TITRATION OF THE RECOMBINANT BOVINE SOMATOTROPIN DOSAGE THAT MAXIMIZES THE ANABOLIC RESPONSE IN FEEDLOT STEERS

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ABSTRACT

The objective of this study was to determine the minimum dosage of recombinant bovine somatotropin (bST) required to elicit maximum depression in plasma urea nitrogen (PUN), an indicator of anabolic activity. Twenty-four steers (389 kg) were blocked by weight into six pens. Six steers were placed on each of the following bST doses: 0, 8, 16 and 32 mg bST/d. Treatments were administered once daily via subcutaneous injections for 21 d. Steers were weighed and jugular blood samples were taken on d 0, 1, 4, 7, 10, 13, 16 and 21 at 1400, approximately 4 h after feeding. Delta PUN = PUN - d 0 PUN. There was no dose x time interaction (P = .94) in DPUN. Maximum reduction in DPUN with bST occurred by d 7 (P < .05). Linear (P < .01) and quadratic (P < .05) orthogonal contrasts indicated that DPUN depression increased with bST administration, with maximal reduction calculated to occur with 23 mg (59 μg/kg) bST/d. There was no further decrease in DPUN with 32 than with 16 mg bST, indicating that the minimum daily dose is at least 16 mg but no more than 23 mg. A similar dose response was observed in daily gain. Results from this study indicate that bST reduced PUN in a dose-dependent manner and that 41 to 64 μg/kg body weight maximized the anabolic effect of bST in growing steers.

(Key Words: Bovine Somatotropin, Plasma Urea Nitrogen Depression, Anabolic Activity.)


Introduction

Many of the anabolic effects of estrogens and somatotropin are similar in growing cattle and sheep (e.g., improved daily gain and feed conversion, decreased urinary N, plasma urea N (PUN) and amino acid N, increased N retention and lean tissue deposition) (Oltjen and Lehmann, 1968; Preston, 1968, 1975, 1987; Davis et al., 1970a,b; Grebing et al., 1970; Trenkle and Topel, 1978; Heitzman, 1979; Goldberg et al., 1980; Buttery, 1983; Muir et al., 1983; Basson et al., 1985; Johnsson et al., 1985; Eisemann et al., 1986; Gopinath and Kitts, 1986; McShane et al., 1989a,b). It has been postulated that the anabolic effect of estrogen is mediated through an increase in somatotropin secretion (Preston, 1975, 1987; Trenkle, 1976; Heitzman, 1979, 1981; Gopinath and Kitts, 1984). Several studies, however, have concluded that the relationship between estrogen and somatotropin is additive (Wolfrom and Ivy, 1985; Wolfrom et al., 1985; Ivy et al., 1986; Roche and Quirke, 1986; Wagner et al., 1988a,b). These studies were not obviously conducted at the optimum dosage for each hormone, however.

Because PUN depression has been suggested to be an early indicator of anabolic activity (Preston, 1968), the objective of this experiment was to determine the minimum dose of recombinant bovine somatotropin (bST) required to maximize PUN depression; plasma bST and gain also were evaluated.
Materials and Methods

Twenty-four British crossbred yearling steers (389 kg) were blocked by weight into six pens with four head per pen. Steers had ad libitum access to a diet balanced to meet or exceed NRC (1984) requirements (Table 1). Because steers were group-fed, individual feed intake data were not obtained. One steer in each pen was allotted randomly to one of the following treatments (six steers/treatment): 0, 8, 16 or 32 mg of bST<sup>5</sup>-head<sup>-1</sup>-d<sup>-1</sup>. The bST injection vehicle was a 0.03 M sodium bicarbonate in 0.15 M sodium chloride buffer (pH = 9.4) Injection solutions were prepared every 5 or 6 d. Treatments were administered once daily at 1400 via s.c. injections in the front shoulder, alternating injection site every other day. The trial was conducted for 21 d. Steers were weighed and blood samples were taken via jugular venipuncture on d 0, 1, 4, 7, 10, 13, 16 and 21 at 1400, approximately 4 h after feeding. Samples were collected in tubes containing disodium EDTA, transported on ice and centrifuged; the plasma was stored refrigerated or at -20°C until it was analyzed for PUN (within 1 to 2 d) and bST, respectively. Hematocrits were determined using an Autocrit<sup>6</sup> centrifuge. Samples were analyzed for PUN using a spectrophotometric assay (Chaney and Marbach, 1962; Searle, 1984).

Plasma samples were analyzed for bST via a double-antibody radioimmunoassay (Hancock, 1989). Briefly, 100 µl of chloramine T radiolabeled [<sup>125</sup>I]bST (10,000 cpm) was added to tubes with 100-µl aliquots of standards (.39 to 100 ng bST/ml), control plasma pools of known concentration or unknown samples. The first antibody (diluted 1:40,000 with 2% normal rabbit serum) was added (100 µl) and the tubes were incubated at room temperature for 20 h. Sheep antirabbit gamma globulin (diluted 1:16) was added (100 µl) and incubated at 4°C for 24 h. Cold phosphate-buffered saline was added (2 ml) and tubes were centrifuged for 30 min at 2,000 × g. Radioactivity in the pellet was determined and unknown concentrations were determined by log/logit analysis. Initial qualifications were performed by determining percentage recovery (avg 108%), curve displacement and parallelism. Quality control was maintained by plotting standard curves, recording total counts, binding percentage, slopes and intercepts. The lower limit of sensitivity of the assay was 0.1 ng bST/ml (95% binding). Intra-assay CV between duplicates was less than 10%, and the interassay CV between low (1.3 ng/ml), medium (15 ng/ml) and high (38.5 ng/ml) plasma pool samples was 11, 1.8 and 3.3, respectively.

Data were analyzed by analysis of variance as a split plot in time design for delta PUN (DPUN) and hematocrit determinations using a GLM procedure (SAS, 1985). Effects included in the main plot analysis were treatment (bST dosage), pen and the treatment × pen interaction. The latter term was used as the error term to test main plot effects. Subplot effects included time, treatment × time interaction and the residual effects, which were used as the error term to test subplot effects. Treatment and time differences were tested by protected least significant difference (LSD) test.

Multiple regression analysis was performed to orthogonally partition the treatment and time effects to determine the optimum dosage of bST required for maximal depression in PUN. The optimum dosage of bST was calculated by solving for the first derivative of the resulting regression equation and then solving for zero.

Daily gain was determined for the overall 21-d period and the model for daily gain

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<table>
<thead>
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<th>TABLE 1. COMPOSITION OF DIET&lt;sup&gt;a&lt;/sup&gt;</th>
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<td>Ingredient</td>
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<td>Steam-flaked sorghum grain</td>
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<td>Cottonseed hulls</td>
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<td>Trace mineral premix</td>
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<td>Tylan premix</td>
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<sup>a</sup>Formulated to contain 14.8% CP, 3.24 Mcal DE/kg; 3.1% crude fat, 36% NDP; 30% roughage equivalent; 62% Ca; 34% P; 10% Na; 81% K; 15% S; 40 ppm Zn; 5,214 IU/kg vitamin A on a dry matter basis.

<sup>b</sup>As-fed basis; diet contained 66.2% dry matter.
Treatment effects were tested by LSD and orthogonally partitioned. Day 0 PUN concentrations also were evaluated for initial differences using this model.

Results and Discussion

Initial differences were observed \((P = .04)\) in PUN concentrations (12.5, 11.1, 11.2 and 10.0 mg/dl, SE = .57, on d 0 for steers on the 0, 8, 16 and 32 mg bST treatments, respectively). Therefore, DPUN calculated as PUN concentration on d 0 was utilized to evaluate the effect of bST on PUN depression.

There was no dose \(\times\) time interaction \((P = .94)\) in the DPUN response (Figure 1). Partition of the time effects \((P < .0001)\) indicated linear, quadratic, cubic and quartic \((P < .005)\) effects in DPUN over time. As days on trial increased, PUN depression increased, with maximal reduction in DPUN occurring by d 7 \((P < .05)\).

As cited by Bauman and McCutcheon (1986), daily somatotropin dosages of 50 to 400 \(\mu g/\text{kg}\) have been used in growing cattle and sheep, resulting in improvements in growth rate and nitrogen retention; however, there has been only one reported dose-response study (Ivy et al., 1986). In our study, main effect DPUN levels were \(-2.13, -3.56, -4.37\) and \(-4.29\) for 0, 8, 16 and 32 mg bST/d, respectively. Linear \((P < .01)\) and quadratic \((P < .05)\) orthogonal contrasts indicated that DPUN depression increased with increasing bST dosage through the 16-mg dosage. The optimum dosage \((D)\) of bST required for maximal PUN reduction was calculated by solving for the first derivative of the quadratic regression equation \(\{\text{DPUN} = .00455(D^2) - .213(D) - 2.134; \text{SE} = .46, r^2 = .51\}\) and then solving for zero. The horizontal tangent (derivative = 0) is defined as the minimum value of the function in the immediate neighborhood of the transition point (Thomas and Finney, 1984) and was assumed to be the maximum dose needed to achieve the maximal effect. Maximal PUN reduction was calculated to occur with 23 mg of bST/d. Because DPUN was not lower with 32 than with 16 mg bST, the optimum daily dose is 16 mg but no more than 23 mg bST (average dose of 41 to 59 \(\mu g/\text{kg} \cdot \text{d}^{-1}\)).

No treatment \((P = .70)\) or treatment \(\times\) time \((P = .14)\) effects were observed in hematocrit percentages. The mean hematocrit for the experiment was 44.1%.

Daily gain for the 21-d period was .21, .32, .83 and .73 kg/d, respectively, for 0, 8, 16 and 32 mg bST/\(\text{head} \cdot \text{d}^{-1}\). There was a significant linear effect \((P < .05)\) and a trend for a quadratic effect \((P = .19)\) in daily gain with increasing bST dose. Similar to the DPUN response, as bST dose increased, daily gain increased through 16 mg; these gains were higher \((P < .05)\) than for the control steers. Maximal increases in daily gain were calculated to occur with 25 mg bST/d \(\{\text{daily gain} = -.001075(D^2) + .0528(D) + .1615; \text{SE} = .19, r^2 = .35\}\). Because no further increase in daily gain was attained with 32 than with 16 mg bST, the minimum daily dose is the least 16 mg, but no more than 25 mg. These results support the minimum dose obtained with the PUN depression response.

Depression in PUN appeared to be a valid early indicator of anabolic activity. As reviewed by Galbraith (1980), endogenous urea production is dependent primarily on amino acid deamination (Sykes, 1978). Therefore, PUN may be reduced as utilization of amino acids for protein deposition increases or protein turnover decreases (Millward et al., 1976; Gopinath and Kitts, 1986). Thus, a PUN reduction would indicate a protein sparing effect (Donaldson and Heitzman, 1983). As stated by Lobely et al. (1985), “improvements in nitrogen retention based on changes in tissue metabolism rather than intake must be accompanied by a decrease in the catabolism of amino acids and an alteration in the rate of
protein synthesis or protein degradation or both."

Results of our study are in accordance with those of Ivy et al. (1986), who titrated pituitary-derived bST dosages of 0, 6, 12 and 24 mg in steers (356 kg) for 42 d and found an optimum dosage of 15 mg (42 µg/kg) bST/d for maximal improvement in gain and feed conversion. They observed a linear decrease in blood urea nitrogen with increasing level of bST, however, rather than the quadratic decrease that we observed, perhaps because their maximum dosage was lower than ours.

Plasma bST concentrations are shown in Figure 2. A 95% confidence interval around the mean plasma bST concentration of all steers on d 0 and for the 0 mg bST treatment over time was determined to have lower and upper limits of 0 and 45 ng/ml, respectively. Biological variation was large (SE = 10 ng/ml) and plasma bST concentrations in the control steers were greater than reported concentrations in untreated steers (Moseley et al., 1982; Grigsby and Trenkle, 1986; Breier et al., 1988; Wagner et al., 1988a,b). On d 7, two control steers had circulating bST concentrations of 95 and 165 ng/ml; excluding these values, the mean would be 9.4 ng/ml. The episodic bST release and plasma bST elevation that may be related to the handling of the steers for daily injections complicates the interpretation of plasma bST data (Graner, 1985). Nevertheless, plasma bST concentrations generally were higher for steers receiving 32 mg bST/d. Because the daily dose of bST required for maximal PUN depression, one index of anabolic activity, was determined to be between 16 and 23 mg, the elevation in plasma bST on d 7 for the 32-mg treatment may reflect an excess of bST above that required for the anabolic response. All blood samples were collected 24 h after bST injection, when most of the previously injected bST should have cleared from the blood (J. F. Wagner, personal communication).

Implications
Depression in plasma urea N, an indicator of anabolic activity, and daily gain both increased with increasing bST administration in growing steers. Maximal reduction in PUN and increased daily gain occurred with at least 16 mg but no more than 25 mg of bST/d (41 to 64 µg-kg⁻¹-d⁻¹).

Literature Cited
TITRATION OF BOVINE SOMATOTROPIN


