the response to supraphysiological doses of bST.

Production Carry-Over Effects

As expected, milk yields during early lactation in the second year of study tended to be increased over the previous year, especially for the control, .6-, and 1.8-g groups (Figure 1).
However, pretreatment milk yields of cows in the 3.0-g group were 5.8 kg/d lower in the 2nd yr than the 1st (Table 3). A significant portion of the decrease in pretreatment milk production in the 3.0-g group was attributable to the health of 3 cows that had an average milk yield decline of 14.5 kg/d from the 1st to the 2nd yr of study. These 3 cows had compromising health disorders limiting their production: lameness, mastitis, or spinal injury incurred during estrus. In contrast, the remaining 6 cows in the 3.0-g group averaged 5.6 kg/d above the previous year’s early lactation performance.

With the start of treatment during the 2nd yr, 3.0-g treated cows responded with an average of approximately 12 kg/d increase in milk production. These data indicate that lower early lactation milk yields in the 3.0-g group were not due to an impaired ability of the mammary gland to synthesize milk. Moreover, pretreatment serum bST concentrations averaged 1.0 ± .45 ng/ml during the second lactation and did not differ among treatments. Therefore, if exogenous bST had a negative feedback effect on pituitary bST production during the 1st yr (not examined), that effect did not persist into the pretreatment period of the 2nd yr.

Pretreatment milk production during the 2nd yr also was significantly affected by the number of days open during the first lactation of study \((P = .01, \text{Table 3})\). Cows that had conceived during the 1st-yr treatment period produced 10.4 kg/d more FCM during the pretreatment period (first 60 d) of the 2nd yr than during the equivalent period of the 1st yr. In contrast, cows bred during the 1st-yr pre-
TABLE 3. Effect of administration of high doses of somatotropin and time of conception during the previous year on production in the subsequent pretreatment period.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Year</th>
<th>Control</th>
<th>.6 g</th>
<th>1.8 g</th>
<th>3.0 g</th>
<th>Estimated pooled SE</th>
</tr>
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<tbody>
<tr>
<td>Number of cows</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>P</td>
<td>14</td>
<td>8</td>
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<td>9</td>
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<tr>
<td>Pretreatment</td>
<td></td>
<td>5</td>
<td>2</td>
<td>4</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Pretreatment</td>
<td>Trt</td>
<td>9</td>
<td>6</td>
<td>3</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>3.5% FCM, kg/d</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1^2</td>
<td></td>
<td>25.9</td>
<td>25.5</td>
<td>26.4</td>
<td>27.6</td>
<td>1.28</td>
</tr>
<tr>
<td>Diff^3</td>
<td></td>
<td>35.2^a</td>
<td>38.3^a</td>
<td>32.0^a</td>
<td>20.9^b</td>
<td>1.69</td>
</tr>
<tr>
<td>P^+</td>
<td></td>
<td>8.6</td>
<td>3.4</td>
<td>-4</td>
<td>-1.5</td>
<td>2.62</td>
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<tr>
<td>D^4</td>
<td></td>
<td>9.3</td>
<td>10.1</td>
<td>6.4</td>
<td>.2</td>
<td>2.62</td>
</tr>
<tr>
<td>Net energy intake, Mcal/d</td>
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<td>27.7</td>
<td>29.1</td>
<td>26.7</td>
<td>26.7</td>
<td>1.36</td>
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<td>32.9</td>
<td>39.0</td>
<td>39.1</td>
<td>33.2</td>
<td>1.24</td>
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<tr>
<td>P^+</td>
<td></td>
<td>5.1</td>
<td>9.4</td>
<td>12.2</td>
<td>8.8</td>
<td>1.23</td>
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<tr>
<td>Net energy balance, Mcal/d</td>
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<td>.83</td>
<td>2.41</td>
<td>.97</td>
<td>-1.45</td>
<td>.909</td>
</tr>
<tr>
<td>Diff^3</td>
<td></td>
<td>-1.42^a</td>
<td>2.73^b</td>
<td>7.24^c</td>
<td>9.49^a</td>
<td>1.037</td>
</tr>
</tbody>
</table>

^a Least squares means within row differ (P < .10).
^1 Pretreatment was from calving through 60 d of lactation. P = Conception during pretreatment period. Trt = Conception during treatment period.
^2 Statistical model included treatment (T), lactation parity (L), and T x L interaction.
^3 Model included T, L, T x L, and 1st-yr pretreatment production as covariate.
^4 Model included T, L, T x L, period when conception occurred (C), and T x C interaction.
^* Significant effect of timing of conception during the previous year (P = .01).

Treatment period produced only 2.5 kg/d more FCM than in the equivalent period of the 2nd yr. During each year, cows were dried off at approximately 60 d prior to expected calving or when average milk production for an injection cycle decreased to 5 kg/d. Therefore, dry-off due to stage of gestation occurred later in lactation for those animals conceiving during the treatment period. The cows with later conception dates may have been better able to replenish body stores prior to calving than animals that conceived earlier.

After one lactation of bST treatment, cows in the .6- and 1.8-g groups consumed numerically more feed energy during the pretreatment period than controls (Table 3). Net energy balance was positive and higher in bST cows than controls during the second lactation pretreatment period and by difference from one year to another (Table 3). These differences in energy balance may reflect compensation for the lower body fat stores in animals of the higher dose groups. At the end of each lactation, when body condition scores of some control cows were too high (over 4), bST-treated cows tended to be leaner, but within the expected range (approximately 3.5) according to the system of Wildman et al. (29). The perceived overall difference in body fat probably reflected the known antilipogenic, anti-insulin effects of bST.

Feed Intake and Nutrient Balances

Dry matter intake was increased significantly during the first treatment period by approximately 3 kg/d among all bST groups (Table 2). During the 2nd yr, DMI was numerically elevated. Overall net energy intake was increased approximately 6 and 5.5 Mcal/d among bST-treated cows during the treatment period of the 1st and 2nd yr, respectively (Table 2). Milk energy was increased approxi-
mately 6 Mcal/d among bST-treated cows, and responses did not differ among bST doses (Table 2). Increases in milk yield because of bST are not immediately associated with changes in energy intake (1, 21). The pattern of delayed increase in energy intake noted in bST-treated animals is consistent with the feeding behavior associated with the normal lactation curve; i.e., maximum feed intake follows milk yield. During the initial weeks of treatment, the milk energy response resulted in decreased calculated energy and protein balances: approximately 2 to 5 Mcal/d and up to 600 g/d, respectively (Figure 2). Subsequent elevations of energy intake in sometribove groups balanced earlier energy losses, and no
overall differences were noted in average energy balance, similar to previous results (1, 2, 3, 25). This sequence was repeated during the 2nd yr, although calculated energy balance was numerically increased approximately 2.5 Mcal/d, and protein balance was improved by an average of 245 g/d (Table 2). Similarly, gross feed efficiency (kilograms of FCM/megacalories of NE\textsubscript{L} intake) was elevated during the first but not the second treatment period (Table 2). Although this toxicity study was not an efficacy test, the absence of negative effects on calculated efficiency suggests a simple dilution of maintenance energy requirements by bST (1, 21).

**Milk Composition**

Milk composition during either lactation was unaffected by sometribove treatment (Table 2). As lactation progressed, percentage of milk fat and protein increased an average of .5 and .3%, respectively, in both the control and bST-treated animals, and no differences among treatments were noted over 16 cycles of administration. Milk lactose also was not affected by bST. The lack of change in milk composition was consistent with previous long-term studies (1, 2, 3, 22, 26) and further supported the normality of milk from bST-treated cows.

Milk bST concentrations were at or below the limit of sensitivity of the assay for most cows (1 ng/ml). During the weekly sampling period (wk 1 to 5), milk bST tended to be elevated at the midpoint and lower at the end of each injection cycle in the 1.8- and 3.0-g bST groups (Figure 3). During each 14-d injection cycle, blood bST concentrations rose and fell synchronously with the milk response (3). No pattern was present in the milk bST concentrations of the control and .6-g groups (Figure 3) although blood bST concentrations were increased significantly in the .6-g bST group. Chi-square analysis revealed that bST increased the proportion of cows with measurable milk bST (P < .10), although even the 3.0-g dose rarely resulted in concentrations greater than 2 ng/ml (Figure 3). Thus, the 20- to 30-fold increases in blood bST concentrations noted for the 3.0-g group did not dramatically increase milk bST concentrations (Figure 3). These data are similar to those of Schams (25), who was able to elicit an increase in milk bST only after a “provocative” dose of 1.92 g was administered. Hart et al. (13) also noted an increase in the proportion of milk serum samples with detectable bST in bST-treated cows, but Bourne et al. (4) and Mohammed and Johnson (17) reported no change in milk bST concentrations. Overall, these data confirm that bST concentrations in milk are exceedingly small. A proposed commercial dose (.6 g/14 d) did not affect milk bST concentrations.

**Milk Minerals and Nutrient Balance**

Overall milk ash, calcium, phosphorus, magnesium, and zinc concentrations were unaffected by sometribove (Table 4). In spite of transient decreases of up to 40 and 20 g/d in calcium and phosphorus balance, respectively, milk mineral content remained well within normal limits ([8]; Figures 4 and 5). These results demonstrate that, even at the pharmacological bST doses in this study, milk mineral content was normal.

Despite large increases in milk production, overall calcium and phosphorus balances also were unaffected by bST during either year. Similar to energy balance, calcium and phosphorus balances decreased early in the treatment period as milk production increased rapidly, and the rise in feed intake followed several weeks later (Figures 1, 4, and 5). During the period of decreased calcium and phosphorus balances, serum calcium and phosphorus concentrations were unaffected by sometribove ([9]; Figures 4 and 5).

Because milk calcium, phosphorus, magnesium, and zinc concentrations generally were unaffected or increased by bST, the whole-body turnover of these moieties must have increased at least commensurate with the increase in milk secretion. Even during these changes, bST did not affect the incidence of mineral-related metabolic imbalances such as paresis or tetany (7). Although hormones such as parathyroid hormone, which control blood calcium concentrations, were not measured directly, sometribove did not cause any macro- or microscopic lesions or hypertrophy of parathyroid glands. Furthermore, bone radiologic appearance, ash content, and the absence of macroscopic or microscopic lesions all indicated that bST did not cause periosteal new
Figure 3. Effect of 0, .6, 1.8, or 3.0 g of bST injected every 2 wk on average milk and blood serum somatotropin concentrations during the 1st yr of study. Horizontal reference line in milk bST plot is the assay sensitivity limit.
Figure 4. Effect of 0, .6, 1.8, or 3.0 g of bST injected every 2 wk on average milk calcium, serum calcium, and calculated calcium balance during the 1st yr of study.
Figure 5. Effect of 0, 0.6, 1.8, or 3.0 g of bST injected every 2 wk on average milk phosphorus, serum phosphorus, and calculated phosphorus balance during the 1st yr of study.

bone formation, osteoporosis, enchondral changes, or fractures. This total pattern of mineral homeostasis is consistent with a coordinated, controlled response to bST (1, 21).

CONCLUSIONS

The sustained milk response for all bST doses was consistent with good overall health of cows administered bST for two consecutive lactations. Sometribove generally had no effect on milk fat, protein, lactose, calcium, phosphorus, magnesium, or zinc concentrations. Milk serum bST concentrations were increased only at the highest bST doses. No toxic effects of bST were observed, although milk losses due to mastitis obscured the production response in the 3.0-g dose group during the second lactation. Sometribove increased milk production of dairy cows without affecting milk composition or energy intake.

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REFERENCES


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