Comparison of Somatotropin and Growth Hormone-Releasing Factor on Milk Yield, Serum Hormones, and Energy Status

G. E. DAHL,¹ L. T. CHAPIN,¹ M. S. ALLEN,¹ W. M. MOSELEY,² and H. A. TUCKER¹

Department of Animal Science
Animal Reproduction Laboratory
Michigan State University
East Lansing 48824
and
The Upjohn Company
7921 -25-4
Performance Enhancement Research
Kalamazoo, MI 49001

ABSTRACT

Holstein cows received 12 mg/d of growth hormone-releasing factor (continuous i.v. infusion, n = 5), 14 mg/d of bST (single daily i.m. injection, n = 8), or no treatment (controls, n = 8) for 60 d. Compared with controls (31.6 kg/d), bST and growth hormone-releasing factor increased milk yield to 34.2 and 37.0 kg/d, respectively. The increase in milk yield induced by the growth hormone-releasing factor was greater than that for bST. Milk yield was not different among groups following cessation of treatment. Milk energy output was 24.2 Mcal/d in controls, and growth hormone-releasing factor increased milk energy output to 28.5 Mcal/d. Milk energy output of cows receiving bST was 26.1 Mcal/d. Growth hormone-releasing factor increased DMI (23.2 kg/d) over that of controls (21.1 kg/d), whereas bST (21.5 kg/d) did not. Relative to controls, bST increased averaged daily serum somatotropin from 1.3 to 7.6 ng/ml and insulin-like growth factor-I from 67.5 to 116.0 ng/ml. Relative to bST, growth hormone-releasing factor increased serum somatotropin to 16.3 ng/ml and insulin-like growth factor-I to 202.6 ng/ml. Relative to control (115.8 meq/dl) and bST (158.1 meq/dl), growth hormone-releasing factor increased plasma NEFA (230.3 meq/dl). During treatment, calculated energy balance was negative for cows receiving growth hormone-releasing factor but positive for bST and control cows. Milk composition, body condition score, BW, and apparent digestibility of DM were not different among treatments. We conclude that i.v. infusion of 12 mg/d of growth hormone-releasing factor has greater galactopoietic activity than i.m. injections of 14 mg/d of bST. These data support the concept that the galactopoietic effects of growth hormone-releasing factor are mediated via increased secretion of somatotropin and insulin-like growth factor-I.

(Key words: growth hormone-releasing factor, somatotropin, milk yield)

Abbreviation key: BCS = body condition score, bGRF = bovine growth hormone-releasing factor, GRF = growth hormone-releasing factor, IGF-I = insulin-like growth factor-I, ST = somatotropin.

INTRODUCTION

Exogenous growth hormone-releasing factor (GRF) and bST increase milk production in dairy cattle (3, 7, 10, 19). However, the relative galactopoietic potencies of GRF and bST have not been directly compared. Moreover, milk production responses to GRF or bST are dissimilar among studies following withdrawal of treatment. For example, elevation of milk yield in sheep (17) and cows (7, 19) was sustained following withdrawal of GRF. In contrast, milk production declined rapidly after...
withdrawal of bST from ewes (17) and cows (14). Our first objective was to compare the effects of GRF and bST on milk yield during and after treatment. Our approach was to use doses and routes of administration of GRF and bST that in independent studies optimized their respective galactopoietic responses (1, 7).

Potentially, differences in galactopoietic potency between GRF and bST may be explained by differences in response of serum concentrations of somatotropin (ST) or insulin-like growth factor-I (IGF-I). Both GRF and bST increase serum concentrations of ST (3, 7, 14, 19). Indeed, the galactopoietic action of GRF is attributed to increased serum concentrations of ST (7, 8, 20). However, whether GRF and bST at doses and routes of administration that optimize yield of milk each elicit similar responses in serum concentrations of ST is unknown. Thus, our second objective was to compare the effects of GRF and bST on serum concentrations of ST. The putative mediator of bST galactopoietic action at the mammary gland is IGF-I (14). To our knowledge, there has been no comparison of the abilities of GRF and bST to increase serum concentrations of IGF-I. Therefore, a third objective was to compare effects of GRF or bST on serum concentrations of IGF-I.

Differences in nutrient partitioning between GRF and bST may affect their relative galactopoietic potency. Increases in milk production require increased nutrient availability at the mammary gland (2). Potentially, increases in DMI, DM digestibility, or mobilization of tissue stores are sources of energy to support increased milk production (25). Thus, our final objective was to identify the source or sources of energy that support increased milk yield in response to GRF and bST.

MATERIALS AND METHODS

Design and Management

Fifteen multiparous and nine primiparous Holstein cows averaging 77.8 ± 7.3 d of lactation were used in a randomized complete block design with repeated measurement. Eight blocks of three cows each were formed based on parity and pretreatment milk yield between -21 and -17 d of the experiment. Within each block, cows were assigned randomly to treatment (eight cows per treatment). Treatments were continuous i.v. (jugular) infusion of recombinant bovine GRF (1-45) homoserine lactone (bGRF; 12 mg/d); single daily i.m. injection of recombinant bST (14 mg/d); and un.injected, uninfused controls. Fourteen days before treatment began, VETport® infusion catheters (Thermedics, Woburn, MA) were implanted surgically into cows assigned to bGRF treatment as described previously (7). Doses of bGRF and bST were prepared daily in sterile pyrogen-free water. Infusions of bGRF were as previously described (7). Injections of bST were made in the left or right flank region, alternating each day. Treatments were initiated at 0900 h on d 0. Each day of treatment consisted of the 24-h period following 0900 h. Cows were housed in tie stalls, exposed to 24 h/d of light, and milked at 0600 and 1700 h. Milk production was recorded daily, and milk was sampled for 3 consecutive d every 20 d for composition analysis beginning with the -3 to -1 d pretreatment period. Fat, protein, solids, and lactose in milk were measured using an infrared analyzer (Multispec, Wheel-drake, Engl.) at Michigan DHIA (East Lansing). Yield of SCM and output of energy in milk (Mcal/d) were calculated (31). A TMR was fed for ad libitum intake. The TMR was formulated (Table 1) to provide adequate nutrition for a 612.2-kg cow producing 40.8 kg/d of milk containing 3.6% fat and assuming an intake of 24.2 kg/d of DM (25). Feed was offered daily at 0300 and 1200 h. Weight of orts was recorded once daily. Cows were weighed for 3 consecutive d every 20 d beginning on -3 to -1 d. Two experienced examiners scored body condition (BCS) 1 to 5 (32) at -1, 59, and 79 d.

During the study, three cows were removed after they contracted coliform mastitis and ceased to lactate. The three cows were in the bGRF treatment group, although one of the three contracted the mastitis and was removed from study prior to receiving bGRF. The data of these three cows were deleted from all statistical analyses. It should be noted that the incidence of coliform mastitis was elevated in the entire Michigan State University dairy herd at the time of the study.

Feed Digestibility Determination

Between -5 to -1, 55 to 59, and 75 to 79 d, fecal samples were collected every 15 h. Also,
TABLE 1. Feed composition (DM basis) of the diet fed to lactating Holstein cows treated with 14 mg/d of bST or 12 mg/d of recombinant bovine growth hormone-releasing factor (bGRF).1

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alfalfa haylage</td>
<td>30.2</td>
</tr>
<tr>
<td>Corn silage</td>
<td>11.2</td>
</tr>
<tr>
<td>Ground hull corn</td>
<td>37.3</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>9.9</td>
</tr>
<tr>
<td>Whole cotton seed</td>
<td>8.7</td>
</tr>
<tr>
<td>Mineral mix2</td>
<td>2.4</td>
</tr>
<tr>
<td>Salt</td>
<td>.3</td>
</tr>
</tbody>
</table>

1Diet contained calculated values of 17.0% CP, 1.72 Mcal/kg NE<sub>L</sub>, 15% crude fiber, 19.3% ADF, 32.4% NDF, 97% Ca, and .49% P.

2Mineral and vitamin premix contained .89% S, 5709 ppm Zn, 3101 ppm Cu, 7819 ppm Mn, 181 ppm I, 152 ppm Se, 1.74 × 10<sup>6</sup> IU vitamin A, 5.11 × 10<sup>6</sup> IU vitamin D, and 7.3 × 10<sup>6</sup> IU vitamin E/kg of DM.

on each day during fecal collections, feed and ors were sampled from each cow. All fecal, feed, and ors samples were dried at 55°C, ground through a Wiley mill (1-mm screen; Arthur H. Thomas, Philadelphia, PA), and each was composited for each cow. Neutral detergent fiber was determined in duplicate according to Goering and Van Soest (15) with the omission of decahydrogenapthalene, sodium sulfite (which was added to fecal samples), the substitution of trimethylene glycol for 2-ethoxyethanol (5), and the inclusion of α-amylase (29). Acid detergent fiber was determined sequentially on the NDF residue according to Goering and Van Soest (15). Lignin content was quantified by treating the ADF residue with 72% H<sub>2</sub>S<sub>O</sub><sub>4</sub> (15). Crude protein was determined using the method of Hach et al. (16). Dry matter content was determined gravimetrically after drying samples at 100°C for 24 h. Samples were ignited at 500°C for 5 h to determine ash content. Apparent digestibility was calculated using lignin as an intrinsic marker.

Blood Sampling and Analysis

Blood was sampled hourly from an indwelling jugular catheter for 25 h at 1 and 59 d and for 8 h on 60 and 64 d. Also, single samples were collected by coccygeal vessel puncture on d -1, 19, 39, and 79. Blood samples collected for serum were stored at room temperature for 2 to 6 h at 4°C for approximately 15 h. Blood samples collected for plasma were treated with NaF-EDTA and placed on ice immediately after collection. Serum or plasma was harvested after centrifugation for 30 min at 1550 × g and frozen at −20°C until assayed for ST (24), IGF-I (7), and NEFA [NEFA-C kit, Wako Chemicals USA, Dallas, TX; as modified in (23)].

Statistical Analysis

The experiment had nine periods: one pretreatment period (−10 to −1 d), six treatment periods (0 to 9, 10 to 19, 20 to 29, 30 to 39, 40 to 49, and 50 to 59 d), and two posttreatment periods (60 to 69 and 70 to 79 d). All data were subjected to split-block ANOVA with repeated measurement (13). Within-period means were examined using the Bonferroni t test (12).

RESULTS

Pretreatment milk yield (−10 to −1 d; Figure 1) was significant when tested as a covariate; therefore, subsequent milk yields were adjusted by covariance for pretreatment milk yield. Compared with controls (31.6 ± .6 kg/d),
bST and bGRF increased milk production to 34.2 ± 0.6 (P < .06) and 37.0 ± 0.7 (P < .01) kg/d during treatment. Following cessation of treatment (60 to 79 d), there was no difference in milk yield among treatment groups. Yield of SCM of bGRF-treated cows (38.0 kg/d) increased (P < .01) relative to control cows (32.3 kg/d). However, yield of SCM of bST-treated cows (34.8 kg/d) was not different (P > .10) from that of bGRF-treated or control cows. Average percentages of protein (3.2), lactose (4.8), fat (4.2), and solids (12.9) in milk were similar among treatments throughout the study.

Relative to controls, cows receiving bGRF increased (P < .01; Figure 2) energy output in milk during all treatment periods, but cows receiving bST had increased (P < .05) energy output in milk only from 40 to 59 d. Neither bGRF nor bST affected energetic efficiency of milk production adjusted for BW differences (data not shown). During pretreatment, DMI was not different among treatment groups (Figure 3). Relative to control, neither bST nor bGRF affected DMI (Figure 3) or DM digestibility (data not shown) during the treatment.

**Figure 2.** Milk energy output of cows receiving 12 mg/d of recombinant bovine growth hormone-releasing factor (bGRF; •), or 14 mg/d of bST (○), or no treatment (△) for 60 d. Each connected point represents the average of a treatment group (least squares means) within each 20-d period, adjusted by covariance with pretreatment milk energy output. Beginning and end of treatment indicated by the solid and open arrows, respectively. The SE of difference within a period for control versus bST was 1.17 Mcal/d. The SE of difference for all other comparisons within a period was 1.33 Mcal/d.

**Figure 3.** The DMI of cows receiving 12 mg/d of recombinant bovine growth hormone-releasing factor (bGRF; •), or 14 mg/d of bST (○), or no treatment (△) for 60 d. Each point represents the average of a treatment group (least squares means) within each 10-d period, adjusted by covariance with initial BW. Beginning and end of treatment indicated by the solid and open arrows, respectively. The SE of difference within a period for control versus bST was 0.90 kg/d. The SE of difference for all other comparisons within a period was 1.02 kg/d.

### TABLE 2. Body weights (kilograms) of cows receiving 14 mg/d of bST, or 12 mg/d of recombinant bovine growth hormone-releasing factor (bGRF), or serving as controls for 60 d.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>d 1</th>
<th>d 19</th>
<th>d 39</th>
<th>d 59</th>
<th>d 79</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>511.8</td>
<td>513.8</td>
<td>524.3</td>
<td>532.9</td>
<td>540.4&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>bST</td>
<td>535.6</td>
<td>536.0</td>
<td>545.8</td>
<td>556.5</td>
<td>558.0&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>bGRF</td>
<td>557.2</td>
<td>561.8</td>
<td>556.8</td>
<td>579.9</td>
<td>569.2</td>
</tr>
</tbody>
</table>

<sup>1</sup>The SE of difference within a day for control versus bST was 22.3 kg. The SE of difference for all other comparisons within a day was 25.4 kg.

<sup>2</sup>Gains from −1 to 79 d were significant (P < .01) for these groups. The SE of difference for gain for these groups was 4.6 kg. The SE of difference for gain for bGRF-treated cows was 5.8 kg.

TABLE 3. Body condition scores (BCS) (five-point scale) of cows receiving 14 mg/d of bST, or 12 mg/d of recombinant bovine growth hormone-releasing factor (bGRF), or serving as controls for 60 d.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>d -1</th>
<th>d 59</th>
<th>d 79</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.66</td>
<td>1.72</td>
<td>1.94</td>
</tr>
<tr>
<td>bST</td>
<td>1.70</td>
<td>1.61</td>
<td>1.97</td>
</tr>
<tr>
<td>bGRF</td>
<td>1.76</td>
<td>1.58</td>
<td>1.82</td>
</tr>
</tbody>
</table>

The SE of difference within a day for control versus bST was .14. The SE of difference for all other comparisons within a day was .16.

2 Increases in BCS from -1 to 79 d were significant (P < .05) for these groups. The SE of difference for increases in BCS across day for these groups was .09. The SE of difference for increases in BCS across day for bGRF-treated cows was .11.

and posttreatment periods. Initial BW of all cows averaged 531.7 ± 9.2 kg, and BW of cows treated with bGRF or bST did not differ from that of controls within any period (Table 2). However, control and bST-treated cows gained (P < .01) 28.6 ± 4.6 and 22.3 ± 4.6 kg of BW from -1 to 79 d, whereas BW of bGRF-treated cows was unchanged. Initial BW was not different among treatments (Table 3). However, BCS of control and bST-treated cows increased (P < .05) from -1 to 79 d, but BCS of bGRF-treated cows was unchanged. Control and bST-treated cows sustained positive calculated energy balance throughout the study (Table 4). Calculated energy balance was negative for bGRF-treated cows from 0 to 59 d.

Compared with control, bGRF and bST increased mean serum concentrations of ST at 1 and 59 d (Figure 4). Furthermore, bGRF increased serum concentration of ST relative to bST on 1 and 59 d. At 60 d, cows previously treated with bGRF had increased (P < .05) serum concentrations of ST relative to bST and control cows. But by 64 d, serum concentrations of ST were not different among controls and cows previously treated with bGRF or bST.

On -1 d, there was no difference in serum concentrations of IGF-I among treatment groups (Figure 5). Compared with controls, bGRF and bST increased (P < .05) serum concentrations of IGF-I during treatment. Furthermore, concentrations of IGF-I in bGRF-treated cows were greater (P < .05) than in bST-treated cows. Serum concentrations of IGF-I remained elevated at 24 h after cessation of treatment with bGRF or bST (60 d); however, there was no difference in serum IGF-I among treatment groups on 64 or 79 d.

Plasma concentrations of NEFA were not different among treatment groups on -1 d. Although plasma NEFA throughout treatment were numerically and consistently greater for animals given bGRF or bST than for control cows (Figure 6), differences from control were significant (P < .05) only at 19 and 39 d of treatment with bGRF. Plasma concentrations of NEFA did not differ among cows after treatment ended.

DISCUSSION

Increased milk yield in response to bGRF or bST during the 60-d treatment are within the range of responses previously reported (7, 10, 19, 21, 28). Indeed, Peel et al. (27) reported similar increases in milk yield in cows treated with bST at the same production level and stages of lactation as bST-treated cows of the present study. Milk yield did not remain elevated following the cessation of bGRF or bST, whereas in a previous study (7) milk yield...
yield was elevated for at least 15 d following the end of bGRF treatment. Possibly, the earlier stage of lactation or lower BCS of cows in the present study relative to cows in our previous study (7) accounts for the discrepancy in milk yield response after withdrawal of treatment.

Increasing the doses of GRF or bST increases milk yield in association with increases in serum concentrations of ST and IGF-I (7, 8, 18). The doses of bGRF and bST used in the present study were previously shown to optimize the galactopoietic response, albeit in independent experiments (1, 7). In the present study, bGRF elicited a greater increase in serum concentrations of ST and IGF-I than bST. Thus, the ability of bGRF to increase serum concentrations of ST and IGF-I relative to the bST treatment probably explains the larger increases in milk yield with bGRF treatment. Another hypothesis is that the pattern of ST response to bGRF (pulsatile) versus bST (single daily pulse) affects the subsequent galactopoietic response. Indeed, body growth was greater in rats that received ST via a continuous infusion versus single daily injections (6). However, different routes of bST administration did not affect the galactopoietic response in dairy cows (11, 22). Furthermore, increases in nitrogen retention in steers were not different when identical amounts of ST were administered in various patterns each day (24). Therefore, pattern of ST administration does not appear to affect lactational or growth responses in cattle. Rather, the absolute amount of serum ST per day appears to determine the lactational or growth response. One approach to study further the differences in galactopoietic action of GRF and bST would be to match serum concentrations of ST. In a
preliminary study, we found that continuous infusion of 29 mg/d of bST was necessary to attain an increase in serum concentration of ST of 15 ng/ml, thus matching the average serum concentration of ST with 12 mg/d of bGRF (Dahl et al., unpublished observations). However, the galactopoietic effects of similar serum concentrations of ST induced by bST and bGRF treatments are unknown.

In agreement with previous reports (3, 7), milk composition in the present study was unaffected by bGRF or bST treatment. Thus, the pattern of increases in milk energy output induced by bST and bGRF was similar to that for milk yield. Relative to controls, DMI was unaffected by bGRF or bST treatment during any period. Previous reports indicate that bST has no effect on DM digestibility (27, 33). In agreement, bST had no effect on any aspect of DM digestibility that was examined in the present study. In contrast with bST, Tyrrell et al. (30) reported that GRF reduced losses of energy and nitrogen in feces and urine of steers. In the present study, bGRF had no effect on DM digestibility. Thus, neither DMI nor DM digestibility contributed to the increased energy required to support increased milk production. Exogenous GRF (7, 9, 21) and bST (4) increase plasma concentrations of NEFA. In the present study, plasma concentrations of NEFA were generally increased by bGRF or bST, suggesting mobilization of lipid stores (14). Moreover, the negative energy balance experienced by bGRF-treated cows supports the hypothesis that cows in negative energy balance mobilize adipose reserves to sustain increased milk production (26). Conversely, control and bST-treated cows gained BW and BCS in the present study, especially after treatments ceased. The lack of increase in BCS and BW in bGRF-treated cows, coupled with increased concentrations of NEFA, indicates that mobilization of adipose tissue is the likely source of energy to support increased milk production.

It is concluded that the galactopoietic response to continuous i.v. infusion of 12 mg/d of bGRF is greater than that of once daily i.m. injection of 14 mg/d of bST. The greater galactopoietic effects of bGRF relative to bST are probably mediated via increased secretion of ST and IGF-I. Mobilization of adipose tissue reserves likely provided the energy to support increased milk production in bGRF-treated cows.

ACKNOWLEDGMENTS

Acknowledgement is made to the Michigan Agricultural Experiment Station for support of this research.

The authors thank M. R. Zontello and L. F. Krabill (The Upjohn Co.) for assay of ST and IGF-I; W. Claflin and G. Alaniz (The Upjohn Co.) for supervision of Vetport catheter installation; D. Main and K. O’Neill (Department of Animal Science, Michigan State University) for assistance with forage analysis; members of the Animal Reproduction Lab, Michigan State University for assistance in sample collection; and S. F. Cotton for typing this manuscript.

REFERENCES

9 Enright, W. J., L. T. Chapin, W. M. Moseley, S. A.