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Effects of Supplementary Copper Chelates in the Form of Methionine, Chitosan and Yeast on the Performance of Broilers*

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ABSTRACT : An experiment was conducted to investigate the effects of supplemental copper (Cu) chelates (methionine, chitosan and yeast) on the performance, nutrient digestibility, serum IgG level, gizzard erosion, Cu content in the liver and excreta and the level of total cholesterol in breast muscle and serum of broiler chickens. Two hundred and forty hatched broiler chickens (Ross® 208) were assigned to 4 treatments: control, 100 ppm Cu in methionine chelate (Met-Cu), 100 ppm Cu in chitosan chelate (Chitosan-Cu) and 100 ppm Cu in yeast chelate (Yeast-Cu). Each treatment had six replicates of 10 (5 males+5 females) birds each. Weight gain and feed intake tended to be higher in Cu chelate treatments than the control; weight gain was significantly higher in the Met-Cu chelate treatment and feed intake was significantly higher in the Yeast -Cu chelate treatment than the control ($p<0.05$). Feed/gain was significantly different between treatments in which Met-Cu was lowest followed by the control, Chitosan-Cu and Yeast-Cu. DM availability was increased by Cu chelates among which chitosan-Cu showed the highest DM availability. Cu chelates supplementation tended to increase gizzard erosion index, and Cu content in the liver was highest in the Met-Cu treatment. Supplementation of Cu chelates tended to decrease total cholesterol level in breast muscle and serum but tended to increase the level of HDL in serum. It was concluded that dietary supplementation of 100 ppm Cu in chelates increased weight gain, feed intake and DM availability. Met-Cu was more effective than Chitosan-Cu or Yeast-Cu in improving productivity of broiler chickens. (**Key Words :** Chelates, Methionine-Cu, Chitosan-Cu, Yeast-Cu, Broiler)

INTRODUCTION

Copper is a component of various intracellular and extracellular enzymes such as cytochrome oxidase, lysyl oxidase, ceruloplasmin and superoxide dismutase (Klasing, 1998). Cu supplementation at the level of 125-250 ppm improved growth rate and feed conversion ratio in broilers (Baker et al., 1991; Paik, 2001) and pigs (Roof and Mahan, 1982; Cromwell et al., 1989). Pesti and Bakalli (1996) reported that 125-250 ppm of Cu from copper sulfate penthahydrate and citrate copper supplementation reduced cholesterol levels of serum and breast muscle. In other experiment (Chowdhury et al., 2004), however, methionine-Cu linearly increased serum cholesterol level but decreased serum triglycerides level.

It was also observed that supplementation of such high levels of Cu resulted in high level Cu excretion through feces (Paik et al., 1999). Presence of high concentration of Cu in feces inhibits normal fermentation process and its accumulation in the soil causes environmental concern. Copper in the form of chelates, complexes or proteinates was considered for use in animal diet as the alternatives to inorganic sources for solution of these problems. This is probably due to better absorption, which enhances use efficiency (Downs et al., 2000; Yu et al., 2000; Guo et al., 2001).

Metal-amino acid chelates or complexes furnish trace elements that are more efficiently absorbed from gut than those provided by inorganic salts (Wedekind et al., 1992; Aoyagi and Baker, 993a). They also provide readily bioavailable amino acids (Aoyagi and Baker,1993b).

Chitosan was studied as a new physiological material because of its effects against cancer, bacteria mold, hypertension and hypercholesterolemia (Muzzarelli et al., 1990; Kochkina and Chirkov, 2000). Chitosan is produced by removing acetyl group from chitin which has adhesion capacity over 80% with Cu^{2+} in pH 6 (Choi, 1988).

Yeast has high capacity of converting absorbed

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Table 1. Formula and composition of basal diets

Ingredient	Starter	Finisher
	------------------ % ----------------	
Corn	53.02	58.79
SBM-44	34.60	33.40
Corn gluten	4.79	
Animal fat	4.00	4.89
Calphos-18	1.75	1.22
Limestone	0.80	0.97
Salt	0.38	0.26
D,L-methionine (98%)	0.26	0.17
Broiler premix[1]	0.25	0.20
Lysine-HCl (78%)	0.14	0.10
Total	100	100
Additional amounts of each chelates (g/ kg diet)		
Methionine-Cu chelate	0.59	
Chitosan-Cu chelate	1.43	
yeast-Cu chelate	5	
Composition[2]		
ME (kcal/kg)	3,050	3,100
Crude protein (%)	23.03	20.00
Lysine (%)	1.10	1.00
Met+cys (%)	0.86	0.70
Calcium (%)	1.00	0.90
Nonphytate P (%)	0.45	0.35
Total P (%)	0.73	0.62
Copper (mg/kg)	16.55	14.62

[1] Contains per kg premix: vitamin A, 10,000,000 IU; vitamin D_3, 2,500,000 IU; vitamin E, 25,000 mg; vitamin K_3, 1,700 mg; vitamin B_1, 2,000 mg; vitamin B_2, 5,000 mg; vitamin B_6, 3,000 mg; vitamin B_{12}, 16,000 μg; biotin, 84,000 μg; niacin, 34,000 mg; pantothenic acid, 9,000 mg; folic acid, 1,000 mg; Zn. 30,000 mg; Mn, 30,000 mg; Fe, 30,000 mg; Cu, 3,000 mg; I, 660 mg and Se, 180 mg.

[2] Calculated values except copper which was assayed with ICP spectrophotometer.

inorganic minerals into organic mineral form and capacity for absorbing Cu in specific (De Rome and Gadd, 1987; Lin and Kosman, 1990).

Present experiment was conducted to determine effects of supplementary Cu in the form of methionine-Cu (Met-Cu), Chitosan-Cu, Yeast-Cu on the performance of broiler chickens and cholesterol level of serum and breast muscle.

MATERIALS AND METHODS

Preparation of Cu chelates

Methionine-Cu chelate(Met-Cu) : Methionine-Cu chelate (Met-Cu) was produced by reacting D,L-methionine and copper sulfate at 2:1 ratio in molecular base (Lim and Paik, 2003).

Chitosan-Cu chelate (Chitosan-Cu) : One kg chitosan was dissolved in 65 L 4.6% acetic acid. Chitosan solution was mixed with 50 L of 5,000 ppm $CuSO_4$ aqueous solution and 1 N NaOH was added to adjust pH to 6.5 for maximum precipitation. Precipitate was separated, dried in an oven at 50°C for 2 days. The precipitate had molecular weight of 10,000 and 7% of Cu (Lee at al., 2001).

Yeast-Cu chelate (Yeast-Cu) : *Saccharomyces cerevisiae* (wild strain Y3) was incubated with 5 mM of $CuSO_4$ in hydrolyzed tapioca media supplemented with yeast extract (Difco laboratory, Detroit, MI., USA) for 1 day (30°C, pH 4). The product had approximately 2% of Cu (Kim et al., 2001).

Birds and diets

Two hundred forty Ross 208 broiler chicks purchased from a commercial supplier were used in this experiment. The chicks were individually weighed and were assigned to 4 dietary treatments. Each treatment consisted of 6 replications in 6 cages (10 birds per cage, 5 males and 5 females each).

Corn-soybean meal based diets (broiler starter and broiler finisher) in mash form were formulated to meet or exceed the nutrient requirements of broiler chickens (NRC, 1994) (Table 1). Four experimental diets were the control and Cu diets supplemented at the level of 100 ppm Cu in the form of Met-Cu, Chitosan-Cu and Yeast-Cu.

Starter diet was fed to chicks from day-old to 3 weeks followed by feeding a finisher diet for an additional 2 weeks. Birds were maintained on a 24 h constant light schedule. Feed and water were offered for consumption *ad libitum* and body weight gain and feed consumption were recorded weekly.

Metabolic trial

At 5th week of feeding, 5 male birds from each treatment were chosen and housed in the individual metabolic cage and colleted total excreta for the last 3 days, after an adjustment period of 4 days.

Collection of blood, liver and gizzard sample

At the termination of the feeding experiment, 2 birds (one male and one female) from each pen or 12 birds per treatment were sacrificed by cervical dislocation for the collection of blood, livers and gizzards.

Ten ml of blood was collected from each bird in a nonheparinized vacutainer tube (Becton Dickinson, Rutherford, NJ, USA) following heart puncture. Samples were centrifuged (Brushless D.C. Motor Centrifuge VS-6,000 CF, Vision Scientific Co. Ltd., Korea) at 3,000 rpm for 15 minutes. Serum was removed and stored at -20°C in Eppendorf tubes until the analyses of serum total cholesterol, high-density lipoprotein (HDL) and triglyceride (TG).

After blood sampling, livers, breast muscle and gizzard were removed. Liver samples were washed in running tap water and dried immediately at 50°C for 4 days in dry oven. The dried samples were cooled at room temperature, ground, bagged and stored again in a refrigerator until chemical

Table 2. Performance of broilers fed experimental diets

Period		Treatments[1]				SEM
		Control	Met-Cu	Chitosan-Cu	Yeast-Cu	
0 to 3 weeks	Feed intake (g)	1,061.6[b]	1,088.6[ab]	1,119.9[a]	1,106.1[ab]	16.60
	Weight gain (g)	666.5[b]	706.2[a]	698.6[a]	693.5[ab]	9.71
	Feed/gain	1.60	1.54	1.60	1.60	0.021
	Mortality (%)	3.33	3.33	5.00	1.67	2.448
4 to 5 weeks	Feed intake (g)	1587.3	1634.2	1644.8	1671.6	26.82
	Weight gain (g)	821.8[b]	861.8[a]	824.3[b]	821.4[b]	8.52
	Feed/gain	1.93[bc]	1.90[c]	2.00[ab]	2.04[a]	0.029
	Mortality (%)	3.33	0	0	0	1.667
0 to 5 weeks	Feed intake (g)	2,648.9[b]	2,722.8[ab]	2,764.6[ab]	2,777.7[a]	38.17
	Weight gain (g)	1488.2[b]	1568.0[a]	1522.9[ab]	1514.9[b]	15.80
	Feed/gain	1.78[bc]	1.74[c]	1.82[ab]	1.84[a]	0.017
	Mortality (%)	6.67	3.33	5.00	1.67	2.784

[1] Copper treated diets were supplemented with Cu at the level of 100 ppm in the forms of the respective chelates.
[a-c] Means in a row with no common superscripts differ significantly (p<0.05).

Table 3. Nutrients availability of broilers fed experimental diets

	Treatments[1]				SEM
	Control	Met-Cu	Chitosan-Cu	Yeast-Cu	
	(%)				
DM	78.1[c]	81.2[b]	84.0[a]	81.9[b]	0.66
Crude ash	27.2	29.2	26.8	30.1	2.58
Crude protein	64.9	65.7	62.5	65.9	2.54
Crude fat	81.4	83.2	85.5	84.3	1.56
Crude fiber	30.7	28.8	29.6	27.3	2.01
NFE	81.4	83.6	83.7	82.3	0.74

[1] Copper treated diets were supplemented with Cu at the level of 100 ppm in the forms of the respective chelates.
[a-c] Means in a row with no common superscripts differ significantly (p<0.05).

analyses for Cu and fat content. Breast muscle samples were put into individual sample bags and kept in -20°C until analyses.

The collected gizzards were opened and the internal contents were removed. Samples were thoroughly cleaned in running tap water and labeled with code numbers. A three-member panel of judges examined gizzard erosion (ulceration of the lining of the gizzard) visually and determined indices on the basis of a scoring system, 0 for normal, 1 for mild erosion, 2 for moderate erosion and 3 for severe erosion.

Chemical analyses for serum, feed, excreta, liver and breast muscle

Serum samples were analyzed for total cholesterol and HDL concentrations following the methods described by Sigma Chemical Company (1998 and 1999, respectively). For the analysis of Cu concentration in liver, feed and excreta, samples were dry-ashed, treated with HNO_3, solubilized in HCl and filtered through 42 Whatman paper (AOAC, 1990). The samples were then assayed with ICP (Inductively Coupled Plasma) Emission Spectrometer (Model JY, Jobin Yvon, France). Concentration of fat in liver, feed and feces were determined by soxhlet extraction following the principle of proximate analysis (AOAC, 1990).

Breast muscle samples were analyzed for cholesterol concentrations followed the methods of Sale et al. (1984).

Statistical analyses

Data were subjected to analysis of variance using general linear model procedure (SAS Institute, 2000). Significant differences among treatment means were analyzed by Duncan's multiple range test at p<0.05 (Steel and Torrie, 1980)

RESULT

Results of feeding trial are shown in Table 2. There were significant differences among treatments in feed intake, weight gain and feed conversion during the whole period (0-5 wks). Feed intake was highest in Yeast-Cu followed by Chitosan-Cu, Met-Cu and control. Weight gain was highest in Met-Cu followed by Chitosan-Cu, Yeast-Cu and the control. Feed conversion (feed/gain) was lowest in Met-Cu, the control, Chitosan-Cu and Yeast-Cu. Mortality was not significantly different among treatments.

DM availabilities were significantly different among treatments (Table 3). DM availabilities of Cu chelate treatments were significantly higher then that of the control. Availabilities of crude ash, crude fat and NFE tended to be higher and that of crude fiber to be lower in Cu chelate treatments than in the control.

Table 4 shows gizzard erosion, copper content in the liver and excreta, serum HDL and total cholesterol level in serum and breast muscle. Gizzard erosion tended to increase by Cu chelates supplementation but was not significantly different among treatments. Copper contents in the liver were significantly different among treatments. Met-Cu was highest followed by Chitosan-Cu, Yeast-Cu and the control. Copper in excreta was significantly

Table 4. Gizzard erosion index, copper contents in liver and excreta, and total cholesterol level in serum and breast muscle

	Treatments[1]				SEM
	Control	Met-Cu	Chitosan-Cu	Yeast-Cu	
Gizzard erosion index (0-3)	1.42	1.83	2.08	1.75	0.245
Copper contents in liver (mg/kg)	14.7[b]	39.0[a]	22.6[ab]	20.6[ab]	6.14
Copper contents in excreta (mg/kg)	120.3[b]	297.8[a]	314.2[a]	309.1[a]	14.13
Serum					
Total cholesterol (mg/100 ml)	142.00	131.86	134.1	139.7	10.02
HDL (mg/100 ml)	109.18	122.48	121.3	120.6	6.14
Breast muscle					
Total cholesterol (mg/100 ml)	42.22	40.28	41.6	41.4	6.58

[1] Copper treated diets were supplemented with Cu at the level of 100 ppm in the forms of the respective chelates.

[a-c] Means in a row with no common superscripts differ significantly ($p<0.05$).

increased by supplementary Cu chelates. Total cholesterol in serum and breast muscle and serum HDL were not significantly different among treatments.

DISCUSSION

Improvement of weight gain and feed conversion ratio have been reported when broilers were fed diets supplementation with 125-250 ppm of copper in the from of sulfate (Choi and Paik, 1989; Baker et al., 1991). Results of researches conduced with supplementary Cu chelates also have been reported, Paik (2001) reported that supplementation of Met-Cu chelate at the level of 100-125 ppm Cu promoted performance of broilers and pigs. It was also reported that supplementation of 100 ppm of Cu as Met-Cu or Met-Cu-Zn improved performance in broilers (Hong et al., 2002) and egg production was increased by supplementation of 100 ppm of Cu as Met-Cu chelate in layers (Lim and Paik, 2003).

Hawbaker et al. (1961) reported those growth promotion effect of copper was due to antibacterial action. But it has been demonstrated that intravenous injection of Cu stimulates the growth of weaning pigs (Zhou et al., 1994). The result of this experiment indicated that Cu acts significantly to influence the growth regulatory system in many ways. In the present study, Cu chelate treatments tend to be higher than the control in feed intake and weight gain in 0-5 weeks. Especially, Met-Cu chelate treatment was significantly higher than the control in weight gain throughout all the feeding period. Chitosan-Cu was effective in improving performance during starter period but the effectiveness decreased during the later period. Supplementation of Yeast-Cu did not significantly improve the performance. Among the three Cu chelates, Met-Cu was the most effective Cu supplement and 100 ppm Cu as Met-Cu showed significant improvement in the performance of broilers. Effectiveness of Met-Cu may be related to the study of Wang et al. (1987) who reported that the best response to pharmacological levels of Cu feeding was observed with high levels of methionine supplementation. Wedekind et al. (1992) and Aoyagi and Baker (1993a) reported that metal-amino acid chelates or complexes furnish trace elements that are more efficiently absorbed from gut than those of inorganic form.

Since DM availability increased in all Cu chelate treatments, the improvement of performance of broilers may be partly due to the effect of Cu on DM digestibility.

Gizzard erosion index was increased by supplementation of Cu chelates, but the differences were not significant among treatments. Several investigations were conducted about relationship between gizzard erosion and copper supplementation. Fisher et al. (1973) reported that supplementation of 600 ppm Cu sulfate increased the symptom and Miyazki and Umemura (1987) reported toxic effect of $CuSO_4$ induced gizzard erosion, but Hong et al. (2002) reported 100 ppm of Cu supplementation as Cu-Met did not significantly increased gizzard erosion.

Copper contents in the liver and excreta were increased by supplementary Cu chelates. Cu level in the liver of Met-Cu chelate treatment was higher than other chelate treatments. This result indicates that Met-Cu is more readily absorbed and accumulates in the liver than other Cu chelates.

Levels of cholesterol in serum and breast muscle were not significantly different. Bakali et al. (1995) reported 250 ppm of Cu supplementation as $CuSO_4 \cdot 5H_2O$ decreased cholesterol level in serum and breast muscle, but Paik et al. (1999) reported that serum cholesterol was not affected by supplementary 125 ppm Cu as Met-Cu when the control diet contained sufficient level of Cu for normal metabolism. Level of copper in the basal diet of Bakali et al. (1995) was 5 ppm, while that of Paik et al. (1999) was 14 ppm which is more than sufficient to meet NRC (1994) requirement. In the present study, the Cu level of basal diets was 16.6 ppm in starter diet and 14.6 ppm in finisher diet. Deficient or marginally low level of Cu in the diet seems to induce hypercholesterolema (Kim et al., 1992). Paik et al. (1999) also reported supplementation of 125 ppm Cu as Met-Cu tended to increase HDL level in serum, which is in agreement with current data.

In conclusion, supplementation of 100 ppm Cu as chelates increased feed intake, weight gain and DM availability in broilers. Methionine-Cu chelate was the most effective one among three chelates (methionine, chitosan and yeast). Copper content in the liver of broilers was highest in Met-Cu chelate treatment indicating that Met-Cu is more readily absorbed than other forms of Cu chelates.

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