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Effects of supplemental chromium propionate and rumen-protected amino acids on nutrient metabolism, neutrophil activation, and adipocyte size in dairy cows during peak lactation

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ABSTRACT

The objective of this study was to evaluate effects of chromium propionate (CrPr), rumen-protected lysine and methionine (RPLM), or both on metabolism, neutrophil function, and adipocyte size in lactating dairy cows (38 ± 15 d in milk). Forty-eight individually fed Holstein cows (21 primiparous, 27 multiparous) were stratified by calving date in 12 blocks and randomly assigned to 1 of 4 treatments within block. Treatments were control, CrPr (8 mg/d of Cr, KemTRACE brand chromium propionate 0.04%, Kemin Industries Inc., Des Moines, IA), RPLM (10 g/d lysine and 5 g/d methionine intestinally available, from LysiPEARL and MetiPEARL, Kemin Industries Inc.), or CrPr plus RPLM. Treatments were fed for 35 d; blood plasma samples were collected on d 21 and 35 of treatment, and blood neutrophils were isolated from 24 cows for analysis of tumor necrosis factor α (*TNFα*) and interleukin 1β (*IL-1β*) transcript abundance in the basal state and after 12 h of lipopolysaccharide (LPS) activation. Tailhead subcutaneous adipose tissue samples were collected on d 35 for measurement of adipocyte size. Plasma glucose, nonesterified fatty acids, and glucagon concentrations were unaffected by treatments, whereas plasma insulin concentration was increased by RPLM. Basal *TNFα* transcript abundance in neutrophils was not affected by treatment, but basal *IL-1β* transcript abundance was decreased by RPLM and tended to be increased by CrPr. After LPS activation, CrPr increased neutrophil *TNFα* transcript abundance. In addition, RPLM × parity interactions were detected for both *TNFα* and *IL-1β* abundance after LPS activation, reflecting enhanced responses in primiparous cows and attenuated responses in multiparous cows supplemented with RPLM. Adipocyte size was not affected by treatment. Supplemental CrPr and RPLM had minimal effects on metabolism when fed for 35 d near peak lactation but may modulate innate immune function in lactating dairy cows.

Key words: lysine, methionine, chromium, immunity

INTRODUCTION

Chromium is a nutrient that can influence animal metabolism. Supplementation of Cr in cattle has been shown to increase milk production (Hayirli et al., 2001; McNamara and Valdez, 2005; Smith et al., 2005), improve glucose clearance and insulin sensitivity (Bunting et al., 1994; Sumner et al., 2007; Spears et al., 2012), reduce lipolysis (Besong, 1996; Bryan et al., 2004), alter adipose tissue metabolism (McNamara and Valdez, 2005), and modulate immune response (Burton et al., 1993, 1996; Burton, 1995). Although considerable research has been conducted with Cr in cattle, Cr supplementation in cattle diets has only recently been permitted by the US Food and Drug Administration. Currently, Cr-propionate (**CrPr**) is the only source of Cr allowed for supplementation to cattle in the United States, at inclusion rates up to 0.5 mg of Cr/kg of diet. By feeding CrPr to dairy cows from 21 d prepartum to 35 d postpartum, McNamara and Valdez (2005) increased DMI and milk yield in early lactation. Interestingly, they also observed that CrPr dramatically increased adipose tissue lipogenesis compared with that of controls in the postpartum period. Collectively, these results indicate that CrPr may improve production by modulating metabolism of lactating cows.

To improve the performance and productive efficiency of lactating cows, first-limiting AA such as rumen-protected lysine and methionine (**RPLM**) have been commonly supplemented (Chilliard and Doreau, 1997; Leonardi et al., 2003; Berthiaume et al., 2006). A recent meta-analysis (Patton, 2010) indicated that feeding rumen-protected methionine increased milk protein content and yield and slightly increased milk yield across 35 studies in the literature. However, little research has been conducted to determine effects of

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RPLM on glucose or lipid metabolism or immune function in dairy cows. Enhanced metabolic and immune function may contribute to improved milk production.

Currently, there are no reports regarding the interaction between CrPr and RPLM supplementation in dairy cows. We hypothesized that supplementation of both may generate effects superior to supplementing either one alone, in part because enhanced milk production in response to CrPr would increase essential AA requirements. Therefore, the objective of this study was to determine if CrPr and RPLM supplementation affects neutrophil function, adipocyte size, or intermediary nutrient metabolism in dairy cows near peak lactation.

MATERIALS AND METHODS

The Kansas State University Institutional Animal Care and Use Committee approved all experimental procedures. Production responses to these treatments are reported in a companion paper (Vargas-Rodriguez et al., 2014).

Design and Treatments

Forty-eight individually fed Holstein cows (21 primiparous, 27 multiparous, 38 ± 15 DIM, mean ± SD) were stratified by calving date in 12 blocks and randomly assigned to 1 of 4 treatments within block. Treatments were control, CrPr (8 mg/d of Cr in the form of 20 g/d KemTRACE chromium propionate 0.04%; Kemin Industries Inc., Des Moines, IA), RPLM (10 g/d of lysine and 5 g/d of methionine, intestinally available), or both (**CrPr+RPLM**). The RPLM supplement was composed of 48.8 g/d of LysiPEARL and 15.3 g/d of MetiPEARL (Kemin Industries Inc.). Treatments were premixed with ground corn and top-dressed at 200 g/d for 35 d. All cows were fed once daily (1600 h) for ad libitum intake of a diet formulated to meet NRC (2001) nutrient requirements (Table 1). Analysis by the Cornell Net Carbohydrate and Protein System version 6.1 (NDS version 3, Ruminant Management and Nutrition, Reggio Emilia, Italy) estimated metabolizable Met supply at 47 g/d (2.03% of MP) and metabolizable Lys supply at 148 g/d (6.38% of MP) with 22 kg/d DMI in the control diet. The RPLM supplement was predicted to result in Lys and Met supplies of 6.77% and 2.23% of MP, respectively.

Plasma Samples

On d 21 and 35 (1430 h), approximately 14 mL of blood was collected from all cows from the coccygeal vessels into 2 evacuated tubes, one containing K_3-EDTA (for analyses of NEFA, glucagon, insulin, leptin, and adiponectin) and the other containing potassium oxalate with sodium fluoride (for analysis of glucose) as a glycolytic inhibitor (Vacutainer, Becton Dickinson, Franklin Lakes, NJ). Samples were centrifuged at 2,000 × *g* for 15 min immediately after sample collection, and plasma was harvested and frozen at −20°C until analysis. Plasma aliquots for glucagon analysis were added to tubes containing benzamidine (50 m*M* final concentration; Sigma-Aldrich Chemical Co., St. Louis, MO) as a protease inhibitor. Plasma was analyzed for NEFA using an enzymatic colorimetric procedure (NEFA-HR, Wako Chemicals USA, Richmond, VA), glucose by a colorimetric kit (Autokit Glucose; Wako Chemicals USA), glucagon by a radioimmunoassay kit (#GL-32K Millipore Corp., Billerica, MA), and insulin by a bovine-specific sandwich ELISA (#10-1201-01, Mercodia AB, Uppsala, Sweden).

Plasma samples collected on d 35 were analyzed for leptin and adiponectin protein abundance by Western blot. Plasma samples (1 μL) were diluted with 19 μL of Laemmli sample buffer (Bio-Rad, Richmond, CA). The homogenate was heated at 90°C for 5 min, cooled,

Table 1. Ingredient and nutritional composition of the basal diet

Item	Value
Ingredient (% of DM)	
Corn silage	31.5
Alfalfa hay	23.4
Wet corn gluten feed[1]	6.8
Ground corn	23.1
Whole cottonseed	4.6
Mechanically extracted soybean meal[2]	2.1
Solvent-extracted soybean meal	5.1
Ca salts of long-chain fatty acids[3]	0.8
Micronutrient premix[4]	2.6
Nutrient (% of DM unless otherwise noted)	
DM (% as fed)	57.9
OM	91.3
CP	16.7
NDF	31.7
ADF	20.1
fNDF[5]	22.1
NFC	39.8
Ether extract	3.1
Model-predicted ME[6] (Mcal/kg)	2.50

[1]SweetBran (Cargill Inc., Blair, NE).

[2]Soy Best (Grain States Soya, West Point, NE).

[3]Megalac-R (Church & Dwight Co., Princeton, NJ).

[4]Premix consisted of 45.1% limestone, 32.2% sodium bicarbonate, 6.4% magnesium oxide, 5.2% sodium chloride, 5.2% vitamin E premix (44 IU/g), 0.45% vitamin A premix (30 kIU/g), 0.19% vitamin D premix (30 kIU/g), 2.1% 4-Plex (Zinpro Corp., Eden Prairie, MN; contains 2.58% Zn, 1.48% Mn, 0.90% Cu, 0.18% Co, 8.21% Met, and 3.80% Lys), 0.96% selenium premix (600 mg/kg Se), 0.45% Zinpro 100 (Zinpro Corp.; contains 10% Zn and 20% Met), 0.03% ethylenediamine dihydriodide premix (3.65% I), 0.88% Kallsil (Kemin Industries Inc., Des Moines, IA), and 0.88% Myco CURB (Kemin Industries Inc.).

[5]Forage NDF.

[6]ME predicted by CNCPS 6.1 (NDS version 3, Ruminant Management & Nutrition, Reggio Emilia, Italy).