# **Effects of Copper Supplement on Haematological Profiles and Broiler Meat Composition**

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## **ABSTRACT**

It appears that copper (350 mg kg<sup>-1</sup>) supplemented in diet has damaged the gizzard, depressed the feed intake, thus resulting in reduced growth rate of broiler. Copper supplement increased the PCV, Hb and decreased plasma cholesterol and triglyceride significantly. Composition of the breast meat was not affected. Liver accumulated more Cu than the breast muscle, thus indicative of insignificantly higher AAT and AST enzyme system. Plasma proteins and its fraction did not change due to Cu-supplementation.

Key Words: CuSO<sub>4</sub> supplement; Chicken; Haematology; Muscle composition; Enzymes

#### INTRODUCTION

Copper (Cu) is an essential nutrient and it has been reported that sulfate form (CuSO<sub>4</sub>) is more effective than oxide (CuO) (Cromwell *et al.*, 1989). Its level 125 to 250 ppm as CuSO<sub>4</sub> improves growth and feed efficiency in broiler (Choi & Paik, 1989; Baker *et al.*, 1991). Cu supplement also reduces the incidence of oily bird syndrome (Maurice *et al.*, 1981). However, its excess in the diet depresses growth and feed efficiency in broilers (Funk & Baker, 1991), damages both gizzards (Robbins & Baker, 1980) and liver functions (Chen *et al.*, 1996, 1997 a, b). Independent of changes in blood lipids, difference in fatty acid composition of cardiac tissue may be gender dependent (Srinivasan *et al.*, 1987). Lee *et al.* (1986) demonstrated gender linked effects on the basal activities of lipogenic enzymes in response to starvation and re-feeding in rats.

Chiou *et al.* (1999) demonstrated that high Cu (500 mg kg<sup>-1</sup>) in diet have damaged duodenal villi, therefore, have impact on nutrient absorption, depress food intake resulting in poor growth performance of broiler chickens. Inclusion of 500 mg kg<sup>-1</sup> of Cu in the diet did not disturb normal protein metabolism of broiler chicken (Chiou *et al.*, 1999).

Chen *et al.* (1996) observed an increase in the enzymes level as a result of increase in the level of dietary Cu in country chicken and pullet chickens. No such report is published that deals with the supplement Cu that is higher than 250 ppm and less than 500 ppm to see its effects on the haematochemicals and hormonal profiles in broiler chicken. Therefore, an experiment was designed to monitor the effect of CuSO<sub>4</sub> supplementation on the performance, haematology and hormonal profiles of broiler chickens.

#### MATERIALS AND METHODS

Sixty day-old broiler chicks were raised in battery incubator for three weeks with starter ration (ME 3,050 KCal kg<sup>-1</sup> ). Birds were divided into two groups. Group I birds were served normal diet (10 mg Cu kg<sup>-1</sup> of diet ). Requirement for Cu is 8 mg kg-1 of diet for broiler (NRC, 1994), and supplement level up to 10 mg kg<sup>-1</sup> of diet was given to exclude any possibility of causing deficiency in these birds. Group II birds were fed additional 350 mg CuSO<sub>4</sub>.5H<sub>2</sub>O kg<sup>-1</sup> diet. All birds were given starter ration for first four weeks and then finisher ration for the last two weeks. Water was offered ad libitum. Weekly body weight gain and feed intake were measured. At the end of experiment, 10 birds from each group were randomly selected for collection of blood, breast muscles and liver. Gastrointestinal tract was collected for the gross pathological lesions. The blood samples were collected in a centrifuge tubes having EDTA for separation of plasma. Small aliquots of plasma were made according to the requirement of the tests and were frozen at -20°C for further analysis. Microcentrifuge tubes were filled with whole blood estimation of packed cell volume and haemoglobin was estimated by Sahli's apparatus (Zia-ur-Rahman et al., 1981). Plasma glucose, total proteins, albumin, cholesterol, triglyceride, aspartate aminotransferase (AST) and alanine aminotransferase (AAT) were estimated using commercial kits (Abbott Laboratories). Meat samples were collected and analysed by AOAC methods (1990) for proximate analysis. Moisture contents were determined by drying the samples of known weight in an oven at 105°C till a constant weight is achieved. Nitrogen contents were determined by Kjeldahl's method and protein contents were worked out by

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N x 6.25. Ether was used as solvent for the analysis of ether extract content. Ash was determined using muffle furnace at 600°C (AOAC, 1990). Cu was determined in the blood, breast muscles and liver samples after digestion of these materials with nitric acid and perchloric acid (1:1) (Zia-ur-Rahman & Akhtar, 1993). The final volume was adjusted to 25 mL with demineralized water and Cu concentration was read by using atomic absorption photometer. All data collected were analysed using student t-test (SAS, 1985) at a significance level of P> 0.05..

## RESULTS AND DISCUSSION

Feed intake, live body weight and feed conversion ratio have been presented in Table I. Birds fed CuSO<sub>4</sub> (350 mg kg<sup>-1</sup> of diet) showed significantly depressed feed intake bird<sup>-1</sup> and poor feed conversion ratio. Therefore, birds in group II showed significant decrease in growth performance as compared to bird fed normal diet. Chen et al. (1996, 1997 a,b) reported that body weight gain were reduced significantly by the supplementation of of Cu @ 500 mg kg<sup>-</sup> <sup>1</sup> in the diet of country chicken and layer pullets. Paik *et al.* (1999) demonstrated that supranormal level of Cu gave different response in different species. Kassim and Suwanpradit (1996) reported that Cu at a level of 375 mg kg<sup>-1</sup> increased total sulfur amino acid requirement. In the present study diet was supplemented with Cu @ 350 mg kg-1 of diet that is almost similar to the Kassim and Suwanpradit (1996) and much lower than Chen et al. (1996, 1997 a, b), however, it led to significant decrease in live weight gain in group II. It could be assumed from these observations that if an antioxidant is given in supra supplement amount, we may speculate its adverse action.

Table I. Effect of normal and CuSO<sub>4</sub> (350 mg kg<sup>-1</sup>) supplement on the growth performance of six week old broiler

Parameters	Group-I (Control)	Group-II (CuSO <sub>4</sub> )
Feed intake (g/bird/day)	113.0 ± 4.4	108.4 ± 3.1*
Live Weight Gain (g/day)	$63.5 \pm 3.2$	$58.6 \pm 2.7*$
Feed Conversion Ratio	$1.77 \pm 0.8$	$1.8 \pm 0.5$
Average Dressing Percentage	$72.6 \pm 2.6$	$68.8 \pm 4.5*$
Average Heart Weight (g)	$7.32 \pm 1.2$	$6.57 \pm 0.9*$
Average Liver Weight (g)	$50.33 \pm 4.4$	$47.5 \pm 3.2*$
Average Gizzard Weight (g)	$37.0 \pm 3.7$	$34.0 \pm 2.1*$
Abdominal fat pad (% b/w)	$2.35 \pm 0.06$	$1.68 \pm 0.4 *$

<sup>\*</sup> Significantly different (P>0.05) from the control values.

Vogt *et al.* (1981) and Burnell *et al.* (1988) reported that Cu has antimicrobial actions thus has growth stimulating action. It has also been demonstrated that intravenous injection of Cu stimulates growth of weanling pigs (Zhou *et al.*, 1994). CuSO<sub>4</sub> in the feed, depressed intake of feed and live weight gain in the present study (Table I). It is not clear whether, low feed intake in CuSO<sub>4</sub> fed birds caused a decrease in body weight gain or it may

have been due to the adverse effects of CuSO<sub>4</sub> on the Gastro-intestinal tract. Grossly, pathophysiological observations showed obvious lesion such as gizzard erosion. However, no gross pathological changes were observed on the GI-tract of birds supplemented with CuSO<sub>4</sub>. Jensen *et al.* (1991) observed oral lesion and damage to gizzard when birds were given Cu more than 169 mg kg-1 in the diet. Chiou et al. (1999) demonstrated that 250 mg kg<sup>-1</sup> Cu in the diet did not significantly influence broiler performance, however, high dietary Cu supplementation of 500 mg kg<sup>-1</sup> in the broiler diet significantly influenced the morphology of the GI-tract. Chiou et al. (1999) demonstrated that high level of Cu supplement up to 500 mg kg<sup>-1</sup> of diet severely depressed villi height not only in duodenum but also in the jejunum. Therefore, it is concluded that even though any gross lesion on the G.I-tract was not observed but supplementation of Cu (350 mg kg<sup>-1</sup>) might have demonstrated its effect and have caused thickening of the muscular layers in the small intestine of the birds to reduce absorption thus reduce weight gain in group II. Abdominal fat pad on the basis of percentage of body weight was 2.30 and 1.88 in control and Cu supplemented birds, respectively (Table I). Bakalli et al. (1995) reported that supplementation with Cu reduces plasma triglyceride, while Konjufva et al. (1997) observed no change in triglyceride in other species. Liver Cu concentration was significantly higher in CuSO<sub>4</sub> treated birds (33.3  $\pm$  3.6 mg kg<sup>-1</sup>, DM) as compared to control birds (16.8  $\pm$  2.6 mg kg<sup>-1</sup>, DM). A decrease in the plasma glucose concentration in Cu-supplemented birds might be due to decreased feed intake, increased packed cell volume, and haemoglobin (Table II).

Table II. Haematological, biochemical and enzymatic profile of normal and CuSO<sub>4</sub> supplemented (350 mg kg<sup>-1</sup>) broilers

Parameters	Group-I (Control)	Group-II (CuSO <sub>4</sub> )
Packed Cell Volume (%)	$28.5 \pm 0.9$	30.4 ± 1.3*
Haemoglobin (g/dl)	$6.4 \pm 1.0$	$7.5 \pm 0.8*$
Glucose (mg/dl)	$160.2 \pm 6.9$	$148.8 \pm 4.8*$
Total Proteins (g/dl)	$3.8 \pm 0.50$	$3.5 \pm 0.44$
Albumin (g/dl)	$1.3 \pm 0.21$	$1.4 \pm 0.18$
Total Cholesterol ( mg/dl)	$144.7 \pm 2.2$	$118.5 \pm 4.0*$
Plasma Triglyceride (mg/dl)	$73.0 \pm 3.5$	$60.2 \pm 1.3*$
Aspartate Aminotransferase (U/L)	$170.0 \pm 15.0$	$198.6 \pm 10*$
Alanine aminotransferase (U/L)	$8.5 \pm 0.9$	$10.5 \pm 1.0*$

<sup>\*</sup> Significantly different at (P>0.05) from control values.

In Cu supplemented birds, Cu plays a major role as cofactor in hematogenesis (Chiou *et al.*, 1999). Cu is one of the most critical trace elements in livestock because it is necessary for haemoglobin formation, iron absorption from GI-tract and iron mobilization from tissue stores (Mpofu *et al.*, 1999). Plasma cholesterol and triglyceride concentrations were significantly reduced, however, fat percentage of breast muscle (Table III) was unaffected by CuSO<sub>4</sub> supplementation. Paik *et al.* (1999) demonstrated

that high level of Cu reduced glutathion (GSH) that reduced stimulation of HMG-CoA reductase activity to reduce cholesterol synthesis. Bakalli *et al.* (1995) reported that Cu supplementation reduced plasma triglycerides but not by others (Konjufva *et al.*, 1997; Paik *et al.*, 1999). Supplement of Cu at a dose of 300 mg kg<sup>-1</sup> significantly increased the AAT and AST enzyme system. These observations are in agreement with Chiou *et al.* (1997) and Chen *et al.* (1996, 1997 b). These authors reported an increase in enzymes level as a result of very high doses of CuSO<sub>4</sub>. Higher level of Cu accumulation might have damaged the liver to increase these enzymes.

Table III. Composition of breast meat and copper contents of liver and breast meat of birds fed normal and supplemented with 350 mg CuSO4 in the diet

Parameters	Group-I (Control)	Group-II (CuSO <sub>4</sub> fed)
Dry Matter (%)	28.09	26.85
Crude Protein (%)	22.15	21.78
Fat (%)	3.80	3.1
Ash (%)	1.13	1.38
Breast meat	$1.76\pm0.05$	2.59±0.04*
Liver (mg kg <sup>-1</sup> DM)	17.0±1.8	31.2±1.5*

<sup>\*=</sup> Significantly different from control group

## **CONCLUSION**

It was concluded that supplementation of Cu with high doses at a rate of 350 mg kg<sup>-1</sup> of diet have significantly reduced the production performance of broiler. The decrease in the performance was due to decreased absorption of nutrient by the gastrointestinal tracts, reduced giblet weight, mobalization of fat and these facts are further supported by alteration in the haematochemical parameters in group II.

## REFERENCES

- AOAC, 1990. Official Methods of Analysis of the Association of Official Analytical Chemists. 15th Ed. Association of Official Analytical Chemists. Inc. Arlington, Virginia, 22201, U.S.A.
- Bakalli, R.I., G.M. Pesti, W.L. Ragland and V. Konjufca, 1995. Dietary copper in excess of nutritional requirement reduces plasma and breast muscle cholesterol of chickens. *Poult. Sci.*, 74: 360–65.
- Baker, D.H., J. Odle, M.A. Funk and T.M. Wieland, 1991. Bioavailability of copper in cupric oxide, cuprous and in a copper-lysine complex. *Poult. Sci.*, 70: 177–9.
- Burnell, T.W., G.L. Cromwell and T.S. Stahly, 1988. Cited by J. Gohl in Bottom Line of Nutrition. Feed stuff, June 13, pp. 16–8.
- Chen, K.L., C.P. Wu and J.J. Lu, 1996. Effects of dietary copper levels on performance, tissues and serochemistry value of Taiwan country chicken. J. Biomass Energy Soc. Chin., 15: 70–5.
- Chen, K.L., C.L. Chen, T.E. Lien and Y.M. Horng, 1997a. Effect of dietary levels of copper on tissue residue and serochemistry value of growers. *Taiwan. J. Vet. Med. Anim. Husb.*, 67: 45–50.
- Chen, K.L., C.P. Wu and C.L. Chen, 1997b. Effects of dietary levels of copper on performance and intestinal structure in growers. *J. Chia-Yi Inst. Agric. Technol.*, 50: 31–9.
- Chiou, P.W.S., K.L. Chen and B. Yu, 1997. Toxicity, tissue accumulation and residue in egg and excreta of copper in laying hens. *Anim. Feed Sci. Technol.*, 67: 49–60

- Chiou, P.W.S., C.L. Chen and C.P. Wu, 1999. Effects of high dietary copper on the morphology of gastro-intestinal tract in broiler chickens. Asian-Austalasian J. Anim. Sci., 12: 548–53.
- Choi, Y.J. and I.K. Paik, 1989. The Effect of Supplementing copper sulfate on the performance of broiler chicken. Korean. *J. Anim. Nutr. Feed.*, 13: 193–200.
- Cromwell, G.L., T.S. Sthly and H.J. Mongue, 1989. Effect of source and level of copper on performance and liver copper stores in weanling pigs. *J. Anim. Sci.*, 67: 2996–3002.
- Funk, M.A. and D.H. Baker, 1991. Toxicity and tissue accumulation of copper in chicks fed casein and soy-based diets. J. Anim. Sci., 69: 4505–11
- Jensen, L.S., P.A. Runn and K.N. Dolson, 1991. Induction of oral lesion in broiler chicks by supplementation the diets with copper. *Avian Dis.*, 35: 963–73
- Kassim H. and S. Suwanpradit, 1996. The influence of copper on the total sulphur amino acids requirement of broiler during two growing periods. *Asian-Australasian. J. Anim. Sci.*, 9: 359–62.
- Konjufva, V.H., G.M. Pesti and R.L. Bakalli, 1997. Modulation of cholesterol levels in broiler meat by dietary garlic and copper. *Poult.* Sci., 76: 1264–71.
- Lee, V.M., B. Szepesi and R.J. Hansen, 1986. Gender linked differences in dietry induction of hepatic glucose-6-phosphate dehydrogenase, 6phosphogluconate dehydrogenase and malic enzyme in rat. *J. Nutr.*, 116: 1547–54.
- Maurice, D.V., J.E. Jones and N.J. Bherer, 1981. Interaction of roxarsone and copper in broiler chicks. *Poult. Sci.*, 60: 1695.
- Mpofu, I.D.T., L.R. Ndlovu and N.H. Casey, 1999. The Copper, Cobalt, Iron, Selenium and Zinc Status of cattle in the Sanyati and Chinamhora small holder grazing areas of Zimbabwe. Asian-Australasian J. Anim. Sci., 12: 579–84.
- NRC, 1994. *Nutrient Requirement of Poultry*. National Academy of Science, Washington, D.C.
- Paik, I.K., S.H. Seo, J.S. Um, M.B. Chang and B.H. Lee, 1999. Effects of supplementary copper-chelate on the performance and cholesterol level in plasma and breast muscle of broiler chickens. *Asian-Australasian J. Anim. Sci.*, 12: 794–8.
- Robbins, K.R. and D.H. Baker, 1980. Effect of the sulfur amino acid level and source on the performance of chicks fed high level of copper. *Poult. Sci.*, 59: 1246–53.
- SAS, 1985. SAS User's Guide; Statistics. Statistical Analysis System Institute Inc., Cary, NC.
- Srinivasan, S.R., C. Amos, R. Albares, R.R. Bhandaru, E.R. Daffen, L.S. Webber and G.S. Berenson, 1987. Influence of serum lipo-proteins and carbohydrate metabolism on erythrocyte membrane composition in children. Bogaluse heart study. *Metabolism*, 35: 466–72.
- Vogt, H., S. Matthes and S. Harnisch, 1981. Preservatives organic acids in broiler and laying rations. Conf. on Feed Additives, Budapest, Hungary.
- Zhou, W., E.T. Kornegay, M.D. Lindemann, J.W.G.M. Swinkels, M.K. Welton and E.A. Wong, 1994. Stimulation of Growth by Intravenous Injection of Copper in Weanling pigs. J. Anim. Sci., 72: 2395–2403.
- Zia-ur-Rahman, A.H. Gilani, M.D. Ahmad and R.H. Siddiqui, 1981. Adaptability of crossbred heifers to summer as measured by erythrocyte count, haemoglobin concentration and packed cell volume. *Pakistan Vet. J.*, 1: 106–10.
- Zia-ur-Rahman and M.S. Akhtar. 1993. Trace elements concentration in the liver, kidney and serum of healthy animals. *Int. Symp. Trace Elements and Liver Diseases*, Karachi, Pakistan. Pp: 44–5.

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