EFFECTS OF DIETARY LEVELS OF CHROMIUM PICOLINATE ON GROWTH PERFORMANCE, CARCASS QUALITY AND SERUM TRAITS IN BROILER CHICKS

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Summary

An experiment was conducted to evaluate the effects of dietary levels of chromium in the form of chromium picolinate on growth performance, nutrient utilizability, carcass composition, serum traits, and *in vitro* lipolysis and lipogenesis in adipose tissues of Arbor Acre broiler chicks. Experimental diets containing six different levels of chromium (0, 100, 200, 400, 600 and 800 ppb) were fed for 6 weeks. Individual treatment had six replicates of eight birds each and their average initial weight was 59.2 g. Dietary addition of chromium did not affect growth performance and nutrient utilizability. However, mortality appeared to be reduced with addition of chromium to the diet. It was obvious that chromium supplementation significantly decreased serum cholesterol and increased serum HDL cholesterol ($p < 0.05$), but serum insulin, glucose, triglyceride and non-esterified fatty acid concentrations were inconsistent among dietary supplementation levels of chromium. The *in vitro* lipolysis and lipogenesis in adipose tissues were significantly influenced by dietary addition of chromium ($p < 0.05$). Chicks fed diets containing 200 or 400 ppb chromium showed the highest protein content and the lowest fat content in their carcass.

(**Key Words** : Chromium Picolinate, Mortality, Cholesterol, Insulin, Lipolysis, Lipogenesis, Carcass Composition, Broiler Chicks)

Introduction

Chromium^{+3} is generally regarded as an essential micronutrient capable of controlling glucose and lipid metabolism. But most feedstuffs are deficient in chromium, and moreover its digestion and utilization in the digestive tract are low (Schroeder, 1971). The absorption and utilization of chromium may be dependent upon its association with an organic molecule (Mertz, 1969; Votava et al., 1973; Offenbacher and Pi-Sunyer, 1980). One of the most absorbable and effective chromium compounds in the digestive tract is known as chromium picolinate.

Abraham et al. (1980) reported that chromium reduced cholesterol-induced atherosclerotic plaques in rabbits. Samsell and Spears (1989) indicated that chromium supplementation lowered fasting plasma glucose concentrations in lambs fed a low fiber diet. Chang et al. (1992) suggested that calves fed corn silage following market-transit stress may be deficient in chromium, and supplemental chromium decreased serum cortisol and improved immune status. Currently, the NRC (1994) does not recommend dietary chromium supplementation in poultry diet, even though Jensen et al. (1978) and Steele et al. (1977) have determined that chromium is biologically active in poultry and pigs. Poultry diets primarily composed of ingredients from plant origin may be subjected to chromium deficiency.

In recent studies by Page et al. (1993), chromium picolinate decreased backfat thickness and serum cholesterol in pigs. However, further information on effects of chromium picolinate on nutrient digestibility, carcass quality and mortality is needed.

Therefore, the purposes of this investigation were; 1) to determine the optimum levels of dietary chromium to maximize broiler performance, 2) to investigate the effect

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of varying chromium levels in the diet on the content of serum traits and lipogenic and lipolytic activity in adipose tissues.

Materials and Methods

Experimental design :

Treatments were experimental diets supplemented at 0, 100, 200, 400, 600 and 800 ppb of chromium in the form of chromium picolinate. Arbor Acre broiler chicks were used as experimental subjects. Until reaching to three days of age, experimental animals were raised on commercial diet in the battery with temperature control and then randomly assigned into individual treatment consisting of 6 pens of 8 chicks. Broiler chicks were housed in wire cages during 6 weeks of feeding trial.

Experimental diets :

For starting period (1~3 weeks), birds were fed *ad libitum* a corn-soybean meal based diet containing 23% crude protein, 3,200 ME kcal/kg, and 0, 100, 200, 400, 600 and 800 ppb chromium. For finishing period (4~6 weeks), the corn-soybean meal based diets formulated to contain 20% crude protein, 3,200 ME kcal/kg, and the same levels of chromium as in the starting period were fed. The formula and chemical composition of basal diets of starting and finishing diets are presented in table 1. All other nutrients were included to meet or exceed the nutrient requirement suggested by National Research Council (NRC, 1984).

Feeding trial :

All the chicks were raised in battery cages made of steel wire and housed in a room with 24 hours light and air ventilation. During the 3 day pre-experimental period, broiler chicks were fed on a commercial diet. Experimental diets and drinking water were provided *ad libitum* during the entire experimental period. Body weight and feed intake were recorded weekly on group basis to calculate body weight gain and feed conversion. During the feeding trial, deaths were counted to obtain mortality.

Metabolic trial :

Three chicks at three and six weeks of age were randomly housed in a metabolic cage individually, and experimental diets and water were fed *ad libitum*. After four days of adaptation, total excreta were collected four times a day for the three consecutive days by avoiding the contamination of foreign materials such as feed, feathers and scales. Samples were pooled and dried in an air-forced drying oven at 60℃ for 72 hours to gain constant dry weight. All the samples prepared in this way were ground with 1 mm mesh Wiley mill for chemical analysis.

TABLE 1. FORMULA AND CHEMICAL COMPOSITION OF THE BASAL DIETS FOR TRIAL

Nutrient levels	Starter (1-3 weeks)	Finisher (4-6 weeks)
Ingredient (%)		
Corn, yellow	54.81	63.20
Soybean meal	27.44	22.80
Fish meal	5.00	3.60
Corn gluten meal	5.00	3.70
Tallow	5.00	4.00
Limestone	1.92	1.90
Salt	0.30	0.30
Vit.-min. mix.[1]	0.45	0.45
Methionine	0.03	0.05
Antibiotics	0.05	0.05
Total	100.00	100.00
Chemical composition[2]		
ME (kcal/kg)	3,200	3,205
Crude protein (%)	23.05	20.00
Lysine (%)	1.23	1.03
Methionine (%)	0.50	0.41
Calcium (%)	1.00	0.91
Avail. Phosphorus (%)	0.40	0.45

[1] Vit.-min. mixture contains followings in 1 kg: vitamin A, 10,000 IU; vitamin D_3, 1,500 IU; vitamin K_3, 5 mg; vitamin E, 15 mg; vitamin B_2, 8 mg; vitamin B_{12}, 0.008 mg; Ca-d-pantothenate, 8 mg; niacin, 25 mg; folic acid, 0.4 mg; biotin, 0.2 mg; choline, 500 mg; pyridoxine, 1 mg; B.H.T., 125 mg; Co, 0.85 mg; I, 1.29 mg; Zn, 100 mg; Mg, 110 mg; Cu, 8.75 mg; Se, 0.15 mg; Fe, 35 mg.

[2] Calculated values.

Carcass analysis :

Six chicks from each treatment were sacrificed by cervical dislocation and then frozen for determination of final carcass composition. Carcass samples were freeze-dried (ISE, Korea), ground and analyzed by AOAC (1990) methods.

Blood analysis :

Blood samples of three respective chicks from each treatment were drawn into tubes after decapitation and were centrifuged (Hanil, Korea) at 3,000 rpm for 20 minutes. Supernatants (serum) were poured into other sterile tubes for analysis of glucose, insulin, non-esterified fatty acid (NEFA), triglyceride (TG), total cholesterol and high density lipoprotein (HDL) cholesterol.

Chemical analysis :

A proximate composition of experimental diets and excreta were analyzed according to the methods by AOAC (1990). Serum glucose, NEFA, triglyceride, total cholesterol and HDL cholesterol of three chicks per treatment were analyzed by enzymatic kits (Asan Co., Korea), and serum insulin was analyzed with RIA (Radioimmuno Assay) kit (Diagnostic Products Co., USA). All samples were analyzed in duplicate in a single assay to avoid inter-assay variation.

Determination of lipogenic and lipolytic activity :

Measurement of lipogenic activity

Adipose tissues from three chicks at 6 weeks of age were sliced with scissors to be 10~200 mg and were incubated for 120 minutes at 37℃ in 3 ml of medium under a gaseous atmosphere of 5% CO_2. The incubation medium (Krebs-Ringer bicarbonate buffer) also contained 25 mM (N-[2-Hydroxyethyl] piperazine-N'-[2-ethanesulfonic acid]) (HEPES), 5.0 mM glucose, 3% bovine serum albumin and 0.5 μCi [^{14}C] glucose. Incubations were terminated by placing vials on ice. After taking out a tissue slices from medium, total lipids were extracted by the method of Dole and Meintz (1960). After the extracts were dried, radioactivity incorporated into total lipid slices was determined by a liquid scintillation counter (Model : LC 100C).

Measurement of lipolytic activity :

Lipolytic activity was measured with Krebs-Ringer bicarbonate buffer (KRB) with one-half the indicated Ca^{++} containing 4% fatty-acid-pool Fraction V bovine serum albumin and 5.56 mM glucose. Incubations were terminated by placing vials on ice. The medium was filtered through cheese cloth to remove the tissue and was stored at −20℃ until analysis. Non-esterified fatty acids in the medium were extracted and titrated according to method by Kelly (1965).

Statistical analysis :

Statistical analysis for the data was carried out by comparing means according to Duncan's multiple range test (Duncan, 1955), using General Linear Model (GLM) Procedure of SAS (1985) package program.

Results and Discussion

Growth performance

Table 2 summarized body weight gain, feed intake and feed conversion of broilers fed diets supplemented with chromium at varying levels during the entire experimental period (1-6 weeks). Body weight gain was found to be similar among control and chromium supplemented groups. Feed intake was significantly different among treatments ($p < 0.05$); chicks received diets containing 200 ppb of chromium showed the highest feed intake among treatments.

TABLE 2. EFFECTS OF DIETART CHROMIUM LEVELS ON BODY WEIGHT GAIN, FEED INTAKE AND FEED CONVERSION IN BROILER CHICKS (1-6 WEEKS)

Treatment Cr[1] (ppb)	Initial body weight (g/bird)	Final body weight (g/bird)	Body weight gain (g/bird)	Feed intake (g/bird)	Feed conversion	Mortality[3] (%)
0	59.4 ± 0.35[2]	2,123.4 ± 59.5	2,063.9 ± 59.2	4,008.7 ± 89.2[b]	1.94 ± 0.02	12.50
100	58.9 ± 0.11	2,064.6 ± 56.3	2,005.8 ± 56.3	4,015.6 ± 116.8[b]	2.00 ± 0.01	6.25
200	59.4 ± 0.28	2,110.5 ± 72.7	2,051.1 ± 72.4	4,153.3 ± 149.6[a]	2.02 ± 0.10	4.15
400	59.3 ± 0.15	2,135.7 ± 54.7	2,076.3 ± 54.7	4,014.1 ± 108.3[b]	1.93 ± 0.04	4.15
600	58.9 ± 0.25	2,160.1 ± 37.3	2,057.3 ± 35.7	4,134.0 ± 168.2[ab]	2.00 ± 0.05	2.10
800	59.5 ± 0.19	2,121.5 ± 49.8	2,062.0 ± 49.8	4,010.7 ± 62.4[b]	1.95 ± 0.03	0.00

[1] Chromium was added at 0, 100, 200, 400, 600 and 800 ppb as chromium picolinate.
[2] Values are mean ± SE; 6 pens/treatment.
[3] Calculated based on treatment unit.
[ab] Means with different superscripts within the same column are significantly different ($p<0.05$).

Feed conversion was not significantly different among treatments. Steele and Rosebrough (1979) reported that chromium from $CrCl_3 \cdot 6H_2O$ improved the growth rate of turkey poults. Chromium from $CrCl_3 \cdot 6H_2O$ also has been shown to stimulate the growth of rats fed a low protein diet (Mertz and Roginski, 1969). According to

Ward et al. (1993), dietary inclusion of chromium (0, 200, or 400 μg Cr/kg diet) as chromium picolinate did not affect body weight gain, feed intake, feed conversion of growing broiler chicks. These reports were consistent with our results except for feed intake response.

Picolinic acid was demonstrated to have no relationship with growth performance of pigs (Page et al., 1991). Hill et al. (1987) also observed no effect of picolinic acid on weight gain, feed efficiency, or zinc status of weanling pigs.

Mortality

The highest mortality was observed in chicks fed control diet, and the lowest mortality was found in chicks fed 800 ppb chromium supplemented group. Schroeder et al. (1965) reported that chromium reduced mortality from an epidemic of pneumonia in female rats. Mertz and Roginski (1969) also found that chromium supplementation reduced mortality in stressed rats subjected to the additional stress of acute haemorrhage. Mowat et al. (1993) reported that Cr^{+3} (as a high-chromium yeast supplement) reduced circulating cortisol and alleviated shipping stress morbidity of calves during a post-stress feeding trial. This might imply, to some extent, chromium could enhance immune ability of animals under various stress and diseases.

Nutrient utilizability

The effects of chromium on the utilizability of the dry matter, crude protein, crude fat and crude ash are summarized in table 3. In starting period (1-3 weeks), the utilizability of dry matter and crude protein showed no significant differences among treatments. Crude fat and crude ash utilizability was affected by levels of chromium in the diet ($p < 0.05$). Beyond 200 ppb chromium, crude fat utilizability was significantly higher than control diet and 100 ppb chromium. Crude ash utilizability was more increased in chicks fed diets with both 200 and 600 ppb of chromium than control.

In finishing period (4-6 weeks), the utilizability of dry matter and crude protein were not affected by levels of chromium. The utilizability of crude fat and crude ash showed significant differences among treatments ($p < 0.05$); at 200 ppb chromium, crude fat utilizability was lower than control and 100 ppb chromium level, whereas crude ash utilizability was lower in control and 600 ppb chromium than 100 ppb chromium.

TABLE 3. EFFECT OF DIETARY CHROMIUM LEVELS ON THE NUTRIENT UTILIZATION OF BROILER CHICKS (%)

Treatment Cr[1](ppb)	Dry matter	Crude protein	Crude fat	Crude ash
		At 3 weeks of age		
0	75.0±2.95[2]	64.5±2.05	87.5±1.49[b]	28.7±4.65[ab]
100	76.3±1.25	64.4±3.64	89.9±1.06[ab]	21.4±2.56[b]
200	78.6±1.59	70.7±3.07	91.8±0.86[a]	34.4±3.27[a]
400	77.7±1.02	68.8±2.01	91.4±0.99[a]	27.2±3.58[ab]
600	79.5±1.27	68.8±2.83	92.4±0.53[a]	34.1±3.35[a]
800	78.1±1.08	67.5±2.25	92.5±0.16[a]	29.2±1.71[ab]
		At 6 weeks of age		
0	84.2±2.01	78.0±4.07	96.5±1.12[a]	35.6±5.12[b]
100	84.8±1.00	75.2±2.79	96.9±0.41[a]	45.9±4.14[a]
200	84.3±0.85	77.6±1.55	93.2±0.69[b]	37.9±2.49[ab]
400	83.7±1.11	74.6±3.66	95.8±0.73[ab]	40.8±1.66[ab]
600	83.5±0.72	74.7±2.76	95.0±0.57[ab]	34.2±2.33[b]
800	84.9±1.21	78.8±4.30	95.0±1.30[ab]	42.2±2.37[ab]

[1] Chromium was added at 0, 100, 200, 400, 600 and 800 ppb as chromium picolinate.
[2] Values are mean ± SE; 3 chicks/treatment.
[ab] Means with different superscripts within the same column are significantly different ($p<0.05$).

Total cholesterol and HDL cholesterol in serum

The effects of dietary chromium on the content of total cholesterol and HDL cholesterol in serum of broiler chicks are presented in figure 1.

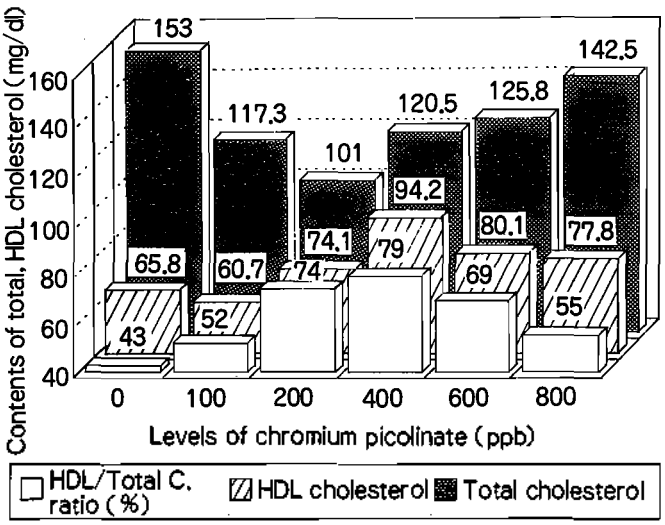

Figure 1. Effects of chromium picolinate levels on serum total cholesterol, HDL-cholesterol concentrations in broiler chicks.

Total cholesterol content in serum was the highest in chicks fed a control diet but the lowest in chicks fed diets supplemented at 200 ppb chromium ($p < 0.05$). Serum cholesterol was reduced in chicks fed diets with chromium.

HDL content in serum was significantly different among treatments ($p < 0.05$). Serum HDL concentration was increased with chromium addition, except for 100 ppb levels of chromium.

The HDL /total cholesterol ratio was also affected by dietary chromium addition ($p < 0.05$). The HDL /total cholesterol ratio was increased as dietary chromium picolinate increased from 0 to 400 ppb chromium and then decreased as dietary chromium picolinate increased from 400 to 800 ppb of chromium. However, chromium supplemented group showed higher HDL /total cholesterol ratio than chicks fed on control diets.

Several studies have shown that chromium supplementation decreased total cholesterol and increased HDL cholesterol (Riales and Albrink, 1981; Mossop, 1983). In monogastric animals supplemented with chromium, reduced serum total cholesterol concentration was one of the most frequently reported responses in lipid metabolism (Mertz, 1993).

Rats fed a low-chromium diet exhibited increased serum cholesterol, aortic lipids and plaque formation (Schroeder and Balassa, 1965), whereas Abraham et al. (1980) provided evidence that chromium not only decreased cholesterol accumulation in rabbits, but that it also increased the removal rate of cholesterol already deposited in the aorta.

Glucose, insulin, triglyceride and non-esterified fatty acid in serum

Table 4 showed serum glucose, insulin, triglyceride and non-esterified fatty acid contents of broiler chicks fed diets containing various levels of chromium. Glucose content in serum was the lowest at both 400 and 600 ppb of chromium ($p < 0.05$).

Insulin content in serum was higher in broiler chicks fed diets supplemented with chromium than in those fed control diet ($p < 0.05$).

Serum triglyceride and non-esterified fatty acid concentrations were not significantly different among treatments.

TABLE 4. EFFECTS OF DIETART CHROMIUM LEVELS ON SERUM GLUCOSE, INSULIN, TRIGLYCERIDE, NEFA CONCENTRATIONS IN BROILER CHICKS

Treatment Cr[1] (ppb)	Glucose (mg/dl)	Insulin (μIU/ml)	Triglycerides (mg/dl)	NEFA[2] (μEq/l)
0	231.4 ± 3.89^{ab}	4.22 ± 0.33^{d}	79.1 ± 13.59	756.1 ± 74.5
100	233.9 ± 5.50^{ab}	6.40 ± 0.77^{b}	77.8 ± 10.29	787.4 ± 40.2
200	230.8 ± 4.64^{ab}	6.79 ± 0.77^{ab}	70.8 ± 6.51	841.2 ± 55.7
400	221.2 ± 2.81^{b}	7.02 ± 0.80^{a}	75.6 ± 4.65	884.5 ± 68.1
600	222.9 ± 7.82^{b}	7.01 ± 0.72^{ab}	78.4 ± 3.90	790.0 ± 85.5
800	240.4 ± 3.34^{a}	5.10 ± 0.27^{c}	81.8 ± 10.95	789.2 ± 56.1

[1] Chromium was added at 0, 100, 200, 400, 600 and 800 ppb as chromium picolinate.
[2] Non-esterified fatty acid.
^{a,b,c} Means with different superscripts within the same column are significantly different ($p<0.05$).

Carcass composition

The effect of dietary chromium levels on carcass composition are presented in figure 2. Crude protein content of carcass was significantly influenced by levels of chromium in diets ($p < 0.05$). Percentage of carcass crude protein was slightly higher in 100, 200 and 400 ppb chromium supplemented groups than in control group.

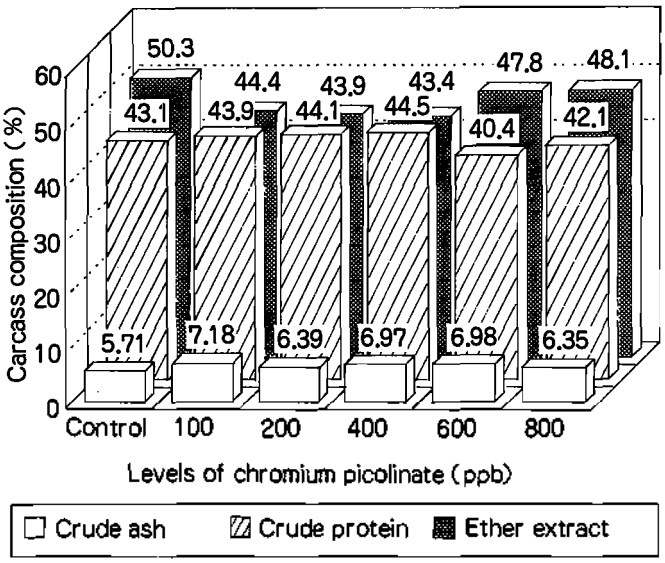

Figure 2. Effects of feeding chromium picolinate on the carcass composition of broiler chicks.

Crude fat composition in carcass was lower in 100, 200 and 400 ppb chromium supplemented group than control and other chromium supplemented groups. Percentage of carcass crude ash was affected by treatments ($p < 0.05$). Crude ash percentage was higher in chromium supplemented groups than control group.

Chromium picolinate has been reported to increase both lean body mass and body fat loss in weight-training men (Evans, 1989). 200 ppb of chromium as chromium picolinate was regarded to be optimum level in diet to have favorable effects on muscling and fat of growing-finishing pigs (Page et al., 1993). Considering that body composition could be altered by a proportional increase in net protein synthesis and (or) by a reduction in lipogenesis. It is plausible that dietary chromium addition should have a positive effect on carcass characterics of broiler.

Lipolytic and lipogenic activity

The effects of dietary chromium picolinate on lipolysis and lipogenesis in adipose tissue *in vitro* are presented in table 5. Lipolysis and lipogenesis in adipose tissues were significantly different among treatments ($p < 0.05$).

TABLE 5. EFFECT OF DIETARY CHROMIUM LEVELS ON LIPOLYTIC AND LIPOGENIC ACTIVITY IN ADIPOSE TISSUES

Treatments Cr[1] (ppb)	Adipose tissue	
	Lipogenic activity[2] (dpm/mg)	Lipolytic activity[3] (dpm/mg)
0	75.2 ± 1.86[a]	38.7 ± 2.16[b]
100	57.2 ± 4.44[bc]	57.6 ± 4.39[a]
200	54.6 ± 3.53[c]	61.1 ± 3.04[a]
400	58.0 ± 3.25[bc]	64.5 ± 2.20[a]
600	65.4 ± 1.70[b]	56.1 ± 2.75[a]
800	64.1 ± 2.24[b]	63.2 ± 2.35[a]

[1] Chromium was added at 0, 100, 200, 400, 600 and 800 ppb as chromium picolinate.
[2] nmol glucose incorporated into total lipids/mg.
[3] neq non-esterified fatty acid (NEFA) released/mg.
[a,b,c] Means with different superscripts within the same column are significantly different ($p<0.05$).

Non-esterified fatty acid uptake by adipose tissues cultured with chromium picolinate was decreased, which means that lipogenesis was blocked while lipolysis was stimulated, compared with control whereas glucose incorporation into adipose tissues cultured with chromium picolinate was increased, which means that protein synthesis was stimulated.

Consequently, decrease in carcass fat may result from fat synthesis inhibition, fat mobilization or a combination of two.

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