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Effects of supplementing dairy cows with chromium propionate on milk and tissue chromium concentrations

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ABSTRACT

Eight primiparous and 8 multiparous Holstein cows were used to determine the effects of Cr supplementation, in the form of Cr propionate (Cr Prop), on milk and tissue Cr concentrations. Cows were randomly assigned by parity to one of 2 diets: 1) control diet or 2) 2 mg of supplemental Cr/kg of DM. The level of Cr Prop supplemented exceeded by 4-fold the concentration of 0.5 mg of Cr/kg permitted by the FDA. Experimental diets were fed from approximately 30 d prepartum until at least 91 d postpartum, resulting in a minimum of 121 d of exposure to supplemental Cr. The control prepartum and postpartum diets analyzed 0.48 and 0.38 mg of Cr/kg of DM, respectively. Milk samples were obtained from the a.m. milking on d 0 (colostrum), 7, 14, 21, 28, 42, 56, 77, and 90 and on the final day of the study for Cr analysis. Cows were harvested after lactating for a minimum of 91 d and samples of liver, kidney, semitendinosus muscle, and fat were obtained for Cr analysis. Chromium was measured using electrothermal atomic absorption spectrophotometry. Milk Cr concentration averaged 1.7 ng/mL and was affected by day of lactation but not by Cr or a Cr × day interaction. Supplementation of 2 mg of Cr/kg of DM increased kidney Cr by approximately 3-fold and liver Cr concentrations by approximately 2-fold. Chromium concentrations in muscle and fat were not affected by Cr supplementation. In summary, supplementation of Cr Prop at a level of 2 mg of Cr/kg of DM did not affect Cr concentration in milk, muscle, or fat, the major bovine products consumed by humans.
Key words: chromium, dairy cattle, milk, tissue

INTRODUCTION

Chromium functions by potentiating the action of insulin. Research suggests that Cr enhances insulin action by binding to a low molecular weight oligopeptide (chromodulin) that amplifies insulin receptor tyrosine kinase activity (Vincent, 2001). Furthermore, low molecular weight Cr-binding compounds with the ability to potentiate insulin action have been isolated from bovine colostrum (Yamamoto et al., 1988) and bovine liver (Davis and Vincent, 1997).

Traditionally, it has generally been assumed that practical diets fed to cattle contain sufficient Cr to meet dietary requirements. However, in recent years, Cr supplementation has been shown to affect glucose metabolism in cattle (Bunting et al., 1994; Stahlhut et al., 2006), increase milk production in dairy cows (Hayirli et al., 2001; McNamara and Valdez, 2005), and enhance immune responsiveness and disease resistance, particularly in stressed cattle (Spears, 2000).

The effects of Cr supplementation of cattle diets on Cr concentrations in milk and edible tissues have received little attention. Chromium analysis of milk and tissues is challenging because of the extremely low concentrations present and because of potential Cr contamination during collection, storage, and preparation of samples for analysis (NRC, 2005). Hayirli et al. (2001) reported that milk Cr concentrations of dairy cows were not affected by supplementation with 0.25 to 0.96 mg of Cr/kg of DM from Cr methionine. However, the milk Cr concentrations reported in this study were at least 30-fold higher than values previously reported for cow's milk (Cocho et al., 1992) and human milk (National Academies, 2001). The high milk Cr concentrations observed in this study were likely attributed to Cr contamination of samples, or the inductively coupled plasma emission spectrometry procedure used not being sensitive enough to accurately detect the low concentrations of Cr present, or both. Supplementation of a relatively low (0.2 mg of Cr/kg of DM) concentration of Cr from a high Cr yeast did not affect tissue Cr concentrations in growing-finishing steers (Chang et al., 1992). To evaluate the safety of supplemental Cr propionate (**Cr Prop**) in regard to potential Cr residues in milk and meat, Cr was supplemented to dairy cows for a minimum of 121 d in the present study. The concentration of Cr Prop supplemented exceeded the concentration permitted by the FDA by 4-fold.

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Table 1. Composition of TMR

Ingredient, % DM	Prepartum	Postpartum
Grass hay, chopped	30.0	—
Corn silage	39.0	25.8
Concentrate mix[1]	29.0	36.0
Alfalfa silage	—	15.7
Soybean hulls	—	7.0
Whole cottonseed	—	13.5
Corn-Cr premix[2]	2.0	2.0

[1]Described in Table 2.

[2]Ground corn premix supplied supplemental Cr for Cr propionate treatment.

Table 2. Ingredient composition of concentrate mixes

Ingredient, % of DM	Prepartum	Postpartum
Ground corn	70.1	65.7
Soybean meal	28.6	19.5
Nutrimax bypass protein[1]	—	9.1
Salt	0.6	1.4
Magnesium oxide	0.3	—
Calcium carbonate	—	2.7
Sodium bicarbonate	—	1.1
Vitamin-mineral premix[2]	0.4	0.5

[1]Blend of nonruminant animal and plant protein sources (Nutrimax, Greensboro, NC).

[2]Contained per kilogram of premix: zinc (as zinc sulfate) 38,700 mg; manganese (as manganese sulfate) 38,700 mg; copper (as copper sulfate) 11,800; iron (as ferrous sulfate) 9,650 mg; iodine (as EDDI) 700 mg; cobalt (as cobalt carbonate) 590 mg; selenium (as sodium selenite) 250 mg; vitamin A 5,896,000 IU; vitamin D_3 1,474,000 IU, and vitamin E 17,688 IU.

MATERIALS AND METHODS

Care, handling, and sampling of cows in this study were approved by the North Carolina State University Animal Care and Use Committee. Eight primiparous and 8 multiparous Holstein cows were randomly assigned by parity to treatments with 4 primiparous and 4 multiparous cows per treatment. Treatments consisted of the following diets: 1) control (no supplemental Cr) and 2) 2 mg of supplemental Cr/kg of DM as Cr Prop (KemTrace Chromium Propionate, Kemin Agri Foods North America Inc., Des Moines, IA).

Cows were housed in a covered free-stall barn equipped with individual Calan gate feeders (American Calan, Northwood, NH). Prior to initiation of the study, cows were adjusted to the Calan gate feeders. Experimental diets were fed from approximately 30 d prepartum until at least 91 d postpartum, resulting in a minimum of 121 d total exposure to supplemental Cr Prop. Ingredient composition of the prepartum and lactation TMR is shown in Table 1. Composition of the concentrate mixes is presented in Table 2. Supplemental Cr was provided in a corn-premix supplied at 2% of diet DM. Chemical composition of the TMR is shown in Table 3. The control prepartum and postpartum TMR analyzed 0.48 and 0.38 mg of Cr/kg of DM, respectively. Chromium-supplemented diets analyzed 2.66 and 2.77 mg of Cr/kg for the prepartum and postpartum periods, respectively. Diets were formulated to meet or exceed nutrient requirements for dry and lactating dairy cows (NRC, 2001). Cows had ad libitum access to feed during the pre- and postpartum periods. Samples of TMR were collected weekly. Following DM determination at 60°C in a forced air oven, samples were ground in a Wiley mill to pass a 2-mm screen and then composited over a 28-d period for Cr analysis. Composite samples of TMR used for Cr analysis were further composited over the entire prepartum and postpartum periods for chemical analysis. Chemical analysis of diets was performed at a commercial laboratory (Dairy One Cooperative Inc., Ithaca, NY).

Cows were moved to maternal pens 1 to 2 d before calving but continued to receive their experimental diet. They were returned to their original pen, with Calan Gate feeders (American Calan, Northwood, NH), 1 d after calving. Cows were milked twice daily at approximately 0600 and 1700 h. Milk samples were obtained from the a.m. milking on d 0 (colostrum), 7, 14, 21, 28, 42, 56, 77, and 90 and on the final day of the study for Cr analysis. On d 90 of lactation, milk samples were also obtained from the p.m. milking. Udders were washed and milk samples were collected manually from the teat streak canal into acid-washed plastic bottles. This prevented possible Cr contamination of milk caused by contact with metal during milking. Milk samples were initially stored at the dairy unit at 4°C. Within 2 d of collection, milk samples were transported to the laboratory where they were stored at −20°C until analyzed for Cr.

All cows were slaughtered at a commercial abattoir after lactating for a minimum of 91 d (range of 91 to 133 d). All cows were slaughtered on the same day, and the ear tag from each cow remained with the carcass during processing. Samples of liver, kidney, semitendinosus muscle, and fat were obtained from a similar location in each animal for Cr determination. The weight of tissue sample obtained for fat, liver, muscle, and kidney was approximately 60, 150, 200, and 140 g, respectively. Tissue samples were placed in large Whirlpak bags (Nasco, Fort Atkinson, WI) and placed on ice. Upon arrival at the laboratory, tissue samples were frozen at −20°C until processing.

Cr Analysis

Milk samples were thawed and vigorously shaken by hand and then a 10-mL sample was removed using a

Table 3. Chemical analysis of TMR[1]

	Prepartum		Postpartum	
Item	Control	Cr Prop	Control	Cr Prop
DM,%	53.3	55.7	52.6	52.9
CP, % DM	12.0	11.5	18.4	18.7
ADF, % DM	25.0	24.6	20.7	20.2
NDF, % DM	42.5	44.4	31.7	29.7
Ca, % DM	0.31	0.40	0.80	0.90
P, % DM	0.34	0.35	0.34	0.37
Mg, % DM	0.27	0.25	0.22	0.24
K, % DM	1.53	1.50	1.04	1.11
Na, % DM	0.09	0.07	0.30	0.30
S, % DM	0.18	0.17	0.21	0.22
Fe, mg/kg of DM	262	254	250	297
Zn, mg/kg of DM	67	68	103	102
Cu, mg/kg of DM	21	17	28	27
Mn, mg/kg of DM	63	56	84	90
Mo, mg/kg of DM	0.7	0.7	0.6	0.4
Cr, mg/kg of DM	0.48	2.66	0.38	2.76

[1]Control = no supplemental Cr; Cr Prop = 2 mg of supplemental Cr/kg of DM as Cr propionate (KemTRACE Chromium Propionate, Kemin Agri Foods North America Inc., Des Moines, IA).

serological plastic pipette. The 10-mL samples were added to 50-mL polypropylene microwave vessels followed by the addition of 8 mL of trace metal grade nitric acid (Trace Metal grade, Fisher Scientific, Raleigh, NC). Samples were then allowed to predigest at room temperature for at least 8 h followed by microwave digestion at 110°C for 30 min. Following cooling, vessels were centrifuged for 10 min at 1,000 × *g* to pull down condensation from the side of the vessels and then brought up to a final volume of 20 mL with deionized water in volumetric flasks.

Tissue samples were allowed to thaw at room temperature. Once thawed, samples were removed from Whirlpak bags and thoroughly rinsed with deionized water. Samples were then removed from tissues using sterile plastic knives to prevent Cr contamination. An innermost specimen (portion of tissue never exposed at the time of slaughter collection) was obtained from each tissue to eliminate or reduce the chance of Cr contamination from slaughter house equipment. Samples of liver, kidney, and muscle were dried and then wet ashed in 15-mL polypropylene microwave vessels using the procedure described for milk. Fat samples were ashed without drying because they liquefied when dried.

Chromium was measured by electrothermal atomic absorption spectrophotometry (model 6701/6601, Shimadzu, Kyoto, Japan). The method of standard addition (**SAM**) was used for each milk and tissue sample to remove matrix effects. Calibration standards containing 10, 15, 20, and 30 ng of Cr/mL were made up in 5% (vol/vol) nitric acid using acid-washed volumetric flasks. All SAM samples were diluted on the day of analysis as follows: 100 μL of each standard solution containing 10, 15, 20, and 30 ng of Cr/mL were pipetted into individual 12 × 0.75 mm polypropylene tubes followed by the addition of 100 μL of digested sample. Each mixture was then thoroughly vortexed and capped until analyzed later that day. The 30 ng/mL standard solution was used only in the analysis of milk because Cr concentrations were extremely low in milk samples. Twenty-microliter samples of the sample and standard mixtures were injected into pyrolytically coated graphite tubes using an auto sampler. All measurements were done in duplicate with coefficients of variation no greater than 5.0% and mean peak area determined. Once all SAM standard solutions were measured for each sample, mean peak area absorbencies were plotted against the concentration of added Cr and the unknown or standard reference material Cr level was determined by extrapolation of the SAM line back to the negative horizontal axis. Bovine muscle obtained from the National Institute of Standards and Technology (Gaithersburg, MD) and certified to contain 71 ± 3.8 ng of Cr/g was used as a reference standard for tissues. Nonfat milk powder, certified to contain 2.6 ± 0.7 ng of Cr/g, was used as a reference standard for milk Cr analysis. When ashed and analyzed in the same manner as experimental samples, the certified muscle and nonfat milk powder analyzed 74.9 ± 2.8 ng of Cr/g and 2.72 ± 0.31 ng of Cr/mL, respectively.

Statistical Analysis

Data were analyzed statistically as a randomized block design by ANOVA using the MIXED procedure of SAS (SAS Institute, 2002). The model included treat-

Table 4. Main effects of supplemental Cr and parity on milk Cr concentrations

Item	Milk Cr, ng/mL
Treatment[1]	
Control	1.59
Cr Prop	1.80
SE	0.13
Parity	
Multiparous	1.56
Primiparous	1.83
SE	0.13

[1]Control = no supplemental Cr; Cr Prop = 2 mg of supplemental Cr/kg of DM as Cr propionate (KemTRACE Chromium Propionate, Kemin Agri Foods North America Inc., Des Moines, IA).

ment, block (parity), and treatment × block. Milk Cr concentrations from samples collected on d 0, 7, 14, 21, 28, 42, 56, 77, and 90 (a.m. sample) were also analyzed as repeated measures. The model included treatment, day, block, and all possible interactions.

RESULTS AND DISCUSSION

General

The number of days cows were fed treatment diets before calving ranged from 8 to 54. Days from the initiation of treatments to calving averaged 28.4 for controls and 33.4 for Cr-supplemented cows. Total (prepartum and postpartum) length of exposure to dietary treatments ranged from 125 to 148 d.

One multiparous cow in the Cr treatment was found not to be pregnant after the study started. The herd veterinarian concluded that the cow was misdiagnosed as being pregnant. No data from this cow were included in statistical analysis. One control and 1 Cr-supplemented cow were diagnosed with a displaced abomasum, and surgeries were performed at the North Carolina State Veterinary Hospital (Raleigh). A control cow exhibited signs of milk fever and was given an intravenous calcium solution. Two cows in the Cr treatment were treated for mastitis, and 1 Cr-supplemented cow was treated for a laceration on its right rear foot. All cows that were treated recovered. Removing data from cows that received medical treatments from statistical analysis did not affect statistical interpretation of the results. Therefore, data presented in this paper include all cows that calved.

Milk Cr Concentrations

Milk Cr concentrations were affected by day of lactation ($P = 0.01$) but not by Cr supplementation from Cr Prop (treatment; $P = 0.30$), treatment × day ($P = 0.76$), treatment × parity ($P = 0.51$), parity ($P = 0.17$), or parity × day ($P = 0.36$). Main effects of supplemental Cr and parity on milk Cr concentrations are shown in Table 4. Across all sampling days, milk Cr averaged 1.7 ng/mL. Reported Cr concentrations in human milk have ranged from 0.09 to 1.56 ng/mL (Cocho et al., 1992; National Academies, 2001). Cocho et al. (1992) measured Cr in cow's milk using electrothermal atomic absorption spectrophotometry and found 0.83 ng/mL. Hayirli et al. (2001) reported milk Cr concentrations in early-lactation dairy cows of approximately 55 ng/mL using an inductively coupled argon plasma emission spectroscopy method. The much higher milk Cr concentrations observed in the aforementioned study compared with those in the present study may relate to Cr contamination of milk samples during collection or preparation of samples for Cr analysis or the analytical procedure used to measure Cr. Inductively coupled plasma emission spectroscopy is less sensitive than electrothermal atomic absorption for measuring low concentrations of Cr (NRC, 2005). Milk Cr concentrations tended ($P = 0.17$) to be slightly higher for primiparous cows compared with multiparous cows.

Milk Cr concentrations by sampling day including the 2 d (d 90 p.m. and final samples) not used in the repeated measures analysis are presented in Table 5. Analysis of milk Cr by sampling day also indicated that Cr Prop supplementation did not affect milk Cr concentrations on any sampling day. This is consistent with studies in humans indicating that milk Cr is not affected by dietary Cr intake (Kumpulainen et al., 1980; Anderson et al., 1993).

Day of lactation affected ($P = 0.01$) milk Cr concentrations (Figure 1). Chromium concentrations in milk did not differ among sampling days in early lactation (d 0, 7, 14, 21, and 28). After d 28, milk Cr concentrations declined, with the lowest concentrations observed on d 77 of lactation. Milk Cr concentrations on d 42 were lower ($P < 0.05$) than those observed on d 0, 14, and 21. On d 56 of lactation, Cr concentrations were lower ($P < 0.05$) than on d 0. Milk Cr concentrations on d 77 were lower ($P < 0.05$) than concentrations on d 0, 7, 14, 21, and 28. On d 90 of lactation, Cr concentrations differed ($P < 0.05$) from d 0 and 21. To our knowledge, this is the first study that has examined the effect of stage of lactation on milk Cr levels in dairy cows. In humans, stage of lactation did not affect milk Cr concentrations (Casey and Hambridge, 1984). The greater volume of milk production in lactating dairy cows compared with humans may explain this discrepancy.

Tissue Cr Concentrations

Tissue Cr concentrations are shown in Table 6. Long-term Cr supplementation from Cr Prop at 2 mg of Cr/

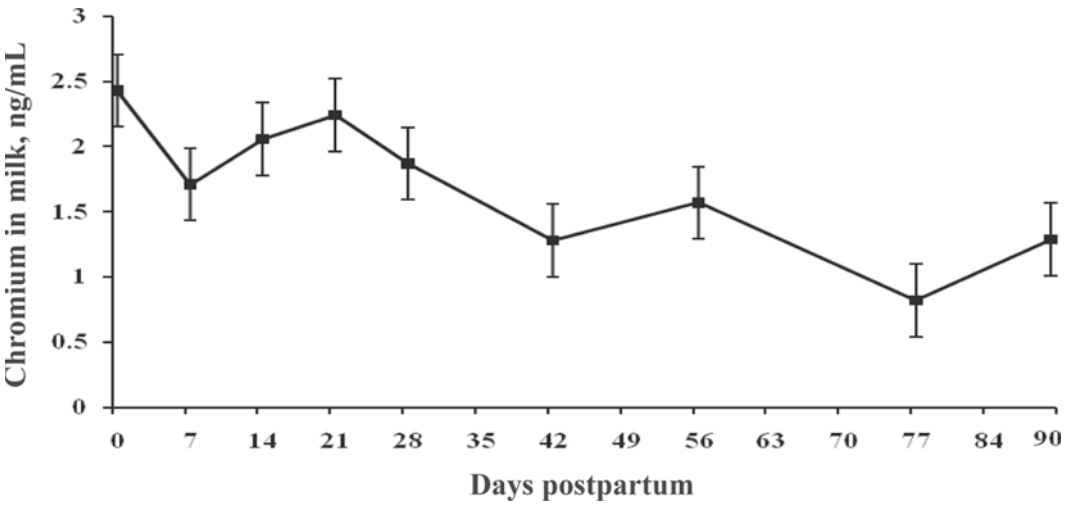

Figure 1. Effect of stage of lactation on milk Cr concentrations.

kg of DM increased ($P < 0.001$) kidney Cr concentration by approximately 3-fold (45 vs. 135 ng of Cr/g of DM) and liver Cr by roughly 2-fold (54 vs. 98 ng/g of DM). Semitendinosus muscle and fat Cr concentrations were not affected by Cr supplementation. The addition of 0.2 mg of Cr/kg of DM as high Cr yeast to a basal diet containing 1.86 mg of Cr/kg of DM did not affect rib lean, rib fat, liver, or kidney Cr concentrations in growing-finishing steers (Chang et al., 1992). Supplementing pigs with 0.3 mg of Cr (as Cr picolinate)/kg of diet for 50 d increased kidney and liver Cr concentrations by 109 and 41%, respectively (Anderson et al., 1997). Heart and longissimus muscle concentrations in pigs were not affected by dietary Cr. In turkeys, supplementation with much higher concentrations of Cr (25 to 200 mg/kg), as Cr chloride, increased Cr concentrations in a dose-dependent manner in breast, leg, gizzard, heart, liver, and kidney after 5 wk (Anderson et al., 1989). Consistent with other studies, increases in tissue Cr in turkeys following supplementation was highest in kidney followed by liver. Although statistically significant, increases in Cr concentrations in other tissues were small relative to those observed in kidney and liver. In rats, tissue responses to dietary Cr were affected by tissue and supplemental Cr source (Anderson et al., 1996). When supplemented to a low Cr diet at a level of 5 μg of Cr/g, none of the supplemental forms of Cr evaluated in this study increased gastrocnemius muscle Cr concentrations.

The concentration of Cr Prop supplemented in the present study was well below the maximum tolerable level for cattle that has been estimated at 100 mg of Cr/kg of DM (NRC, 2005). However, the 2 mg of Cr/kg of DM supplemented in the present study exceeded the concentrations of Cr that have been supplemented in previous production studies with dairy cattle. Hayirli

Table 5. Effect of supplemental Cr and day of lactation on milk Cr concentrations (ng/mL of Cr)

	Treatment[1]			
Day	Control	Cr Prop	SE	*P*-value
0	2.03	2.93	0.76	0.43
7	1.93	1.46	0.22	0.17
14	2.19	2.01	0.20	0.55
21	1.96	2.53	0.43	0.37
28	1.54	2.21	0.34	0.20
42	1.12	1.41	0.35	0.58
56	1.65	1.38	0.23	0.42
77	0.81	0.81	0.19	0.99
90 (a.m.)	1.10	1.46	0.33	0.46
90 (p.m.)	1.85	1.57	0.35	0.59
Final	1.79	1.75	0.43	0.95

[1]Control = no supplemental Cr; Cr Prop = 2 mg of supplemental Cr/kg of DM as Cr propionate (KemTRACE Chromium Propionate, Kemin Agri Foods North America Inc., Des Moines, IA).

Table 6. Effect of supplemental Cr on tissue Cr concentrations (ng of CR/g of DM) in dairy cows

	Treatment[1]			*P*-value		
Tissue	Control	Cr Prop	SE	Treatment	Parity	Treatment × parity
Muscle	32.6	33.6	6.5	0.91	0.92	0.41
Fat	35.8	27.6	9.7	0.56	0.43	0.36
Liver	54.2	97.3	3.5	0.01	0.85	0.77
Kidney	45.5	134.3	9.1	0.01	0.35	0.10

[1]Control = no supplemental Cr; Cr Prop = 2 mg of supplemental Cr/kg of DM as Cr propionate (KemTRACE Chromium Propionate, Kemin Agri Foods North America Inc., Des Moines, IA).

et al. (2001) supplemented lactating dairy cows with 0, 3.7, 7.7, or 15.7 mg of Cr/d from Cr methionine. Based on feed intakes, these levels would have corresponded to approximately 0, 0.25, 0.45, and 0.96 mg of supplemental Cr/kg of DM. In this study, the optimal level of supplemental Cr, based on milk production, was 0.45 mg of Cr/kg of DM (Hayirli et al., 2001). In other studies with lactating dairy cows, the concentration of Cr supplemented has ranged from approximately 0.20 mg/kg of DM (Al-Saiady et al., 2004) to 0.65 mg/kg of DM (Sadri et al., 2009). Based on research in sows fed graded levels of Cr picolinate (Lindemann et al., 2004), the increases in kidney and liver Cr observed in the present study attributed to Cr supplementation would have been considerably less if Cr had been supplemented at only 0.5 mg/kg of DM or less.

CONCLUSIONS

This study indicates that supplementing dairy cows with Cr Prop at a concentration of 2 mg of Cr/kg of DM for more than 121 d did not affect Cr concentrations in milk, muscle, or fat. These represent the major products derived from dairy cows that are consumed by humans. Chromium supplementation did result in increases in liver and kidney Cr. Consumption of beef liver and kidney by humans would generally be low. Assuming a maximum intake by humans of 100 g (wet wt) of liver and 50 g (wet wt) of kidney, consumption of liver and kidney from cows supplemented with 2 mg of Cr/kg of DM (as Cr Prop) would increase Cr intake by 1.3 and 0.9 μg/d, respectively. The adequate intake of Cr in adult humans has been estimated at 25 μg/d for females and 35 μg/d for males (National Academies, 2001). Thus, it would appear that supplementation of dairy cow diets with 2 mg of Cr/kg of DM, in the form of Cr Prop, would have minimal effect on total Cr intake by humans. The level of Cr supplemented in the present study had a wide safety margin (4 to 10×) relative to the concentrations of supplemental Cr that have been used in most previous studies with cattle. Supplementation of Cr Prop at levels that would be recommended for dairy cattle should not affect entry of Cr into the human food chain from milk and meat.

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