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Impact of Chromium Propionate Supplementation and Days of Adaptation on Energy Status in Newly Weaned Steer Calves*

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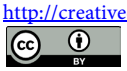

Abstract

The objective of this research was to evaluate the influences that supplemental dietary chromium propionate (CrP; 0 or 0.4 mg·kg^{-1} added to the total diet on a DM basis) has on plasma indicators of energy status in newly weaned steers upon introduction to the feedlot. For this experiment single source, Angus steers (n = 28; BW = 289 ± 12.0 kg) from a ranch in Western South Dakota were weaned and immediately shipped 579 km to the Ruminant Nutrition Center (RNC) in Brookings, SD. Steers were allotted to one of four 7.6 m × 7.6 m pens (2 pens/diet; 7 steers/pen) at 4 d post-arrival to the RNC and test diets were initiated. No anabolic implant was used in this study. Subsequent BW measurements were obtained at 1400 h, to accommodate a post-prandial timing for blood sampling. This was 4 h after initial access to feed, and immediately prior to the afternoon feed delivery. Weights and blood sampling occurred on d 5, 12, 19, and 33. Whole blood samples were collected from all steers via jugular venipuncture and separated as plasma. There were no diet *x* day interactions (P ≥ 0.51) for plasma glucose, insulin, or urea-N. Plasma glucose, insulin, and urea-n levels were similar between diets (P ≥ 0.35). Plasma glucose and urea-n levels were not different across days (P ≥ 0.59). Insulin levels differed as a result of days of adaptation and were greatest (P = 0.01) on d 12 regardless of diet. There tended (P = 0.12) to be a diet *x* day interaction for NEFA levels. Plasma NEFA levels tended to be lower (P = 0.13) for calves fed CrP on d 5, and were greater (P = 0.09) on d 12 in calves fed CrP. The shift in NEFA on d 12 coincided with the spike in insulin levels. Both events occurred at the time that NEg intake was approaching the acclimated plateau and neither event impacted glucose status. In non-ruminants, elevated insulin

*This experiment was funded by the South Dakota Agriculture Experiment Station and the Beef Nutrition Program, South Dakota State University, Brookings, SD. Chromium Propionate was provided compliments of Kemin Industries, Des Moines, IA.

concentrations decrease circulating NEFA levels. We detected minimal differences in regard to plasma indicators of lipid metabolism in this study due to chromium supplementation. These data indicate that ruminants may differ from non-ruminants in the regulation and maintenance of glucose status and body fat catabolism during the post-absorptive state.

Keywords

Beef Steers, Chromium, Feedlot Adaptation, Insulin, Newly Weaned

1. Introduction

The trace mineral Cr is recognized for its potential to aid in cellular uptake of glucose as well as amino acids in insulin-sensitive tissues of non-ruminants. Chromium enhances the insulin receptor so that it more favorably binds insulin which in turn promotes nutrient uptake by the cell. The transition from suckling calf on the prairie to feeder calf in the feedlot is stressful. Transportation of beef cattle results in significant periods of feed and water deprivation, resulting in many days of sub-maintenance intake [1]. Newly arrived feeder calves are often subject to a variety of other stressors such as weaning, comingling with other groups of cattle and unfamiliar feed sources during the immediate days pre- and post-arrival at the feedlot [1]. Stressful situations result in tissue depletion and urinary excretion of Cr in humans [2] [3]. Under these conditions, the reestablishment of Cr status achieved through Cr supplementation could potentially result in improved nutrient uptake and may allow calves to be less dependent upon catabolism of body fat to maintain a positive energy balance. The objective of this research was to evaluate the influence that Cr supplementation and days of adaptation have on plasma indicators of lipid metabolism and energy status in newly weaned steers during the feedlot receiving phase.

2. Materials and Methods

2.1. Animal Care and Use

This experiment was conducted at the Ruminant Nutrition Center (RNC) in Brookings, SD from October to December 2014. All experimental procedures used in this study were approved by the South Dakota State University Institutional Care and Use Committee.

2.2. Treatments and Diets

Dietary treatments were: 0 added Cr (CON); or 0.4 mg·kg^{-1} added Cr to the total diet on a DM basis (CrP) as chromium propionate (KemTRACE 0.4% Chromium Propionate, Kemin Industries, Des Moines, IA). The basal diet was formulated to contain, 12% CP and provide 1.06 Mcal/kg NEg, the diet included 45% roughage (grass hay and sorghum silage), dry rolled corn and dried distill-

ers grains plus solubles (Table 1). The dietary supplement was fortified with vitamins and minerals to meet or exceed NASEM requirements [4] for receiving steer calves and provided 25 mg·kg^{-1} monensin in the complete diet (Table 2). For Cr inclusion, chromium propionate was added to a pelleted supplement (Table 2). Diets were mixed with a reel-type mixer. Feed bunks were managed daily so that only trace amounts (≤0.2 kg) of feed were present in the bunk at the time of feeding daily. Steers were fed twice daily; with 50% of the daily feed allotment being fed in the morning and 50% in the afternoon. Individual feed ingredients were sampled weekly and analyzed for DM [5], CP [6], NDF and ADF [7], and ash [5] throughout the experiment. Diet compositions were calculated based on actual feed ingredient inclusion rates and assayed values for each ingredient.

Table 1. Actual diet formulations and compositions[1,2].

Item	%	SD		%	SD
1 to 8 d					
Sorghum Silage	33.77	1.01	DM	55.6	0.47
Grass Hay	14.21	0.63	CP	12.2	0.05
Dry Rolled Corn	30.54	0.19	NDF	31.9	0.10
Dried Distillers	8.38	0.08	ADF	19.2	0.43
Basal Supplement[3]	11.31	0.09	Ash	7.3	0.03
Treatment Supplement[4]	1.79	0.01			
			Monensin, mg/kg	23	
			NE_g, Mcal/kg[5]	1.07	
9 to 33 d					
Sorghum Silage	29.24	0.82	DM	63.7	0.90
Grass Hay	14.95	0.19	CP	12.8	0.20
Dry Rolled Corn	32.78	0.51	NDF	28.4	0.78
Dried Distillers	9.01	0.12	ADF	16.0	1.16
Basal Supplement[3]	12.09	0.18	Ash	8.1	0.22
Treatment Supplement[4]	1.93	0.03			
			Monensin, mg/kg	25	
			NE_g, Mcal/kg[5]	1.10	

[1]All values except DM on DM basis. [2]Based on weekly ingredient analyses. [3]Pelleted supplement formulated to supply 25 mg·kg^{-1} of monensin and provided minerals and vitamins to meet or exceed NRC requirements. [4]Pelleted supplement for chromium inclusion to provide: 0 or 0.4 mg·kg^{-1} added Cr to the total diet on a DM basis as chromium propionate (KemTRACE 0.4% Chromium Propionate, Kemin Industries, Des Moines, IA). [5]Based upon tabular NE_g values for each ingredient.

Table 2. Formulas for supplement batches[1].

Ingredient	Basal Supplement, kg	
Ground Corn	479.3	
Soybean Meal	334.7	
Limestone	67.6	
Trace Mineralized Salt	23.6	
Microingredients[2]	1.8	
	Treatment Supplement, kg	
	Control	Cr Propionate
Wheat Middlings	453.5	453.5
Ground Corn	453.5	449.4
Chromium Propionate[3]	-	4.1

[1]As is basis. [2]Contained: 185 g (Rumensin 90, Elanco Animal Health, Greenfield, IN), vitamins A & E, zinc hydroxychloride and tri-basic copper chloride. [3]KemTRACE 0.4% chromium propionate, Kemin industries, Des Moines, IA.

2.3. Cattle

A single source, Angus steers (n = 28; BW = 289 ± 12.0 kg) were weaned and shipped 579 km to the RNC on October 29, 2014 for use in this study. Upon arrival at the RNC, all steers were provided long-stem hay and unlimited access to water. On the following morning October 30, 2014, steers were individually identified, vaccinated (Bovishield Gold 5 and Ultrabac 7, Zoetis Animal Health, Kalamazoo, MI), weighed, treated for parasites (Cydectin, Boehringer Ingelheim, St. Joseph, MO) and again received long-stemmed grass hay. Processing BW for steers indicated a 2% shrink (1-(processing BW/pay weight at the ranch)) due to weaning and transportation. During the 3 d period between processing and initiating test diets, all steers were maintained in pens and introduced to the CON diet using standard RNC procedures for receiving calves.

Using the processing BW, steers were allotted to one of four 7.6 m × 7.6 m pens (2 pens/diet; 7 steers/pen; 14 steers/diet) at 4 d post-arrival to the RNC and test diets were initiated. No anabolic implant was used in this study. Subsequent BW measurements were obtained at 1400 h, to accommodate a post-prandial timing for blood sampling. This was 4 h after initial access to feed, and immediately prior to the afternoon feed delivery. Weights and blood sampling occurred on d 5, 12, 19, and 33 following treatment initiation.

2.4. Blood Collection

Whole blood samples were collected from all steers via jugular venipuncture using an 18-gauge needle and 10 mL K_3EDTA evacuated tubes during the weighing process. Samples were centrifuged at 2000 × g for 20 min at 4˚C for plasma recovery. Plasma was aliquoted (n = 5) and stored in 12 × 75 mm borosilicate glass tubes at −20˚C until subsequent analysis.

2.5. Plasma Analyses

Plasma glucose (GLS) concentrations were determined via a glucose oxidase procedure [8] utilizing a commercial reagent (Liquid Glucose Oxidase Reagent Set; Pointe Scientific, Canton, MI). The standard curve constructed for the GLS assay was 0 to 125 $mg \cdot dL^{-1}$. The sensitivity of this assay was 1 $mg \cdot dL^{-1}$. All samples were measured in triplicate using 10 μL of plasma; and allowing 5% variation between high and low replicate determinations. Intraassay and Interassay CV were less than 10%.

Plasma insulin concentrations were determined via RIA using a commercially available Porcine Insulin RIA kit, that possessed 90% cross-reactivity with bovine insulin (Cat # PI-12K; Millipore, Inc., St. Charles, MO). Bovine insulin (Sigma I5500; Sigma-Aldrich Inc.) was used as the standard. The total binding was 60 %. The detectable range for the insulin assay was from 3.125 to 200 $\mu U \cdot mL^{-1}$. The sensitivity for the insulin RIA was 1.611 $\mu U \cdot mL^{-1}$. All samples were measured in duplicate using 100 μL aliquots of plasma; the intra-assay CV for the insulin assay was 5.94% and the interassay CV was 8.13%.

Plasma urea nitrogen (PUN) concentrations were determined by a method originally described by [9] using sodium phenate and sodium hypochlorite. The standard curve constructed for the PUN assay was 0 to 20 $mg \cdot dL^{-1}$. All samples were measured in triplicate using 20 μL of plasma; and allowing 5% variation between high and low replicate determinations. Intra-assay and Inter-ASSAY CV were less than 10%.

Plasma NEFA concentrations were determined via acyl-CoA synthetase, acyl-CoA oxidase, and peroxidase using a commercially available kit (NEFA HR (2); Wako diagnostics, Richmond, VA) in 96-well microtiter plates [10]. The standard curve used for the NEFA assay was 0 to 1 $mmol \cdot L^{-1}$. All samples were run in triplicate using 5 μL of plasma; and allowing 5% variation between high and low replicate determinations. Intra-assay and inter-assay CV were less than 10%.

2.6. Statistical Analyses

Diet and time effects on plasma variables were analyzed using the PROC MIXED procedure of SAS 9.4 (SAS Inst. Inc., Cary, NC) specific for repeated measures, with the fixed effects of diet, day and diet *x* day. A repeated statement was used and included day as the repeated variable. The covariance structure used was compound symmetry [11]. Individual steer served as the experimental unit for testing plasma variables in response to Cr supplementation and days of adaptation to the feedlot. All results are reported as least-squares means. Data were separated using the PDIFF option of SAS if a significant preliminary F-test was detected. An α level of 0.10 was used to determine significance, with tendencies discussed at P-values between 0.10 and 0.15.

This study was not designed to test animal performance responses to treatment. However, animal performance was noted and analyzed to provide context to plasma variables responses. Statistical parameters for steer performance were generated using the PROC GLM model of SAS 9.4 (SAS Inst. Inc., Cary, NC) as

appropriate for a randomized compete block design with main effects of diet: (CON or CrP) and block pen (n = 2). Pen was the experimental unit for steer performance data.

3. Results and Discussion

3.1. Steer Performance

At 3 d post-arrival to the RNC, DMI were at maintenance (Figure 1). Weather conditions throughout this receiving study were unremarkable and intakes were good. One steer from CON was diagnosed with pink-eye on d 5, was treated and returned to his home pen. There were no indications of Bovine Respiratory Disease Complex (BRD) among these steers and no other apparent indications of health problems.

On d 9, the sorghum silage source was switched for the remainder of the experiment. There was a difference in the moisture content of the new silage source. The diet compositions presented in (Table 1) reflect the impact of this change on true diet formulations.

There were no differences ($P > 0.20$, Table 3) for ADG (1.63 ± 0.03 kg), DMI (7.22 ± 0.12 kg), or G:F (0.226 ± 0.01 kg) between diets in this 33 d study.

3.2. Plasma Constituents

There were no diet x day interactions ($P \geq 0.51$) for plasma GLS, insulin or PUN. Plasma GLS concentrations were similar between diets ($P = 0.35$, Figure 2), as well as for days of adaptation in the feedlot ($P = 0.73$). When supplementing Cr as chromium propionate to non-stressed growing heifers [12] reported no differences in serum GLS concentrations at 2 h post-prandial on d 21 and 42. Bernhard *et al.* [13] detected no differences for serum GLS concentrations sale barn acquired steers supplemented with Cr as chromium propionate at 1 h post-prandial after 56 d of supplementation. Likewise, [14] detected no differences in sera parameters when CrP was fed.

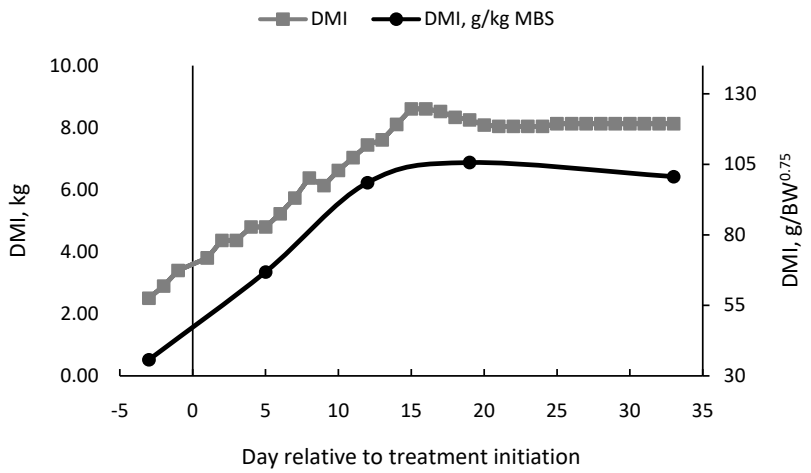

Figure 1. Daily dry matter intakes as kg and g/BW, $kg^{0.75}$ throughout the 33 day receiving study.

Table 3. Effects of chromium propionate supplementation on receiving cattle performance.

	Chromium[1]			
Item	CON	CrP	SEM[2]	P = [3]
Processing BW, kg[4]	288	290	0.2	0.07
d 5 BW, kg[5]	297	300	0.8	0.20
Processing to 5 d				
ADG, kg	0.98	1.13	0.072	--
DMI, kg	4.43	4.43	-	--
G:F	0.221	0.255	0.0161	--
F/G	4.53	4.00	0.194	--
d 12 BW, kg	319	320	2.7	--
6 to 12 d				
ADG, kg	3.21	2.88	0.275	--
DMI, kg	6.37	6.37	-	--
G:F	0.504	0.452	0.0432	--
F/G	2.01	2.21	0.175	--
d 19 BW, kg	334	334	0.3	--
13 to 19 d				
ADG, kg	2.17	2.01	0.344	--
DMI, kg	8.26	8.32	0.109	--
G:F	0.263	0.241	0.0381	--
F/G	3.80	4.16	0.592	--
d 33 BW, kg	350	348	1.0	--
20 to 33 d				
ADG, kg	1.10	1.00	0.046	--
DMI, kg	8.23	7.97	0.154	--
G:F	0.134	0.125	0.0080	--
F/G	7.55	7.94	0.427	--

[1]No added Cr "CON"; added 0.4 mg·kg^{-1} Cr to the total diet on a DM basis "CrP" (Kem-TRACE 0.4% Chromium Propionate, Kemin Industries, Des Moines, IA). [2]SE of treatment means (n = 2 pens/diet mean). [3]Probability; P > 0.20 not indicated. [4]Processing BW was recorded at 0700h 4 d prior to treatment initiation, all cattle were maintained on basal diet prior to treatment initiation. [5]All subsequent BW d 5, 12, 19 and 33 were recorded at 1400 h, ~4 h after access to their morning feed delivery.

Plasma insulin levels were similar (P = 0.46) between diets (**Figure 3**). This is consistent with [12] who fed supplemental Cr as chromium propionate to non-stressed growing heifers. There were no differences in serum insulin concentrations at 2 h post-feeding on d 21 and 42 relative to Controls [12]. Bernhard *et al.* [13] indicated that serum insulin levels were not different for chromium propionate supplemented steers at 1 h post-prandial on d 56 post-arrival. In the present study, plasma insulin levels peaked (P = 0.01, **Figure 3**) at 12 d post-treatment initiation for both CON and CrP steers. This is likely due to increased DMI relative to maintenance intake. By d 12 post-treatment initiation cattle had transitioned from a catabolic state where they were consuming 0.82 ×

maintenance at d -3, to 1.56 × maintenance at d 5 and subsequently 2.42 × maintenance by d 12. In Figure 1, DMI is expressed as kg/d, and g/BW, $kg^{0.75}$ via this graphical representation of intake one can see that there was approximately a 47% increase in DMI when expressed as g/BW, $kg^{0.75}$. Growth and growth efficiency variables for both groups also improved drastically during the 7 d period between d 5 and 12. Elevated plasma insulin concentrations could potentially be caused by the increased flow of blood metabolites as cattle were reaching their maximum voluntary intake. This relative to metabolite demands for growth, could potentially trigger endogenous insulin secretions.

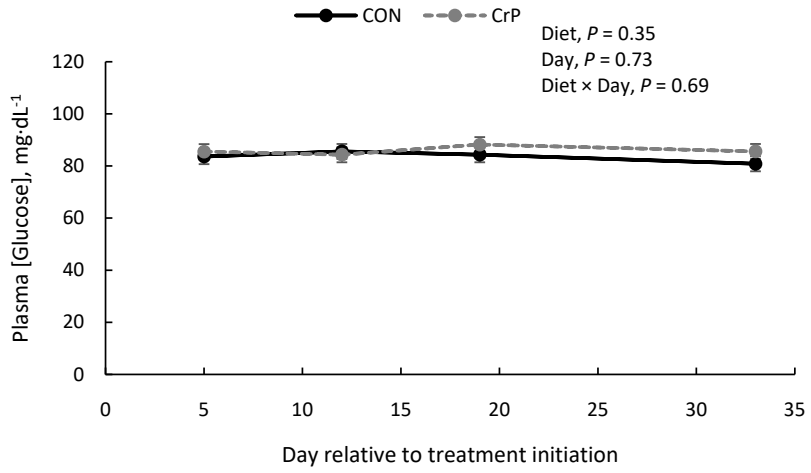

Figure 2. Effect of days of adaption and chromium propionate supplementation on plasma glucose concentrations (Standard error of diet by day treatment means = 2.900; n = 14 steers/treatment); no added chromium "CON", or 0.4 $mg \cdot kg^{-1}$ Cr added to the total diet on a DM basis as chromium propionate (KemTRACE 0.4% Chromium Propionate, Kemin Industries, Des Moines, IA) "CrP".

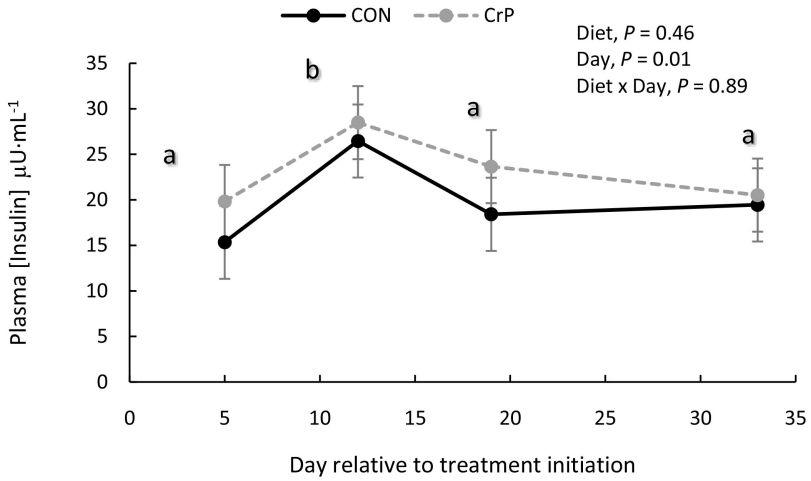

Figure 3. Effect of days of adaption and chromium propionate supplementation on plasma insulin concentrations (Standard error of diet by day treatment means = 4.017; n = 14 steers/treatment); no added chromium "CON", or 0.4 $mg \cdot kg^{-1}$ Cr added to the total diet on a DM basis as chromium propionate (KemTRACE 0.4% Chromium Propionate, Kemin Industries, Des Moines, IA) "CrP"; ^{a,b}Indicates mean values between days without a common superscript differ ($P < 0.05$).

Plasma urea-N concentrations were maintained in a narrow range over time (P = 0.59) and were similar (P = 0.90) between treatments (Figure 4). The ratio of dietary TDN and DIP was constant between diets, DMI were equal, and there is no expectation that Cr would impact the microbial efficiency of nitrogen fixation. Orr *et al.* [15] reported that increased PUN in steers could potentially be attributed to bovine rhinotracheitis virus infection and the subsequent catabolism of body protein in response to stress. Montgomery *et al.* [16] demonstrated that PUN concentrations were greater for heifers treated for apparent BRD then those not treated at least once. This aspect of disease influence on PUN could not be evaluated in the present study due to lack of apparent respiratory issues noted, regardless of diet (Figure 4).

There tended (P = 0.12) to be a diet x day interaction for plasma NEFA concentrations (Figure 5). Circulating concentrations of plasma NEFA were similar (P > 0.20) between diets on d 19 and 33. Plasma NEFA levels tended to be lower (P = 0.13) for calves fed CrP on d 5 and were greater (P = 0.09) on d 12 in calves fed CrP. This shift in circulating NEFA concentrations on d 12 coincided with a spike in plasma insulin concentrations. Both these events occurred at the time that NEg intake was approaching the acclimated plateau and neither event impacted glucose status. The authors speculate that the concentration of hormone sensitive lipase (HSL) relative to NEg intake may potentially have been a better indicator of body fat catabolism and energy status than GLS or insulin in newly weaned calves. The catabolism of body fat is enhanced by HSL activity and results in elevated NEFA concentrations. It would be useful to know the relationship of HSL and NEFA under these experimental conditions.

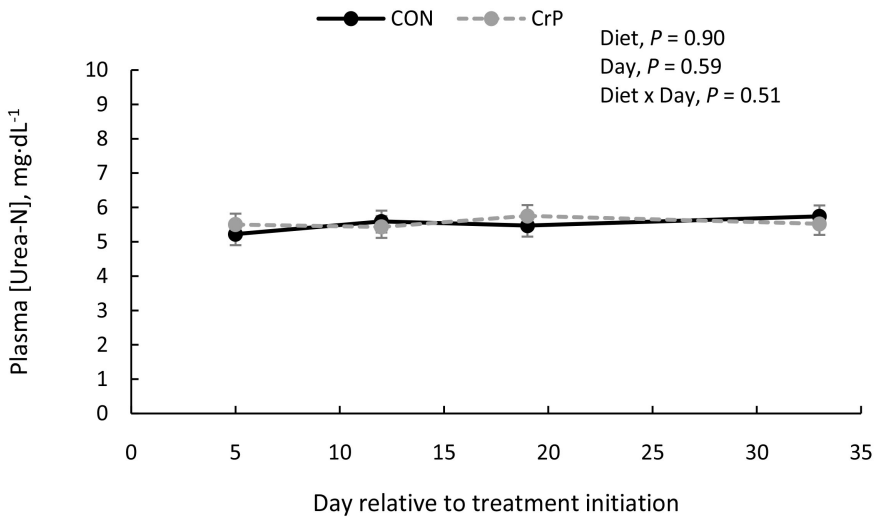

Figure 4. Effect of days of adaption and chromium propionate supplementation on plasma urea nitrogen concentrations (Standard error of diet by day treatment means = 0.318; n = 14 steers/treatment); no added chromium "CON", or 0.4 mg·kg^{-1} Cr added to the total diet on a DM basis as chromium propionate (KemTRACE 0.4% Chromium Propionate, Kemin Industries, Des Moines, IA) "CrP".

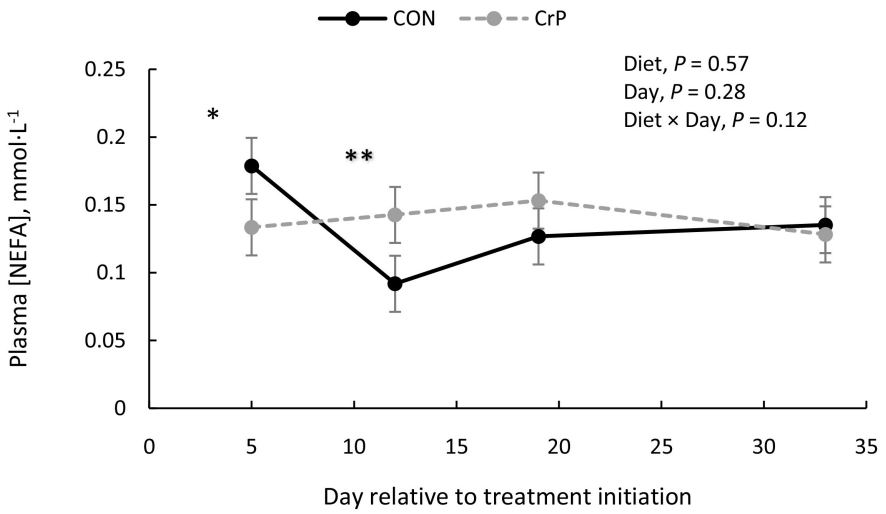

Figure 5. The effect of days of adaption and chromium propionate supplementation on plasma NEFA concentrations (Standard error of diet by day treatment means = 0.021; n = 14 steers/treatment); no added chromium "CON", or 0.4 mg·kg^{-1} Cr added to the total diet on a DM basis as chromium propionate (KemTRACE 0.4% Chromium Propionate, Kemin Industries, Des Moines, IA) "CrP"; * Indicates that Treatment means tended to differ (P = 0.13) on day 5, and ** Indicates that Treatment means differ (P = 0.09) on day 12.

In acclimated ruminants, one of the more consistent findings in Cr research is that Cr supplementation produces lower post-prandial circulating NEFA concentrations. Spears *et al.* [12] indicated that supplementing Cr as chromium propionate to non-stressed growing heifers resulted in no differences in serum NEFA concentrations at 2 h post-feeding after feeding Cr for 21 and 42 d. In auction barn acquired steers supplemented with Cr as chromium propionate, [13] reported that serum NEFA concentrations were less for Cr supplemented steers at 1 h post-feeding after 56 d on test. In sheep, [17] demonstrated that non-stressed, Cr supplemented wethers had lower post-prandial NEFA concentrations when compared to Controls. Additional factors such as stress, weight loss as shrink, and the process of acclimation to feed may alter the influence of Cr on lipid oxidation.

4. Implications

We observed minimal differences in regard to plasma indicators of lipid metabolism in this study. Ideally, an initial blood sample would have been collected prior to dietary treatment initiation for all steers. However, these were healthy calves, and there were no apparent cases of BRD. In the present study, being weaned and shipped 579 km may not have been a sufficient stressor to severely impact Cr status. Overall, DMI for all steers was at 1.11 × maintenance by 3 d post-arrival to the feedlot. All blood metabolite variables were within the physiological ranges expected of this class of cattle. It is unclear why CrP steers had elevated NEFA concentrations that coincided with a spike in insulin for both CON and CrP diets on d 12. This event occurred as cattle were approaching

maximum voluntary intake and did not impact glucose status. In non-ruminants elevated insulin concentrations decrease circulating NEFA levels. A novel finding in this research is that ruminants may differ from non-ruminants in the regulation and maintenance of glucose status and body fat catabolism during the post-absorptive state. The lack of continuity seen in studies supplementing Cr to growing ruminants may be due to differences in the degree of stress, the potential for lean tissue accretion, the supply, and demand of metabolites needed for growth, and finally, the energy density of the diet and sampling window relative to the inflection point on maximum voluntary intake.

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Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

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