Effects of supplemental chromium propionate and rumen-protected amino acids on productivity, diet digestibility, and energy balance of peak-lactation dairy cattle

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ABSTRACT

Chromium (Cr) feeding in early lactation increased milk production in some studies, but responses to dietary Cr during peak lactation have not been evaluated. Furthermore, interactions of essential amino acids (AA) and Cr have not been explored. Our objective was to evaluate responses to CrPr (KemTRACE chromium propionate 0.04%, Kemin Industries Inc., Des Moines, IA) and rumen-protected Lys (LysiPEARL, Kemin Industries Inc.) and Met (MetiPEARL, Kemin Industries Inc.) and their interaction in peak-lactation cows. Forty-eight individually fed Holstein cows (21 primiparous, 27 multiparous, 38 ± 15 d in milk) 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), RPLM (10 g/d of Lys and 5 g/d of Met, intestinally available), or CrPr plus RPLM. Treatments were premixed with ground corn and top-dressed at 200 g/d for 35 d. Diets consisted of corn silage, alfalfa hay, and concentrates, providing approximately 17% crude protein, 31% neutral detergent fiber, and 40% nonfiber carbohydrates. Dry matter intake (DMI) significantly increased with the inclusion of CrPr (22.2 vs. 20.8 ± 0.67 kg/d), and energy-corrected milk (ECM) yield tended to increase. In addition, CrPr increased milk protein yield and tended to increase DMI in primiparous cows but not in multiparous cows. A CrPr $\times$ week interaction was detected for milk lactose content, which was increased by CrPr during wk 1 only (4.99 vs. $4.88 \pm 0.036\%$). As a proportion of plasma AA, lysine increased and methionine tended to increase in response to RPLM, but the inclusion of RPLM decreased N efficiency (milk protein N:N intake). Digestible energy intake, gross energy digestibility, and energy balance were not affected by treatments. We observed no treatment effects on feed efficiency or changes in body weight or body

condition score. In summary, feeding CrPr increased DMI and tended to increase ECM in cows fed for 5 wk near peak lactation, with primiparous cows showing greater responses in DMI and milk protein yield than multiparous cows.

Key words: lysine, methionine, chromium, essential amino acid

INTRODUCTION

After parturition, cows must adapt to milk secretion, but their daily DMI rarely matches the nutrient demands for that activity (Dalbach et al., 2011). Because of these extremely high nutrient requirements, cows near peak lactation are most likely to experience AA deficiencies, which can limit peak milk and, in turn, decrease whole-lactation productivity.

Chromium (Cr) is involved in many metabolic functions (Mertz, 1993; Bryan et al., 2004); it activates certain enzymes and stabilizes AA and nucleic acids (NRC, 1997; Khalili et al., 2012). Some studies utilized supplemental Cr in diets for lactating cattle and reported increases in milk production (Hayirli et al., 2001; McNamara and Valdez, 2005), whereas others detected enhanced immune responsiveness and disease resistance, particularly in animals under stress conditions (Spears et al., 2012).

It is also known that Cr can potentiate the action of insulin by binding to intracellular insulin receptor sites and promoting signal transduction (Kegley et al., 2000), thereby enhancing carbohydrate metabolism. In addition, Cr can alter protein synthesis (Gentry et al., 1999); although the mechanisms underlying this effect are not completely understood, the effect of Cr on insulin sensitivity has been clearly demonstrated in cattle (Sumner et al., 2007), and insulin signaling promotes protein synthesis. However, there is currently no information about interactions between AA nutrition and Cr supplementation in dairy cattle. Therefore, a critical need exists to further explore responses to Cr in the presence and absence of supplemental AA near peak lactation.

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MATERIALS AND METHODS

The Kansas State University Institutional Animal Care and Use Committee approved all experimental procedures.

Design and Treatments

Forty-eight lactating Holstein cows (21 primiparous and 27 multiparous, 38 ± 15 DIM) were used in a randomized complete block design with 4 treatments. The cows were stratified by calving date in 12 blocks, and assigned randomly to treatments within block.

All cows were housed in tie-stalls and individually fed a common diet (Table 1). Analysis by the Cornell Net Carbohydrate and Protein System version 6.1 (NDS version 3, Ruminant Management & Nutrition, Reggio Emilia, Italy) estimated metabolizable Lys supply at 148 g/d (6.38% of MP) and metabolizable Met supply at 47 g/d (2.03% of MP) with 22 kg/d DMI in the control diet. Treatments were premixed with ground corn and offered as a top-dress at a rate of 200 g/cow daily for 35 d. Treatments were control, Cr propionate (**CrPr**; 8 mg/d Cr in the form of 20 g/d KemTRACE chromium propionate 0.04%, Kemin Industries Inc., Des Moines, IA), rumen-protected lysine and methionine (**RPLM**; 10 g/d lysine and 5 g/d 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.), and was predicted to provide Lys and Met supplies of 6.77 and 2.23% of MP, respectively. Cows were milked 3 times daily (0300, 1100, and 1900 h) and fed once daily (1600 h) for ad libitum intake, targeting 10% daily refusals.

Sample and Data Collection

Feed offered and feed refused were measured for each cow daily to determine DMI. Milk yield was recorded for each cow daily. Body weights and BCS (Wildman et al., 1982) were measured on d 1 and 35. Milk samples were collected 3 d per week for milk composition analysis. Samples of feed ingredients were collected weekly and frozen for analysis. On d 19 to 21 and d 33 to 35, samples of TMR and feed refusals were collected daily and fecal samples were collected at 9-h intervals, representing every 3 h of a 24-h period.

On d 21 and 35 (1430 h), approximately 7 mL of blood was collected from the coccygeal vessels into evacuated tubes containing K3-EDTA (Vacutainer, Becton Dickinson, Franklin Lakes, NJ). Samples were centrifuged at $2,000 \times g$ for 15 min immediately after sample collection, and plasma was harvested and frozen at -20°C until analysis.

Sample Analysis

Samples of diet ingredients, TMR, and feed refusals were dried in a 55°C forced air oven for 48 h, composited by collection period (d 21 vs. 35), ground through a 1-mm screen (Wiley mill, Arthur H. Thomas, Swedesboro, NJ), and analyzed for DM, OM, CP, NDF, and ether extract (**EE**). The DM content was determined by drying at 105°C in a forced-air oven for 16 h. Ash concentration was determined after 4 h of oxidation at 500°C in a muffle furnace. Nitrogen content was determined by oxidation and detection of N_2 (Leco Analyzer, Leco Corp., St. Joseph, MI). Concentration of NDF was determined using an Ankom Fiber Analyzer (An-

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
Gross energy, Mcal/kg	4.11
Digestible energy, ⁶ Mcal/kg	3.34
ME, ⁷ Mcal/kg	2.92
NE _L , ⁸ Mcal/kg	1.87
Model-predicted ME, ⁹ Mcal/kg	2.50

¹SweetBran (Cargill Inc., Blair, NE).

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

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

⁴Premix 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 dihydride premix (3.65% I), 0.88% Kallsil (Kemin Industries Inc., Des Moines, IA), and 0.88% Myco CURB (Kemin Industries Inc.).

⁵Forage NDF.

⁶Digestible energy (DE) = (gross energy (GE) intake – GE in feces)/DMI.

⁷ME = $[1.01 \times (\text{DE, Mcal/kg}) - 0.45] + 0.0046 \times (\text{ether extract, \%} - 3)$.

⁸NE_L = $0.703 \times \text{ME (Mcal/kg)} - 0.19 + [(0.097 \times \text{ME, Mcal/kg} + 0.19)/97] \times [\text{ether extract, \%} - 3]$.

⁹ME predicted by CNCPS 6.1 (NDS version 3, Ruminant Management & Nutrition, Reggio Emilia, Italy).

kom Technology, Fairport, NY) including amylase and sodium sulfite (Van Soest et al., 1991). Crude fat was determined by ether extraction (AOAC International, 2000; method 920.9).

Milk samples were analyzed for concentration of fat, true protein, lactose (B-2000 Infrared Analyzer; Bentley Instruments, Chaska, MN), MUN (MUN spectrophotometer, Bentley Instruments), and somatic cells (SCC 500, Bentley Instruments) by Heart of America DHIA (Manhattan, KS). Plasma free AA were determined by HPLC as previously described (Brake et al., 2013). Plasma AA concentrations were expressed as a proportion of total free AA.

Energy Balance

The concentration of acid detergent insoluble ash (**ADIA**) was determined (Van Soest et al., 1991) in dried samples of feed ingredients, refusals, and feces. This parameter was used as an endogenous indigestible marker to estimate fecal output (Cochran et al., 1986). The gross energy (**GE**) content of feed, top-dressed treatments, refusals, and dried fecal samples were determined by bomb calorimetry. Intake of GE was calculated as GE in feed offered (calculated from analysis of feed ingredients) minus GE in refusals; fecal GE was subtracted to determine digestible energy (**DE**) intake. After calculating DE concentration of the diets by cow (DE intake/DMI), ME and NE_L concentrations were calculated using the following formulas (NRC, 2001): ME (Mcal/kg of DM) = $[1.01 \times (\text{DE, Mcal/kg}) - 0.45] + 0.0046 \times (\text{EE, \%} - 3)$, and NE_L (Mcal/kg) = $0.703 \times \text{ME (Mcal/kg)} - 0.19 + [(0.097 \times \text{ME, Mcal/kg} + 0.19)/97] \times (\text{EE, \%} - 3)$. Intakes of ME and NE_L were then determined by multiplying these concentrations by DMI. Milk energy output (Mcal) was calculated as $9.29 \times \text{kg of milk fat} + 5.47 \times \text{kg of milk protein} + 3.095 \times \text{kg of milk lactose}$, and maintenance energy requirements (Mcal/d) estimated as $0.08 \times \text{kg of BW}^{0.75}$ (NRC, 2001). To determine NE_L balance, milk and maintenance energy were subtracted from NE_L intake.

Statistical Analysis

One cow on CrPr+RPLM developed severe mastitis on d 20 of treatment and was subsequently removed from the study. No data were collected from or analyzed for this cow. Milk and DMI data were averaged by week before analysis. Data were analyzed using the Mixed Procedure of SAS (version 9.2; SAS Institute, 2011) to assess the fixed effects of parity (primiparous vs. multiparous), time, CrPr, RPLM, and 2-, 3-, and 4-way interactions, and the random effect of block. With the exception of CrPr $\times$ RPLM, interactions were removed

from models when $P > 0.30$. Repeated measures over time within cow were modeled with an autoregressive [AR(1)] covariance structure. Denominator degrees of freedom were estimated using the Kenward-Rogers method. Significance was declared at $P \leq 0.05$ and tendencies at $0.05 < P < 0.10$. Treatment means were separated with pair-wise *t*-tests when interactions were significant.

RESULTS AND DISCUSSION

Dry matter intake was significantly increased by CrPr ($P < 0.05$) but was not significantly affected by RPLM when fed for 5 wk near peak lactation (Table 2). Although neither RPLM nor CrPr significantly altered yields of milk or milk components, CrPr tended to increase ECM ($P = 0.09$) by 6% (Table 2). In addition, we detected parity $\times$ CrPr interactions for both DMI ($P = 0.06$) and milk protein yield ($P = 0.04$), in both cases indicating positive responses to CrPr in primiparous cows but not in multiparous cows (Figure 1A and B).

Several lines of evidence indicate that Cr supplementation during the periparturient period improves DMI and milk production (Hayirli et al., 2001; McNamara and Valdez, 2005). Hayirli et al. (2001) reported that supplementation of 0, 3.9, 8.3, and 16.5 mg of Cr/d from Cr-methionine resulted in a linear increase in prepartum DMI. Besong et al. (1996) observed increased milk yield in the first 8 wk of lactation in cows supplemented with 0.8 mg of Cr/kg of DMI as Cr-picolinate. Smith et al. (2005) found that supplementation of 0.03 or 0.06 mg of Cr/kg of $BW^{0.75}$ increased DMI and milk yield in early lactation. In heat stress conditions, Cr supplementation from 3 wk prepartum through 12 wk postpartum improved postpartum DMI and increased milk yield by 6.7, 12.3, and 16.5% at 4, 8, and 12 wk postpartum, respectively (Soltan, 2010). In these studies, feed efficiency was essentially unaffected, because the increases in milk yield and DMI in response to Cr supplementation paralleled each other.

The interaction of RPLM and CrPr affected milk protein content ($P = 0.04$, Table 2). Somewhat counterintuitively, in the absence of CrPr, RPLM decreased milk protein content ($P < 0.01$), but no effect of RPLM was detected in the presence of CrPr ($P = 0.77$). Rumen-protected lysine and methionine also decreased the efficiency of N utilization for milk protein ($P = 0.05$). Rumen-protected methionine (**RPM**) supplementation has been reported to increase milk protein content in many studies (Doreau and Chilliard, 1997; Leonardi et al., 2003; Pacheco et al., 2006), but not in others (Papas et al., 1984; Davidson et al., 2008). Fewer studies (Armentano et al., 1997; Rulquin and Delaby,

Table 2. Effects of chromium propionate (CrPr) and rumen-protected lysine and methionine (RPLM) on intake, productivity, and milk composition of lactating dairy cows

Item	Control		RPLM		SEM	P-value		
	Control	CrPr	Control	CrPr		CrPr	RPLM	CrPr × RPLM
DMI, kg/d	19.9	22.2	21.7	22.3	1.10	<0.05	0.18	0.23
Milk yield, kg/d	40.5	43.7	42.4	43.3	1.44	0.14	0.61	0.39
Milk fat, %	4.20	4.13	3.95	3.97	0.15	0.88	0.19	0.74
Milk protein, %	2.75	2.67	2.62	2.68	0.04	0.66	0.09	0.04
Milk lactose, %	4.90	4.99	4.89	4.90	0.04	0.26	0.24	0.37
MUN, mg/dL	13.2	14.0	13.8	13.1	0.56	0.89	0.70	0.09
SCC linear score	1.59	1.10	1.58	1.65	0.50	0.66	0.56	0.56
Fat yield, kg/d	1.68	1.81	1.66	1.70	0.07	0.27	0.37	0.58
Protein yield, kg/d	1.12	1.16	1.12	1.17	0.03	0.22	0.93	0.86
Lactose yield, kg/d	2.01	2.17	2.09	2.15	0.07	0.15	0.74	0.54
Milk N efficiency, ¹ %	30.2	27.6	27.0	27.1	1.17	0.19	<0.05	0.15
ECM, ² kg/d	43.1	46.3	43.1	44.9	1.47	0.09	0.62	0.65
ECM/DMI	2.18	2.08	2.02	2.02	0.08	0.53	0.16	0.56

¹N efficiency = milk protein N:N intake.

²ECM = (0.327 × milk yield) + (12.95 × fat yield) + (7.65 × protein yield); Dairy Record Management Systems (2013).

1997) observed increased milk protein yield. Dietary supplementation of methionine or both methionine and lysine can significantly increase N utilization efficiency

(Wang et al., 2010), but Nofstger and St-Pierre (2003) did not observe an improvement in N efficiency when they evaluated supplemental methionine in a digestibility trial. Likewise, varying results have been reported for milk and milk fat yields. In light of the inconsistent data, a meta-analysis (Patton, 2010) was conducted to investigate the effect of RPM supplementation on production. Those results indicated that addition of RPM to diets increased milk protein, both as percentage (0.07%) and yield (27 g/d), and slightly increased milk yield. However, DMI and milk fat percentage were slightly decreased. Therefore, although research is lacking on the simultaneous addition of both rumen-protected lysine and RPM on milk components, the decreased milk protein content by RPLM in our study was unexpected.

We observed a CrPr × week interaction ($P = 0.04$) for lactose content, reflecting significantly greater lactose content (4.99 vs. $4.86 \pm 0.036\%$) in response to CrPr during wk 1, with no differences observed from wk 2 on. Previous work has demonstrated enhanced glucose utilization in cattle supplemented with Cr (Sumner et al., 2007), consistent with the view that Cr acts primarily as an insulin sensitizing agent. However, we are not aware of other studies in lactating cows reporting increased milk lactose content in response to supplemental Cr, so it is somewhat unclear what caused this response or why it was transient.

Intake of GE and DE and digestibility of GE and DM were similar across treatments ($P > 0.10$), and no treatment effects were detected for NE_L balance, BW change, or BCS change (Table 3). The negative values for BW and BCS changes, which suggested that cows were in a catabolic state, appear to conflict with the positive calculated NE_L balances. However, BW and

Figure 1. Interactions of chromium propionate (CrPr) and parity for (A) milk protein yield and (B) DMI. Supplements were fed for 35 d near peak lactation, and DMI and milk production responses were analyzed by week throughout the study. Values are LSM $\pm$ SEM; $n = 10$ to 13. Color version available in the online PDF.

Table 3. Effects of chromium propionate (CrPr) and rumen-protected lysine and methionine (RPLM) on energy balance of lactating dairy cow

Item ¹	Control		RPLM		SEM	<i>P</i> -value		
	Control	CrPr	Control	CrPr		CrPr	RPLM	CrPr × RPLM
GE _{intake} , Mcal/d	92.8	100.8	98.1	101.1	4.18	0.10	0.42	0.46
DE _{intake} , Mcal/d	76.1	82.5	80.8	81.2	3.31	0.20	0.52	0.25
GE _{digested} , %	81.4	82.0	82.2	81.1	1.43	0.86	0.97	0.51
DM digestibility, %	81.0	81.7	82.6	80.9	1.35	0.67	0.72	0.33
NE _L balance, Mcal/d	3.3	6.3	7.7	6.7	1.89	0.56	0.15	0.25
BW change, kg/28 d	−31.6	−25.2	−24.1	−17.7	9.88	0.53	0.46	0.99
BCS change, per 28 d	−0.34	−0.35	−0.29	−0.32	0.09	0.75	0.61	0.92

¹GE = gross energy; DE = digestible energy.

BCS change data covered the entire 35-d experiment, whereas NE_L balance was only determined on d 21 and 35. It is likely that many cows (mean 38 DIM at the start and 73 DIM at the end of the study) moved from negative energy balance to positive energy balance during the course of the study. Nevertheless, the accuracy of calculated NE_L balance may have been limited due to biased estimations of fecal output with the ADIA marker. Dietary ADIA concentrations were relatively low in this experiment, potentially increasing variability in ADIA intake estimates, which could result in underestimated fecal mass and overestimated supply of energy. The magnitude of this potential bias was estimated by comparing NE_L values calculated from measured energy digestibility versus model-derived NE_L values based on ingredient characterization alone (Table 1). This comparison suggested that marker-

based digestibility analysis may have overestimated ME supply by approximately 17%. Regardless, relative treatment differences were valid because of the common basal diet, and the lack of an effect of Cr on energy balance is consistent with previous findings in early lactation (Smith et al., 2005; Sadri et al., 2009).

Plasma AA profiles are presented in Table 4. The proportion of lysine significantly increased ($P = 0.05$) and that of methionine tended to increase ($P = 0.07$) in response to RPLM. On the other hand, the proportion of threonine was significantly decreased by RPLM ($P < 0.01$). A tendency for a CrPr × RPLM interaction ($P = 0.06$) was observed for tryptophan, reflecting a decreased proportion of tryptophan by CrPr in the presence of RPLM ($P = 0.03$) but not in the absence of RPLM ($P = 0.64$). The plasma lysine and methionine responses to RPLM were less than might have been

Table 4. Effects of chromium propionate (CrPr) and rumen-protected lysine and methionine (RPLM) on plasma amino acids on lactating dairy cows

AA, molar % of total AA	Control		RPLM		SEM	<i>P</i> -value		
	Control	CrPr	Control	CrPr		CrPr	RPLM	CrPr × RPLM
Glycine	15.57	15.07	14.98	13.92	1.10	0.48	0.42	0.80
Valine	12.19	12.19	11.90	11.07	0.65	0.47	0.28	0.53
Alanine	10.88	11.84	11.72	11.53	0.43	0.37	0.52	0.18
Glutamine	8.70	7.97	8.95	9.44	0.58	0.81	0.09	0.22
Leucine	8.20	8.08	8.11	7.89	0.45	0.66	0.71	0.91
Isoleucine	6.82	6.63	6.77	6.71	0.40	0.72	0.96	0.86
Threonine	4.52	4.72	4.17	3.97	0.24	0.99	0.02	0.25
Citrulline	4.40	4.65	4.08	4.18	0.34	0.32	0.23	0.81
Serine	3.95	3.96	3.99	3.81	0.17	0.58	0.73	0.55
Arginine	3.61	4.07	4.01	3.94	0.21	0.30	0.45	0.16
Lysine	3.34	3.37	3.80	3.59	0.17	0.55	0.05	0.47
Glutamate	3.02	2.91	2.78	2.85	0.28	0.91	0.47	0.67
Tyrosine	2.14	2.15	2.30	2.23	0.10	0.75	0.22	0.65
Histidine	2.10	2.09	1.89	2.05	0.07	0.28	0.09	0.25
Asparagine	2.06	1.85	2.20	2.22	0.24	0.63	0.18	0.55
Phenylalanine	1.97	1.96	2.13	2.03	0.08	0.43	0.11	0.52
Taurine	1.75	1.90	1.58	1.80	0.17	0.27	0.41	0.83
Ornithine	1.73	1.68	1.77	1.73	0.10	0.56	0.64	0.97
Tryptophan	1.63	1.68	1.73	1.53	0.07	0.18	0.69	0.06
Methionine	0.95	0.99	1.06	1.01	0.04	0.93	0.07	0.12
Aspartate	0.32	0.38	0.33	0.34	0.02	0.13	0.51	0.28
Total AA (mM)	2.47	2.39	2.14	2.39	0.10	0.39	0.11	0.11

expected, given the lack of increased milk protein yield. We observed increases of approximately 10% in lysine and 6% in methionine as a proportion of AA in response to estimated supplementation of 10 and 5 g/d, respectively. Similar supplementation rates have increased plasma concentrations by more than 30% (Rogers et al., 1987), although without any change in milk protein yield in mid-lactation cows producing approximately 30 kg of milk/d. Compared with our work, the cows evaluated by Rogers et al. (1987) may have been more responsive to AA supplementation (in terms of plasma concentrations) because of lower microbial supply of AA and stable AA demands for protein synthesis. Few studies have evaluated responses to supplemental AA in cows during the transition from the catabolic state in early lactation to an anabolic state. Previous work has demonstrated that muscle repletion occurs during this stage of lactation and has suggested that increases in intestinally available methionine may enhance this process (Phillips et al., 2003). Whether or not such a response interferes with potential milk protein responses to bypass essential AA is unknown and may be a fruitful area of investigation, especially considering the complex interactions of AA supply and insulin signaling to influence both muscle deposition and milk protein synthesis (Bequette et al., 2001).

CONCLUSIONS

The supplementation of CrPr increased DMI and tended to increase ECM yield of peak-lactation cows when fed for a 5-wk period, and DMI and milk protein yield were particularly enhanced in primiparous cows. The inclusion of RPLM increased lysine and tended to increase methionine as a proportion of plasma AA but decreased the efficiency of N utilization for milk protein. These findings indicate that responses to dietary Cr in the dairy cow are not limited to early lactation.

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