PHYSIOLOGY AND MANAGEMENT

Stimulation of Milk Yield and Feed Intake by Bovine Placental Lactogen in the Dairy Cow

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ABSTRACT

A 6 x 6 Latin square design was used to test the effects of recombinant bovine placental lactogen on milk yield, milk composition, feed intake, and blood hormone and metabolite levels in nonpregnant lactating cows. The six treatments (5, 10, 20, and 40 mg/d of placental lactogen, water as negative control, and 20 mg/d of bST as positive control) were administered by subcutaneous injection twice daily for 9 d. Blood samples were taken during the last 5 d of the treatment period. The three highest doses of placental lactogen increased milk yield, and there was a linear dose effect, although placental lactogen was less potent than bST. Milk concentrations of lactose, protein, and fat were not altered by any of the treatments. Dry matter intake was increased by two of the doses of placental lactogen, but not by bST. Blood urea N concentration was decreased in a dose-dependent manner by placental lactogen and was also decreased by bST. Similarly, serum insulin-like growth factor-I was increased in a dose-dependent manner by placental lactogen and was also increased by bST. Plasma concentrations of NEFA and glucose were increased by bST, but placental lactogen had little or no effect on either of these parameters. Thus, placental lactogen appears to act, in part, as a weak somatotropin agonist; however, it also appears to have specific activities, e.g., stimulating feed intake.

(Key words: bovine placental lactogen, bovine somatotropin, lactation, feed intake)

Abbreviation key: bPL = bovine placental lactogen, bPRL = bovine prolactin, BUN = blood urea N, IGF = insulin-like growth factor, IGFBP-2 = insulin-like growth factor binding protein-2, rbPL = recombinant bPL, T3 = triiodothyronine.

INTRODUCTION

Bovine placental lactogen (bPL) is a 200 amino acid protein that is produced by binucleate cells of the bovine trophoblast (9). This hormone is part of the somatotropin and prolactin gene family based on structural and functional similarities to the pituitary hormones, somatotropin and prolactin. Bovine placental lactogen has 50 and 22% sequence homology with bovine prolactin (bPRL) and bST, respectively (21), and, thus, appears to have arisen from duplication of the prolactin gene. In addition, bPL has a small aminoterminal disulfide loop, which is characteristic for mammalian prolactins but absent in somatotropins (17). However, unlike pituitary bST and bPRL, native bPL is heavily glycosylated. A single N-linked oligosaccharide chain and an undetermined number of O-linked carbohydrate chains increase the apparent molecular weight of the native molecule by about 10,000 (8, 22).

In keeping with its prolactin-like structure, bPL displays lactogenic activity both in vitro and in vivo. It binds to the prolactin receptor in the rabbit (10) and bovine (6) liver radioreceptor assays. It also stimulates lactogenesis in mammary explants from rabbits in midpregnancy (4) and is equipotent to bPRL and human somatotropin in the Nb2 lymphoma proliferation assay (7, 20). Both bPRL and bPL have a similar low degree of homology with bST, but bPL also displays somatogenic properties. It binds to the somatotropin receptor in rabbit (10) and bovine (7, 25) liver and is
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equiopotent to bST in an in vitro bioassay using mouse 3T3-L1 cells (7). Also, bPL is equi- 
potent to bST in stimulating weight gain when administered to mature female rats (7). Thus, 
available evidence indicates that bPL, potential- 
tially has both somatogenic and lactogenic 
properties in vivo, depending on the specificity 
of the somatotropin and prolactin receptors.

In the dry cow, injections of recombinant 
bPL (rbPL) stimulated a small increase in 
circulating insulin-like growth factors (IGF)-I 
and IGF-II and appeared to stimulate increased 
N retention (5). However, unlike bST, it did 
not increase blood glucose or insulin concen- 
trations and did not appear to alter irreversible 
loss of NEFA (5). These results suggested that 
rbPL was not acting solely as a somatotropin 
agonist and that it might have specific effects. 
Specific binding sites for bPL are found in the 
uterine endometrium of pregnant cows (13), 
although the incidence of these binding sites in 
other tissues and in the nonpregnant animal are 
unknown at present. However, it may be in- 
ferred that specific high affinity sites for this 
hormone are present because rbPL is very 
rapidly cleared from the circulation and has a 
half-life about one-third that for bST (5).

Dairy cows are normally lactating during 
the first 7 mo of gestation, and concurrent 
pregnancy hastens the decline in milk yield 
and appears to alter milk composition (12). 
Thus, one objective of this study was to deter- 
mine whether rbPL had an effect on milk yield 
and milk composition when given to cows in 
postpeak lactation. In addition, a number of 
blood hormone and metabolite levels were 
measured to help assess the effect of rbPL on 
intermediary metabolism.

MATERIALS AND METHODS

Hormones

Recombinant bPL (lot number 910313-3, 
Monsanto Co., St. Louis, MO) was dissolved 
in sterile water to a nominal concentration of 
30 mg/ml and filter sterilized (.22 μm). The 
absolute concentration of this stock solution 
was determined by reverse-phase HPLC using 
rbPL standards (5 to 30 μg per injection). The 
rbPL stock solution was diluted to the final 
working concentrations (2.5, 5, 10, and 20 mg/ 
ml) with sterile water. Recombinant bST 
([Ala1, Val127], Monsanto lot number M906-
020) was suspended in a minimal volume of 
water, and several drops of 1 M NaOH were 
added to solubilize the bST. This solution was 
diluted to a nominal concentration of 25 mg/ml 
with sterile water, sodium bicarbonate was 
added to give a final concentration of 25 mM, 
and the solution then was filter sterilized. The 
absolute concentration of bST was also deter- 
mined by reverse-phase HPLC, and the stock 
solution was diluted to the final working con- 
centration of 20 mg/ml with 25 mM sodium 
bicarbonate. The working solutions of rbPL 
and bST and the water control were divided 
into aliquots in 3-ml syringes (1 ml per syr- 
inge) and stored frozen at -20°C for up to 3 
mo.

Animals and Experimental Conduct

Nonpregnant, second lactation Holstein 
cows, 90 to 150 d in milk and producing >20 
kg/d of milk, were used. Cows were housed in 
individual tie stalls at Monsanto's Dardenne 
Dairy Center and were weighed weekly 
throughout the duration of the study. All cows 
were fed a total mixed diet consisting of 19.5% 
(DM basis) alfalfa hay, 26.5% corn silage, 
13% whole cotton seed, 5% beet pulp, and 37% 
concentrates. The diet (18% CP, 1.75 
Mcal of net energy/kg of DM, 21% ADF) was 
formulated to meet or exceed 1989 NRC (16) 
requirements. Cows were offered the diet for 
ad libitum intake, and daily consumption was 
recorded. The experimental design was a 6 × 6 
Latin square, balanced for possible carry-over 
effects, with a 9-d treatment period followed 
by a 5-d washout interval between treatments. 
The six treatments were a water negative 
control, four doses of rbPL (5, 10, 20, and 40 mg/ 
d), and a bST (20 mg/d) positive control. 
Injections were made subcutaneously in the 
postscapular region, on alternating sides, twice 
daily (0730 to 0830 h and 1930 to 2030 h). 
Blood samples were taken once per day (im- 
mediately before the a.m. injection) during the 
last 5 d of each treatment period. Cows were 
milked twice per day (1000 to 1100 h and 
2200 to 2300 h), and a sample of milk (20 ml) 
from the a.m. milking was analyzed for per- 
centage of fat, lactose, and protein by infrared 
spectroscopy (Multispec Infrared Analyzer, 
Berwind Instrument Group, York, Engl.).
Assays

Somatotropin. Serum somatotropin was measured by radioimmunoassay using an antiserum raised in rabbits (lot number R608-6/83, Monsanto Co.). Recombinant bST (Monsanto Co.) was used for standard and radiolabel and was radioiodinated by a chloramine T procedure. Bound label was precipitated by goat anti-rabbit gamma globulin (Linco Research, Inc., St. Louis, MO) and polyethylene glycol (Sigma Chemical Co., St. Louis, MO). The sensitivity of the assay was approximately 1 ng/ml. Recovery of augmented bST for several concentrations was 95%, and the intraassay and interassay coefficients of variation were 11.6 and 6.0%, respectively.

Insulin and IGF-I. Serum insulin and IGF-I were assayed exactly as described by Vicini et al. (24). Intraassay and interassay coefficients of variation were 12.1 and 9.1% for insulin and 11.0 and 9.0% for IGF-I.

Triiodothyronine. Serum triiodothyronine (T3) concentration was measured by using a solid phase radioimmunoassay kit (Diagnostic Products, Inc., Los Angeles, CA) validated for use with bovine serum. Recovery of augmented T3 in bovine serum was 104%, and the sensitivity limit of the assay was .8 ng/dl. Intraassay and interassay coefficients of variation were 5.0 and 6.6%, respectively.

IGF Binding Protein-2. Serum IGF binding protein-2 (IGFBP-2) concentrations were determined using a specific homologous radioimmunoassay for bovine IGFBP-2 (5). All samples were run in one assay; the intraassay coefficient of variation was 6.3%.

Blood Metabolites. The concentration of NEFA in serum was measured by using a NEFA-C kit (Wako Chemical Co., Dallas, TX). Blood urea N (BUN) and plasma glucose concentrations were measured by using a Dimension Clinical Chemistry Analyser (E. I. du Pont Nemours and Co., Wilmington, DE). The instrument was calibrated for bovine samples before use, and standard bovine ranges were established for use in quality control evaluation.

Statistical Methods

Individual cow treatment period averages for milk yield, milk composition, DMI, and blood data were examined by analysis of variance [general linear models procedure of SAS (19)] using treatment, period, and cow as class variables. No evidence of carry-over effect was detected ($P > .05$) for any of the parameters tested. When the main effect of treatment was significant at $P \leq .05$, pairwise treatment comparisons were made. In addition, linear and quadratic trend effects of rbPL were tested.

RESULTS

Milk Yield, Milk Composition, and DMI

The data for milk yield, milk composition, and DMI were analyzed using values from all 9 d of treatment. As expected, milk yield following administration of bST was dramatically increased over that of the control (20%, Table 1). The three highest doses of rbPL also increased ($P < .05$) milk yield, although the magnitude of increase stimulated by rbPL was less than for bST, i.e., a 2.8-kg increase (8.2%) with the 40-mg dose of rbPL (Table 1). Nevertheless, there was a linear dose effect of rbPL ($P < .002$). There was no change ($P > .05$) in milk composition (percentage of lactose, protein, or fat; Table 2), although percentage of fat was numerically lower during treatment with any of the doses of rbPL.

Dry matter intake was not altered by treatment with bST; however, two of the rbPL doses increased ($P < .05$) and DMI.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Milk yield</th>
<th>DMI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>34.29a</td>
<td>24.19a</td>
</tr>
<tr>
<td>rbPL, 5 mg/d</td>
<td>35.44ab</td>
<td>24.51a</td>
</tr>
<tr>
<td>rbPL, 10 mg/d</td>
<td>36.43bc</td>
<td>25.18bc</td>
</tr>
<tr>
<td>rbPL, 20 mg/d</td>
<td>35.92bc</td>
<td>24.58bc</td>
</tr>
<tr>
<td>rbPL, 40 mg/d</td>
<td>37.13c</td>
<td>25.27c</td>
</tr>
<tr>
<td>bST, 20 mg/d</td>
<td>41.21d</td>
<td>24.53ab</td>
</tr>
<tr>
<td>SB</td>
<td>.49</td>
<td>.25</td>
</tr>
</tbody>
</table>

Values with different superscripts differ ($P < .05$).

Values are least squares means (n = 6) of the 9-d average for each cow.

There was a linear dose effect of rbPL for both milk yield ($P < .002$) and DMI ($P < .05$).
TABLE 2. The effect of recombinant bovine placental lactogen (rbPL) and bST on milk composition.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% Lactose</th>
<th>% Protein</th>
<th>% Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.8</td>
<td>3.2</td>
<td>3.0</td>
</tr>
<tr>
<td>rbPL, 5 mg/d</td>
<td>4.7</td>
<td>3.2</td>
<td>2.7</td>
</tr>
<tr>
<td>rbPL, 10 mg/d</td>
<td>4.8</td>
<td>3.2</td>
<td>2.7</td>
</tr>
<tr>
<td>rbPL, 20 mg/d</td>
<td>4.7</td>
<td>3.2</td>
<td>2.8</td>
</tr>
<tr>
<td>rbPL, 40 mg/d</td>
<td>4.8</td>
<td>3.2</td>
<td>2.7</td>
</tr>
<tr>
<td>bST, 20 mg/d</td>
<td>4.8</td>
<td>3.2</td>
<td>2.8</td>
</tr>
<tr>
<td>SE</td>
<td>0.016</td>
<td>0.024</td>
<td>0.073</td>
</tr>
</tbody>
</table>

1Values are least squares means (n = 6) of the 9-d average for each cow.

doses (10 and 40 mg/d) increased (P < .05) intake relative to the control by 4.1 and 4.5%, respectively (Table 1), and there was an rbPL dose effect (P < .02). Interestingly, the doses of rbPL that stimulated the greatest increases in DMI also stimulated the largest increases in milk yield (Table 1). The increase in energy intake was highly correlated with increased energy secreted as milk (r = .94, P < .02) (Figure 1). The same plot also demonstrates that increased milk yield stimulated by bST was not associated with an elevated energy intake.

Blood Metabolite Concentrations

Plasma glucose concentration was increased (P < .001) by bST treatment but was unaffected by most of the doses of rbPL. Glucose was elevated by the 20-mg/d dose of rbPL (P < .05) (Figure 2), but there was no apparent dose dependence. The concentration of serum NEFA also was elevated (P < .01) by bST, whereas this parameter was unchanged by any of the rbPL doses (Figure 2). Blood urea N was decreased by treatment with both rbPL and with the two highest doses of rbPL (P < .001) (Figure 2). There was a negative linear dose effect (P < .001) of rbPL on the concentration of BUN.

Serum bST, rbPL, T₃, and Insulin

Serum bST concentrations were elevated (P < .05) in the bST-treated group. By contrast, endogenous bST concentrations were decreased (P < .05) in three of the groups treated with rbPL (Table 3). The concentration of rbPL in serum samples from cows in all of the groups being treated with this hormone was below the assay detection limit (<1 ng/ml). The concentrations of T₃ and of insulin in serum were not different (P > .05) for any of the treatment groups (Table 3).

Serum IGF-I and IGFBP-2

Serum IGF-I concentrations were elevated (P < .05) by all of the treatments, although the greatest increase was stimulated by bST (Figure 2). There was a linear dose effect (P < .001) of rbPL on the serum concentration of this growth factor. The serum concentration of IGFBP-2 was dramatically decreased (P < .0001) by bST, and there was a linear dose-related decrease (P < .01) during treatment with rbPL (Table 3). Treatment with bST decreased (P < .0001) serum IGFBP-2 more than equivalent or higher doses of rbPL.

DISCUSSION

From a previous study (5), it was known that the half-life of intravenously injected rbPL was approximately 7 min; thus, injections of this hormone were made twice per day to ensure that rbPL was present in the circulation for more than a single interval each day.
Figure 2. Blood concentrations of a) glucose, b) NEFA, c) blood urea N (BUN), and d) insulin-like growth factor-I (IGF-I) in nonpregnant, lactating cows treated with recombinant bovine placental lactogen (bPL) and bST. The concentrations of blood metabolites and of IGF-I were measured in blood samples taken during the last 5 d of the 9-d treatment period. The x-axis indicates treatment group (control, bPL, or bST-treated) and the dose (milligrams per day) of hormone administered. Values shown are least squares means (n = 6) of the 5-d average from each cow. Error bars show the pooled standard error of the least squares means. Treatments lacking a common superscript letter (a,b,c,d) differ (P ≤ .05).

Nonetheless, the concentration of rbPL was undetectable (<1 ng/ml) in blood samples taken 12 h after injection, whereas there was a small increase in the concentration of somatotropin in those animals treated with bST. These results confirm our previous findings (5) that the half-life of rbPL is considerably less than that of bST. However, despite the rapid clearance of rbPL, the blood concentrations of IGF-I, IGFBP-2, and urea N were altered by treatment with rbPL. In addition, the concentration of endogenous bST in the circulation was decreased by the three lowest doses of rbPL.

There is no evidence in the data from this study to suggest that rbPL affects milk composition, although the treatment periods may have been too short for changes to occur. The milk composition data does indicate, however, that percentage of milk fat was lower than is normally expected. All of the cows remained healthy throughout the study and showed no signs of ruminal or nutritional dysfunction. There was no obvious reason for the low fat
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TABLE 3. The effect of recombinant bovine placental lactogen (rbPL) and bST on blood concentrations of insulin, insulin-like growth factor binding protein-2, triiodothyronine, and somatotropin in nonpregnant, lactating cows.¹²

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Insulin (µU/ml)</th>
<th>IGFBP-2²</th>
<th>T₃ (ng/ml)</th>
<th>bST</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>14.6</td>
<td>454ᵈ</td>
<td>1.61</td>
<td>1.23ᵇ</td>
</tr>
<tr>
<td>rbPL, 5 mg/d</td>
<td>15.1</td>
<td>426ᵈ</td>
<td>1.55</td>
<td>0.65ᵃ</td>
</tr>
<tr>
<td>rbPL, 10 mg/d</td>
<td>15.2</td>
<td>418ᵈ</td>
<td>1.62</td>
<td>0.66ᵃ</td>
</tr>
<tr>
<td>rbPL, 20 mg/d</td>
<td>17.0</td>
<td>379ᵇ</td>
<td>1.59</td>
<td>0.69ᵃ</td>
</tr>
<tr>
<td>rbPL, 40 mg/d</td>
<td>13.7</td>
<td>379ᵇ</td>
<td>1.59</td>
<td>0.88ᵇ</td>
</tr>
<tr>
<td>bST, 20 mg/d</td>
<td>19.5</td>
<td>167ᵃ</td>
<td>1.80</td>
<td>2.44ᶜ</td>
</tr>
</tbody>
</table>

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¹,ᵇ,c,dValues with different superscripts differ (P < .05).

IGFBP-2 = Insulin-like growth factor binding protein-2; T₃ = triiodothyronine.

²Values are least squares means (n = 6) of the 9-d average for each cow.

³There was a linear dose effect of rbPL for IGFBP-2 (P < .01).

tests, but the resident herd average was also low (usually <3.4%), and the average for the cows used in this study during their first lactation was 3.1%.

Although the galactopoietic effects of bST are well known and have been extensively studied, the exact mechanisms by which bST stimulates lactation are still poorly understood. Evidence to date suggests that bST has direct effects on tissues, such as adipose, as well as indirect actions that are mediated through the somatomedins and IGFBP (1, 24). Nonetheless, all of these actions, direct or indirect, must originate with bST binding to its receptor on target tissues. Recombinant bPL binds to the bST receptor with approximately threefold lower affinity than bST (7). Thus, it would be predicted that rbPL would act as a weak somatotropin agonist. Data from this experiment suggest that this, in part, may be the case. Concentration of BUN was decreased by both hormones (Table 2), although rbPL was less potent than bST. Serum IGF-I was elevated by rbPL also in a dose-dependent manner, although, once again, rbPL was less potent than bST. Furthermore, rbPL decreased the concentration of IGFBP-2, but, again, the decrease was less than that elicited by bST. The increased milk yield stimulated by rbPL may, therefore, have been mediated, in large part, by a somatogenic mechanism.

However, not all of the effects of bST were mimicked by rbPL. Increased milk yield stimulated by bST treatment also increases irreversible loss of NEFA through decreased lipogenesis and, to a lesser extent, through increased lipolysis (1). These combined effects lead to an elevated concentration of serum NEFA. This effect of bST was observed in the present experiment, but there was no indication that serum NEFA was increased by any of the rbPL doses. It is unlikely that differences in energy balance accounted for the differential NEFA response, because all treatment groups were in positive energy balance. Furthermore, serum NEFA was also elevated by bST and unaffected by rbPL in dry cows (5). Another well-documented effect of bST in lactating cows has been a decrease in insulin sensitivity (1, 11), resulting in an increased circulating concentration of glucose. Most of the doses of rbPL had no effect on serum glucose concentration, although this parameter was elevated by the 20-mg/d dose of rbPL. Presently, we are unable to determine whether the response to rbPL at either 20 or 40 mg/d was aberrant or whether the glucose response to this hormone is biphasic.

Another indication that rbPL was not acting solely as a weak somatotropin agonist is the observation that DMI was elevated by this hormone. Lactating cows treated with bST increased feed intake, but only after approximately 8 to 10 wk of continuous treatment (2). In the short term, treatment with bST does not stimulate an increase in DMI, as seen in this study, even though milk yield is dramatically elevated. Placental lactogen also binds to prolactin receptors (6), and exogenously administered prolactin has been shown to increase feed
intake in rats (14) and in one species of bird (3). However, when pituitary-derived bPRL was administered (120 mg/d) to lactating dairy cattle for 14 d before and after peak milk yield, there was no effect on net energy intake (18). Thus, rbPRL may have specific effects on feed intake that could be mediated through the putative placental lactogen receptor (13) rather than through either somatogenic or lactogenic mechanisms.

Finally, endogenous bST concentrations (Table 3) suggest that administration of rbPRL down-regulated the secretion of this pituitary hormone. The mechanism responsible for this effect is presently unknown. However, there is good evidence for a short-loop negative feedback of somatotropin on its own secretion (15); thus, because rbPRL is a somatotropin agonist, it may have had a direct effect on bST secretion. It is unknown whether the effect of rbPRL on lactation was modulated by the apparently decreased secretion of bST.

In summary, short-term administration of rbPRL increased milk yield in a dose-dependent manner, although rbPRL was less potent than bST. Changes in the concentration of serum IGF-I and BUN, as well as the increased milk yield, suggested that rbPRL was acting, in part, as a weak somatotropin agonist. However, unlike bST, rbPRL did not appear to be lipolytic and did not alter insulin sensitivity. In addition, there was an acute increase in DMI in response to administration of rbPRL. Although the twice daily spike of rbPRL imposed in the present experiment was not physiologic, the results could be interpreted to suggest that endogenous bPRL has effects on both nutrient intake and maternal partitioning of nutrients.

ACKNOWLEDGMENTS

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