Effects of fat-enriched diet and methionine on insulin sensitivity in lactating cows

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ABSTRACT: The hyperinsulinemic–euglycemic clamp (EGC) technique was used to investigate the effects of calcium salts of long-chain fatty acids (LCFA-Ca) and rumen-protected Met (RPM) on insulin sensitivity in the peripheral tissues of lactating cows. Six multiparous Holstein cows were used in a 3 × 3 Latin square experiment in each 14-d period. Dietary treatments were 0 (RPM0), 20 (RPM20), and 60 (RPM60) g/d of RPM, supplemented with a diet containing 1.5% of LCFA-Ca equal to 110% of the cows’ ME requirement. And as a control for the 3 LCFA-Ca–containing diets, a dietary treatment without LCFA-Ca (Con) was also included. After a 10-d adaptation period, milk samples were collected for 4 d, and EGC experiments were performed on d 14 of each treatment period. Insulin solution was infused through a jugular vein catheter at a rate of 0.1, 0.2, 0.3, and 0.4 milliunits·kg BW–1·min–1 for 30 min and then at a rate of 0.5 milliunits·kg BW–1·min–1 for 60 min. Glucose solution was variably infused to maintain plasma glucose at steady state through the same catheter. Blood samples for measurements were taken using the contralateral catheter. Plasma total cholesterol, cholesterol ester, free cholesterol, and phospholipid concentrations in RPM0 and RPM20 were higher than those in Con, whereas the concentrations in RPM60 were low at the same degree of those in RPM0 (P < 0.05). Plasma Met concentration was greatest in RPM60 (P < 0.05). In the EGC experiment, the glucose infusion rate was greater in RPM60 than in RPM0 and RPM20 and an effective concentration of insulin resulting in 50% maximal glucose infusion rate was lower in RPM60 compared with RPM0 (P < 0.05), indicating that insulin sensitivity was intensified in RPM60. Although the insulin sensitivity evaluated from the EGC data in RPM0, RPM20, and RPM60 was not different from Con, a slight decline was observed in RPM0 and insulin sensitivity in RPM60 was higher than Con. Our results from the EGC experiment demonstrated that the feeding RPM lead to increased insulin sensitivity, which suggests that dietary Met affects lipid metabolism via insulin action in lactating dairy cows fed a LCFA-Ca–containing diet.

Key words: dairy cattle, insulin sensitivity, long-chain fatty acids, methionine

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INTRODUCTION

Dietary Met causes several biochemical and physiological responses. In dairy cows, supplementation with a methionine hydroxyl analog increases milk fat synthesis with hypertriglyceremia (Huber et al., 1984). Conversely, an insufficiency of Met during the periparturient period results in the development of hepatic lipodosis (Shibano and Kawamura, 2006). Methionine addition to the diet increased plasma chylomicron concentrations in preruminant calves (Auboiron et al., 1994). These reports suggest that Met enhances hepatic very low density lipoprotein (VLDL) secretion and intestinal secretion of chylomicron. Methionine supplementation increased VLDL in calves fed lipid-restricted or control diets but the same effect was absent with lipid-enriched diets, suggesting that Met intake was insufficient relative to fat intake (Auboiron et al., 1995). In our previous study (Fukumori et al., 2012), the supplementation with calcium salts of long-chain fatty acids (LCFA-Ca) to the total mixed ration (TMR) increased plasma concentrations of triglycerides, nonesterified fatty acids, and total cholesterol. The addition of rumen-protected Met (RPM) depressed hyperlipidemia when cows were fed the LCFA-Ca–containing TMR, but the lipid-lowering effects were not shown when cows consumed RPM with the control TMR (without LCFA-Ca). These different effects of Met on lipid metabolism are likely dependent on fat intake level. Lower plasma lipid profiles seem to be a result of the alterations of lipid utilization in the peripheral and mammary tissues. However, peripheral blood data alone is insufficient for the evaluation of lipid utilization, endocrine function, and relationship to productivity. Insulin is one of the most important hormones controlling nutrient metabolism. For lipid metabolism of ruminants, insulin infusion exerted a lipogenic action in wethers (El-Sabagh et al., 2015). Additionally, the relationship between insulin action and Met intake are unclear in dairy cattle. Osorio (2014) reported that feeding RPM improved voluntary DMI and milk yields of protein and fat and produced a faster recovery to a positive energy balance in peripartal cows. He also determined the relationship between Met and endocrine function, but the equivocal results suggested further extensive studies. Therefore, the objective of this study was to evaluate the effects of feeding a fat-enriched diet and RPM on insulin sensitivity and lipid metabolism in lactating cows fed LCFA-Ca.

MATERIALS AND METHODS

The procedures used in the present study were performed in accordance with the principles and guidelines for animal use issued by the National Institute of Livestock and Grassland Science Animal Care Committee, which were formulated to comply with Japanese regulations.

Animals and Treatments

Six multiparous Holstein cows (63.2 ± 1.8 d in milk and 556.2 ± 24.3 kg initial BW) were managed in individual tie stalls, allowed free access to water and mineral blocks, and fed twice daily (0900 and 1800 h). Their diet (TMR) contained 1.5% (DM) of LCFA-Ca–containing diets (Megalac R; Church and Dwight Co., Inc., Princeton, NJ). Declared fatty acids contained 26% palmitic acid, 4% stearic acid, 33% oleic acid, 32% linoleic acid, and 5% linolenic acid.

Table 1. Ingredients of the basal diet

<table>
<thead>
<tr>
<th>Item</th>
<th>Con</th>
<th>RPM0</th>
<th>RPM20</th>
<th>RPM60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingredient (% DM basis)</td>
<td></td>
<td>35.1</td>
<td>35.1</td>
<td>35.1</td>
</tr>
<tr>
<td>Corn silage</td>
<td>9.8</td>
<td>9.8</td>
<td>9.8</td>
<td>9.8</td>
</tr>
<tr>
<td>Alfalfa hay cubes</td>
<td>6.1</td>
<td>6.1</td>
<td>6.1</td>
<td>6.1</td>
</tr>
<tr>
<td>Sudan grass hay</td>
<td>8.5</td>
<td>8.5</td>
<td>8.5</td>
<td>8.5</td>
</tr>
<tr>
<td>Mixed concentrate(^3)</td>
<td>28.9</td>
<td>28.9</td>
<td>28.9</td>
<td>28.9</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>8.8</td>
<td>8.8</td>
<td>8.8</td>
<td>8.8</td>
</tr>
<tr>
<td>Beet pulp pellet</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Calcium salts of fatty acids(^4)</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Molasses feed(^5)</td>
<td>2.8</td>
<td>2.8</td>
<td>2.8</td>
<td>2.8</td>
</tr>
<tr>
<td>Chemical composition (DM basis)(^6)</td>
<td>16.0</td>
<td>15.8</td>
<td>15.8</td>
<td>15.8</td>
</tr>
<tr>
<td>CP, %</td>
<td>3.1</td>
<td>4.6</td>
<td>4.6</td>
<td>4.6</td>
</tr>
<tr>
<td>Crude fat, %</td>
<td>2.6</td>
<td>2.7</td>
<td>2.7</td>
<td>2.7</td>
</tr>
<tr>
<td>ME, Mcal/kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) Con = dietary treatment without LCFA-Ca.
\(^2\) LCFA-Ca = calcium salts of long-chain fatty acids; RPM0 = 0 g/d of rumen-protected Met; RPM20 = 20 g/d of rumen-protected Met; RPM60 = 60 g/d of rumen-protected Met.
\(^3\) Mixed concentrate contained 55% corn grain, 20% corn gluten feed, 10% soybean meal, and 8% other.
\(^4\) Megalac R (Church and Dwight Co., Inc., Princeton, NJ). Declared fatty acids contained 26% palmitic acid, 4% stearic acid, 33% oleic acid, 32% linoleic acid, and 5% linolenic acid.
\(^5\) A product of Fuji Development Co., Ltd., Chiba, Japan.
\(^6\) Estimated values from the NRC (2001).
Hyperinsulinemic–Euglycemic Clamp

At 1300 h on d 14 of each experimental period, tissue responsiveness to insulin was determined using a hyperinsulinemic–euglycemic clamp (EGC) technique according to the method of Sano et al. (1992). Cows were fitted with catheters (14 G; Medikit Co. Ltd., Tokyo, Japan) into their both jugular veins. Insulin solution (Novolin R100 [recombinant DNA origin]; Novo Nordisk Pharma Ltd., Bagsvaerd, Denmark) was continuously infused during 4 sequential 30-min periods at the infusion rate of 0.1, 0.2, 0.3, and 0.4 milliunits·kg BW\(^{-1}\)·min\(^{-1}\), respectively, and then, thereafter, during a 90-min period, at the rate of 0.5 milliunits·kg BW\(^{-1}\)·min\(^{-1}\) into a jugular vein catheter using a multichannel peristaltic pump. Glucose solution (50%, wt/vol; Otsuka Pharmaceutical Factory, Inc., Tokushima, Japan) was infused through the same catheter at variable rates to maintain plasma glucose concentration at steady state by another peristaltic pump. The plasma glucose concentration was monitored at 5-min intervals during the EGC experiment. Blood samples (1 mL) were taken using the contralateral catheter and immediately centrifuged 10,000 \times g for 30 s at room temperature, and glucose concentrations of the obtained plasma were measured using a glucose analyzer (YSI 2300 STAT PLUS; YSI Life Sciences, Yellow Springs, OH). The glucose infusion rate (GIR) was also adjusted and recorded during the 210-min period of the insulin infusion.

Sampling

The blood samples (8 mL) for laboratory analysis were taken at 15-min intervals from 15 to 210 min relative to the insulin infusion, immediately placed in tubes containing heparin (10 units/mL of blood; Wako Pure Chemical Industries Ltd., Osaka, Japan) and aprotinin (500 Kallikrein Inhibitor Units/mL of blood; Sigma-Aldrich Inc., Tokyo, Japan), and centrifuged at 1,500 \times g for 20 min at 4°C. Plasma samples were stored at –80°C.

Milk samples were collected for the last 4 d of each period, preserved with sodium azide, and stored at 4°C until analysis.

Sample Analysis

Milk samples were measured for fat, protein, lactose, total solids, and solids-not-fat by infrared analysis (Milko-Scan 1344 A/BN.; Foss Electric Company, Inc., Hillerod, Denmark).

Plasma triglyceride, phospholipid, total cholesterol, free cholesterol, albumin, and urea nitrogen concentrations were determined using an automated biochemistry analyzer (Beckman Coulter, Inc., Tokyo, Japan). Plasma cholesterol ester concentration was calculated according to the following formula: cholesterol ester (mg/dL) = (total cholesterol – free cholesterol) \times 1.68 (Auboiron et al., 1995).

Plasma hormone concentrations were determined using time-resolved fluorimunoassay (2030 ARVO X4; PerkinElmer Inc., Waltham, MA). Plasma ghrelin (Sugino et al., 2004), insulin, glucagon (Fukumori et al., 2013), and IGF-1 (Laarman et al., 2012) concentrations were measured as described previously (Fukumori et al., 2013). Plasma GH concentration was measured using bovine GH (National Institute of Diabetes and Digestive and Kidney Diseases, Torrance, CA), europium-labeled bovine GH, anti-monkey γ-globulin (Nordic-MUBio, Susteren, Netherlands), and anti-bovine GH (National Institute of Diabetes and Digestive and Kidney Diseases). The intra- and interassay CV were 2.0 and 1.7%, respectively. Least detectable dose and 50% inhibitory concentration in this assay system were 1.25 and 0.0135 ng/mL, respectively.

Calculation and Statistical Analysis

In the EGC experiment, the GIR in each step of insulin infusion (0.1 to 0.5 milliunits·kg BW\(^{-1}\)·min\(^{-1}\)) were used for statistical analysis. The value of the last 5 min (25 to 30 min) was adopted in each step at 0.1 to 0.4 milliunits·kg BW\(^{-1}\)·min\(^{-1}\), and the values of the last 30 min (60 to 90 min) were averaged and adopted in the step at 0.5 milliunits·kg BW\(^{-1}\)·min\(^{-1}\). The plasma insulin concentration resulting in 50% maximal GIR was obtained from a second-order polynomial plot of individual dose–response curves for GIR vs. plasma insulin concentration, as described as Sano et al. (1992).

Treatment differences were evaluated using the PROC MIXED of SAS (SAS Inst. Inc., Cary, NC) with the model including cow and period as a random effect and treatment as a fixed effect for statistical analysis of milk production, plasma concentrations of metabolites and hormones, maximal GIR, and effective concentration of insulin resulting in 50% maximal GIR. For statistical analysis of GIR and plasma concentrations of glucose and insulin during the EGC experiment, the model included treatment and insulin infusion rate and their interaction as a fixed effects and cow and period as random effects. Comparisons between treatments were done using Dunnett and Tukey tests. Significant differences were set at \(P < 0.05\).

RESULTS

Milk Yield and Milk Composition

Milk yield and composition are presented in Table 2. Milk yield and fat corrected milk yield were
Methionine supply and insulin sensitivity

Table 2. Milk yield and milk composition in lactating cows. Data are presented as least squares means (LSM) and SE of the LSM (SEM).

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment1</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Con</td>
<td>RPM0</td>
<td>RPM20</td>
<td>RPM60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milk yield, kg/d</td>
<td>32.6</td>
<td>32.2</td>
<td>33.1</td>
<td>32.2</td>
<td>1.28</td>
<td>0.718</td>
</tr>
<tr>
<td>Fat corrected milk</td>
<td>32.9</td>
<td>33.8</td>
<td>34.6</td>
<td>32.8</td>
<td>1.79</td>
<td>0.533</td>
</tr>
<tr>
<td>yield, kg/d</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat corrected milk</td>
<td>24.0</td>
<td>24.6</td>
<td>25.1</td>
<td>24.0</td>
<td>1.20</td>
<td>0.555</td>
</tr>
<tr>
<td>yield, Meal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milk composition</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat, %</td>
<td>4.17</td>
<td>4.25</td>
<td>4.33</td>
<td>4.09</td>
<td>0.283</td>
<td>0.617</td>
</tr>
<tr>
<td>Protein, %</td>
<td>3.25</td>
<td>3.15</td>
<td>3.23</td>
<td>3.19</td>
<td>0.100</td>
<td>0.010</td>
</tr>
<tr>
<td>Lactose, %</td>
<td>4.44</td>
<td>4.42</td>
<td>4.40</td>
<td>4.40</td>
<td>0.047</td>
<td>0.578</td>
</tr>
<tr>
<td>Total solids, %</td>
<td>12.9</td>
<td>12.8</td>
<td>12.9</td>
<td>12.7</td>
<td>0.323</td>
<td>0.533</td>
</tr>
<tr>
<td>Solids-not-fat, %</td>
<td>8.69</td>
<td>8.58</td>
<td>8.63</td>
<td>8.59</td>
<td>0.106</td>
<td>0.155</td>
</tr>
<tr>
<td>BW, kg</td>
<td>575</td>
<td>555</td>
<td>558</td>
<td>560</td>
<td>24.8</td>
<td>0.003</td>
</tr>
<tr>
<td>BW change, kg/wk</td>
<td>1.23</td>
<td>4.37</td>
<td>3.86</td>
<td>4.03</td>
<td>2.32</td>
<td>0.619</td>
</tr>
</tbody>
</table>

* a–bTreatment means in a row that do not share a common letter are different (P < 0.05).

1Con = dietary treatment without LCFA-Ca; RPM0 = 0 g/d of rumen-protected Met; RPM20 = 20 g/d of rumen-protected Met; RPM60 = 60 g/d of rumen-protected Met.

not different among the treatments. Milk protein and solids-not-fat contents were lower in RPM0 than Con, whereas protein content recovered in RPM20 (P < 0.05). The treatment effects were not observed in milk fat, lactose, and total solids contents. The BW were greatest in Con (P < 0.05), but weekly BW change was unaffected by the treatment.

**Plasma Concentrations of Metabolites and Hormones**

Plasma concentrations of metabolites and hormones during the insulin preinfusion period are presented in Table 3. Plasma concentrations of phospholipids, total cholesterol, cholesterol ester, and free cholesterol were higher in RPM0 and RPM20 than Con (P < 0.05), whereas those concentrations in RPM60 were lower than in RPM0 (P < 0.05) and recovered at the same degree of those in Con. Plasma Met concentration was greatest in RPM60 (P < 0.05). Plasma IGF-1 and ghrelin concentrations were higher in Con than in LCFA-Ca diets (P < 0.05), but plasma ghrelin concentration in cows fed RPM recovered at the same level of cows fed Con. The effect of treatment was not detected in plasma concentrations of glucose, triglyceride, NEFA, ketone bodies, albumin, urea nitrogen, insulin, glucagon, and GH.

**The Hyperinsulinemic–Euglycemic Clamp Experiment**

In the EGC experiment, 5-step insulin infusions were used to investigate the insulin sensitivity because a single infusion dosage cannot describe the exact tissue responsiveness (Sano et al., 1992). The time course of plasma glucose, insulin, and glucagon concentrations and GIR are presented in Fig. 1 and the summarized data are presented in Table 4. Plasma insulin concentration increased by insulin infusion (P < 0.05) and was higher in RPM0 compared with Con and RPM60 throughout the 5-step infusion period (P < 0.05; Table 4); however, the total dosage of insulin was similar (44.7 milliunits for Con, 41.7 milliunits for RPM0, 41.9 milliunits for RPM20, and 42.1 milliunits for RPM60, respectively). Plasma glucose concentrations were successfully maintained close to the initial concentration (P = 0.883) by various rates of the glucose clamp. The GIR through the 5-step insulin infusion period and maximal GIR did not differ between the Con and LCFA-Ca–containing diets, but those in RPM60 were greater compared with the RPM0 and RPM20 (P < 0.05) diets. Calculated insulin concentration resulting in 50% maximal GIR in RPM60 was lower than in RPM0. Plasma glucagon concentration and maximal GIR was unaffected by insulin and glucose infusions.
Methionine is the primary limiting AA for milk production in lactating dairy cows (Schwab et al., 1976), hence the numerous studies that have investigated the effects of Met supplementation on milk performance. Furthermore, Met restriction may induce liver lipidosis in dairy cows (Shibano and Kawamura, 2006) and supplementation with Met enhanced hepatic VLDL secretion in calves (Auboiron et al., 1995). In rodent models, Met and choline deficiency induces steatohepatitis by inadequate lipoprotein secretion (Yao and Vance, 1988; Rizki et al., 2006). On the other hand, our previous study observed that RPM decreased plasma lipid concentrations when cows consumed a high-fat diet (Fukumori et al., 2012), but the mechanisms of Met remained unclear.

The present study found that feeding LCFA-Ca increased plasma phospholipids, total cholesterol, cholesterol ester, and free cholesterol concentrations and that the increase was attenuated by RPM. The effects of protein source on lipid metabolism have been observed in numerous animal models because the plant proteins that these animals consume generally have a low Met content. In particular, soybean protein has hypocholesterolemic action but is not seen in high-fat diets (Nagata et al., 1980). Some rodent studies observed that addition of Met to plant protein induced hypocholesterolemia (Moundras et al., 1995; Taniguchi et al., 2008; Yang and Kadowaki, 2011), which is consistent with our present data. Moundras et al. (1995) reported that adding Met to a soy protein–based diet decreased serum cholesterol levels, particularly the low-density and high-density lipoproteins. Yang and Kadowaki (2011) reported that rice protein decreased hepatic secretions of total lipid and VLDL into circulation but that with the addition of Met, their levels recovered to those of casein protein (Met-rich protein). On the other hand, bile flow and biliary outputs of bile acids, cholesterol, and phospholipids were stimulated by adding Met to a rice protein–supplemented diet. Therefore, the cholesterol-lowering effects of Met should be considered, not only for the increased lipoprotein uptake in peripheral tissues but also for an increase in the fecal excretion of fatty acids. These experiments in rodent and in vitro models suggest that AA imbalances in the amount of lipid ingested might be involved in lipid utility and animal health. Further studies are required to clarify the relationship between the protein sources or AA balance to lipid metabolism in dairy cows.

The present study demonstrated, first, the effects of feeding LCFA-Ca and RPM on insulin sensitivity. Insulin sensitivity was estimated using the EGC technique. As a result, although the fat-including diets did not alter the EGC properties, the supplement with 60 g/d of RPM increased GIR and decreased the insulin concentration resulting in a half-maximal GIR, which suggests that insulin sensitivity was increased by feeding a threshold level of RPM. Both dietary energy density and energy intake level could affect insulin action (Sano et al., 1992), but ME intake of cows were maintained constant throughout the experimental period, supporting the alteration that insulin sensitivity was caused by RPM ingestion. Unfortunately, the present study could not describe the effect of RPM on insulin sensitivity in the Con diet because the extend

**DISCUSSION**

Methionine is the primary limiting AA for milk production in lactating dairy cows (Schwab et al., 1976), hence the numerous studies that have investigated the effects of Met supplementation on milk performance. Furthermore, Met restriction may induce liver lipidosis in dairy cows (Shibano and Kawamura, 2006) and supplementation with Met enhanced hepatic VLDL secretion in calves (Auboiron et al., 1995). In rodent models, Met and choline deficiency induces steatohepatitis by inadequate lipoprotein secretion (Yao and Vance, 1988; Rizki et al., 2006). On the other hand, our previous study observed that RPM decreased plasma lipid concentrations when cows consumed a high-fat diet (Fukumori et al., 2012), but the mechanisms of Met remained unclear.

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experiment changed lactation stage, which alters physiological state such as insulin sensitivity. Osorio (2014) reported that feeding RPM to peripartal cows improved a faster recovery to a positive energy balance and tended to decrease plasma concentration of IL-6, which is secreted by the adipose and develops insulin resistance. This preferred effect of Met in dairy cows might be related to improved insulin sensitivity.

Insulin is one of the most important hormones in the regulation of nutrient metabolism. In lipid metabolism, insulin enhances lipoprotein lipase activity (Faulconnier et al., 1994) and fatty acid synthesis (Vernon et al., 1991a) and inhibits lipolysis (Vernon et al., 1991b). The present study focused on the effects of adding Met on the endocrine response and tried to explain the lipid-lowering effects of Met in cows fed a LCFA-Ca–containing diet. The GIR represents the sum of increased glucose utilization and decreased endogenous glucose production and gives a measure of the overall effect of insulin on glucose metabolism (Weekes et al., 1983). Although increased GIR in RPM60 cannot be used to infer enhanced lipid metabolism, insulin mediates the Met-induced alteration of plasma lipid profiles. Plasma concentrations of cholesterol esters and phospholipids were decreased through feeding with RPM. Insulin is known to increase low-density lipoprotein (LDL) receptor expression (Gopalakrishnan and Chandra, 2006) and the activity of LDL uptake into the cells (Ramakrishnan et al., 2012). Therefore, feeding RPM may enhance the removal of cholesterol esters and phospholipids from circulation.

A previous study showed that the combined supplements of LCFA-Ca and RPM increased plasma ghrelin concentration (Fukomori et al., 2012). However, in the present study, similar increases in plasma ghrelin and GH concentrations were not observed. The early lactating period of dairy cows is a period characterized by plasma glucose and insulin concentration reduction and an elevated plasma GH concentration (Lucy, 2008). Low plasma insulin concentration reduces glucose uptake by insulin-sensitive tissues (adipose and muscle) and enhances glucose uptake by the mammary gland, a non-insulin-sensitive tissue (Bauman and Currie, 1980; Lucy, 2008). In the present study, RPM60 improved insulin sensitivity in the absence of any adverse effect on milk production. Dietary Met deficiency markedly increased energy expenditure (Hasek et al., 2010), which suggests that Met improves the utilization efficacy of nutrients. As insulin is a key signal of nutrient state, improved insulin sensitivity seems to be favorable for the recovery of lost body stores and the prevention of metabolic disorders during the early lactating period. Unfortunately, however, the present 14-d trial was too short to evaluate these effects. A higher plasma glucose concentration due to RPM supplementation may also reflect an improved nutrient state.

In conclusion, we demonstrated that the addition of Met to a diet containing LCFA-Ca improves insulin sensitivity in lactating dairy cows without adverse effects on milk production. Our results suggest that increasing insulin sensitivity partly relates to the Met-induced decrease in plasma lipid profiles.

**LITERATURE CITED**


