Growth hormone response to growth hormone-releasing hormone in beef cows divergently selected for milk production

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ABSTRACT: In dairy cattle, increased circulating growth hormone has been associated with selection for greater milk yield. This study tested the hypothesis that beef cows divergently selected for milk production would have differing GH responses to a challenge dose of GHRH. Growth hormone response to a challenge of GHRH was measured in 36 Angus-sired cows ranging from 6 to 10 yr of age. The cows were classified as high milking (n = 16) or low milking (n = 20), on the basis of their sires’ milk EPD. Mean milk EPD (in kilograms) were 16.6 and −14.4 for high and low milking cows, respectively. Milk production was estimated by the weigh-suckle-weigh procedure. Blood samples were taken immediately before and 10 min after a clearance dose of 4.5 μg GHRH/100 kg BW (injected i.v.) and, 3 h later, immediately before and 10 min after a challenge dose of either 1.5 or 4.5 μg GHRH/100 kg BW. Each animal received both challenge doses, and the doses were randomly assigned across 2 d of blood collection. Serum concentrations of GH and IGF-I were measured by RIA. Serum IGF-I was measured in the baseline blood sample on d 1 of blood collection. A positive relationship (r = 0.35; P = 0.03) was observed between the cows’ rankings for each dose of GHRH; that is, high responders to the low dose were high responders to the high dose. Growth hormone response to the 4.5 μg /100 kg BW challenge dose of GHRH was positively related to sire milk EPD (R2 = 0.09; P = 0.03). Response of GH to the 1.5 μg GHRH/100 kg BW challenge dose also tended to be related (P = 0.08) to sire milk EPD of high milking cows. In addition, IGF-I concentrations of high milking cows were inversely related (R2 = 0.24; P = 0.04) to sire milk EPD. Growth hormone response to GHRH challenge may have potential as an additional tool in the evaluation of milk production in beef cattle.

Key Words: Beef Cattle, Growth Hormone-Releasing Hormone, Milk Production, Selection


Introduction

Profitability in a cow-calf operation is highly related to calf weaning weight. Weaning weight of a beef calf is largely dependent on the dam’s milk production. Gleddie and Berg (1968) estimated that milk yield of the dam accounts for over 60% of the variation in ADG to weaning. However, selection for greater milk production has been limited, in part because of the small number of criteria generally available to the producer. Measurement of actual milk production is typically laborious and time-consuming. Additionally, criteria based on indirect genetic merit, such as sire milk EPD, often measure the weight of the calf and do not directly measure actual milk production. Rupert et al. (1999) demonstrated a positive relationship between sire milk EPD and actual milk production; however, selection criteria are also needed that more directly relate to the dam’s production.

Recently, we have observed that beef bulls with a greater GH response to GHRH had higher postweaning ADG (Connor et al., 1999, 2000) and that beef heifers with greater GH response to GHRH at weaning had lower postweaning ADG (Auchtung et al., 2001). It is known that greater rates of gain (above 1.0 kg/d) during the prepubertal period in heifers lead to decreased mammary secretory tissue and increased mammary adipose tissue (Sejrsen et al., 1982), as well as to decreased subsequent milk production (Hohenboken et al., 1995).
Therefore, greater GH response to GHRH, which relates to lower postweaning gain in beef heifers and potentially greater mammary gland growth, may be related to higher milk production.

The objective of this study was to determine whether GH response to GHRH is related to differences in milk production among mature beef cows as estimated by sire milk EPD and the weigh-suckle-weigh procedure. Our approach was to evaluate GH response to GHRH in lactating beef cows divergently selected for sire milk EPD and relate GH response to sire milk EPD, actual milk production, and IGF-I.

Materials and Methods

Animals

The 36 Angus-sired cows used for this study were part of a larger study performed at the Oklahoma Agricultural Experiment Station, Stillwater, OK. The cows ranged from 6 to 10 yr of age. The high-milk EPD cows (n = 16) were the progeny of high-milk EPD sires and averaged 592 kg BW (SD = 61) at the time of the GHRH challenge. The low-milk EPD cows (n = 20) were sired by low-milk EPD sires and averaged 607 kg BW (SD = 43) at the time of the GHRH challenge. Body condition score (1 to 9; Richards et al., 1986) averaged 5.3 (SD = 0.6) for high-milk EPD cows and 5.5 (SD = 0.6) for low-milk EPD cows at the time of the GHRH challenge. All cows were offspring of Angus-Herford or Angus-Herford-Brahman cows, which had been randomly assigned to be bred either to high- or low-milk EPD sires. High-milk EPD sires averaged 16.6 kg of milk EPD, whereas low-milk EPD sires averaged −14.4 kg of milk EPD. Cows calved in the spring of 1999 (average calving date = 03/04/99), at which time male calves were castrated, body weights were measured on cows and calves, and cows were scored for body condition. The cows and calves were pastured on native grasses on the North Lake Carl Blackwell Range, in Oklahoma. Animal procedures were approved by the University of Maryland and Oklahoma State University Animal Care and Use Committees.

Milk Yield Estimation

Milk yield was estimated by a weigh-suckle-weigh procedure, performed at monthly intervals seven times, from birth to weaning, as described by Minick (1999). Briefly, on the afternoon of the day preceding the milk yield estimate, cows and calves were separated. At 0545 on the day of the estimation, calves were paired with their dams and allowed to nurse until satiated. When the calves stopped nursing they were separated from their dams until 1145. Cows were weighed and their body condition scores were estimated. The purpose of this preliminary separation and suckling period was to empty the udder and minimize variation among cows at the start of the initial 6-h separation period. At 1145, calves were individually weighed, returned to their dams to nurse, and again weighed after they completed nursing. This weigh-suckle-weigh procedure was repeated at 1745 to obtain the second 6-h estimation. Pre-nursing weight was subtracted from post-nursing weight, and that was taken as an estimate of the 6-h milk production for each dam. The two 6-h estimates were added together and multiplied by 2 to get the estimated 24-h milk production of each dam. Area under the lactation curve was estimated as described by Minick (1999) using the method of Jenkins and Ferrell (1984). Briefly, the amount of milk produced, Y(n), was divided by day in lactation, n, and the natural log of that value was regressed on day of lactation to estimate parameters (a and k) of the curve.

\[ \log_e [Y(n)/n] = (\log_e [1/a]) - kn \]

The lactation curve defined by those parameters was Y(n) = n/ae^kn and the curve was used to estimate the time of peak lactation (1/k) and yield at peak lactation (1/ake) for each cow. Area under the lactation curve will be subsequently referred to as total milk production.

GHRH Challenge

At an average of 218 d postpartum, the cows received two challenge doses (1.5 and 4.5 μg /100 kg BW) of a bovine GHRH (1-30) analog (Pharmacia & Upjohn, Kalamazoo, MI) in a complete randomized design. Administration of GHRH was as previously described (Auchtung et al., 2001; Connor et al., 2000). The final concentration of GHRH was 15 μg/mL in 0.9% physiological saline with 0.1% BSA. The GHRH challenge was performed after the last milk yield estimate to ensure that the present study did not interfere with data collection in the larger study.

GH Assays

Blood samples were stored at room temperature for 4 to 6 h and then at 4°C for up to 30 h. Serum was obtained from whole blood after centrifugation (1,850 × g, 20 min, 4°C) and stored at −20°C until assayed for GH. Serum GH concentrations were determined by RIA (Connor et al., 1999). Mean concentrations of the low, medium, and high controls were 1.5, 10.9, and 44.1 ng/mL, respectively. Mean intraassay coefficients of variation (three assays) were 14.1, 12.9, and 15.0% for low, medium, and high control pools, respectively. Mean interassay coefficients of variation for low, medium, and high control pools were 7.4, 13.1, and 11.3%, respectively. Assay sensitivity averaged 0.2 ng/mL.

IGF-I Assays

Insulin-like growth factor-I assays were run on the serum harvested from baseline blood samples collected on d 1 of the GHRH challenge. Serum IGF-I concentra-
tions were determined by RIA (Auchtung et al., 2001). Briefly, 100 μL of 0.2 M glycylglycine HCl (pH 2.1) was added to 100 μL of plasma in polypropylene tubes and mixed, resulting in an overall pH < 3.5. The samples were incubated at room temperature for 36 h. A 25-μL aliquot was then assayed in duplicate. The mean intraassay coefficient of variation (two assays) was 2.6%, and the interassay coefficient of variation was 13.8%. Assay sensitivity averaged 4.3 ng/mL.

Statistical Analyses

Statistical analyses were performed using the SAS System v. 6.12 (SAS Inst. Inc., Cary, NC). Milk production differences among the cows from low- vs high-milk EPD sires were determined using a mixed model. The model included category of EPD and milk yield as variables. Pearson’s correlation was used to calculate correlation coefficients of actual milk production with sire milk EPD. The MIXED procedure was used to determine the dose response to the two challenge doses of GHRH, where dose and GH response were variables in the model. Spearman’s rank correlation was used to evaluate consistency of the GH responses to the clearance dose over the 2 d of GHRH challenge and the GH responses to the two GHRH challenge doses. Linear regression was used to determine the relationships between GH response to 1.5 and 4.5 μg GHRH/100 kg BW vs sire milk EPD, estimated milk production, and IGF-I. Because GH status at time of GHRH challenge may affect the GH response (Løvendahl et al., 1991; Connor et al., 2000), baseline GH concentration was evaluated as a covariate in each model involving the GH response to GHRH. The UNIVARIATE procedure was performed on the GH responses to both challenge doses of GHRH to identify outliers.

Results

General

Total milk production was positively correlated to sire milk EPD (r = 0.46; P = 0.005) for the 36 cows in this study. Average 24-h milk production (P = 0.03), yield at peak lactation (P = 0.02), and total milk production (P = 0.02) were higher in cows from high-milk EPD sires than in cows from low-milk EPD sires. Time of peak lactation (in days) averaged 71.3 ± 3.2 and 88.0 ± 13.8 for high and low milking cows, respectively, and did not differ (P = 0.30) among the two groups. Cows responded in a dose-dependent manner to the two GHRH challenge doses (P < 0.05; Table 1). The mean GH concentrations before and after injection of GHRH are presented in Table 1. Growth hormone responses to the clearance dose over the two consecutive days of GHRH challenge were positively correlated (r = 0.52; P < 0.01). Ranked GH responses to the two GHRH challenge doses were also positively correlated (r = 0.35, P = 0.03); that is, high GH responders to the low dose of GHRH were high GH responders to the high dose of GHRH. Serum GH concentrations following the 4.5 μg GHRH/100 kg BW clearance dose returned to baseline prior to administration of the challenge doses (Table 1).

GH Response to the 4.5 μg/100 kg BW Dose of GHRH is Related to Milk Production

Response of GH to the 4.5 μg/100 kg BW dose of GHRH was positively related to sire milk EPD in a model that included both high and low milking cows (R² = 0.09; P = 0.03; Figure 1). There also tended to be a positive relationship (P = 0.08) between GH response to the 1.5 μg/100 kg BW dose of GHRH and sire milk EPD in a model that included only cows from high-milk EPD sires.

Growth hormone response to the 4.5 μg/100 kg BW dose of GHRH was also positively related to average 24-h milk yield (R² = 0.12; P = 0.04; Figure 2). Due to the distribution of the data in Figures 1 and 2, the GH responses to both challenge doses were subjected to a test for outliers and none were identified, thus no data were excluded. Inclusion of the corresponding baseline

### Table 1. Mean serum GH concentrations (ng/mL) before (baseline) and after (10 min postinjection) GHRH challenge in Angus-sired cows (n = 36)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>SD</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Low milk EPD cows (n = 20)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline GH, clearancea d 1</td>
<td>2.0a</td>
<td>1.1</td>
<td>0.9</td>
<td>4.4</td>
</tr>
<tr>
<td>Baseline GH, clearance d 2</td>
<td>0.9a</td>
<td>0.4</td>
<td>0.3</td>
<td>1.7</td>
</tr>
<tr>
<td>Postinjection GH, clearance d 1</td>
<td>19.4a</td>
<td>33.1</td>
<td>0.9</td>
<td>75.3</td>
</tr>
<tr>
<td>Postinjection GH, clearance d 2</td>
<td>7.2a</td>
<td>5.9</td>
<td>1.4</td>
<td>23.9</td>
</tr>
<tr>
<td>Baseline GH, 1.5 challenge</td>
<td>2.0a</td>
<td>2.1</td>
<td>0.6</td>
<td>9.6</td>
</tr>
<tr>
<td>Baseline GH, 4.5 challenge</td>
<td>1.7a</td>
<td>1.2</td>
<td>0.5</td>
<td>4.1</td>
</tr>
<tr>
<td>Postinjection GH, 1.5 challenge</td>
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<td>4.5</td>
<td>0.7</td>
<td>20.1</td>
</tr>
<tr>
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<td>11.2a</td>
<td>18.6</td>
<td>0.5</td>
<td>73.8</td>
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<tr>
<td><strong>High milk EPD cows (n = 16)</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline GH, clearance d 1</td>
<td>4.3b</td>
<td>5.8</td>
<td>1.0</td>
<td>23.3</td>
</tr>
<tr>
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<td>0.3</td>
<td>2.9</td>
</tr>
<tr>
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<td>1.5</td>
<td>170.3</td>
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<tr>
<td>Postinjection GH, clearance d 2</td>
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<td>70.8</td>
<td>1.5</td>
<td>138.9</td>
</tr>
<tr>
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<td>1.2</td>
<td>0.8</td>
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</tr>
<tr>
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<td>3.1</td>
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<td>12.3b</td>
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<td>1.3</td>
<td>38.3</td>
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</tbody>
</table>

a,b,c,dMeans without a common superscript letter differ (P < 0.05) as determined by ANOVA.

4.5 μg/100 kg BW dose of GHRH 3 h before challenge.

micrograms per 100 kg BW.

W,x,y,zMeans without a common superscript letter differ (P < 0.05) as determined by ANOVA.
Positive relationship between GH response to 4.5 μg /100 kg BW of GHRH during lactation and sire milk EPD in Angus-sired cows (n = 36). GH concentration in the statistical analyses did not improve the relationships of GH response to GHRH to any of the measures of milk production.

A positive relationship was detected between GH response to 4.5 μg of GHRH/100 kg BW and yield at peak lactation (R² = 0.15; P = 0.01). In addition, GH response to 4.5 μg of GHRH/100 kg BW tended to be positively related to total milk production (R² = 0.09; P = 0.06).

Relationship of IGF-I to Estimates of Milk Production

Serum IGF-I (±SE) averaged 64.2 ± 5.2 ng/mL for high milking cows and 59.3 ± 3.3 ng/mL for low milking cows. Serum IGF-I was inversely related (R² = 0.24; P = 0.04; Figure 3) to sire milk EPD in a model including only cows from high-milk EPD sires. Numerically, the relationship between IGF-I and sire milk EPD was inverse when all cows were added to the analysis, but the relationship was not significant. Serum IGF-I was also not related to any estimations of actual milk production (P > 0.20). Regression of baseline GH on serum IGF-I also gave an inverse relationship (R² = 0.10; P = 0.03).

Discussion

Recently, we reported that GH response to GHRH is inversely related to ADG in beef heifers (Auchtung et al., 2001). Evidence that lower prepubertal gain is related to greater subsequent milk production (Hohenbocken et al., 1995) led us to hypothesize that greater GH response to GHRH would be indicative of higher milk production. Indeed, in the present study, we observed a positive relationship between GH response to GHRH in mature beef cows and estimates of their milk production. Our results confirm what has previously been observed in dairy cattle. Growth hormone response to GHRH was greater during lactation in dairy cows selected for higher milk production (Lukes et al., 1989) and in dairy calves selected for greater milk yield potential (Løvendahl et al., 1991). Additionally, dairy bulls with higher genetic merit for milk production had greater GH responses to GHRH (Zinn et al., 1994).
The correlation we report between total milk production and sire milk EPD ($r = 0.46$) was similar to (Gray et al., 1998) or higher than previous studies (Diaz et al., 1992; Marshall and Long, 1993; Marston et al., 1992). The higher correlation in our study is possibly due to the range of sire milk EPD, as compared with the other studies, and the number of cows that were analyzed. Our results identifying differences in genetic merit for milk production concur with those of Buchanan and Gosz (1998) for average 24-h milk production and Rupert et al. (1999) and Minick (1999) for total milk production.

Although GH response to GHRH had a positive relationship with both sire milk EPD and estimates of actual milk production, it was not as strong as the relationship between sire milk EPD and actual milk production. This may be due to the fact that the cows were near the end of lactation at the time of the GHRH challenge. Bourne et al. (1977) and Vines et al. (1977) found that there is a greater GH response to TRH during early lactation than late lactation. Greater GH responses to a secretagogue may allow for increased detection of differences among animals and therefore strengthen any relationship to actual milk production.

In a recent study (Auchtung et al., 2001), performance of heifers was related more strongly to the lower dose of GHRH, whereas the performance of the mature cows in this study was related more strongly to the higher dose of GHRH. The mature cows also had lower baseline concentrations of GH than the heifers of our other study. We would expect such a reversal based on the observations of Lapierre et al. (1992) and Plozuzek and Trenkle (1991). They found that aging decreases the concentration of GH in the blood and also decreases the GH response to a secretagogue, such as GHRH.

In the present study, we observed an inverse relationship between GH and IGF-I concentrations. There was also a significant inverse relationship between IGF-I and sire milk EPD in high milking beef cows. These observations are consistent with the results of various studies in dairy cattle (Ronge et al., 1988; Vicini et al., 1991; Sharma et al., 1994). Although it seems that IGF-I is likely useful as a selection criterion, its concentrations can be influenced by physiologic state. For example, during early lactation, cows are typically in negative energy balance and there is an uncoupling of the GH/IGF-I axis (Cohick, 1998). At the time of GHRH challenge, cows in the present study were in late lactation and likely not in negative energy balance. However, subtle effects of nutrient partitioning on the somatotropic axis cannot be completely excluded. Given the inverse relationship observed between GH and IGF-I and the significant relationship between IGF-I and milk EPD, characterization of the cow’s IGF-I status for use with the GH response to GHRH would likely improve the producer’s ability to identify cows with greater potential for milk production.

Løvendahl et al. (1994) reported that GH response to GHRH is highly heritable in cattle. Our findings suggest that milk EPD are related to GH response in beef cattle. Further, bulls with higher GH response are known to have higher postweaning ADG (Connor et al., 1999, 2000) and heifers have lower postweaning ADG (Auchtung et al., 2001). Currently, we are attempting to determine if the GH response to GHRH at weaning, in heifers, is predictive of future lactational performance. This information would strengthen the argument for the use of GH response to GHRH in addition to the existing selection criteria for beef heifers.

When considering the use of GH response to GHRH as an additional management tool, other factors need to be addressed, such as the impact on reproductive performance. Van Oijen et al. (1993) questioned the recommendations that support selection for high milk production in beef cows because of the effects on reproductive rates and biological efficiencies. However, Beal et al. (1990) and Montano-Bermudez and Nielsen (1990) observed that cows differing in level of milk production had no significant differences in subsequent reproductive performance. Before GH response to GHRH could be adopted on a large scale as a selection indicator, monitoring of reproductive efficiencies in selected animals would be warranted. However, given the previous lack of correlation of increased milk yield with detrimental effects on reproduction, it is possible that GH response would show a similar lack of correlation with decreased reproductive performance.

**Implications**

The beef industry may benefit from additional criteria for selecting of cows with greater milk production potential. Increased milk production relates to increased weaning weight, which is a major goal of a successful beef cow-calf operation. Growth hormone response to growth hormone-releasing hormone is positively related to milk production in the mature beef cow. Thus it may be useful, in conjunction with insulin-like growth factor-I status and non-invasive tools, such as sire milk expected progeny differences, to enhance the efficiency of beef production.

**Literature Cited**


