Effects of growth hormone-releasing hormone treatment on milk production and plasma hormones and metabolites in lactating Japanese Black cows under negative energy balance

H. Shingu,*2 K. Hodate,† S. Kushibiki,‡ E. Touno,* A. Oshibe,* Y. Ueda,*3 M. Shinoda,*4 and S. Ohashi§

*Department of Animal Production and Grasslands Farming, National Agricultural Research Center for Tohoku Region, Morioka, Iwate, 020-0198, Japan; †School of Veterinary Medicine, Kitasato University, Towada, Aomori, 034-8628, Japan; ‡Department of Animal Physiology and Nutrition, National Institute of Livestock and Grassland Science, Tsukuba, Ibaraki, 305-0901, Japan; and §Kanazawa Institute of Technology, Hakusan, Ishikawa, 924-0838, Japan

ABSTRACT: The current study was performed to clarify the effects of GHRH treatment on milk production and plasma hormones and metabolites in lactating Japanese Black cows (a beef breed) under negative energy balance (EB). Ten multiparous lactating beef cows were offered a normal-energy diet daily (110% of ME requirements for maintenance and lactation) until 5 d in milk (DIM) to standardize the cows before dietary treatment. From 6 DIM to the final days (63 DIM) of the experiment, the cows were allotted to experimental dietary treatments: 5 cows were offered a diet formulated for 130% [high-energy diet (HED)] and the remaining 5 cows were offered a diet formulated for 80% [low-energy diet (LED)] of ME requirements for maintenance and lactation. In addition, all cows received daily subcutaneous injections of 3 mg of bovine GHRH from 36 to 56 DIM (GHRH treatment period). Differences in BW of HED- and LED-fed cows at 63 DIM were +28.4 and −7.2 kg compared with BW at 6 DIM, and HED- and LED-fed cows were under positive EB (+23.7 MJ/d) and negative EB (−11.6 MJ/d) throughout the experiment period. Treatment with GHRH increased (P < 0.01) the average daily milk yield to 6.2 kg in HED-fed cows compared with a milk yield of 5.3 kg for 7 d before the GHRH treatment period (pretreatment period); LED-fed cows had no increase in milk production from GHRH treatment. Plasma GH, IGF-1, insulin, and glucose concentrations increased (P < 0.05) after GHRH treatment in both HED- and LED-fed cows; GHRH treatment also induced an increase (P < 0.05) in the net area under the curve of plasma insulin after glucose challenge in both HED- and LED-fed cows. Plasma urea N concentrations were decreased (P < 0.05) by GHRH treatment in HED-fed cows, but not in LED-fed cows. Plasma NEFA concentration was unaffected by GHRH treatment in both HED- and LED-fed cows. We conclude that GHRH treatment of lactating Japanese Black cows stimulates endogenous GH and subsequent IGF-1 secretion and might induce an increase in insulin resistance, irrespective of EB; however, compared with lactating dairy cows, both galactopoietic and lipolytic effects of GHRH might be insufficiently exerted under negative EB in lactating beef cows.

Key words: beef breed, energy balance, growth hormone, growth hormone-releasing hormone, insulin, lactation

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INTRODUCTION

In lactating ruminants, GH exerts galactopoietic and lipolytic effects, resulting in the facilitation of preferential partitioning of nutrients to the mammary glands for milk production (Bauman and Currie, 1980). In general, the milk response to GH treatment depends on the energy balance (EB) of lactating dairy cows (Bauman, 1992). In dairy cows under positive EB, GH and GHRH treatments induce galactopoietic and lipolytic effects. In contrast, in dairy cows under negative EB, as is often seen in early lactation, a negligible response or no response of milk production to GH and GHRH treatments is observed. However, reports that GH could exert a galactopoietic effect in dairy cows under negative EB are emerging (see the review by Carriquiry et al., 2008). Thus, dairy breeds under negative EB as well as under positive EB may have additional catabolic action.

Japanese Black cows, a beef breed, have less milk production, with less plasma GH and NEFA and greater insulin concentrations, compared with Holstein cows (Shingu et al., 2002). Moreover, this beef breed has a smaller proportion of somatotrophs in the adenohypophysis (Matsuzaki et al., 2001), a greater ratio of muscle to bone (Zembayashi, 1987), and a greater fat percentage in carcass than dairy breeds (Ozutsumi et al., 1984), indicating that lactating Japanese Black cows have greater anabolic, rather than catabolic, actions compared with Holstein cows. Treatment of beef cows fed to meet requirements with GHRH enhanced milk yield and increased plasma GH, IGF-1, and insulin, as in dairy cows; in contrast to dairy cows, there was little change in plasma NEFA (Shingu et al., 2004). However, it is not known whether GHRH would exert the same effect in beef cows under negative EB. The aim of the current study was to examine the effects of GHRH treatment on milk production and blood hormones and metabolites in lactating Japanese Black cows under negative EB.

MATERIALS AND METHODS

All experimental procedures were approved by the Animal Care Committee of the National Agricultural Research Center for the Tohoku Region, according to the Guide for the Care and Use of Experimental Animals (Consortium, 1988).

Animal Management and Feeding Regimen

Ten multiparous Japanese Black cows (8 in the second lactation and 2 in the third lactation) were used from 6 to 63 d in milk (DIM) and housed in a stanchion barn with free access to trace mineralized salt and water. After calving, all cows were offered the same lot of diet formulated for 110% of ME requirements for maintenance and milk production until 5 DIM; experimental dietary treatment was begun on 6 DIM. The types of the diets were total mixed rations formulated for 130% (high-energy diet: HED) and 80% (low-energy diet: LED) of ME requirements for maintenance and milk production, and the cows were offered either the HED (n = 5) or LED (n = 5) twice daily at 0830 and 1630 h until 63 DIM; each diet group contained 1 cow in the third lactation.

The ingredients in the HED vs. LED diets were as follows (DM basis): timothy hay (8.5 MJ/kg, 8.1% CP), 27.3 vs. 35.5%; corn silage (10.4 MJ/kg, 8.5% CP), 5.9 vs. 5.9%; alfalfa hay cube (8.5 MJ/kg, 17.0% CP), 13.0 vs. 17.8%; concentrate (12.6 MJ/kg, 20.1% CP), 35.0 vs. 21.1%; beet pulp (11.8 MJ/kg, 12.6% CP), 18.8 vs. 18.9%; and urea, 0 vs. 0.8%. In the Japanese Feeding Standard for Beef Cattle (2000), daily requirements for maintenance of mature beef cows (ME_{M}) are defined as 37.89, 41.88, 45.74, 49.51, and 53.17 MJ at 350, 400, 450, 500, and 550 kg of BW, respectively, and the daily requirement for lactation (ME_{T}) of lactating beef cows is defined as 5.52 MJ/kg of milk. By using the relational expression and cow BW and daily milk yield, total daily ME of cows to meet the requirements perfectly (ME_{T}) was expressed as the sum of ME_{M} and ME_{L}. The theoretical ME to meet the requirement in cows on the HED [ME_{T(HED cows)}] and LED [ME_{T(LED cows)}] was calculated by multiplying the ME_{T} by 1.3 and 0.8. On the other hand, based on the HED and LED containing nutritional values of 10.67 and 10.03 MJ/kg of DM, the amounts of HED and LED (kg of DM) were expressed as ME_{T(HED cows)} divided by 10.67 and as ME_{T(LED cows)} divided by 10.03. Moreover, when the nutritional value of diet residues was defined as ME_{R}, EB was calculated by using the equation ME_{T(HED cows) or LED cows} − (ME_{T} + ME_{R}), MJ/d. The amounts of HED and LED were adjusted on the day after BW measurement on 1330 h at 5, 9, 13, 18, and 25 DIM. In addition, the cows were weighed weekly to check the changes in BW and on the day before glucose challenge to adjust the dose of glucose injected. After calving, the cows were milked twice daily at 0600 and 1630 h in a 2 × 3 tandem milking parlor (Orion Machinery Co. Ltd., Nagano, Japan).

Experimental Procedures

The experimental period, ranging from 29 to 63 DIM, was divided into 3 parts: a pretreatment period (29 to 35 DIM), a GHRH treatment period (36 to 56 DIM), and a posttreatment period (57 to 63 DIM). At 1200 h, all animals received daily subcutaneous injections of 3 mg of bovine GHRH analog, A15-DAbGHRH (see Shingu et al., 2004), during the GHRH treatment period. The powdered GHRH was dissolved in 5 mL of physiological saline at 1145 h on the date of use. Blood samples (7 mL) were collected from a jugular vein with heparinized syringes (65 U of heparin, Terumo Corp., Tokyo, Japan) at 1145 and 1155 h during the periods of pretreatment (29, 31, 33, and 35 DIM), GHRH treatment (37, 39, 42, 44, 46, 49, 51, 53, and 56 DIM), and posttreatment (57, 59, 61, and 63 DIM) to measure...
plasma concentrations of hormones and metabolites. In addition, glucose challenges were conducted during the periods of pretreatment (32 DIM) and GHRH treatment (52 DIM) to examine endogenous insulin secretion. On each challenge day, a 75-mm-long indwelling catheter was inserted into a jugular vein at 0945 h for glucose injection and blood collection, after which the cows were left undisturbed until the final sampling time (1400 h). Blood samples (3 mL) were collected at −30, −15, 0 (1200 h), 5, 10, 15, 20, 30, 45, 60, 90, and 120 min after intravenous glucose injection (112.5 mg/kg of BW; Otsuka Pharmaceutical Co. Ltd., Tokyo, Japan) via heparinized syringes.

**Analytical Methods**

Collected blood samples were immediately chilled on ice and centrifuged at 1,600 × g at 4°C for 25 min. The plasma samples were stored at −30°C until analysis. Plasma GH and IGF-1 concentrations were determined by RIA (see Shingu et al., 2004). Plasma insulin concentration was measured by using a RIA kit, Insulin Eiken RIA Kit (Eiken Chemical Co. Ltd., Tokyo, Japan; see Shingu et al., 2004). Intra and interassay CV were 7.2 and 9.7% for GH, 4.2 and 12.2% for IGF-1, and 1.9 and 7.7% for insulin, respectively; sensitivities were 0.5 ng/mL for GH, 1.0 ng/mL for IGF-1, and 1.0 µU/mL for insulin. Plasma glucose, NEFA, and urea N concentrations were determined by using colorimetric kits, Glucose 2-HA, Wako NEFA-HA Tests, and UN-HA (Wako Pure Chemical Industries Ltd., Osaka, Japan), using a Hitachi 7070 autoanalyzer (Hitachi Ltd., Tokyo, Japan). Plasma concentrations of the metabolites were measured at the same time; the intraassay CV were 1.8% for glucose, 4.4% for NEFA, and 2.4% for urea N, respectively.

**Calculations**

The percentage changes in mean daily milk yield during the GHRH treatment and posttreatment periods over the average milk yield during the pretreatment period (100%) were calculated. Moreover, as an index of the amount of insulin secretion in the glucose challenge, the net insulin area under the response curve (AUC) was calculated.

**Statistical Analysis**

Data were analyzed by using the GLM procedure (repeated method; SAS Inst. Inc., Cary, NC). Differences were considered significant at \( P < 0.05 \). The statistical model was

\[
Y_{ijk} = \mu + \alpha_i + \gamma_{ij} + \beta_k + \alpha\beta_{ik} + \varepsilon_{ijk},
\]

where \( Y_{ijk} \) are data; \( \mu \) is the overall mean; \( \alpha_i \) are effects attributable to diet; \( \gamma_{ij} \) are effects attributable to individual cows (repeated treatment); \( \beta_k \) are effects attributable to period; \( \alpha\beta_{ik} \) are effects attributable to the interaction between diet and period; and \( \varepsilon_{ijk} \) are residuals. The significance of differences among means of items measured was determined by using the Tukey multiple range test.

The randomized complete block model was

\[
Y_{ij} = \mu + \alpha_i + \beta_j + \varepsilon_{ij},
\]

where \( Y_{ij} \) are data; \( \mu \) is the overall mean; \( \alpha_i \) are effects attributable to diet; \( \beta_j \) are effects attributable to cows; and \( \varepsilon_{ij} \) are residuals.

In addition, to examine significant changes in milk yield during the GHRH treatment and posttreatment periods compared with average milk yield during the pretreatment period, data were analyzed by using the CONTRAST statement with GLM procedures (SAS Inst. Inc.).

**RESULTS**

**EB and Milk Production**

The HED- and LED-fed cows weighed an average of 443 and 456 kg at 6 DIM \( (P > 0.05) \). On the final day of the experiment, HED-fed cows had increasing BW \((+28.4 \text{ kg})\), whereas LED-fed cows lost 7.2 kg compared with BW at 6 DIM. Throughout the experimental period, average diet intake was 9.6 and 5.7 kg/d on a DM basis and the average EB was +23.7 and −11.6 MJ/d \( (P < 0.001) \) in HED- and LED-fed cows, respectively.

Administration of GHRH increased \( (P < 0.01) \) milk yield of HED-fed cows from 5.3 (during the pretreatment period) to 6.2 kg/d \((+{16.4\%})\); there was no change in milk yield of LED-fed cows (from 3.5 to 3.3 kg/d, −3.9%) during GHRH treatment (Figure 1). During the posttreatment period, milk yield of HED-fed cows remained similar to that during GHRH treatment (5.9 kg/d, +10.3% of milk yield during the pretreatment period). In contrast, there was a considerable decrease \( (P < 0.05) \) in milk yield of LED-fed cows during the posttreatment period (2.8 kg/d, −20.4% of milk yield during the pretreatment period).

**Plasma Concentrations of Hormones and Metabolites**

Changes in plasma hormone and metabolite concentrations during the experimental period are shown in Table 1. In both HED- and LED-fed cows, GHRH treatment increased \( (P < 0.05) \) plasma concentrations of GH, IGF-1, insulin, and glucose compared with those during the pretreatment period. The HED-fed cows had a decrease \( (P < 0.05) \) in plasma urea N concentration during GHRH treatment, although there was little change in urea N concentration in LED-fed cows. Plasma NEFA concentrations were unaffected by administration of GHRH in both HED- and LED-fed cows.
Figure 1. Changes in the daily milk yield in HED-fed cows (cows offered a high-energy diet to meet 130% of ME requirements, n = 5; open circles) and LED-fed cows (cows offered a low-energy diet to meet 80% of ME requirements, n = 5; open triangles). Values are the mean ±1 SE. All cows received daily subcutaneous injections of 3 mg of GHRH from 36 to 56 d in milk. For milk yield during the experimental period (from 29 to 63 d in milk), closed symbols indicate significant increases or decreases (P < 0.05) in milk yield, compared with the average milk yield during the pretreatment period.

Table 1. Changes in plasma concentrations of hormones and metabolites in HED- and LED-fed cows by GHRH treatment

<table>
<thead>
<tr>
<th>Hormone or metabolite</th>
<th>Diet</th>
<th>Period</th>
<th></th>
<th></th>
<th>Pooled SE</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pretreatment</td>
<td>GHRH treatment</td>
<td>Posttreatment</td>
<td></td>
<td></td>
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<tr>
<td>GH, ng/mL</td>
<td>HED</td>
<td>1.27</td>
<td>3.77</td>
<td>2.05</td>
<td>0.25</td>
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<td></td>
<td>LED</td>
<td>1.31</td>
<td>2.80</td>
<td>1.77</td>
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<td>IGF-1, ng/mL</td>
<td>HED</td>
<td>51.1</td>
<td>128.6</td>
<td>69.4</td>
<td>4.5</td>
<td>*** NS NS</td>
</tr>
<tr>
<td></td>
<td>LED</td>
<td>45.6</td>
<td>126.9</td>
<td>52.7</td>
<td></td>
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<tr>
<td>Insulin, µU/mL</td>
<td>HED</td>
<td>30.9</td>
<td>58.9</td>
<td>36.5</td>
<td>1.9</td>
<td>*** *** **</td>
</tr>
<tr>
<td></td>
<td>LED</td>
<td>19.7</td>
<td>33.4</td>
<td>17.9</td>
<td></td>
<td></td>
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<tr>
<td>Glucose, mg/dL</td>
<td>HED</td>
<td>62.0</td>
<td>73.6</td>
<td>68.8</td>
<td>1.3</td>
<td>*** NS *</td>
</tr>
<tr>
<td></td>
<td>LED</td>
<td>63.7</td>
<td>67.8</td>
<td>62.3</td>
<td></td>
<td></td>
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<tr>
<td>NEFA, µEq/L</td>
<td>HED</td>
<td>141</td>
<td>132</td>
<td>126</td>
<td>7</td>
<td>** *** NS</td>
</tr>
<tr>
<td></td>
<td>LED</td>
<td>250</td>
<td>239</td>
<td>210</td>
<td></td>
<td></td>
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<tr>
<td>Urea N, mg/dL</td>
<td>HED</td>
<td>17.7</td>
<td>13.4</td>
<td>16.0</td>
<td>0.4</td>
<td>* * NS</td>
</tr>
<tr>
<td></td>
<td>LED</td>
<td>19.7</td>
<td>18.8</td>
<td>18.0</td>
<td></td>
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</tr>
</tbody>
</table>

*a–c Means with different superscripts in the same row are significantly different (P < 0.05).
1HED = cows offered a high-energy diet to meet 130% of ME requirements, n = 5; LED = cows offered a low-energy diet to meet 80% of ME requirements, n = 5.
2For GHRH treatment: daily subcutaneous injections of 3 mg of GHRH (see Shingu et al., 2004).
3For period: pretreatment, 29 to 35 d in milk; GHRH treatment, 36 to 56 d in milk; and posttreatment, 57 to 63 d in milk.
4P = period, D = diet; *P < 0.05, **P < 0.01, ***P < 0.001, and NS = not significant (P > 0.05).
Insulin Secretion at Glucose Challenge

At glucose challenge, almost all plasma insulin concentrations during the GHRH treatment period were greater ($P < 0.05$) than those during the pretreatment period in both HED- and LED-fed cows (Figure 2). Likewise, insulin AUC during the GHRH treatment period was greater ($P < 0.05$) than that during the pretreatment period in HED- (5,341 ± 441 vs. 2,278 ± 435 µU·min·mL$^{-1}$) and LED-fed cows (3,701 ± 350 vs. 2,415 ± 265 µU·min·mL$^{-1}$).

DISCUSSION

In general, milk production in lactating dairy cows peaks before DMI peaks. This indicates that the dairy breed is likely in negative EB even at the peak of milk production. In contrast, Japanese Black cows (a beef breed), with less milk production, have a markedly earlier peak in milk yield (at 2 to 3 wk in milk) and turning point from negative to positive EB, compared with Holstein cows (Shingu and Hodate, 2001). This shows that this beef breed has sufficient DMI to support maintenance and lactation even at early lactation. In addition, in the current study, differences were observed in relative BW (+28.4 vs. −7.2 kg) and calculated average EB (+23.7 vs. −11.6 MJ/d) in HED- and LED-fed cows during the experimental period, which means that the HED- and LED-fed cows were under positive and negative EB, respectively, during the pre-GHRH treatment period as well as after it. Moreover, compared with dairy breeds, this beef breed has physiological characteristics of less plasma GH and greater insulin concentrations during lactation (Shingu et al., 2002), a smaller proportion of somatotrophs in the adenohypophysis (Matsuzaki et al., 2001), a greater ratio of muscle to bone (Zembayashi, 1987), and a greater fat percentage in carcass (Ozutsumi et al., 1984). We concluded, based on the hormonal and morphological profiles, that Japanese Black cows were in greater anabolic status relative to dairy cows during lactation.

Administration of GHRH enhances milk yield and endogenous secretions of GH, and subsequently IGF-1, in lactating dairy cows. During lactation, GH exerts galactopoietic and lipolytic effects, leading to a preferential partitioning of nutrients to the mammary glands and an increase in milk production through orchestrated changes in the metabolism (Bauman and Currie, 1980). The increased milk production during GH treatment is attributable to IGF-1-mediated effects (Peel and Bauman, 1987; Burton et al., 1994). In addition to the physiological role of the GH–IGF-1 axis, in lactating dairy cows, a status of depressed insulin secretion appears to contribute to the stability of milk production (Butler et al., 2003; Rhoads et al., 2004). Similarly, GH and GHRH treatments also induce an increase in milk yield with enhancement of blood GH and IGF-1 concentrations in the beef breeds Angus, Charolais, Simmental (Armstrong et al., 1995) and Japanese Black (Shingu et al., 2004) offered diets sufficient to meet ME requirements. Thus, exogenous GHRH might exert galactopoietic effects on beef cattle as well as on dairy cattle when they are offered a diet with an adequate energy level. In the current study, increased milk yield by GHRH treatment persisted until the first half of the post-GHRH treatment period in HED-fed cows. In GHRH-treated beef cows offered enough of the diet to meet requirements, the status of increase in milk yield continued with increased blood IGF-1 even after cession of the GHRH treatment (Shingu et al., 2004). The continuance of greater milk yield during the post-
GHRH treatment period in HED-fed cows might be due to the increased plasma IGF-1.

Overall, in lactating dairy cows, the magnitude of milk response to GH depends on the amounts of feeding (McCutcheon et al., 1989; Bauman, 1992); the milk yield response with GH treatment might be negligible in chronically underfed cows. In underfed dairy cows, however, GH and GHRH treatments have been reported to increase milk yield (McGuire et al., 1992; Lapierre et al., 1995) and to increase GH and IGF-1 secretions (Lapierre et al., 1995). Those results indicate the possibility that, to some degree, exogenous GH and GHRH exert a galactopoietic effect in lactating dairy cows even under negative EB. On the other hand, the LED-fed cows in the present experiment, under negative EB, had increases in plasma GH and IGF-1 concentrations after GHRH treatment, but no apparent enhancement of milk production during GHRH treatment. It seems that the mammary gland might be less responsive or even resistant to GH in the beef cows under negative EB. Moreover, in the current study, irrespective of EB, GHRH treatment induced increases in blood insulin concentration and insulin AUC, corresponding with the previous results in beef cows treated with GH (Armstrong et al., 1995) and GHRH (Shingu et al., 2004), as well as in dairy cows treated with GH. In addition, GHRH treatment induced an increase in plasma glucose concentration, which is consistent with results for GH-treated lactating Holstein cows (Hodate et al., 1991). These results indicate that, irrespective of EB, GHRH treatment might induce insulin resistance in lactating Japanese Black cows.

In general, GH exerts galactopoietic and lipolytic effects in lactating dairy cows under positive EB. In addition, even when administration of GH induces negative EB in lactating dairy cows, a chronic increase in plasma NEFA concentration with enhancement of milk production has been observed (see the review by Peel and Bauman, 1987). In lactating Japanese Black cows under negative EB, however, GHRH treatment induced no increase in NEFA concentration. As in HED-fed cows, plasma NEFA concentration was scarcely affected by GHRH treatment in the beef cows under zero EB (Shingu et al., 2004). Thus, compared with the lactating dairy breed, GHRH might be less capable of lipid mobilization for milk production in this beef breed.

Changes in plasma urea N concentration are closely related to the amounts of N resources in the rumen and the amination and deamination of AA taking place in the liver and muscle. Lactating dairy cows treated with GH have reduced plasma urea N concentrations compared with control cows (Cheli et al., 1998; Santos et al., 2000); the decreased plasma urea N concentration under GH treatment might reflect the results of additional consumption of AA to enhance milk protein production in dairy cows. In the current study, administration of GHRH induced a decrease in plasma urea N concentration in beef cows under positive EB; in beef cows under negative EB, the treatment caused no change in plasma urea N concentration without an increase in milk yield. The results indicate that the increased endogenous GH and subsequent IGF-1 secretions after GHRH treatment induced improved AA utilization in peripheral tissues, irrespective of EB, but that because of the lack of N resources to meet the minimum requirement, the deamination of AA for additional milk protein production might have been markedly reduced in the cows under negative EB.

In summary, daily consecutive GHRH treatment ranging from 36 to 56 DIM stimulated endogenous GH and subsequent IGF-1 secretions and enhanced insulin resistance in Japanese Black cows, not only under positive EB, but also under negative EB. However, considering that GHRH is less capable of lipid mobilization for milk production in the beef breed compared with the dairy breed, irrespective of EB, the series of results in the current study provide supportive evidence that lactating Japanese Black cows have physiological characteristics of greater anabolic action compared with dairy cows.

**LITERATURE CITED**


