Effects of excessive energy intake and supplementation with chromium propionate on insulin resistance parameters in nonlactating dairy cows

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ABSTRACT: The objective was to compare insulin resistance parameters in cows with adequate or excessive energy intake as well as in cows with excessive energy intake receiving Cr supplementation as chromium propionate. Thirteen multiparous, nonlactating Gir × Holstein cows were ranked by BW and BCS and assigned to 1 of 3 dietary treatments on d 0: 1) diet to meet their ME requirements without Cr supplementation (MAN; n = 4), 2) diet to exceed their ME requirements without Cr supplementation (HIGH; n = 4), and 3) HIGH with 2.5 g/d of chromium propionate (HIGHCR; n = 5, with 10 mg of Cr/cow daily). Diets were formulated to provide 100% of daily ME requirements of MAN and 177% of daily ME requirements of HIGH and HIGHCR cows and offered twice daily via individual self-locking head gates from d 0 to 88. Cow BW and BCS were recorded on d 0 and 88 of the experiment. Blood samples were collected before and 2 h after the morning feeding twice weekly. Preprandial revised quantitative insulin sensitivity check index (RQUICKI) was determined using serum glucose, insulin, and NEFA concentrations obtained before feeding. Glucose tolerance tests (GTT) were performed on d 32 and 88 by infusing cows with 0.5 g of glucose/kg of BW whereas blood samples were collected at –15, 0, 10, 20, 30, 45, 60, and 90 min relative to infusion. Change in BCS tended to be greater in HIGH and HIGHCR (P = 0.09) compared with MAN cows. Within samples collected twice weekly, serum concentrations of glucose, insulin (beginning on d 14 of the experiment), and NEFA (preprandial samples only) were greater (P ≤ 0.05) in HIGH compared with HIGHCR cows and tended to be greater in HIGH compared with MAN cows (P ≤ 0.10) but did not differ (P ≥ 0.52) between HIGHCR and MAN cows. Moreover, HIGH cows had reduced RQUICKI compared with MAN (P = 0.02) and HIGHCR cows (P = 0.05) whereas RQUICKI was similar between MAN and HIGHCR cows (P = 0.53). Within samples collected during the GTT, mean serum insulin concentrations and insulin:glucose ratio were greater (P < 0.01) in HIGH compared with HIGHCR cows, tended (P ≤ 0.09) to be greater in HIGH compared with MAN cows, and were similar (P ≥ 0.16) between HIGHCR and MAN cows. Serum glucose concentrations were greater (P < 0.01) for HIGH compared with MAN and HIGHCR cows 20 min relative to infusion. In conclusion, chromium propionate supplementation prevented the increase in insulin resistance caused by excessive energy intake in nonlactating dairy cows.

Key words: chromium, dairy cows, energy intake, insulin resistance

INTRODUCTION

Nutritional management directly impacts production efficiency of dairy systems (Butler, 2005; Eastridge, 2006) whereas insufficient or excessive nutrient intake can be detrimental to performance and welfare of dairy cattle (Van Saun and Sniffen, 1996). As an example, dairy cows often experience increased insulin resistance during the postpartum period, which is largely due to deficient energy intake (Cronjé, 2000;
Pires et al., 2007; Sinclair, 2010). In turn, excessive energy intake leads to obesity, a common concern among late-lactating and nonlactating dairy cows (Van Saun and Sniffen, 1996) that has also been related with insulin resistance in ruminants (McCann and Reimers, 1986; McCann et al., 1986). This syndrome, characterized by persistent hyperglycemia despite increased insulin secretion, has been negatively associated with milk production, reproduction, and health parameters of dairy cattle (Adamiak et al., 2005; LeBlanc, 2010).

Chromium is known to be a component of the glucose tolerance factor that facilitates the action of insulin on body cells (Mertz, 1992). Accordingly, Cr supplementation has been shown to reduce insulin resistance in periparturient dairy cows under negative energy balance, particularly when an organic source of Cr is used (Subiyatno et al., 1996; Hayirli et al., 2001). However, no research has yet evaluated this relationship in dairy cows with excessive energy intake. Based on this information, we hypothesized that excessive energy intake promotes insulin resistance in nonlactating dairy cows and organic Cr supplementation is an alternative to alleviate this outcome. Hence, this experiment compared insulin resistance parameters, including serum concentrations of glucose, insulin, and NEFA, in cows with adequate or excessive energy intake as well as in cows with excessive energy intake receiving or not Cr supplementation as chromium propionate.

**MATERIALS AND METHODS**

This experiment was conducted at the São Paulo State University–Lageado Experimental Station, located in Botucatu, São Paulo, Brazil. The animals used were cared for in accordance with the practices outlined in the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 1999).

**Animals and Diets**

Thirteen nonlactating, nonpregnant, and ovariectomized Gir × Holstein cows (mean ± SE; BW = 687 ± 26 kg and BCS = 3.7 ± 0.1) were assigned to the experiment (d 0 to 88). On d 0, cows were ranked by BW and BCS (Wildman et al., 1982) and assigned to 1 of 3 dietary treatments in a manner in which all treatment groups had equivalent initial average BW and BCS: 1) diet to meet their ME requirements without Cr supplementation (MAN; n = 4), 2) diet to exceed their ME requirements without Cr supplementation (HIGH; n = 4), and 3) HIGH with 2.5 g/daily of chromium propionate (HIGHCR; n = 5, with 10 mg of Cr/cow daily; KemTrace 0.4% Cr; Kemin Agrifoods South America, Indaiatuba, São Paulo, Brazil). All dietary treatments were formulated to exceed CP, mineral, and vitamin requirements (NRC, 2001), hence focusing treatment differences on ME intake.

Cows were maintained in a single drylot with ad libitum access to water and a commercial mineral mix without the inclusion of Cr (7.7% Ca, 4.0% P, 3.0% Na, 0.20% K, 0.20% Mg, 2.0% S, 0.002% Co, 0.03% Cu, 0.002% I, 0.02% Mn, 0.13% Zn, and 0.02% F) from d –15 to 88. Cows were individually offered a total-mixed ration (TMR) through self-locking head gates twice daily (0700 and 1900 h). Diet composition was (DM basis) 31.5% of coast-cross [*Cynodon dactylon* (L.) Pers.] hay, 39.3% of ground corn, 11.8% of soybean meal, 14.8% of citrus pulp, 1.7% of limestone, and 0.9% of a commercial mineral mix (18% Ca, 10.7% Na, 8% P, 1.2% S, 0.5% Mg, 0.13% Cu, 0.007% Co, and 0.007% I). Samples of the offered TMR were collected weekly, pooled into 1 sample, and analyzed for nutrient content by a bromatology laboratory (São Paulo State University, Botucatu, Brazil). Calculations of ME and NEm used the equation proposed by the NRC (2001). Nutritive value (DM basis) of the TMR was 31% NDF, 46% non-fibrous carbohydrates, 30% starch, 2.65 Mcal/kg of ME, 1.74 Mcal/kg of NEm, and 15.2% CP.

From d –15 to 0, all cows received (DM basis) 6.3 kg/d of the TMR without Cr supplementation as an adaptation period. From d 0 to 88, MAN cows continued to receive (DM basis) 6.3 kg/d whereas HIGH and HIGHTMR cows received 11.2 kg/d of the TMR. Based on the NRC (2001) and initial BW and BCS, ME requirements of cows was 16.7 Mcal/d. Hence, diets were initially formulated to provide 100% of daily ME requirements of MAN cows and 177% of daily ME requirements of HIGH and HIGHTMR cows. Chromium propionate (2.5 g of KemTrace 0.4% Cr; Kemin Agrifoods South America) was mixed with 97.5 g of semolina and top-dressed daily into the TMR of HIGHTMR cows during the morning feeding. Semolina was also top-dressed into the TMR of HIGH and MAN cows (97.5 g during the morning feeding) but without the addition of the chromium propionate. All cows completely consumed their TMR within 2 h after feeding.

**Sampling.** Cow BW and BCS were recorded on d 0 and 88 of the experiment. Furthermore, BCS was evaluated by the same 2 evaluators throughout the experiment, which were blinded to distribution of cows across treatments. Blood samples were collected immediately before (0 h) and 2 h after the morning feeding twice weekly (Mondays and Fridays) during the experiment for determination of serum glucose, insulin, and NEFA (0 h only) concentrations. Concentrations of glucose, NEFA, and insulin obtained before feeding (0 h) were used to determine preprandial revised quantitative insulin sensitivity check index (RQUICKI). This methodology has been used to estimate insulin sensitivity in dairy cows (Hol-
Glucose tolerance tests (GTT) were performed on d 32 and 88 by intravenously infusing cows with 0.5 g of glucose/kg of BW. More specifically, cows were weighed the day before each GTT and were not offered the dietary treatments 12 h before and during the GTT. Cows were fitted with indwelling jugular catheters according to the procedures described by Curley et al. (2008) immediately before infusion and received a 50% dextrose solution (Glicose 50%; Laboratório Prado S.A., Curitiba, Brazil) according to their BW. Catheters were removed after infusion was complete. Blood samples were collected at −15, 0, 10, 20, 30, 45, 60, and 90 min relative to infusion and analyzed for serum concentrations of glucose and insulin. During each GTT, area under the curve (AUC) for glucose and insulin were calculated with the trapezoidal method (Shiang, 2004) whereas insulin:glucose ratio (I:G) was determined by dividing insulin and glucose concentrations within each sampling time (Bernhard et al., 2012). Glucose clearance rate and half-life were calculated with the equations described by Bernhard et al. (2012), using incremental serum glucose concentrations between 30 and 90 min postinfusion during the GTT.

During the weekly or GTT blood collections, samples were obtained from either the coccygeal vein or artery into commercial blood collection tubes (Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, NJ), placed immediately on ice, maintained at 4°C for 24 h to allow clotting, and centrifuged at 3,000 × g at 4°C for 30 min for serum collection. Harvested serum was stored frozen at −20°C until further processing. Glucose was determined using a quantitative colorimetric kit (Katal Biotecnológica Ind. Com. Ltda., Belo Horizonte, Brazil). Insulin was determined using Coat-A-Count kits (DPC Diagnostic Products Inc., Los Angeles, CA) solid phase 125I RIA previously used for bovine samples (Moriel et al., 2008). However, insulin concentrations in samples collected on d 60 of the experiment were unexpectedly below the minimum detectable concentrations of the assay and hence removed from the insulin analysis whereas the RQUICKI values from d 60 were also discarded. Concentrations of NEFA were determined using an enzymatic colorimetric kit (Randox Brasil Ltda., São Paulo, Brazil). The intra- and interassay CV were, respectively, 6.4 and 5.7% for glucose, 6.3 and 7.7% for insulin, and 3.8 and 9.2% for NEFA.

**Statistical Analysis.** All data were analyzed using cow as the experimental unit, cow(treatment) as random variable, with the MIXED procedure of SAS (SAS Inst., Inc., Cary, NC; version 9.3) and Satterthwaite approximation to determine the denominator df for the tests of fixed effects. The model statement used for analysis of BW and BCS change as well as initial, average, and final BCS and BW during the experiment contained the effects of treatment. The model statement used for serum glucose and insulin obtained during the weekly collections contained the effects of treatment, day, hour, and all resultant interactions whereas the model statement used for serum NEFA and RQUICKI contained the effects of treatment, day, and the interaction. The model statement used for serum glucose, serum insulin, and I:G obtained during the GTT contained the effects of treatment, day (d 32 and 88), hour, and all resultant interactions whereas the model statement used for glucose and insulin AUC, glucose clearance rate, and glucose half-life contained the effects of treatment, day (d 32 and 88), and the interaction. The specified term for the repeated statement was day for the weekly collections and hour for the GTT, with cow(treatment) as subject whereas the covariance structure used was compound symmetry, which provided the best fit for these analyses according to the Akaike information criterion. Results are reported as least square means and separated using PDIFF. Significance was set at $P \leq 0.05$, and tendencies were determined if $P > 0.05$ and $P \leq 0.10$. Results are reported according to treatment effects if no interactions were significant or according to the highest-order interaction detected.

**RESULTS AND DISCUSSION**

During the experimental period, BW change was greater ($P \leq 0.05$) for HIGHCR compared with HIGH and MAN cows (Table 1) but similar ($P = 0.38$) between HIGH and MAN cows. Average and final BW during the experimental period and final BW were similar ($P \geq 0.80$) among all treatments (Table 1). These results were unexpected based on the planned differences in energy intake between HIGHCR and HIGH compared with MAN cows. According to the average BW of each treatment group during the experiment (Table 1), the NRC model (NRC, 2001), and TMR intake and nutritional value, daily ME intake was 29.7 Mcal for HIGHCR and HIGH and 16.7 Mcal for MAN. Hence, daily ME intake was 166, 172, and 93% of the daily ME maintenance requirements of HIGH, HIGHCR, and MAN cows during the experiment, respectively. Nevertheless, changes in BW include synthesis of body tissues as well as fluctuations in feed and water consumption whereas BCS reflects the body tissue status without being influenced by the content of the gastrointestinal tract. Hence, BCS better indicates nutritional status than BW changes in dairy cattle (West et al., 1990; Moallem et al., 2000). Accordingly, BCS change during the experiment tended to be greater...
in HIGH and HIGHCR \((P = 0.09)\) compared with MAN cows (Table 1) although no treatment effects were detected \((P ≥ 0.34)\) for average and final BCS (Table 1). These results indicate that HIGH and HIGHCR cows, but not MAN cows, gained body tissues during the experimental period, corroborating with the designed differences in ME intake among treatments.

Within samples collected twice weekly, a tendency for a treatment effect was detected for serum glucose \((P = 0.09; \text{Table 2})\). Mean glucose concentrations were greater \((P = 0.05)\) in HIGH compared with HIGHCR cows and tended to be greater in HIGH compared with MAN cows \((P = 0.10)\) but did not differ \((P = 0.63)\) between HIGHCR and MAN cows. A treatment \(×\) day interaction was also detected \((P < 0.01)\) for serum insulin concentrations within samples collected twice weekly (Fig. 1). Serum insulin concentrations were often greater for HIGH compared with MAN and HIGHCR cows beginning on d 14 of the study. In fact, mean insulin concentrations from d 14 to 88 of the experimental period were greater \((P = 0.03)\) in HIGH compared with HIGHCR cows and tended to be greater \((P = 0.10)\) in HIGH compared to MAN cows but were similar \((P = 0.52)\) between HIGHCR and MAN cows (11.0, 5.1, and 6.7 \(\mu\)IU/mL for HIGH, HIGHCR, and MAN cows, respectively; SEM = 1.7; treatment effect, \(P = 0.08)\). Circulating concentrations of insulin and glucose in cattle are influenced positively by energy intake (Vizzarra et al., 1998; Butler, 2003). Therefore, the greater serum concentrations of these substances detected in HIGH compared with MAN cows could be attributed to the greater energy intake of HIGH cows. However, serum insulin and glucose concentrations were also greater in HIGH compared with HIGHCR cows despite their similar energy intake as well as similar between HIGHCR and MAN cows. Hence, treatment effects detected herein for serum insulin and glucose concentrations from samples collected twice weekly cannot be exclusively attributed to rate of energy intake.

Within samples collected twice weekly but before the morning feeding (0 h), a tendency for a treatment effect was detected \((P = 0.08)\) for serum NEFA con-

### Table 1. Body weight and BCS of nonlactating dairy cows receiving diet to meet their ME requirements without Cr supplementation (MAN; \(n = 4\)), diet to exceed their ME requirements without Cr supplementation (HIGH; \(n = 4\)), or HIGH with 2.5 g/d of chromium propionate (HIGHCR; \(n = 5\), with 10 mg of Cr/cow daily)\(^1\)

<table>
<thead>
<tr>
<th>Item</th>
<th>HIGH</th>
<th>HIGHCR</th>
<th>MAN</th>
<th>SEM</th>
<th>(P)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body weight, kg</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial BW (d 0), kg</td>
<td>702</td>
<td>658</td>
<td>707</td>
<td>48</td>
<td>0.72</td>
</tr>
<tr>
<td>Final BW (d 88), kg</td>
<td>722</td>
<td>693</td>
<td>721</td>
<td>49</td>
<td>0.88</td>
</tr>
<tr>
<td>Average BW during experiment, kg</td>
<td>712</td>
<td>675</td>
<td>714</td>
<td>49</td>
<td>0.80</td>
</tr>
<tr>
<td><strong>BW change, kg</strong></td>
<td>20.5(^a)</td>
<td>35.0(^b)</td>
<td>14.0(^a)</td>
<td>4.9</td>
<td>0.03</td>
</tr>
<tr>
<td><strong>BCS(^2)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial BCS (d 0)</td>
<td>4.00</td>
<td>3.85</td>
<td>3.75</td>
<td>0.23</td>
<td>0.77</td>
</tr>
<tr>
<td>Final BCS (d 88)</td>
<td>4.25</td>
<td>4.10</td>
<td>3.69</td>
<td>0.25</td>
<td>0.34</td>
</tr>
<tr>
<td>Average BCS</td>
<td>4.12</td>
<td>3.97</td>
<td>3.72</td>
<td>0.23</td>
<td>0.51</td>
</tr>
<tr>
<td><strong>BCS change</strong></td>
<td>0.25</td>
<td>0.25</td>
<td>–0.06</td>
<td>0.12</td>
<td>0.09</td>
</tr>
</tbody>
</table>

\(^a,b\) Means with different superscripts differ \((P < 0.05)\).

\(^1\) During the experimental period (d 0 to 88), MAN cows received (DM basis) 6.3 kg/d of a total-mixed ration (TMR) whereas HIGH and HIGHCR cows received 11.2 kg/d of the same TMR. Cows individually received the TMR through self-locking head gates twice daily (0700 and 1900 h), which were initially formulated to provide 100% of daily ME requirements of MAN cows and 177% of daily ME requirements of HIGH and HIGHCR cows. Chromium propionate (2.5 g of KemTrace 0.4% Cr; Kemin Agrifoods South America, Indaiatuba, São Paulo, Brazil) was mixed with 97.5 g of semolina and top-dressed daily into the TMR of HIGHCR cows.

\(^2\) According to Wildman et al. (1982).

### Table 2. Serum parameters and revised quantitative insulin sensitivity check index (RQUICKI) of nonlactating dairy cows receiving diet to meet their ME requirements without Cr supplementation (MAN; \(n = 4\)), diet to exceed their ME requirements without Cr supplementation (HIGH; \(n = 4\)), or HIGH with 2.5 g/d of chromium propionate (HIGHCR; \(n = 5\), with 10 mg of Cr/cow daily)\(^1\)

<table>
<thead>
<tr>
<th>Item</th>
<th>HIGH</th>
<th>HIGHCR</th>
<th>MAN</th>
<th>SEM</th>
<th>(P)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Weekly collections(^2)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum glucose, mg/dL</td>
<td>95.6(^a)</td>
<td>91.1(^b)</td>
<td>92.7(^ab)</td>
<td>1.3</td>
<td>0.09</td>
</tr>
<tr>
<td>Serum NEFA, mmol/L</td>
<td>0.176(^a)</td>
<td>0.139(^b)</td>
<td>0.144(^ab)</td>
<td>0.012</td>
<td>0.08</td>
</tr>
<tr>
<td>RQUICKI</td>
<td>0.578(^a)</td>
<td>0.780(^b)</td>
<td>0.841(^b)</td>
<td>0.072</td>
<td>0.05</td>
</tr>
<tr>
<td><strong>Glucose tolerance test(^3)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose (area under the curve, mg/dL·min)</td>
<td>18,638</td>
<td>17,708</td>
<td>16,971</td>
<td>1,650</td>
<td>0.45</td>
</tr>
<tr>
<td>Insulin (area under the curve, mg/dL·min)</td>
<td>6,324(^a)</td>
<td>3,668(^b)</td>
<td>4,918(^ab)</td>
<td>1,394</td>
<td>0.04</td>
</tr>
<tr>
<td><strong>Glucose clearance rate, %/min</strong></td>
<td>0.949</td>
<td>1.163</td>
<td>1.049</td>
<td>0.173</td>
<td>0.61</td>
</tr>
<tr>
<td><strong>Glucose half-life, min</strong></td>
<td>101.7</td>
<td>77.4</td>
<td>78.1</td>
<td>21.9</td>
<td>0.68</td>
</tr>
</tbody>
</table>

\(^a,b\) Means with different superscripts differ \((P < 0.05)\).

\(^1\) During the experimental period (d 0 to 88), MAN cows received (DM basis) 6.3 kg/d of a total-mixed ration (TMR) whereas HIGH and HIGHCR cows received 11.2 kg/d of the same TMR. Cows individually received the TMR through self-locking head gates twice daily (0700 and 1900 h), which were initially formulated to provide 100% of daily ME requirements of MAN cows and 177% of daily ME requirements of HIGH and HIGHCR cows. Chromium propionate (2.5 g of KemTrace 0.4% Cr; Kemin Agrifoods South America, Indaiatuba, São Paulo, Brazil) was mixed with 97.5 g of semolina and top-dressed daily into the TMR of HIGHCR cows.

\(^2\) Blood samples collected immediately before (0 h) and 2 h after the 0700 h feeding twice weekly (Mondays and Fridays) during the experiment for determination of serum glucose, insulin, and NEFA (0 h only) concentrations.

\(^3\) Glucose tolerance tests were performed on d 32 and 88 by intravenously infusing cows with 0.5 g of glucose/kg of BW. Blood samples were collected at ~15, 0, 10, 20, 30, 45, 60, and 90 min relative to infusion and analyzed for serum concentrations of glucose and insulin. Area under the curve for glucose and insulin were calculated with the trapezoidal method (Shiang, 2004). Glucose clearance rate and half-life were calculated with the equations described by Bernhard et al. (2012).
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concentration (Table 2). During the experimental period, preprandial serum NEFA concentration was greater ($P = 0.04$) in HIGH compared with HIGHCR cows and tended to be greater in HIGH compared with MAN cows ($P = 0.08$) but did not differ ($P = 0.79$) between HIGHCR and MAN cows. A treatment effect was also detected ($P = 0.05$) for RQUICKI (Table 2) calculated from samples collected twice weekly. Based on serum glucose, insulin, and NEFA concentrations obtained before the morning feeding, HIGH cows had reduced RQUICKI, which means reduced preprandial insulin sensitivity and hence increased basal insulin resistance (Kahn, 1978), compared with MAN ($P = 0.03$) and HIGHCR cows ($P = 0.05$; Table 1) whereas RQUICKI was similar between MAN and HIGHCR cows ($P = 0.55$). Contrarily to glucose and insulin, circulating NEFA concentrations in cattle are negatively associated with energy intake (Grummer, 1995) but were greater in HIGH compared to HIGHCR and MAN cows despite their similar or greater energy intake, respectively. Nevertheless, insulin resistance results in increased mobilization of body fat reserves and subsequent NEFA release into the circulation to compensate for the reduced uptake of glucose by body tissues (Barbour et al., 2002). Hence, results from samples collected twice weekly indicates that HIGH cows had greater basal insulin resistance compared with HIGHCR and MAN cows, and treatment effects detected for serum insulin and glucose should also be attributed to hyperglycemia and increased insulin secretion typical of this syndrome. These results also indicate that chromium propionate supplementation prevented the increase in basal insulin resistance in cows consuming excessive energy, based on the similar RQUICKI and serum concentrations of insulin, glucose, and NEFA between HIGHCR and MAN cows. Moreover, insulin resistance may impair BW gain as a physiological defense against excessive obesity (Saltiel, 2012). Therefore, the similar BW gain between HIGH and MAN cows despite their different ME intake or the reduced BW gain in HIGH compared with HIGHCR cows despite their similar ME intake (Table 1) may also be associated with the greater insulin resistance of HIGH cows compared with HIGHCR and MAN cohorts.

Within samples collected during the GTT, a treatment × hour interaction was detected for serum insulin ($P = 0.05$; Fig. 2). Serum insulin concentrations were greater ($P \leq 0.01$) in HIGH compared with HIGHCR cows beginning 10 min after infusion, greater ($P \leq 0.05$) for HIGH compared with MAN cows at 10, 20, and 90 min relative to infusion, and greater ($P \leq 0.03$) for MAN compared with HIGHCR cows at 30 and 45 min relative to infusion. Accordingly, insulin AUC was greater ($P = 0.01$) in HIGH cows compared to HIGHCR cows but similar ($P \geq 0.18$) between HIGH and MAN cows and between MAN and HIGHCR cows (treatment effect, $P = 0.04$; Table 2). A tendency for a treatment × hour interaction was detected ($P = 0.10$) for serum glucose concentrations (Fig. 3) during the GTT, which were greater ($P < 0.01$) for HIGH compared with MAN and HIGHCR cows at 20 min relative to infusion. However, no treatment effects were detected ($P \geq 0.45$) for glucose AUC, clearance rate, and half-life (Table 2). Nevertheless, a treatment × hour interaction was detected for serum I:G ($P = 0.02$; Fig. 4) during the GTT. Serum I:G, which is negatively associated with insulin sensitivity during a GTT (Kegley et al., 2000), was greater ($P \leq 0.05$) for HIGH compared with HIGHCR cows at –15, 10, and beginning at 30 min relative to infusion, greater ($P \leq 0.03$) for HIGH compared with MAN cows at 10, 60, and 90 min relative to infusion, and greater ($P \leq 0.02$) for MAN compared with HIGHCR cows at 30 and 45 min relative to infusion.
Furthermore, mean serum insulin concentrations and I:G during the GTT were greater \((P < 0.01)\) in HIGH compared with HIGHCR cows, tended \((P \leq 0.09)\) to be greater in HIGH compared with MAN cows, and were similar \((P \geq 0.16)\) between HIGHCR and MAN cows (58.4, 35.0, and 45.4 μIU/mL of insulin, SEM = 4.8, and 0.305, 0.189, and 0.238 of I:G, SEM = 0.024, for HIGH, HIGHCR, and MAN cows, respectively). Collectively, treatment effects detected during the GTT for serum insulin, glucose, and I:G further support the outcomes detected during the weekly collections. The overall differences between MAN and HIGH indicate that excessive energy intake reduced insulin sensitivity in cattle following a glucose challenge. The differences in the same parameters between HIGHCR and HIGH cows in addition to the similar responses between HIGHCR and MAN cows also indicate that chromium propionate supplementation prevented the decrease in insulin sensitivity caused by excessive energy intake in cattle receiving a glucose challenge.

Although others reported that deficient energy intake also impairs insulin and glucose metabolism in dairy cattle (Cronjé, 2000; Pires et al., 2007; Sinclair, 2010), this research demonstrates that cattle consuming excessive energy experience reduced basal insulin sensitivity or reduced insulin sensitivity following a GTT. The physiological reason to why excessive energy intake causes insulin resistance in cattle is still not fully comprehended but may be attributed to the increased lipogenesis (Ballard et al., 1972; Prior and Scott, 1980; Vernon, 1980), given that lipids interfere with the binding of insulin to its cellular receptors (Lewis et al., 2002; Ferezou and Bach, 1999). Moreover, increased circulating NEFA concentrations caused by insulin resistance can further intensify this syndrome in dairy cattle (Pires et al., 2007), given that cellular NEFA metabolism interferes with the intracellular insulin receptor signaling cascade (Lewis et al., 2002). Hence, development of insulin resistance in the HIGH cows evaluated herein can be attributed, at least partially, to concurrent increases in lipogenesis and lipolysis caused by excessive energy intake.

Supporting our results regarding chromium propionate supplementation, research with cattle receiving growing diets also reported that supplemental Cr enhances glucose metabolism parameters (Chang and Mowat, 1992; Mowat et al., 1993). Others also reported that supplemental Cr enhanced insulin sensitivity and glucose metabolism following GTT in with periparturient dairy cows (Subiyatno et al., 1996; Hayirli et al., 2001) or growing cattle (Kegley et al., 2000; Summer et al., 2007). During the GTT in the present study, I:G was occasionally reduced in HIGHCR cows compared with MAN cows. Furthermore, insulin AUC was reduced in HIGHCR compared with HIGH and MAN cows, despite similar treatment effects on glucose AUC, glucose clearance rate, and glucose half-life. Hence, chromium propionate supplementation appears to prevent the decrease in insulin sensitivity caused by excessive energy intake and perhaps even optimize this response in cattle receiving a GTT. Chromium is a critical component of the glucose tolerance factor that facilitates the action of insulin on body cells (Mertz, 1992), and Cr supplementation has been shown to enhance glucose metabolism in ruminants (Kegley et al., 2000; McNamara and Valdez, 2005; Summer et al., 2007). More specifically, Cr modifies glucose metabolism through chromodulin, an oligopeptide that binds with high affinity to 4 chromic ions and enables Cr to be involved in the autoamplification of insulin signaling, maintaining the active conforma-
tion of insulin receptors and promoting greater glucose uptake (Vincent, 2000, 2001). To our knowledge, this is the first research demonstrating that excessive energy intake also results in increased basal insulin resistance in dairy cattle and chromium propionate supplementation prevents this outcome. Hence, results from this experiment suggest that chromium propionate supplementation prevents the negative interference of excessive circulating lipids, such as serum NEFA reported herein, on the activity of insulin on target receptors and cells. Nevertheless, excessive energy intake and chromium propionate supplementation may also impact insulin sensitivity parameters in adipose and other body tissues through immunological signals such as proinflammatory cytokine response (Wellen and Hotamisligil, 2005; Shi et al., 2006). However, this experiment was not designed to investigate the specific cellular mechanisms associating excessive energy intake, chromium propionate supplementation, and insulin sensitivity in nonlactating dairy cows. Therefore, additional research is still warranted to further comprehend the physiological mechanisms responsible for the outcomes observed herein.

In conclusion, results from this experiment indicate that nonlactating dairy cows consuming excessive energy experience reduced insulin sensitivity during routine management or on a GTT compared to cows consuming adequate amounts of energy, characterizing a state of insulin resistance. In addition, supplementation of chromium propionate prevents the aforementioned decrease in insulin sensitivity in cows consuming excessive energy. Hence, nutritional management including adequate energy intake and chromium propionate supplementation should be adopted to prevent the incidence of insulin resistance in nonlactating dairy cows, which has been negatively associated with productive, reproductive, and health parameters of dairy cattle (Adamiak et al., 2005; LeBlanc, 2010).

**LITERATURE CITED**


