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Effects of Dietary Supplementation of Copper Chelates in the Form of Methionine, Chitosan and Yeast in Laying Hens

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ABSTRACT : An experiment was conducted to investigate the effects of dietary supplementation of copper chelates in the form of methionine, chitosan and yeast on the performance of laying hens. Four hundred ISA Brown layers, 84 wks old, were assigned to 4 treatments: control, 100 ppm Cu in methionine chelate (Met-Cu), 100 ppm Cu as chitosan chelate (Chitosan-Cu) and 100 ppm Cu as yeast chelate (Yeast-Cu). Each treatment had five replicates of 20 hens. Hen-day and hen-housed egg production and egg weight were significantly ($p<0.05$) increased by Met-Cu supplementation. The increase by Chitosan-Cu and Yeast-Cu supplementation was not significant. Contrast of the control vs. Cu chelates showed egg weight was significantly ($p<0.05$) increased by Cu chelate supplementation. Soft-shell egg production was significantly ($p<0.05$) reduced by supplementation of Cu chelates. Met-Cu treatment showed the lowest incidence of soft egg production. Gizzard erosion index was increased by Cu chelate supplementation. Crude fat in liver, total cholesterol in yolk and Cu content in liver and yolk were not significantly influenced by Cu chelate supplementation. It was concluded that dietary supplementation of 100 ppm Cu as Met-Cu significantly increased egg production and egg weight. Cu-Met chelate was also effective in reducing soft-shell egg production but increased gizzard erosion index. (**Key Words :** Egg Production, Chelates, Copper, Methionine-Cu, Layer)

INTRODUCTION

Copper (Cu) is an essential mineral which serves as a cofactor in many enzyme system, such as cytochrome oxidase, lysyl oxidase, ceruloplasmin and superoxide dismutase (Klasing, 1998). It has been known that high level Cu (125-250 ppm) in diet improves performance and feed conversion ratio in broilers (Baker et al., 1991; Paik, 2001) and pigs (Roof and Mahan, 1982; Cromwell et al., 1989; Paik, 2001). Pesti and Bakalli (1998) reported dietary supplementation of 250 ppm of Cu in the form of sulfate pentahydrate improved egg production and Lim and Paik (2003) also reported that egg production was increased by supplementary copper methionine chelate.

Additional benefit of high level Cu supplementation was reduced cholesterol levels of serum and breast muscle (Pesti and Bakalli, 1996) and reduced cholesterol levels of egg yolk (Pesti and Bakalli, 1998).

It was also observed that such high levels of Cu supplementation increased copper concentration in feces (Paik et al., 1999). High level of Cu in feces inhibits normal fermentation process and its accumulation in the soil causes environmental concern. Supplementation of copper in the form of chelates, complexes or proteinates was considered for use in animal diet as alternatives to inorganic source to solve these problems. Ammermann et al. (1995) reported that relative bioavailability estimates of organic Cu sources ranged from 88 to 147% of the response to cupric sulfate in poultry, swine, sheep and cattle.

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, 1993a). They also provide readily bioavailable amino acids (Aoyagi and Baker, 1993b).

Chitosan was studied as a new physiological material because it is known to have anticancer, antibacterial and antimold activity, and to alleviate hypertension and reduce serum cholesterol level (Muzzarelli et al., 1990; Kochkina and Chirkov, 2000). Chitosan is produced by removing acetyl group from chitin. Chitin has adhesion capacity of over 80% with Cu+2 in pH 6 (Choi, 1988).

Yeast has high capacity in converting absorbed inorganic minerals into organic form and some strains were found to have high capacity for absorbing Cu in specific (De Rome and Gadd, 1987; Lin and Kosman, 1990).

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Table 1. Formula and composition of basal diet

	Percent of mix
Corn	65.07
Soybeen meal	20.00
Limestone	9.10
Wheat bran	2.89
Animal fat	1.50
Tricalcium phosphate (18% P)	0.75
Salt	0.32
Layer primix[1]	0.17
D, L-methionine (98%)	0.12
Lysine-HCl (78%)	0.08
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 (cal/kg)	2,800
Crude protein (%)	15.0
Arginine (%)	0.82
Lysine (%)	0.70
Methionine (%)	0.37
Met and cyst (%)	0.55
Calcium (%)	3.80
Non-phytate P (%)	0.25
Total P (%)	0.49
Salt (%)	0.36
Copper (ppm)	20.5

[1] Provides per kg diet: vitamin A, 10,000 IU; vitamin D_3, 2,500 IU; vitamin E, 15 IU; vitamin K3, 2 mg; vitamin B1, 1.5 mg vitamin B2, 4 mg; vitamin B6, 3 mg; vitamin B12, 3 μg; Pantothenic acid, 8 mg; niacin, 25 mg; folic acid, 0.5 mg Zn, 52.5 mg; Mn, 52.5 mg; Fe, 52.5 mg; Cu, 5.25 mg; I, 1.155 mg; Co, 0.315 mg; Se, 0.315 mg.

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

Present experiment was conducted to determine effects of dietary supplementation of methionine-Cu chelate (Met-Cu), chitosan-Cu chelate (Chitosan-Cu) and yeast-Cu chelate (Yeast-Cu) on laying performance, lipid parameters and copper content in the liver and egg yolk.

MATERIALS AND METHODS

Preparation of Cu chelate

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 et 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).

Experimental diet

A basal diet was formulated to meet or exceed the nutrient requirements listed in NRC (1994) (Table 1). Four experimental diets were control, methionine-Cu chelate (Cu-Met), chitosan-Cu chelate (Chitosan-Cu) and yeast-Cu chelate (Yeast-Cu) supplemented diet. Each treated diet was supplemented with Cu at the level of 100 ppm in the form of Met-Cu, Chitosan-Cu and Yeast-Cu, respectively.

Feeding regimen

Four hundred ISA brown layers of 84-wks old were assigned to four dietary treatments. Each treatment consisted of 5 replications of 10 cages (2 birds per cage). Diets were presented in mash form, and feed and water were given *ad libitum* during the 8 wk experimental period. The house for birds was provided with programmed lighting of 16:00 h per day.

Parameters of production performance and eggshell strength

The number of total eggs, broken and soft shell eggs and egg weight were determined on a daily basis. Feed consumption was measured weekly. On 6th day of each week, all eggs, except soft and broken eggs, were collected to measure eggshell strength. Eggshell strength was measured using Compression Test Cell in Texture Test Systems (Model T2100C, Food Technology Corp., Rockville, MD). Copper contents in egg yolk were measured using ICP (inductively coupled plasma) emission spectrometer (Model JY-24, Jobin Yvon, Longjumeau, Cedex, France).

Cholesterol level and copper content in egg yolk

Egg yolks were pooled in each treatment after measurement of eggshell strength, and stored at 20°C until analysis. Egg yolk cholesterol was measured following the methods of Zlatkis et al. (1953) using spectrophotometer (UVIKON 933, KONTRON Instruments, Via G Fantoli 16/15 20138, Milano, Italy) after saponification following the methods of Abell et al. (1952).

Gizzard erosion index, copper and crude fat content in liver

After 8 wks on experimental diets, fifteen birds per treatment (three hens from each replication) were killed by cervical dislocation, and liver and gizzard were removed for further test. The gizzards were opened and the internal

Table 2. Laying performance of hens fed diets supplemented with different forms of Cu chelates

	Treatments[1]				SEM
	Control	Met-Cu	Chitosan-Cu	Yeast-Cu	
Hen-day egg production (%)	59.86[b]	62.47[a]	60.33[ab]	60.76[ab]	0.787
Hen-housed egg production (%)	59.55[b]	62.47[a]	60.29[ab]	60.30[ab]	0.792
Egg weight* (g)	70.59[b]	71.74[a]	71.11[ab]	70.73[ab]	0.235
Feed intake (g/hen/day)	119.03	120.40	119.77	119.47	0.861
Feed conversion ratio (g/100 g egg mass)	2.85	2.74	2.81	2.80	0.038

[1] Each treated diet was supplemented with Cu at the level of 100 ppm in the form of each chelate.
[a-c] Means with different superscripts in the same row differ ($p<0.05$).
* $p<0.05$, Contrast control vs. Cu chelate treatments.

Table 3. Soft and broken egg production, eggshell strength and gizzard erosion index of hens fed diet supplemented with different forms of Cu chelates

	Treatments[1]				SEM
	Control	Met-Cu	Chitosan-Cu	Yeast-Cu	
Soft egg production* (%)	0.73[a]	0.31[b]	0.38[b]	0.43[ab]	0.114
Broken egg production (%)	3.03[ab]	3.62[ab]	4.28[a]	2.40[b]	0.458
Broken egg and soft egg production (%)	3.76[ab]	3.93[ab]	4.66[a]	2.83[b]	0.493
Eggshell strength (g/egg)	3,515.25	3,569.04	3,520.59	3,515.25	41.899
Gizzard erosion index*	0.80	1.60	1.33	1.13	0.261

[1] Each treated diet was supplemented with Cu at the level of 100 ppm in the form of each chelate.
[a-c] Means with different superscripts in the same row differ ($p<0.05$).
* $p<0.05$, Contrast control vs. Cu chelate treatments.

contents were removed. Liver samples were thoroughly cleaned in running tap water. Gizzard erosion indices were determined on the basis of a scoring system, 0 for normal, 1 for mild erosion, 2 for moderate erosion and 3 for severe erosion. Collected livers were dried, and copper contents were measured by same procedure as the measurement of copper in egg yolk. Concentration of fat in liver was determined by Soxhlet extraction following the principle of proximate analysis (AOAC, 1990).

Statistical analyses

Data were subjected to analysis of variance using general linear model procedure (SAS Institute, 2000). Significant differences among treatment means were measured by Duncan's multiple range test at $p<0.05$ (Steel and Torrie, 1980). Contrast was performed between control vs Cu treatments to compare the effects of copper supplementation.

RESULTS

Results of the feeding experiment are shown in Table 2. There were significant differences among treatments in hen-day and hen-housed egg production and egg weight. Met-Cu treatment was significantly higher than the control in egg production and egg weight. Contrast of control vs. Cu chelate treatments showed Cu treatments were significantly higher than the control in egg weight but the increases by chitosan-Cu and yeast-Cu supplementations were not significant. There were no significant differences among treatments in feed intake and feed conversion ratio.

Result of broken and soft egg production, eggshell strength and gizzard erosion index are shown in Table 3. Soft egg production was significantly reduced by supplementation of Cu chelates. Supplementation of Met-Cu chelate treatment was lowest followed by Chitosan-Cu, Yeast-Cu and the control in soft-egg production. However, broken egg production and broken egg and soft egg production were lowest in Yeast-Cu and highest in Chitosan-Cu. Eggshell strength was not significantly different among treatments. Gizzard erosion index was not significantly different among treatments but contrast of control vs. Cu chelate treatments showed Cu treatments were significantly higher than the control.

Table 4 shows crude fat in the liver, total cholesterol in yolk and Cu content in the liver and egg yolk. Cu chelates supplementation did not significantly influence these parameters.

DISCUSSION

In the present study, it is clear that supplementation of copper chelates increases egg production and egg weight. Especially, Met-Cu chelate increased 4.4% in hen-day egg production and 4.7% in hen-housed egg production over the control. Pesti and Bakalli (1998) reported the results of two layer experiments in which egg productions were linearly increased as the supplementary Cu in the form of Cu sulfate pentahydrate increased from 0 to 125 and 250 mg/kg diet. Jackson (1977) also reported that 256 mg/kg Cu

Table 4. Crude fat in liver, total cholesterol in egg yolk and copper contents of liver and egg yolk of hens fed diet supplemented with different forms of Cu chelates

	Treatments[1]				SEM
	Control	Met-Cu	Chitosan-Cu	Yeast-Cu	
Crude fat in liver (%)	19.24	21.41	19.70	19.06	3.256
Total cholesterol in egg yolk (ppm)	17.63	19.02	16.76	18.27	0.760
Cu content	---------- ppm ----------				
Liver	30.23	32.99	33.62	31.83	3.740
Egg yolk	3.79	3.78	4.51	3.66	0.400

[1] Each treated diet was supplemented with Cu at the level of 100 ppm in the form of each chelate.

supplementation increased egg production. Other studies reported 200 ppm copper supplementation as sulfate form increased egg production, but when Cu supplementation was over 400 ppm, egg production was decreased (Chiou et al., 1997; Chiou et al., 1998).

Lim and Paik (2003) reported that supplementation of 100 ppm copper in the form of methionine increased hen-day and hen-housed egg production which is in agreement with the result of the present study.

In the present study, Yeast-Cu and Chitosan-Cu were not as effective as Met-Cu in egg production. 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.

In the previous study, soft egg production tended to be reduced by supplementation of 100 ppm Cu-Met chelate (Paik, 2001; Lim and Paik, 2003). In the present study, significant reduction in soft egg production was observed by Met-Cu and chitosan-Cu supplementation.

Result of gizzard erosion was not significantly different among treatments in the present study, but contrast analyses showed Cu chelates increased gizzard erosion.

Fisher et al. (1973) reported that 600 ppm Cu supplementation increased gizzard erosion in broiler.

In the present experiment, crude fat of liver and cholesterol in egg yolk were not affected by supplementation of copper chelates. Pesti and Bakalli (1998) also reported supplementation of 125-250 ppm Cu as $CuSO_4$ decreased cholesterol in egg yolk and Pesti and Bakalli (1996) reported Cu 125-250 ppm supplementation from sulfate form decreased cholesterol level in serum and breast muscle in broiler. However, Paik et al. (1999) reported that serum cholesterol was not affected by supplementary 125 ppm Cu as Met-Cu chelate when the control diet contained sufficient level of Cu for normal metabolism. Copper content in basal diet of Pesti and Bakalli (1998) experiment was 6.74 ppm. Copper level of experiment basal diet is over 20 ppm which is 3 times higher than that of Pesti and Bakalli (1998). Deficient or marginally low level of Cu in the diet seems to induce hypercholesterolemia (Kim et al., 1992). Therefore, supplementation of Cu in excessive of level may not influence lipid parameters when the basal diet contains sufficient level of Cu.

Copper content in liver and egg yolk were not significantly different among treatments in present study, Chiou et al. (1997), however, reported feeding a diet supplemented with 200 ppm Cu as copper sulfate for 4 weeks, increased copper content in liver and egg yolk.

Birds used in this experiment were 84 wk when this experiment began. It is a bit old for commercial layer but old layers are more sensitive for observation of supplementary effect on the egg production and eggshell quality. Considering the possibility of Cu deposition in the liver of layers from long term copper supplementation, it may be advisable to supplement Cu-methionine to old layer flock to extend economic life and improve eggshell quality.

It was concluded that dietary supplementation of 100 ppm Cu as Met-Cu significantly increased egg production and egg weight. Cu-Met chelate was also effective in reducing soft shell egg production but increased gizzard erosion index.

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