

Chromium propionate in broilers: human food and broiler safety

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ABSTRACT Chromium propionate (**Cr Prop**) is approved by the U.S. Food and Drug Administration Center for Veterinary Medicine for supplementation to broiler diets up to 0.20 mg Cr/kg diet. A 49-D study was conducted to: 1) determine the safety of Cr Prop when supplemented at 2 and 10 times (\times) the approved feeding level over the normal life span of broilers, and 2) determine the effects of supplementing Cr Prop on Cr concentrations of tissues consumed by humans. On day zero, 216 Ross 708 broilers were stratified by weight within sex and randomly assigned to treatments. Dietary treatments were 0 (control), 0.40, and 2.0 mg supplemental Cr/kg diet from Cr Prop. There were 6 replicate cages each of male and female broilers per treatment. At the end of the study blood was collected for determination of plasma biochemi-

cal measurements and tissue samples were collected for Cr analysis. Supplementing 0.40 mg Cr/kg diet ($2\times$) did not adversely affect broiler performance, mortality, plasma biochemical measurements or Cr concentrations in breast muscle, skin with adhering fat, or liver. Chromium propionate supplemented at 2.0 mg Cr/kg ($10\times$) did not affect Cr concentrations in breast muscle or skin with adhering fat, but increased ($P < 0.05$) liver Cr concentrations. Supplementing Cr Prop at $10\times$ the approved feeding level decreased feed intake and gain in male but not female broilers from days 21 to 49. Results of this study support the safety of Cr Prop in broiler diets, and indicate that Cr Prop supplementation to broiler diets at 2 or $10\times$ the approved feeding level does not present a human health concern.

Key words: chromium, broilers, tissue chromium

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INTRODUCTION

Chromium (**Cr**) functions in insulin-sensitive tissues to potentiate the action of insulin (Brooks et al., 2016). Poultry are more resistant to insulin than mammals (Scanlan, 2009). However, considerable research clearly indicates that insulin plays an important role in glucose homeostasis in poultry (Akiba et al., 1999; Tokushima et al., 2005; Dupont et al., 2008). It is well documented that hormones produced during heat stress and other types of stress reduce insulin sensitivity in broilers (Zhao et al., 2009). Studies (Sands and Smith, 1999; Huang et al., 2016; Sahin et al., 2017) have indicated that Cr supplementation can increase performance of broilers especially when reared under heat stress conditions.

Chromium propionate (**Cr Prop**) has been shown to enhance insulin sensitivity in broilers (Brooks et al., 2016). The U.S. Food and Drug Administration Center for Veterinary Medicine (**FDA**) approved a food additive petition in June, 2016 which allowed the use of

Cr Prop as a source of supplemental Cr in broiler diets (FDA, 2016). Chromium propionate is the only Cr source currently approved in the US for supplementation to broiler diets, and it can be added at concentrations up to 0.20 mg of Cr/kg diet. Feed mixing errors may occasionally occur that result in Cr being supplemented above the approved concentration. Therefore, the FDA food additive petition required a study to demonstrate broiler safety and also human food safety when Cr Prop was provided above the approved concentration of 0.20 mg Cr/kg diet. The present study was conducted to: 1) determine the safety of Cr Prop when supplemented at 2 and 10 times (\times) the approved rate over the normal life span of broilers, and 2) determine the effects of supplementing broiler diets with Cr Prop on Cr concentrations in tissues consumed by humans.

MATERIALS AND METHODS

Experimental Design

The North Carolina State University (**NCSU**) Animal Care and Use Committee approved all care, handling, and sampling procedures utilized in this study. A total of 216-day-old broilers (Ross 708 Strain; NC

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Table 1. Ingredient composition of diets.

Ingredient	Inclusion rate, %		
	Starter	Grower	Finisher
Ground corn	53.16	57.62	59.95
Soybean meal	36.00	30.00	28.50
Fat	3.00	5.00	5.00
Corn-chromium premix ¹	2.00	2.00	2.00
Limestone	2.40	2.20	2.00
Phosphoric acid	1.60	1.40	1.10
L-Lysine	0.38	0.35	0.25
DL-methionine	0.38	0.35	0.25
Threonine	0.10	0.08	0.00
Sodium chloride	0.28	0.25	0.25
Choline chloride	0.20	0.20	0.20
Vitamin premix ²	0.10	0.10	0.10
Mineral premix ³	0.20	0.20	0.20
Sodium bicarbonate	0.20	0.25	0.20

¹Supplied only ground corn in the control treatment and chromium propionate (Cr Prop) mixed with ground corn to provide the appropriate concentration of chromium (Cr) for the Cr treatments.

²Provided per kg of diet: 13,200 IU vitamin A, 4,000 IU vitamin D, 66 IU vitamin E, 39.6 µg vitamin B₁₂, 13.2 mg riboflavin, 110 mg niacin, 22 mg d-pantothenate, 4 mg menadione, 2.2 mg folic acid, 4.0 mg thiamine, 7.9 mg pyridoxine, 253 µg d-biotin, and 100 mg ethoxyquin.

³Provided per kg of diet: 40 mg Zn (from ZnSO₄), 60 mg Mn (from MnSO₄), 80 mg Fe (from FeSO₄ · H₂O), 10 mg Cu (from Cu₂(OH)₃), 1 mg I (from Ca(IO₃)₂), and 0.3 mg Se (from Na₂SeO₃).

State University Hatchery, Raleigh, NC) were selected from a pool of 223 for use in this study. Upon delivery from the hatchery (day 0), birds were sorted by sex, weighed, and identified with numerical neck tags. The healthiest and most uniform birds were stratified within sex by weight and randomly assigned to 1 of 3 treatments. There were 6 replicate cages of female broilers and 6 replicate cages of male broilers per treatment. Each replicate cage consisted of 6 broilers during the starter phase and 4 broilers during the grower and finisher phases. Dietary chromium treatments included: 0 (control), 0.4 (Cr 0.4), and 2.0 (Cr 2.0) mg of supplemental chromium (from KemTRACE Cr Prop; Kemin Agrifoods North America, Des Moines, IA) per kg of diet.

Diets and Housing

Ingredient composition of the starter, grower, and finisher diets is presented in Table 1. The corn-soybean-based diets were formulated to meet or exceed all nutrient requirements of broiler chickens (NRC, 1994). Chemical composition of control diets is shown in Table 2. Experimental diets were mixed at the NCSU Feed Mill (Raleigh, NC). One base mix of the starter, grower, and finisher diets was produced. Treatment diets were prepared by mixing 2% of a corn-Cr Prop premix with 98% base mix. Chromium Prop was mixed with finely ground corn to provide 0, 0.4, or 2.0 mg supplemental Cr/kg of diet, when added at 2% to the diet. Diets were offered ad libitum in mash form, and feed disappearance was measured throughout the study. Fresh water was available at all times via a nipple water line system. Broilers were observed daily for health and wellbeing. Broilers were euthanized as needed for wel-

Table 2. Chemical analysis of control diets (as-fed basis).

	Starter	Grower	Finisher
DM, %	92.9	93.2	92.7
CP, %	24.5	22.4	21.3
NDF, %	8.3	8.9	8.8
Ca, %	0.91	0.95	0.86
P, %	0.70	0.70	0.59
Mg, %	0.16	0.16	0.16
K, %	0.94	0.91	0.92
Na, %	0.15	0.17	0.15
Fe, mg/kg	146	142	165
Zn, mg/kg	79	70	99
Cu, mg/kg	16	16	18
Mn, mg/kg	72	63	71

fare concerns. Necropsies of dead or euthanized broilers were conducted by the Rollins Animal Disease Diagnostic Laboratory, Raleigh, NC.

Chicks were housed in temperature controlled battery cages (Alternative Design Mfg., Siloam Springs, AR) with a 23:1 light:dark cycle. The starter diet was fed from 0 to 21 D, the grower diet from 21 to 42 D, and the finisher diet was offered from 42 to 49 D. Broilers were housed in brooder cages during the 21-D starter phase. At the end of the starter phase, 2 chicks were removed per cage, based on deviation from mean body weight, and the 4 most uniform chicks were transferred to grower cages for the remaining 28 D. Broilers were individually weighed on days 0, 21, 42, and 49 of the study.

Sampling

Samples of experimental diets were collected weekly and composited by phase for Cr and chemical analysis (Tables 2 and 3). Water samples were also collected weekly and composited by phase for chromium analysis.

At the termination of the study (day 49), blood was collected from the wing vein in heparinized tubes from 1 broiler in each replicate cage for determination of plasma biochemical measurements. Following blood collection, broilers were euthanized via cervical dislocation. The chick selected in each cage for harvesting was the one with the closest weight to the mean weight of birds in the cage. Samples of liver (left lobe), breast muscle (right *pectoralis major*), and skin with adhering fat (skin and fat covering the right breast muscle) were obtained from a similar location in each broiler for Cr analysis. Titanium knives and plastic forceps were used

Table 3. Analyzed chromium (Cr) concentrations in experimental diets from composite samples.

Treatment	Supplemental Cr ¹ , mg/kg	Analyzed Cr, mg/kg diet (as-fed basis)		
		Starter	Grower	Finisher
Control	0.00	0.349	0.357	0.345
Cr 0.4	0.40	0.803	0.719	0.698
Cr 2.0	2.00	2.483	2.627	2.342

¹Chromium propionate (Kemin Agrifoods North America, Des Moines, IA) was supplemented to provide 1, 0.4, or 2.0 mg Cr/kg diet.

to collect tissues to prevent Cr contamination. Samples were rinsed with deionized water, blotted dry with paper towels, placed in Whirlpak bags, and placed on ice. Upon returning to the laboratory, samples were stored at -20°C until analysis.

Laboratory Analysis

Feed samples from each treatment were composited into the 3 feeding phases prior to analysis. Composite samples of control diets were analyzed for chemical components at a commercial laboratory (Dairy One Cooperative Inc., Ithaca, NY). Composite feed samples were dried for 48 h at 55°C in a forced air oven and then prepared for Cr analysis by wet ashing with trace metal grade nitric acid (Trace Metal Grade, Fisher Scientific, Raleigh, NC). Water samples were analyzed for Cr without ashing. Breast muscle and liver samples were partially thawed, rinsed, blotted dry, and then sectioned with a titanium knife for DM analysis prior to Cr determination. The DM was determined by drying at 55°C in a forced air oven for 48 h. Skin+fat was cut with titanium scissors and analyzed for Cr on a wet tissue basis. All tissues were prepared for Cr analysis as described for feed samples.

Chromium was determined via electrothermal atomic absorption spectrophotometry (Model 6701, Shimadzu, Kyoto, Japan). The method of standard addition was used for each feed and tissue sample to remove matrix effects as described by Lloyd et al. (2010). Bovine liver (SRM 1577C) obtained from the National Institute of Standards and Technology (Washington, DC) and certified to contain 53 ± 14 ng of Cr/g was used as a reference standard.

Plasma was analyzed for an avian chemistry panel that included glucose, uric acid, urea-nitrogen, phosphorus, calcium, total protein, albumin, globulin, cholesterol, alkaline phosphatase (AP), aspartate aminotransferase (AST), lactate dehydrogenase (LD), and creatine kinase (CK). Plasma analysis was performed at the Clinical Pathology Laboratory at the NCSU Veterinary School.

Statistical Analysis

Data were analyzed statistically as a completely randomized design by analysis of variance using the Proc Mixed procedure of SAS (2002). The model included sex, treatment, and sex \times treatment. Differences among treatment means were determined using the LSD test when the overall treatment or sex \times treatment interaction was significant ($P < 0.05$).

RESULTS

Chromium Analysis of Diets

The Cr concentration of the control diets averaged 0.35 mg Cr/kg diet (Table 3). Diets supplemented with

Table 4. Mortalities recorded for broiler chickens fed diets with varying amounts of supplemental chromium (Cr).¹

	Treatment		
	Control	Cr 0.4	Cr 2.0
Initial number of birds	72	72	72
Mortality diagnosis ²			
Leg abnormalities	2	1	1
Congestive heart failure	0	1	0
Yolk peritonitis	0	0	1
Cannibalism or injury	1	0	0
Idiopathic	0	1	1
Total mortality			
Number per treatment	3	3	3
% per treatment	4.2	4.2	4.2

¹Chromium propionate was supplemented to provide 0, 0.4, or 2.0 mg Cr/kg diet.

²Necropsies were conducted by Rollins Animal Disease Diagnostic Laboratory, Raleigh, NC.

Cr averaged 0.74 and 2.48 mg Cr/kg diets for the Cr 0.4 and Cr 2.0 diets, respectively. Analyzed diet Cr concentrations aligned with calculated concentrations for each of the treatments. Water samples collected during the starter, grower, and finisher phase analyzed 0.70, 1.48, and 1.49 μg Cr/L, respectively.

Mortality

Throughout the study, 9 broilers either died or were euthanized for welfare concerns. The descriptions of mortalities by the diagnostic laboratory ranged from idiopathic to leg abnormalities (Table 4). Mortality was similar across treatments.

Performance

Performance of broilers during the starter phase is presented in Table 5. Body weight gain and feed intake of broilers during the starter phase was not affected ($P > 0.05$) by dietary Cr or a sex \times treatment interaction. Feed/gain tended (treatment $P = 0.06$) to be greater for Cr-supplemented broilers. However, individual comparisons among treatments did not differ ($P > 0.05$).

Broiler performance for the combined growing and finishing phase is shown in Table 6. Final body weights ($P = 0.03$) on day 49 and gain ($P = 0.003$) during the growing and finishing phase were affected by a sex \times treatment interaction. Chromium supplementation at 0.4 or 2.0 mg/kg diet did not affect final body weight or gain in female broilers. Male broilers supplemented with 2.0 mg Cr/kg diet had lower ($P < 0.05$) final body weights than those supplemented with 0 or 0.4 mg Cr/kg diet. Weight gain during the growing and finishing phase was lower ($P < 0.05$) in male broilers supplemented with 2.0 mg Cr/kg compared with controls. Gain and final body weights of male broilers supplemented with 0.4 mg Cr/kg diet did not differ from controls. Feed intake during the growing and finishing phase was also affected ($P = 0.03$).

Table 5. Effect of supplemental chromium¹ (Cr) on performance of broilers during the starter phase (day 0 to 21 post-hatch).

	Treatment			SEM	Sex			SEM	P values		
	Control	Cr 0.4	Cr 2.0		Male	Female	Treatment		Sex	Sex × treatment	
Body weight, g											
Day 0	46.7	46.8	46.9	0.2	46.9	46.7	0.2	0.82	0.18	0.99	
Day 21	879.6	852.9	851.5	11.7	857.8	864.9	9.5	0.18	0.60	0.84	
Gain, g/d	39.4	38.4	38.1	1.6	36.8	38.8	1.3	0.37	0.31	0.36	
Feed intake, g/d	54.1	53.5	53.4	0.8	54.0	53.3	0.7	0.81	0.42	0.28	
Feed/gain	1.37	1.39	1.40	0.01	1.41 ^a	1.37 ^b	0.0	0.06	0.003	0.25	

¹Chromium propionate was supplemented to provide 0, 0.4, or 2.0 mg Cr/kg diet.

^{a,b}Means in a row not sharing a common superscript letter differ ($P < 0.0$).

Table 6. Effect of supplemental chromium¹ (Cr) on performance of broilers during the growing and finishing phase (day 21 to 49 post-hatch).

	Treatment			SEM	Sex			SEM	P values		
	Control	Cr 0.4	Cr 2.0		Male	Female	Treatment		Sex	Sex × treatment	
Body weight, g											
Day 21	887	857	864	14.4	866	873	12	0.31	0.67	0.54	
Day 49	3,447	3,389	3,277	37	3,599	3,143	30	0.009	0.001	0.03	
Males	3,747 ^a	3,618 ^a	3,432 ^b	52							
Females	3,147	3,159	3,123	52							
Gain g/d	91.2 ^a	90.1 ^a	85.7 ^b	1.1	97.1	81.0	0.9	0.005	0.001	0.003	
Males	102.1 ^a	98.5 ^{a,b}	90.6 ^b	1.6							
Females	80.4	81.6	80.9	1.6							
Feed intake, g/d	168.1	166.7	161.3	2.3	172.9	157.8	1.9	0.11	0.001	0.03	
Males	180.1 ^a	174.1 ^a	164.4 ^b	3.2							
Females	155.9	159.3	158.2	3.2							
Feed/gain	1.85	1.86	1.89	0.01	1.80	1.97	0.01	0.06	0.001	0.36	

¹Chromium propionate was supplemented to provide 0, 0.4, or 2.0 mg Cr/kg diet.

^{a,b}Means in a row not sharing a common superscript letter differ ($P < 0.05$).

by a sex × treatment interaction. Male broilers receiving 2.0 mg Cr/kg diet had lower ($P < 0.05$) feed intake than unsupplemented controls and birds supplemented with 0.4 mg Cr/kg diet. Feed intake of female broilers was not affected by dietary Cr. Feed/gain was lower ($P = 0.001$) in males than females but was not affected by treatment ($P = 0.06$) or a sex × treatment interaction.

Tissue Chromium Concentrations

Breast muscle Cr concentration from 1 broiler in the control treatment appeared to be contaminated, and was not included in the statistical analysis. The muscle Cr concentration in this broiler was over 5-fold higher (406 vs. 72 ng/g DM) than the next highest muscle Cr value in this treatment. Skin + adhering fat from 1 broiler in the 2.0 mg Cr/kg diet treatment also appeared to be contaminated, and was not included in the statistical analysis. Chromium concentration in skin + adhering fat from this broiler was almost 4-fold higher (528 vs. 136 ng/g) than the next highest value in this treatment. Contamination of biological samples with Cr during collection, storage, and preparation for analysis can be a problem when conducting Cr research (NRC, 2005).

Tissue Cr concentrations were not affected by sex or a sex × treatment interaction (Table 7). Chromium concentrations of breast muscle and skin + adhering fat were not affected by the addition of 0.4 or 2.0 mg Cr/kg diet from Cr Prop. Liver Cr concentrations were higher ($P < 0.05$) in broilers supplemented with 2 mg Cr/kg diet than in controls and birds receiving 0.4 mg Cr/kg diet. Chromium concentrations in liver did not differ between controls and birds supplemented with 0.4 mg Cr/kg diet.

Plasma Biochemical Measurements

Plasma biochemical measurements conducted at the end of the 49-D study are shown in Table 8. Plasma cholesterol was higher ($P < 0.05$) in broilers supplemented with 2.0 mg Cr compared with controls and those supplemented with 0.4 mg Cr/kg diet. Plasma glucose, uric acid, calcium, phosphorus, total protein, albumin, and globulin concentrations were not affected ($P > 0.05$) by treatment. Plasma enzymes measured were not affected by treatment. However, creatine kinase was affected ($P = 0.05$) by a sex × treatment interaction. Male broilers supplemented with 0.4 or 2.0 mg Cr/kg diet had lower ($P < 0.05$) plasma creatine kinase activity than controls. Plasma creatine kinase

Table 7. Effect of supplemental chromium¹ (Cr) on tissue chromium concentrations in broilers.

	Treatment			SEM	Sex		SEM	P values		
	Control	Cr 0.4 ng Cr/g	Cr 2.0		Male	Female		Treatment	Sex	Sex × treatment
Breast muscle ²	36.6	37.9	38.4	4.3	39.5	35.8	3.6	0.96	0.47	0.30
Skin+fat ³	45.3	51.3	65.4	10.6	44.9	63.2	8.9	0.42	0.15	0.97
Liver ²	33.4 ^a	39.9 ^a	94.0 ^b	3.7	56.5	55.0	3.0	0.0001	0.74	0.39

¹Chromium propionate was supplemented to provide 0, 0.4, or 2.0 mg Cr/kg diet.

²Dry matter basis.

³As-is basis.

^{a,b}Means in a row not sharing a common superscript letter differ ($P < 0.05$).

Table 8. Effect of supplemental chromium¹ (Cr) on plasma clinical chemistry measurements in broilers.

	Treatment			SEM	Sex		SEM	P Values		
	Control	Cr 0.4	Cr 2.0		Male	Female		Treatment	Sex	Sex × treatment
Glucose ²	236.7	239.5	235.0	3.9	242.1	232.1	3.5	0.52	0.004	0.71
Uric acid ²	3.55	4.03	4.24	0.34	3.33	4.54	0.28	0.36	0.005	0.49
Phosphorus ²	7.48	7.02	7.03	0.20	6.77	7.58	0.18	0.08	0.002	0.82
Calcium ²	10.99	10.71	10.66	0.23	10.57	11.00	0.19	0.55	0.12	0.26
Protein ³	2.94	2.83	3.06	0.09	2.84	3.05	0.08	0.26	0.06	0.59
Albumin ³	1.22	1.21	1.32	0.04	1.20	1.29	0.04	0.06	0.04	0.33
Globulin ³	1.75	1.65	1.76	0.07	1.66	1.78	0.05	0.45	0.11	0.93
Cholesterol ²	119.3 ^a	112.3 ^a	135.7 ^b	5.3	123.3	121.6	4.3	0.01	0.77	0.99
Alkaline phosphatase ⁴	1,645	1,439	1,148	233	1,585	1,236	190	0.34	0.21	0.91
Aspartate aminotransferase ⁴	556	466	506	56	537	481	50	0.37	0.30	0.19
Lactate dehydrogenase ⁴	2,249	1,941	2,371	523	2,337	2,036	466	0.76	0.54	0.48
Creatine kinase ⁴	49,543	35,188	42,256	558	42,422	42,236	4562	0.20	0.98	0.05
Males	61,503 ^a	28,706 ^b	37,056 ^b	802						
Females	37,582	41,670	47,457	777						

¹Chromium propionate was supplemented to provide 0, 0.4, or 2.0 mg Cr/kg diet.

²Expressed as mg/dL.

³Expressed as g/dL.

⁴Expressed as IU/L.

^{a,b}Means in a row not sharing a common superscript letter differ ($P < 0.05$).

activity in female broilers was not affected by treatment. Male broilers had higher ($P = 0.004$) plasma glucose concentrations than females, while plasma uric acid ($P = 0.005$), phosphorus ($P = 0.002$), and albumin ($P = 0.04$) concentrations were higher in females than in male broilers.

DISCUSSION

Chromium propionate was supplemented throughout the typical life span of broilers to provide 2 or 10× the concentration of supplemental Cr approved as the upper feeding level (0.2 mg Cr/kg diet) by FDA. Supplementing 0.4 or 2.0 mg Cr/kg diet for 49 D did not affect Cr concentrations in breast muscle or skin with adhering fat, the major poultry products consumed by humans in the US. Previous studies in pigs, broilers, and cattle (Anderson et al., 1997; Zha et al., 2009; Lloyd et al., 2010) have indicated that supplementing Cr at concentrations ranging from 0.30 to 2.0 mg/kg diet did not affect muscle Cr concentrations. Supplementing 2.0 mg Cr/kg diet resulted in an increase in liver Cr concentrations compared to control broilers. An increase in liver Cr concentrations was also reported in a

study conducted in broilers supplemented for 6 wk with 0.50 mg Cr/kg diet from Cr picolinate (Zha et al., 2009). However, consumption of chicken liver by a majority of humans is estimated to be extremely low. Assuming a maximum intake by humans of 100 g (wet weight) of liver, consumption of liver from broilers supplemented with 2.0 mg Cr/kg DM would increase Cr intake by 1.5 µg/D. The adequate intake of chromium in adult humans has been estimated at 25 µg/D for females and 35 µg/D for males (National Academies, 2001). Therefore, supplementation of Cr from Cr Prop for broilers receiving 2.0 mg Cr/kg diet through 49 days would have minimal effect on the total Cr intake by humans

Chromium supplementation had minimal effects on plasma biochemical measurements. Broilers supplemented with 2.0 mg Cr/kg diet had higher plasma cholesterol concentrations than broilers supplemented with 0 or 0.4 mg Cr/kg diet. Previous studies have indicated variable effects of supplemental Cr on circulating cholesterol in broilers. In some studies (Kim et al., 1996; Sahin et al., 2002) Cr supplementation has decreased serum cholesterol concentrations. However, broilers supplemented with 0.8 mg Cr/kg diet, from either a high-Cr yeast or Cr picolinate, had higher serum

cholesterol concentrations than broilers supplemented with 0.2 or 0.4 mg Cr/kg diet at the end of a 49-D study (Suksombat and Kanchanatawee, 2005). Broilers supplemented for 42 D with 0.2, 0.4, or 0.8 mg Cr/kg diet, from Cr picolinate, also had higher serum cholesterol concentrations than controls in 1 of 2 experiments (Lee et al., 2003). In the present study control broilers had a mean plasma cholesterol concentration of 119.3 mg/dL. Control broilers had serum cholesterol concentrations greater than 150 mg/dL in studies where Cr supplementation decreased serum cholesterol concentrations (Kim et al., 1996; Sahin et al., 2002). In contrast, serum cholesterol in control birds was less than 120 mg/dL in studies where Cr supplementation has either increased (Lee et al., 2003; Suksombat and Kanchanatawee, 2005) or not affected (Zheng et al., 2016) circulating cholesterol concentrations.

Elevation of various plasma or serum enzymes can indicate cellular damage or metabolic disease, causing leakage of the enzyme from a particular organ (Kramer, 1989). Plasma activities of AP, AST, and LD were not affected by Cr. Creatine kinase activity was affected by a sex \times treatment interaction. Chromium supplementation at 0.4 or 2.0 mg/kg diet decreased plasma CK compared with controls in male but not in female broilers. Elevated activity of CK in plasma or serum is a good indicator of muscle damage (Kramer, 1989). It is unclear why plasma CK was higher in control male broilers. The decrease in plasma CK due to Cr addition would not be considered an adverse effect of Cr Prop supplementation.

Bird mortality results in the present study, as well as previously published studies (Jackson et al., 2008; Rajalekshmi et al., 2014; Brooks et al., 2016), support the safety of Cr Prop in broiler diets. Supplementing Cr Prop in broiler diets did not adversely affect bird mortality in any of the studies. The highest concentration of Cr supplemented in these studies ranged from 3 \times (Brooks et al., 2016) to 16 \times (Rajalekshmi et al., 2014) the upper supplemental concentration approved by FDA.

Chromium supplementation at 2.0 mg/kg diet reduced feed intake and gain in male broilers during the growing and finishing phase. The reduced gain observed in male broilers supplemented with 2.0 mg Cr/kg diet can largely be explained by reduced feed intake. It is unclear why feed intake was reduced in male broilers receiving 2.0 mg Cr/kg diet in the present study. In contrast to these results, Cr Prop supplemented to provide up to 1.6 mg Cr/kg (Xiao et al., 2017) or 3.2 mg Cr/kg diet (Rajalekshmi et al., 2014) did not adversely affect gain or feed intake of male broilers. Chromium supplementation at 0.4 mg Cr/kg diet did not adversely affect performance of male broilers. Furthermore, performance of female broilers was not affected by Cr supplementation at 2.0 mg/kg diet. The significant sex \times treatment interaction observed in the present study was unexpected and has not been reported previously. Jackson et al. (2008) evaluated varying concentrations of supplemental Cr

Prop up to 0.8 mg Cr/kg diet in two experiments where male and female broilers were housed in separate pens. Both experiments were large pen studies with 365 birds per treatment and 7 replicate pens per treatment group. Broiler performance in these studies was not affected by a sex \times treatment interaction (Jackson et al., 2008).

CONCLUSIONS

This study was conducted to determine broiler and human food safety if Cr Prop is erroneously supplemented to broiler diets at concentrations that exceed the FDA approved level of 0.20 mg Cr/kg diet. Chromium propionate supplementation to broiler diets at 10 \times the approved feeding level did not affect Cr concentrations in breast muscle or skin with adhering fat. Liver Cr concentrations were increased slightly in broilers supplemented with 2.0 mg Cr/kg but not in those supplemented with 0.40 mg Cr/kg diet. This indicates that Cr Prop supplementation to broiler diets does not present a human health concern. Supplementing Cr Prop during the typical life span of broilers to supply 2 \times the approved feeding did not adversely affect performance, mortality, or plasma biochemical measurements. The present study and previous research supports the safety of Cr Prop in broiler diets.

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