Effects of Dietary Chromium Picolinate Supplementation on Growth Performance and Immune Responses of Broilers

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ABSTRACT : Two experiments were conducted to evaluate the effects of chromium (Cr) on the growth performance, bone trait, serum traits, and immune responses in broilers. The broilers were fed corn-soybean meal basal diet supplemented with Cr at level of 0 (control), 200, 400, or 800 ppb in the form of chromium picolinate (CrPic). The broilers were fed treated diets for 6 weeks in Exp. 1, but the Cr supplement was removed for the last 3 weeks in Exp. 2. Exp. 1 showed that dietary supplement of Cr did not affect growth performance of the broiler, though improved feed efficiency ($p<0.05$) was observed during 0 to 3 weeks. Moreover, serum total ($p<0.05$) and HDL cholesterols ($p<0.06$) were significantly higher in pooled Cr added group at 6 weeks of age, however, the difference was not significant in Exp. 2. The pooled Cr added group in Exp.1 had significantly lower ($p<0.05$) alkaline phosphatase activity and higher ($p<0.09$) calcium at 3 weeks. Significantly lower phosphorus was also observed in Exp. 2. With continued supplement of Cr as in Exp. 1, the alkaline phosphatase activity maintained higher at 6 weeks, as opposed to significantly lower in Exp. 2, which had no further Cr supplement. Higher bone breaking strength was observed in 400 ppb Cr supplemented in Exp. 1, though not significantly different. Serum glucose and triglyceride were not affected by Cr supplement. Antibody against *Infectious Bronchitis* (IB) was significantly ($p<0.05$) higher with 400 ppb Cr supplemented, and anti-*Newcastle disease* (ND) antibody also tended to be higher ($p<0.06$) in pooled Cr added group at 6 weeks of age in Exp. 1. Peripheral blood blastogenesis activity was not different among the treatments. The results suggest that diet supplemented with 400 ppb CrPic may be beneficial to the broilers. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 2 : 227-233)***

Key Words : Chromium Picolinate, Broiler, Growth Performance, Immune Response

INTRODUCTION

Chromium (Cr) was recognized as an essential element of diet in mammals by Schwarz and Mertz in 1957. Since then, many physiological functions of Cr in mammals have been reported, including tolerance factor in maintaining normal blood glucose concentration (Amoikon et al., 1995; Bunting et al., 1994), decreasing lipid accumulation (Anderson et al., 1991), roles in protein synthesis and nucleotides metabolism (Evans and Bowman, 1992; Min et al., 1997), and possible effect in bone calcium metabolism (McCarty, 1995). Chromium content is very low in most feedstuffs, with 37 and 10 ppb in corn and soybean meal, respectively (Fisher et al., 1990). In addition, compound form of Cr affects the rate of absorption. Only 0.3 to 5% of inorganic Cr is absorbed in rats, while 10 to 25% for organic Cr (Anderson and Kozlovsky, 1985; Dowling et al., 1989). The National Research Council (NRC) recommends 300 ppb dietary Cr for laboratory animals (NRC, 1995), however, no figure is listed for poultry (NRC, 1994).

Supplementation of chromium picolinate (CrPic) in diet or drinking water increased daily gain and feed utilization in broilers (Gonzalez et al., 1997; Piao et al., 1997), and improved growth performance of broiler fed low protein diet (80% of NRC recommended level) (Kim et al., 1995). Blood levels of glucose, triglyceride, and free fatty acid were decreased in broilers fed diet supplemented with 800 ppb CrPic (Kim et al., 1995; Kim et al., 1996a; Kim et al., 1996b). Chromium in the forms of yeast or CrPic also tended to decrease mortality (Kim et al., 1996a; Kim et al., 1996b; Hossain et al., 1998) or to increase immune response in broilers (Guo et al., 1999; Luo et al., 1999). However, inconsistent data were also reported (Kim et al., 1996a; Motozone et al., 1998). This research was to investigate the effects of different levels of CrPic supplementation on the growth performance, bone trait, serum traits, and immune responses in broilers during starting and finishing periods.

MATERIALS AND METHODS

Treatment and management

Experiment 1 : This experiment was a completely randomized design with 640 commercial broilers (Avian) allocated to 4 treatments supplemented with 0 (control), 200, 400, or 800 ppb Cr in the form of CrPic (Prince Chromax, Prince Agri Products, Inc., USA). Each treatment consisted of 2 pens with 40 birds in each pen, and the experiment was repeated once. Birds were raised from day-old to 6 weeks of

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age, and were fed *ad libitum* with a corn-soybean meal basal diet containing 4 and 2% fish meal for starting (0-3 weeks) and finishing (3-6 weeks) periods, respectively. The formula and chemical composition of basal diets for starter and finisher are listed in Table 1. All nutrients were supplemented to meet or exceed the requirements recommended by NRC (NRC, 1994). Chromium contents were 4.83 and 3.89 ppm in starting and finishing diets, respectively, as measured by atomic absorption spectrophotometer (Analyst 100, Perkin-Elmer, USA).

The birds were reared with 24 h in light, and the feed intake and body weight were measured weekly. All birds were intramuscularly immunized with killed vaccine of *Newcastle disease* (ND, Clone 30) and *Infectious bronchitis* (IB, M41) at age of 4 days. At 14 day old, the birds were boosted via nasal with live vaccine of ND (Clone 30) and IB (Ma5). All birds' beaks were trimmed at 10 day old. At 3 and 6 weeks of ages, four birds from each pen were randomly selected for blood collection via wing vein for the measurements of serum traits, antibody titers, and peripheral blood blastogenesis. Antibody titers of control serums were collected from four birds at day-old age. At the end of experiment, 2 of each male and female birds in each pen were randomly selected and were sacrificed after 12 h fasting to collect left tibia bone for bone trait measurement.

Table 1. Formula and nutrient composition of basal diets

	Starter (0-3 weeks)	Finisher (3-6 weeks)
Ingredients (%)		
Corn	50.79	59.57
Soybean meal, CP 44%	36.78	31.11
Fish meal	4.00	2.00
Soybean oil	5.80	4.62
DL-Methionine	0.15	0.07
Choline-chloride, 60%	0.02	0.05
Dicalcium phosphate	0.84	0.76
Limestone, pulverized	1.07	1.27
Salt	0.30	0.30
Vitamin premix[a]	0.10	0.10
Trace mineral premix[b]	0.10	0.10
Coccidostat[c]	0.05	0.05
Calculated nutrient composition		
Metabolizable energy (kcal/kg)	3,200	3,200
Crude protein (%)	23.00	20.00
Methionine+Cystine (%)	0.90	0.73
Lysine (%)	1.30	1.08
Calcium (%)	1.00	0.90
Nonphytate phosphorus (%)	0.45	0.35
Analyzed nutrient composition		
Chromium (ppm)	4.83	3.89

[a] Supplemented per kg diet: vitamin A, 8,000 IU; vitamin D_3, 1,200 IU; vitamin E, 40 IU; vitamin K_3, 4 mg; vitamin B_2, 8 mg; pantothenic acid, 24 mg; niacin, 80 mg; vitamin B_{12}, 40 μg, and choline-HCl, 700 mg.
[b] Supplemented per kg diet: Cu, 20 mg; Zn, 100 mg; Fe, 140 mg; Mn, 4 mg; Se, 0.1 mg and I, 0.2 mg.
[c] Supplemented per kg diet: Maduramicin ammonium 5 mg.

Experiment 2 : Two hundred eighty-eight broilers were randomly allocated to each treatment. Starting diets were supplemented with 0 (control), 200, 400 or 800 ppb CrPic. Basal diet without CrPic supplement was used for all finishing periods. Each treatment contained 2 pens with 36 birds each. At ages of 4 and 14 days, all birds were immunized via nasal with ND (B1 strain) live vaccine. Basal diets, management and measurements were the same as in Exp. 1.

Measurements

Bone breaking strength was determined by the modification of Rowland et al. (1968). Fresh tibia was boiled for 5 min to remove muscle and cartilage, soaked in ethanol and ether for 48 h each, and then dried at 105°C for 24 h. The force required to break the tibia was determined using a rheometer (Stable Micro System, TA-HD, UK). Each bone was supported at both ends with 65 mm apart in the middle, and was pressed decently at the rate of 0.5 mm/sec.

Blood samples were coagulated at 4°C overnight, centrifuged at 2,000×g for 10 min to collect the serum. Glucose, alkaline phosphatase (ALP), inorganic phosphorus, triglyceride, total cholesterol, and HDL cholesterol were analyzed by an automated clinical chemistry analyzer (Hitachi 7150, Japan). Serum calcium was determined by atomic absorption spectrophotometer (Analyst 100, Perkin-Elmer). Serum antibodies against ND and IB were assayed by hemagglutination inhibition and enzyme linked immunosorbent assay (ELISA) (ProFLOK, Kirkegaard and Perry Laboratories Inc., USA), respectively. Peripheral blood blastogenesis was measured according to Lee (1978). Briefly, whole blood was diluted 1/50 and incubated in RPMI 1640 medium supplemented with 5% fetal bovine serum (Gibco BLR, Life Technologies, USA). Concanavalin A (Con A, Sigma Chemical Co., USA) at 12 μg/mL was used as a T cell mitogen. After incubation at 41°C in 5% CO_2 for 56 h, 1 μCi of ^{3}H-thymidine (Amersham Pharmacia Biotech., Sweden) was added. Cells were incubated for further 18 h, and were then harvested in glass filter. ^{3}H-thymidine incorporation into cells was measured by liquid scintillation counter (Parkard, USA). Blastogenesis activity was calculated as the cpm of sample with Con A stimulated divided by the cpm of sample without Con A stimulated.

Statistical analysis

Experimental data were analyzed using SAS statistical program (SAS, 1999). General linear model was used to analyze variance, and Duncan's multiple range test was used to determine the treatment differences.

RESULTS

Experiment 1

Feed efficiency was significantly ($p<0.05$) improved in pooled group of Cr supplemented during 0 to 3 weeks of age (Table 2). Other growth performance or bone trait was not affected by adding Cr in both starting and finishing diets during 6 weeks experimental period. Results of serum traits are presented in Table 3. Total and HDL cholesterols were significantly higher ($p<0.05$) in serum from 3 week old broilers fed 400 or 800 ppb Cr as compared with those from control or 200 ppb Cr. At 6 weeks of age, pooled Cr added group had higher levels of total and HDL cholesterols as compared to the control. Serum glucose, ALP, inorganic phosphorus, calcium, and triglyceride were not affected by the supplement of Cr in the diets.

Table 4 lists the effects of CrPic supplementation on antibody titers and blastogenesis activity of broilers. Antibody against IB was significantly ($p<0.05$) higher in 6 week old broiler fed 400 ppb Cr compared with control or 200 ppb. However, Antibody against ND and blastogenesis activity of peripheral blood were not affected by the addition of Cr in the diets.

Experiment 2

The growth performance was not affected in broilers fed diets with different levels of Cr supplement during 0 to 3 weeks of age, and then fed the finishing diet without Cr supplement (Table 5). Bone trait was not affected either. Table 6 presents serum traits of broilers fed different amounts of Cr ALP activity at 6 weeks of age was significantly ($p<0.05$) lower in 400 ppb Cr treatment, compared with control or 200 ppb. Inorganic phosphorus was also significantly lower ($p<0.05$) in pooled Cr added group compared with control at 3 week old, however, the difference did not extent to 6 weeks of age. Other serum traits were not affected by the addition of Cr in the diets. Results of anti-ND titer and blood blastogenesis activity are listed in Table 7. No significant difference was found in the treatment.

DISCUSSION

During 0 to 3 weeks of age, Cr supplemented group improved feed efficiency in Exp. 1, and tended to decrease feed intake in Exp. 2. Improving feed efficiency in broiler has been reported for diets supplemented with CrPic (Sands and Smith, 1999) or yeast Cr (Hossain et al., 1998). However, the effect of Cr on feed intake was not consistent. Most researches found no influence on feed consumption with Cr supplement (Kim et al., 1996a; Kim et al., 1996b; Sands and Smith, 1999), however, supplementation of 1,600 ppb Cr was reported to increase feed intake (Lien et al., 1999). The effect of dietary CrPic supplement on feed consumption of broiler needs further investigation.

Effect of Cr on calcium metabolism was profiled in this experiment. Active osteoblast is secrets ALP into blood (Kaneko, 1980). Consequently, the serum phosphate may increase that decreases serum calcium level, and the bone breaking strength may increase. The ALP activity in serum from 3 week old broilers were all higher than those from 6 weeks, both in Exp. 1 and 2, indicating more active bone growth at younger age. At 3 weeks of age, pooled Cr added group in Exp. 1 had significantly ($p<0.05$) lower ALP, and the trend ($p<0.09$) of higher calcium. The relationship of lower phosphate with dietary Cr added was also supported in Exp. 2. With continued supplement of Cr in Exp. 1, the ALP activity seemed to maintain higher compared with control at 6 weeks of age, and the bone breaking strength increased 1.40 kg with 400 ppb Cr treatment (Table 2).

Table 2. Effects of supplemental chromium picolinate on growth performance of broilers (Exp. 1)

	Control (C)	Cr added (ppb)			Pooled	Significance
		200	400	800	SE	C vs Cr
Initial weight (g)	41.91	41.34	41.88	40.93	0.25	NS
Final weight (g)	2,218.6	2,245.7	2,248.9	2,232.5	34.62	NS
Daily gain (g/day)						
0-3 wk	34.17	34.99	35.16	34.76	0.86	NS
3-6 wk	69.48	69.78	69.94	69.60	1.17	NS
0-6 wk	51.83	52.48	52.55	52.18	0.82	NS
Feed intake (g/day)						
0-3 wk	44.52	45.16	45.07	44.44	1.04	NS
3-6 wk	135.64	134.48	135.58	135.07	3.39	NS
0-6 wk	90.08	89.82	90.32	89.76	2.13	NS
Feed/gain						
0–3 wk	1.30	1.29	1.28	1.28	0.01	$p<0.05$
3-6 wk	1.96	1.93	1.94	1.94	0.03	NS
0-6 wk	1.74	1.71	1.72	1.72	0.02	NS
Bone breaking strength (kg)						
6 wk	15.73	16.05	17.13	16.57	1.68	NS

NS: Not significant.

Table 3. Effects of supplemental chromium picolinate on serum traits of broilers (Exp. 1)

	Control (C)	Cr added (ppb)			Pooled	Significance
		200	400	800	SE	C vs Cr
Glucose (mg/dL)						
3 wk	222.44	219.50	215.31	233.38	5.30	NS
6 wk	255.08	247.01	250.50	246.13	4.22	NS
Alkaline phosphatase (IU/mL)						
3 wk	11.94	11.52	9.71	10.18	1.20	p<0.05
6 wk	4.14	6.49	5.47	5.89	0.82	p<0.10
Inorganic phosphorus (mg/dL)						
3 wk	6.28	6.17	5.84	6.43	0.25	NS
6 wk	6.36	6.52	6.44	6.37	0.17	NS
Calcium (mg/dL)						
3 wk	11.46	11.87	11.58	12.20	0.19	p<0.09
6 wk	12.31	12.11	12.16	12.16	0.46	NS
Triglyceride (mg/dL)						
3 wk	22.44	22.13	22.38	23.63	1.59	NS
6 wk	40.79	36.90	34.88	36.75	2.63	NS
Total cholesterol (mg/dL)						
3 wk	111.19^{ab}	105.38^{b}	120.88^{a}	119.44^{a}	4.29	NS
6 wk	106.13	121.50	113.31	115.88	4.24	p<0.05
HDL cholesterol (mg/dL)						
3 wk	88.38^{ab}	82.31^{b}	95.94^{a}	94.75^{a}	3.58	NS
6 wk	78.52	89.99	82.25	88.50	3.54	p<0.06

^{a,b} Means within the same row without common superscripts differ significantly (p<0.05).
NS: Not significant.

Table 4. Effects of supplemental chromium picolinate on antibody titers and peripheral blood blastogenesis activity of broilers (Exp. 1)

	Control (C)	Cr added (ppb)			Pooled	Significance
		200	400	800	SE	C vs Cr
Newcastle disease (log_2 titer)						
0 wk	5.17					
3 wk	6.44	5.94	6.31	6.19	0.43	NS
6 wk	7.67	8.43	8.40	8.56	0.34	p<0.06
Infectious bronchitis (ELISA titer)						
0 wk	4,124					
3 wk	61	287	-	139	123	NS
6 wk	929^{bc}	593^{c}	1,414^{a}	1,262^{ab}	246	NS
Blastogenesis activity[1]						
3 wk	13.96	23.64	19.65	18.16	7.63	NS
6 wk	18.01	30.61	27.93	25.62	5.72	NS

^{a,b,c} Means within the same row without common superscripts differ significantly (p<0.05).
NS: Not significant.
[1] Values are expressed as (cpm of mitogen stimulated / cpm of non-stimulated)×100%.

Without further Cr supplement as in Exp. 2, the ALP activity in pooled Cr added group was significantly (p<0.05) lower than control, and the increase of bone breaking strength was not observed. Hermann et al. (1997) also found that femur strength of quail was increased with high Cr and low copper in the diet. However, Guo et al. (1999) reported that broilers fed with 0.4, 2.0 or 10 ppm Cr, either in the forms of $CrCl_3$ or yeast Cr, from 3 to 6 weeks of age did not affect ALP activity. More data are needed to clear out the profile of calcium metabolism as affected by Cr supplement.

Serum glucose and triglyceride were not affected by the supplement of Cr up to 800 ppb in the diet, which were similar to the result of Kim et al. (1996a), however, over 1,600 ppb Cr may decrease both traits in broilers (Kim et al., 1996b; Lien et al., 1999). The increases in total and HDL cholesterols were consistent in Exp. 1 and 2, indicating that Cr may influence the cholesterol metabolism in broiler.

Dietary Cr supplement tended to increase anti-ND titer, and supplement of 400 ppb Cr resulted in significant increase of anti-IB titer. Elevated anti-ND titer was reported in broilers under normal environment during 0 to 3 weeks of age (Luo et al., 1999), or under heat stress during 3 to 6 weeks of age with supplement of 2 or 10 ppm Cr, either in

Table 5. Effects of supplemental chromium picolinate on growth performance of broilers (Exp. 2)

	Control (C)	Cr added (ppb)			Pooled	Significance
		200	400	800	SE	C vs Cr
Initial weight (g)	46.78	45.75	44.96	45.67	0.32	NS
Final weight (g)	2,458.4	2,360.9	2,400.0	2,423.5	42.75	NS
Daily gain (g/day)						
0-3 wk	34.76	33.97	33.83	34.15	0.85	NS
3-6 wk	80.09	76.27	78.31	79.07	1.34	NS
0-6 wk	57.42	55.12	56.07	56.61	1.02	NS
Feed intake (g/day)						
0-3 wk	48.66	47.86	45.94	46.84	0.72	$p<0.10$
3-6 wk	151.94	134.61	145.11	151.62	3.83	NS
0-6 wk	100.30	91.23	95.53	99.23	1.83	$p<0.07$
Feed/gain						
0–3 wk	1.40	1.41	1.36	1.37	0.03	NS
3-6 wk	1.90	1.77	1.85	1.92	0.07	NS
0-6 wk	1.75	1.66	1.70	1.75	0.05	NS
Bone breaking strength (kg)						
6 wk	13.31	12.52	13.64	11.93	0.89	NS

NS: Not significant.

Table 6. Effects of supplemental chromium picolinate on serum traits of broilers (Exp. 2)

	Control (C)	Cr added (ppb)			Pooled	Significance
		200	400	800	SE	C vs Cr
Glucose (mg/dL)						
3 wk	245.13	248.50	258.13	237.25	5.88	NS
6 wk	241.13	246.13	245.50	241.50	5.59	NS
Alkaline phosphatase (IU/mL)						
3 wk	11.83	10.63	12.06	10.99	1.40	NS
6 wk	7.15^{a}	6.62^{a}	3.29^{b}	5.17^{ab}	0.87	$p<0.05$
Inorganic phosphorus (mg/dL)						
3 wk	6.59	6.05	6.37	5.72	0.23	$p<0.05$
6 wk	7.47	7.19	7.88	7.43	0.39	NS
Calcium (mg/dL)						
3 wk	13.03	13.60	13.46	13.63	0.44	NS
6 wk	13.23	13.55	12.63	12.45	0.30	NS
Triglyceride (mg/dL)						
3 wk	45.50	55.25	45.38	45.75	4.50	NS
6 wk	63.13	57.13	53.50	51.13	5.17	NS
Total cholesterol (mg/dL)						
3 wk	120.00	136.00	130.00	130.75	8.99	NS
6 wk	134.75	132.63	130.00	129.88	5.32	NS
HDL cholesterol (mg/dL)						
3 wk	57.50	64.75	64.25	64.38	3.21	$p<0.07$
6 wk	60.63	62.13	63.38	62.75	3.01	NS

[ab] Means within the same row without common superscripts differ significantly ($p<0.05$).

NS: Not significant.

the form of $CrCl_3$ or yeast (Guo et al., 1999). Improved immune responses against virulent antigens were also reported in weanling pig (Lee et al., 1997) or calf (Burton et al., 1994; Chang et al., 1996) with dietary Cr supplement. Blastogenesis activity was raised up to 100% in some Cr treated samples, but not significantly different due to high variation. No significant difference in mortality was observed in this experiment, though supportive data were reported (Kim et al., 1996a; Hossain et al., 1998).

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Table 7. Effects of supplemental chromium picolinate on antibody titer and peripheral blood blastogenesis activity of broilers (Exp. 2)

	Control (C)	Cr added (ppb)			Pooled	Significance
		200	400	800	SE	C vs Cr
Newcastle disease (log_2 titer)						
0 wk	3.25					
3 wk	3.50	4.25	4.00	3.57	0.49	NS
6 wk	2.75	2.25	3.00	3.38	0.33	NS
Blastogenesis activity[1]						
3 wk	20.63	10.30	42.27	41.77	7.90	NS
6 wk	20.39	27.37	22.13	31.36	4.06	NS

NS: Not significant.

[1] Blastogenesis activity as Table 4 footnote.

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