



## **Influence of supplementation of copper at two levels and flavomycin on the mineral concentrations in plasma and tibia of broiler chicken**

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### **Abstract**

To determine the influence of copper (Cu) supplementation at two dietary levels and flavomycin on the mineral concentrations in plasma and tibia, a feeding experiment was carried out with 160 day old broiler chicks (VenCobb 400) for a period of 42 days. The birds were divided into four treatment groups (T<sub>1</sub> to T<sub>4</sub>) with four replicates of ten chicks in each. The T<sub>1</sub> was control with no additives and Cu as CuSO<sub>4</sub> at 100 ppm, 200 ppm and flavomycin at 100 ppm were supplemented in the control diet to form treatment T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub> respectively. One bird from each replicate was slaughtered at the end of the experiment, during when plasma and tibial bone were collected for the analysis of mineral contents. Statistical analysis revealed significant (P<0.01) difference in plasma Ca, P, Cu, Fe and Zn and tibial Ca, P, Mg, Fe and Zn concentrations among the treatment groups T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub>. The result indicates that the supplementation of either copper or flavomycin had a positive influence on the mineral concentrations of both plasma and tibia in broilers.

**Keywords:** Copper, Flavomycin, Plasma, Tibia, Minerals, Broiler)

### **Introduction**

Copper sulfate is a naturally-occurring inorganic salt and copper is an essential trace element in livestock and poultry nutrition. It plays a vital role in haemoglobin synthesis, connective tissue maturation, nerve function and bone development. Copper is required for bone formation by promoting structural integrity of bone collagen and for normal elastin formation in the cardiovascular system. The broiler chicken nutritional requirement for copper is approximately 8 ppm (NRC, 1994). Copper is usually fed commercially at much higher pharmacological levels (100 to 300 ppm) because of its growth promoting properties (Bakalli *et al.*, 1995; Pesti and Bakalli, 1996). Traditionally, the source of copper has

been CuSO<sub>4</sub>·5H<sub>2</sub>O due to cost and commercial availability. In recent years, the potential use of Cu as feed supplement has expanded.

Flavomycin (synonyms: moenomycin, and bambermycin) is a glycolipid antibiotic produced by *Streptomyces* species including *S. bambbergiensis*, *S. ghanaensis*, *S. geysirensis*, and *S. ederensis* (Wallhausser *et al.*, 1966).

The product is manufactured as a complex of very similar components, of which moenomycin A, a phosphorus-containing glycolipid, is the main component (Huber *et al.*, 1966; Subramaniam *et al.*,

1997). Its growth promoting activity was repeatedly tested in 48 experiments, and its efficacy in broilers has been maintained 20 years after it was first tested (Dost, 1985). Flavomycin is used only as a growth-promoting antibacterial in animal feeds. The ban of feed antibiotics growth promoters (AGPs) requires seeking of alternatives.

Information on the effectiveness of the copper in comparison with an antibiotic growth promoter in broiler chicken diets is scant and hence the present study was conducted with an aim to examine the influence of supplementation of copper and flavomycin on plasma and bone mineral status of broiler chicken based on maize and soybean meal based diets.

## Materials and Methods

An experiment was carried out for a period of six weeks using one hundred and sixty, day-old broiler chicks (VenCobb 400).

### Experimental design

The chicks were wing banded, weighed individually and randomly allotted to four dietary treatments viz., T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub>. Each group comprised of four replicates of ten birds each.

### Experimental diet

The group T<sub>1</sub> was fed a control ration as per the BIS (1992) and this diet was supplemented with Cu as CuSO<sub>4</sub> at 100 ppm, 200 ppm and flavomycin at 100 ppm level in T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub>, respectively. The birds were fed with standard broiler starter ration up to 4 weeks of age and finisher ration up to 6 weeks of age. All the rations were made isocaloric and isonitrogenous. Feed and water were provided *ad libitum*. Standard managerial practices were followed throughout the experimental period.

### Proximate analysis of the rations

Proximate analysis of the broiler starter and finisher rations were done according to the procedures described by AOAC (1990).

### Collection of blood for plasma separation

Collection of blood was done on 42<sup>nd</sup> day of the trial during slaughter. About 5ml of blood was collected in vial with sodium citrate as anticoagulant agent. 4 ml of blood sample was taken in a centrifuge tube and was centrifuged at 3,000 rpm for 10 minutes. Then the supernatant was separated by sterilized Pasteur pipette in a sterilized vial and was preserved in deep freeze at -20°C. The collected plasma was subjected to estimation of plasma minerals viz., Ca, Mg, Cu, Fe and Zn by Atomic Absorption Spectrophotometer (Perkin Elmer AAS Model 3110), inorganic P (P<sub>i</sub>) using blood analyser (Phosphomolybdate method).

### Collection of Tibial bones

Tibial bones were collected during the time of slaughter and the muscles were removed by boiling the bones on a water bath containing 2 per cent sodium hydroxide at 100°C for 10-15 minutes. Then the bones were kept in incubator at 70°C over night to remove moisture. Tibial bones were then defatted using the soxhlet apparatus and the bones were again subjected to incubation. For mineral analysis, a portion of dried tibial bone samples were ground and subjected to wet digestion, using nitric acid and perchloric acid (2:1). Calcium, Mg, Zn, Cu and Fe content of the digested sample were determined using Atomic Absorption Spectrometer (Perkin Elmer AAS Model 3110) and Phosphorus by colorimetry (ANSA method, AOAC, 1990) using spectrophotometer (Spectronic 20D<sup>+</sup>, spectronic instruments, USA).

### Statistical analysis

Data collected on various parameters were statistically analyzed by Completely Randomized Design (CRD) method as described by Snedecor and Cochran (1994). Means were compared by Duncan's Multiple Range Test (DMRT).

## Results and Discussion

The chemical composition of T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub> rations contained 15.43, 106.49, 212.54 and 18.69 ppm of Cu in broiler starter and 12.67, 101.13, 202.72 and 20.20 ppm of Cu in broiler finisher, respectively (not shown in table).

Table 1. Ingredient composition of the basal diet (%)

Ingredients	Starter	Finisher
Maize	53.01	62.20
Soybean meal	42.12	32.94
Di calcium phosphate	2.00	2.10
Calcite	1.80	1.80
DL-methionine <sup>1</sup>	0.15	0.04
Nicomix BE <sup>2</sup>	0.10	0.10
Trace Mineral mixture	0.04	0.04
Nicomix AB <sub>2</sub> D <sub>3</sub> K <sup>3</sup>	0.03	0.03
Toxin binder <sup>4</sup>	0.25	0.25
Choline chloride	0.15	0.15
Salt	0.25	0.25
Coccidiostat <sup>5</sup>	0.10	0.10
Total	100.00	100.00

<sup>1</sup>DL- methionine containing 99 per cent feed grade. <sup>2</sup>Nicomix BE containing Vitamin B<sub>1</sub>- 4 mg, Vitamin B<sub>6</sub>- 8 mg, Vitamin B<sub>12</sub>- 40 mcg, Niacin- 60 mg, Calcium pantothenate- 40 mg and Vitamin E- 40 mg, per gram. <sup>3</sup>Nicomix AB<sub>2</sub>D<sub>3</sub>K containing Vitamin A- 82500 IU, Vitamin D<sub>3</sub>- 12000 IU, Vitamin B<sub>2</sub>- 50 mg and Vitamin K- 50 mg, per gram. <sup>4</sup>Toxin binder containing hydrated sodium calcium alumino silicate, mannan oligosacchride and Activated charcoal. <sup>5</sup>Coccidiostat containing Salinomycin 12 per cent feed grade. <sup>6</sup>CuSO<sub>4</sub>·5H<sub>2</sub>O containing 25.45 per cent Cu. <sup>7</sup>Flavomycin containing flavophospholipol 40 mg/g.

In addition, the basal diet was supplemented with 39.3 per cent of CuSO<sub>4</sub> (Cu- 100 ppm) for treatment 2 (T<sub>2</sub>), 78.5 per cent of CuSO<sub>4</sub> (Cu-200 ppm) for treatment 3 (T<sub>3</sub>) and flavomycin 10 g/100 kg (100 ppm) for treatment 4 (T<sub>4</sub>) irrespective of the broiler starter or finisher rations.

### Plasma mineral concentration

Statistical analysis revealed significant (P<0.01) difference in plasma mineral (Table 2) concentrations viz., Ca, P, Cu, Fe and Zn among the different treatment groups except plasma Mg concentration.

The plasma Ca concentrations of treatment group supplemented with Cu at 100 ppm (T<sub>2</sub>) level was higher (P<0.01) than that of other three treatment groups. However, the plasma Ca levels of other groups (T<sub>1</sub>, T<sub>3</sub> and T<sub>4</sub>) were statistically similar.

The plasma inorganic P (P<sub>i</sub>) concentration was highest (P<0.01) in 200 ppm Cu supplemented group (T<sub>3</sub>) and was the lowest in control group (T<sub>1</sub>), where as the group supplemented with Cu at 100 ppm and flavomycin had statistically similar inorganic P values.

Plasma Mg levels of the birds maintained on the four dietary treatments were statistically similar, whereas the plasma Cu level was highest (P<0.01) for T<sub>3</sub> and was the lowest for the control.

The present study is in agreement with that of the findings of, Ewing *et al.* (1998) who reported that when broiler chicken were supplemented with 125 ppm of Cu from three different sources such as copper sulphate, copper oxychloride and copper citrate, levels of plasma Cu was significantly higher compared to the control group. Similarly, Samanta *et al.* (2011) observed that broiler chicken supplemented with Cu at 75, 150 and 250 ppm levels as CuSO<sub>4</sub> showed high plasma Cu concentration compared to control birds.

It could be seen from Table 2 that the treatment groups supplemented with either Cu (100 and 200 ppm) or flavomycin (100 ppm) had higher (P<0.01) plasma Fe concentrations than that of the control group (T<sub>1</sub>).

The plasma Zn concentration was highest in flavomycin supplemented group and control group and lowest in group supplemented with Cu at 200 ppm and within the Cu supplemented group, supplementation of Cu at 100 ppm level showed increased (P<0.01) plasma Zn concentration compared to 200 ppm level. That chickens give priorities to their mineral requirements for vital functions in compromise of body growth is indicated by the normal concentrations of the minerals in the plasma of the control birds (Roth, 2003).

Table 2. Mineral concentration of plasma and tibia

Minerals	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	SEM
<b>Plasma</b>					
Ca, mg/dl	11.74 <sup>a</sup>	12.47 <sup>b</sup>	11.72 <sup>a</sup>	11.21 <sup>a</sup>	0.20
P, mg/dl	4.96 <sup>a</sup>	5.52 <sup>b</sup>	6.33 <sup>c</sup>	5.67 <sup>b</sup>	0.17
Mg, mg/dl	1.51	1.69	1.88	1.69	0.14
Cu, ppm	0.39 <sup>a</sup>	1.49 <sup>c</sup>	1.95 <sup>d</sup>	0.80 <sup>b</sup>	0.11
Fe, ppm	2.89 <sup>a</sup>	3.30 <sup>b</sup>	3.30 <sup>b</sup>	3.60 <sup>b</sup>	0.09
Zn, ppm	2.83 <sup>c</sup>	2.45 <sup>b</sup>	2.15 <sup>a</sup>	2.89 <sup>c</sup>	0.09
<b>Tibial</b>					
Ca, %	25.08 <sup>b</sup>	20.89 <sup>ab</sup>	20.93 <sup>ab</sup>	19.22 <sup>a</sup>	0.51
P, %	12.10 <sup>a</sup>	14.24 <sup>b</sup>	12.46 <sup>a</sup>	14.52 <sup>b</sup>	0.44
Mg, %	1.21 <sup>a</sup>	1.34 <sup>b</sup>	1.53 <sup>c</sup>	1.47 <sup>c</sup>	0.03
Cu, ppm	2.36	1.87	2.10	1.62	1.61
Fe, ppm	56.59 <sup>a</sup>	62.68 <sup>b</sup>	68.28 <sup>c</sup>	70.27 <sup>c</sup>	0.36
Zn, ppm	153.73 <sup>a</sup>	180.11 <sup>c</sup>	116.25 <sup>a</sup>	131.64 <sup>a</sup>	5.33

a, b, c – Means bearing different superscripts within the same row differed significantly (P<0.01)

### Tibial mineral concentration

Statistical analysis on tibial mineral concentrations (Table 2) revealed that tibial Ca concentration was highest (P<0.01) for control (T<sub>1</sub>) and lowest (P<0.01) for flavomycin supplemented group (T<sub>4</sub>), whereas the groups supplemented with Cu (T<sub>2</sub> and T<sub>3</sub>) had comparable values.

Tibial P concentration of T<sub>2</sub> and T<sub>4</sub> groups were significantly higher than that of groups T<sub>1</sub> and T<sub>3</sub>.

Tibial Mg and Fe concentrations were higher (P<0.01) for groups supplemented with Cu at 200 ppm (T<sub>3</sub>) and flavomycin supplemented group (T<sub>4</sub>) followed by group supplemented with Cu at 100 ppm (T<sub>2</sub>) and the lowest (P<0.01) for control group (T<sub>1</sub>). There was no significant difference among the treatment groups on tibial Cu concentrations.

The tibial Zn values were highest (P<0.01) for group supplemented with Cu at 100 ppm (T<sub>2</sub>) whereas T<sub>3</sub> and T<sub>4</sub> had lowest values compared to control fed group

(T<sub>1</sub>). It has been observed the present study was in agreement with the findings of Mohanna and Nys (1999) that when the dietary Zn content was greater than the requirement for growth, there was an increased plasma and tibia concentration. The bone is a complex heterogeneous tissue that supports the musculature, and, thus, its growth and development are intimately connected with overall body growth (Loveridge, 1992).

On contrary to the results obtained in the present study, Ledoux *et al.* (1991) reported that concentration of tibial Cu tended to increase from an average of 5.3 to 6.6 ppm as the dietary Cu concentration increased from 150 to 300 ppm.

It could be concluded that supplementation of either copper or flavomycin had some positive influence over the plasma and tibial mineral concentrations which is indirectly necessary for the various vital functions and growth of bones in broilers and hence poultry farmers can use Cu as a feed additive alternative to antibiotic growth promoters.

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