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The Effects of Copper Supplementation on the Performance and Hematological Parameters of Broiler Chickens

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ABSTRACT : To determine the efficiency of copper (Cu) supplementation, a feeding experiment was carried out with 240 day old broiler chicks (vencobb-100). Birds were divided into four dietary treatments: i) C - no additives, ii) T_1-75 mg inclusion of Cu/kg diet, iii) T_2-150 mg inclusion of Cu/kg diet, iv) T_3-250 mg inclusion of Cu/kg diet. The present study was carried out in the Department of Animal Physiology, West Bengal University of Animal and Fishery Sciences, Kolkata, India for a period of 42 days (6 weeks). Growth performance was measured in terms of live weight gain, cumulative feed intake and feed conversion ratio at the end of 21st and 42nd day of the experiment and the result was found to be encouraging for commercial enterprises when the chickens were fed at 150 mg Cu/kg (T_2) of their diet. Excess dietary copper more than 150 mg/kg reduced the haemoglobin (Hb) concentration in blood and resulted in the accumulation of copper in the liver with decreased blood Hb concentration and packed cell volumes (PCV). Copper supplementation increased the total erythrocyte count (TEC) as copper is involved in erythropoiesis. But, from the result it is indicated that the dietary copper concentration could not alter the total leukocyte count (TLC). In case of different leucocytes count (DLC), there were no significant differences observed among the different treated groups. Statistical analysis showed significant ($p<0.01$) difference in plasma concentration of copper, zinc, ferrous and cholesterol among the different copper treated groups. The result indicates that supplementation of copper is an effective way of improving the production performance and haematological parameters in broiler chicken. (**Key Words :** Copper, Haemoglobin, Blood Parameters, Feed Intake, Broiler Chicken)

INTRODUCTION

Copper (Cu) is an integral part of cytochrome system. The enzyme tyrosinase, ascorbic acid oxidase, feroxidase (ceruloplasmin), superoxide dismutase contain copper and their activity dependent on this element (Swenson and Reece, 1996). Copper compound were used for medicinal purpose as early as 4000 BC, it was not until the 1920's that copper has first recognized as an essential nutrient for animal. Copper is required for the activity of different metallo enzymes associated with iron metabolism, elastin and collagen formation, melatonin production and the integrity of central nervous system. It is required for normal red blood cell formation by allowing iron absorption from small intestine and release of iron in tissue into the blood plasma. Copper is required for bone formation by promoting structural integrity of bone collagen and for normal elastin formation in the cardiovascular system. Immuno-regulation of body system is a greatly dependent on copper. Copper deficiency is known to cause anemia, diarrhoea, bone disorder, change in hair and wool pigmentation, cardio vascular disorder and impaired glucose and lipid metabolism. Despite of its wide physiological role copper has great role to lower the plasma and meat cholesterol. Copper promote the low density lipoprotein *in vitro*. Kim et al. (1992) have shown in rats that copper deficiency causes hypercholesterolaemia by increasing hepatic reduced glutathione (GSH) concentration which increase the activity of HMG-CoA reductase which is the primary control point for the cholesterol synthesis. It has been hypothesized that the high concentration of liver Cu regulate cholesterol biosynthesis indirectly by decreasing the reduced form of glutathione (GSH) and increasing the oxidized form of glutathione (GSSG) (Kim et al.,1992; Bakalli et al., 1995).

After the green revolution of India, nation can be kept out of food famine by putting some roughage into the

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hungry stomach, but that cannot build up a healthy nation. For healthy growth and development of body tissues need protein food-specially animal protein which is a rich source of essential amino acids. All the developed countries in the world put special emphasis on the production of more and more protein food from animal origin. Indian diet is highly deficient in animal protein. Malnutrition is a scourge on our population especially among growing children, pregnant woman and nursing mother in rural areas. With the advent of broiler strain, poor and middle class family can take wholesome animal protein at considerably cheaper cost. With the change of food habits, taboos and increase per capita income, the demand of chicken in place of chevon and mutton has increased considerably. Consumer's demands for broiler have increased considerably due to its taste, easy digestibility and palatability which confer the high acceptability.

Therefore, the present study was under taken to examine the effect of different levels of Cu on growth performance and haematological parameters in broiler chicken.

MATERIALS AND METHODS

Experimental stock

Two hundred and forty day old broiler chicks (vencobb-100) were randomly divided in to twelve groups each of 20 chicks (4 treatments×3 replicates). The experiment had a randomized design (Snedecor and Cochran, 1994). Birds were kept in floor pens, on straw bedding and reared under uniform husbandry condition (14h light/d, relative humidity 60% and 25-32°C). The feed and water were given *ad libitum*. The same technician provides feed, water and collected data from the birds during the course of the experiment. The experiment followed the guidelines of "Institutional Animal Ethics Committee (IAEC, WBUAFS, Kolkata)".

Formulation of experimental diets

The basal diet (C) contained 215 g/kg crude protein (CP), 3,050 Kcal/kg ME, 32 g/kg total calcium and 15 g/kg total phosphorus (Table 1). T_1, T_2 and T_3were formulated to contain an additional 75, 150 and 250 mg/kg diet Cu, respectively. Cu sulphate pentahydrate ($CuSO_4$, $5H_2O$) was used as the source of Cu.

Determination of production performance

The feed intake and body weights were registered at weekly intervals and the body weight gain (BWG) and feed conversion ratio (FCR) were calculated to the nearest 1 g accuracy. Mortality was recorded and growth performance was evaluated in terms of live-weight gain, cumulative feed intake and feed conversion ratio (FCR).

Table 1. Composition of the basal diet

Ingredients	
Composition (g/kg)	
Maize	580
Soybean meal (Solvent extract)	120
Soybean (Full fat)	100
De-oiled rice bran (DORB)	70
Fish meal	60
Limestone	20
Oyster shell	15
Marble chips	20
Dicalcium phosphate	10
Sodium chloride	5
DL-methionine	0.160
Choline chloride	0.320
Mineral mixture (Premix-1)	0.150
Vitamin A, B_2, D_3, K (Premix-2)	0.050
Vitamin B complex (Premix-3)	0.060
Calculated composition	
Crude protein (g/kg)	215.0
Crude fibre (g/kg)	22.5
Copper (mg/kg)	8.0
Fe (mg/kg)	80.0
Total calcium (g/kg)	32.0
Total phosphorus (g/kg)	15.0
ME (Kcal/kg)	3,050

Premix-1: Each g of mineral mixture contained: 200 mg of $FeSO_4 \cdot 7H_2O$, 20 mg of $CuSO_4 \cdot 5H_2O$, 200 mg of $MnSO_4 \cdot H_2O$, 150 mg of $ZnSO_4 \cdot 7H_2O$, 1 mg of KI.

Premix-2: Each g of vitamin A, B_2, D_3, K (Spectromix, Ranbaxy) provided: vitamin A (retinol) 540 mg, vitamin B_2 (riboflavin) 50 mg, vitamin D_3 (cholecalciferol) 400 mg, vitamin K (menadione) 10 mg.

Premix-3: Each g of B-Complex provided: vitamin B_1 (thiamine) 2 mg, folic acid 10 mg, pyridoxine HCl 4 mg, Cyanocobalamin 10 μg, nicotinamide 12 mg.

Collection of blood for haemato-biochemical study

Collection of blood was done on 21st and 42nd day of trial. Blood was collected from wing-vein by sterile disposable syringe (Dispovan®-5 ml). About 5ml of blood were collected in heparinised vial for haematological and biochemical estimation. About 1 ml of blood was used for estimation of different haematological parameter *viz*. Haemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC), total leukocyte count (TLC), different leucocytes count (DLC).

Preparation of plasma

4 ml of blood sample was taken in a centrifuge tube and was centrifuged at 1,000 rpm for 15 mins in Remi centrifuge machine (Biswas et al., 2006). Then the supernatant plasma 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

Table 2. The effects of copper supplementation on performance in broiler birds (Mean±SEM; n = 60)

Item / Days	Dietary treatment				Level of significant
	C	T1	T2	T3	
Body weight gain, g					
0-14	343.83±4.97^{ab}	345.42±5.19^{ab}	356.97±6.47^{a}	329.68±1.44^{b}	**
15-42	1,682.96±3.38^{c}	1,695.26±8.83^{c}	1,832.53±8.23^{a}	1,805.89±9.74^{b}	**
0-42	2,026.79±8.35	2,040.68±14.02	2,299.50±14.70	2,135.57±11.18	
FCR, kg/kg					
0-14	1.54±0.01^{a}	1.50±0.02^{ab}	1.46±0.02^{ab}	1.53±0.02^{a}	*
15-42	1.95±0.01^{a}	1.94±0.02^{a}	1.79±0.01^{c}	1.85±0.01^{b}	**
0-42	3.49±0.02	3.44±0.04	3.25±0.03	3.38±0.03	
Cumulative feed intake, g					
0-14	889.91±8.35^{b}	912.08±4.55^{ab}	920.99±7.35^{ab}	928.09±8.54^{ab}	*
15-42	3,275.38±18.57^{b}	3,292.72±16.61^{ab}	3,286.70±8.00^{b}	3,338.22±15.57^{a}	*
0-42	4,164.29±26.92	4,204.80±31.16	4,207.69±15.35	4,266.31±24.11	

C = Control, T_1 = Treatment 1, T_2 = Treatment 2, T_3 = Treatment 3.
Mean bearing different superscript in the same column differ significantly. * p<0.05, ** p<0.01, NS = Non significant.

of different biochemical parameter *viz,* Cu, Fe, Zn and total cholesterol concentration.

Estimation of haematological parameters

The haemoglobin level in blood was estimated using Drabkin's method (cyanmethemoglobin method) as described by Cannan (1958) and the kits were procured from Sigma-Aldrich company. PCV was determined in wintrobe haematocrit tube as per standard method of Jain (1986) and was expressed in terms of %. As the avian erythrocytes were nucleated the mammalian white blood cell diluting fluid could not be used in total leucocyte count because though the erythrocyte may be lysed, the nuclei were left and appeared prominent so it is difficult to distinguish the leucocytes from them. To overcome this difficulty, differential stain was used for counting both erythrocytes and leucocytes as they stain differently and can be counted easily as described by Sastri (1983). A thin tongue shaped blood smear was made from fresh whole blood. After proper drying of the smear methanol fixing was done. DLC was done as per the method described by Schalm and Jain (1975) and was expressed as percentage of total leucocytes.

Estimation of Cu, Zn, Fe and cholesterol in plasma

Plasma Cu, Zn, Fe was estimated by atomic absorption spectrophotometer as described by Mkino and Takahara (1981) and total cholesterol content was determined by the enzymatic method as described by Wybenga et al. (1970).

Statistical analysis

The data were analyzed using statistical software package developed at the computer centre of the Institute following standard procedure for ANOVA (Snedecor and Cochran, 1967) and Duncan's multiple range tests (Duncan, 1955) by comparing means for significant differences.

RESULTS AND DISCUSSION

Live weight, cumulative feed intake and feed conversion ratio have been presented in Table 2. Birds in group T_2 showed a significantly (p<0.01) higher live weight throughout the experimental period. Cu sulphate at 150 mg/kg in the feed depressed the cumulative feed intake and significantly lowered the feed conversion ratio after the 42nd day of experiment in the birds.

Hematological changes (Hb, PCV, TEC and TLC) after

Table 3. The effects of copper supplementation on different hematological parameter in broiler birds (Mean±SEM; n = 60)

Treatments	Hb (g/dl)		PCV (%)		TEC (10^6 cu/mm)		TLC (10^3 cu/mm)	
	21st day	42nd day	21st day	42nd day	21st day	42nd day	21st day	42nd day
C	9.84^{c}±0.10	9.49^{b}±0.10	32^{b}.00±0.68	27.63^{b}±0.75	3.04^{bc}±0.25	3.19^{c}±0.31	30.00±0.81	29.55±1.16
T_1	10.26^{b}±0.13	10.23^{a}±0.13	35.25^{a}±0.37	31.38^{a}±0.65	3.30^{b}±0.26	3.28^{bc}±0.27	26.42±1.35	28.54±0.84
T_2	10.44^{ab}±0.12	10.46^{a}±0.12	33.00^{b}±0.38	29.13^{b}±0.55	4.19^{a}±0.19	4.25^{a}±0.21	28.65±1.10	28.02±0.88
T_3	10.81^{a}±0.14	9.11^{b}±0.20	32.50^{b}±0.42	28.13^{b}±0.79	3.96^{ab}±0.33	4.00^{ab}±0.20	29.13±0.89	27.92±1.13
Level of significant	**	**	**	*	*	*	NS	NS

C = Control, T_1 = Treatment 1, T_2 = Treatment 2, T_3 = Treatment 3.
Mean bearing different superscript in the same column differ significantly. * p<0.05, ** p<0.01, NS = Non significant.

Table 4. The effects of dietary Cu supplementation on different leucocytes count in broiler birds (Mean±SEM; n = 60)

Treatments	Heterophil (%)		Oesonophil (%)		Basophil (%)		Lymphocyte (%)		Monocyte (%)	
	21st day	42nd day	21st day	42nd day	21st day	42nd day	21st day	42nd day	21st day	42nd day
C	32.30±1.76	36.05±1.58	3.25±0.37	2.95±0.44	0.99±0.11	1.10±0.16	55.27±1.26	54.70±1.72	8.20±0.45	5.20±0.40
T_1	33.35±1.80	34.41±1.37	3.48±0.38	3.15±0.45	1.14±0.13	0.97±0.17	53.69±1.49	55.87±1.61	9.35±0.48	5.63±0.32
T_2	32.25±1.60	34.97±1.78	4.02±0.46	4.12±0.46	1.51±0.18	1.00±0.13	53.50±1.50	54.07±1.67	8.72±0.31	5.83±0.26
T_3	30.56±1.05	33.58±1.74	3.76±0.36	2.92±0.36	1.10±0.15	0.80±0.11	55.94±1.99	57.28±1.68	8.64±0.56	5.42±0.30
Level of significant	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

C = Control, T_1 = Treatment 1, T_2 = Treatment 2, T_3 = Treatment 3.
Mean bearing different superscript in the same column differ significantly. * p<0.05, ** p<0.01, NS = Non significant.

the supplementation of copper were presented in Table 3. The average Hb concentration in 3rd week and 6th week of age were showed significant (p<0.01) difference among the group. T_3 group which is treated with 250 mg/kg copper showed significantly high level Hb compared to control and other groups of birds. T_1 and T_2 group showed no significant difference in Hb concentration. The TEC concentrations were significantly (p<0.05) different among the treated groups. In case of different leucocytes count (DLC), there were no significant differences observed among the different treated groups (Table 4). Statistical analysis showed significant (p<0.01) difference in plasma concentration of Cu, Zn, Fe and cholesterol among the different copper treated groups (Table 5). The lowest concentration of total plasma cholesterol observed in T_3 group which was supplemented with 250 mg/kg Cu.

The supplementation $CuSO_4$, $5H_2O$ at 150 mg/kg feed was found to be a positive inducer for the live weight gain in broiler chicks which might be a consequence of the significant reduction of total pathogenic organism of gut interfering in weight gain (Xia et al., 2004). It has also been demonstrated that intravenous injection of Cu stimulates growth of weaning pigs (Zhou et al., 1994). Therefore, birds in group T_2 showed the best growth performance as compared to the birds of other two groups and control group where feed conversion ratio was found to be poor. It is not clear whether variation in feed intake at different level of Cu supplementation caused a significant alteration in growth performance or it might have been due to the adverse effects of Cu sulphate on the gastro intestinal tract. Grossly pathophysiological observations showed no obvious lesions.

The average Hb in 6th week of age were 09.49±0.10, 10.23±0.13, 10.46±0.12, 09.11±0.20 (g/dl) in group C, T_1, T_2 and T_3 respectively. Statistical analysis revealed significant (p<0.01) difference of Hb concentration among different group. The highest Hb concentration was found in T_2 group followed by T_1, C and T_3. T_3 group showed the lowest Hb concentration compared to the other group of birds. Packed cell volume (PCV) in 3rd weeks of age were showed significant (p<0.01) difference in PCV where in 6th weeks of age, the significant level were p<0.05 among the treated group. From the present finding it can be postulated that excess dietary copper more than 250 mg/kg reduced the Hb concentration in blood. Excess of dietary copper results in an accumulation of copper in liver with decrease blood Hb concentration and packed cell volumes (Swenson and Reece, 1996). Ozcelik et al. (2002) reported that similar finding in Wister albino rats.

Statistical analysis showed significant (p<0.01) difference in PCV among the different treated groups. T_1 groups showed highest PCV the compare to other group of bird. T_3 group which is supplied with 250 mg/kg copper showed low level of PCV compared to T_1 and T_2. Present

Table 5. The effects of dietary Cu supplementation on mineral concentration and total cholesterol concentration of broiler birds (Mean±SEM; n = 60)

	Cu (ppm)		Zn (ppm)		Fe (ppm)		Total cholesterol of plasma (mg/100 gm meat)	
	21st day	42nd day	21st day	42nd day	21st day	42nd day	21st day	42nd day
C	0.25^{b}±0.00	0.26^{c}±0.01	1.40^{b}±0.05	1.54^{c}±0.05	1.92^{b}±0.07	2.09^{b}±0.07	54.63^{a} ±0.98	63.76^{ab}±0.65
T_1	0.29^{a}±0.00	0.29^{b}±0.01	1.35^{b}±0.03	2.04^{b}±0.04	2.14^{ab}±0.10	2.23^{ab}±0.05	55.81^{a}±1.12	66.36^{a}±1.55
T_2	0.31^{a}±0.01	0.32^{a}±0.00	1.60^{a}±0.04	2.13^{ab}±0.06	2.32^{a}±0.07	2.12^{b}±0.06	50.44^{b}±0.78	60.87^{b}±0.81
T_3	0.30^{a}±0.01	0.33^{a}±0.01	1.57^{a}±0.06	2.20^{a}±0.03	2.02^{b}±0.08	2.34^{a}±0.03	42.67^{c}±0.38	51.62^{c}±0.71
Level of significant	**	**	*	**	*	*	**	**

C = Control, T_1 = Treatment 1, T_2 = Treatment 2, T_3 = Treatment 3.
Mean bearing different superscript in the same column differ significantly. * p<0.05, ** p<0.01.

finding showed that excess copper reduce the PCV. Swenson and Reece, (1996) also reported that excess dietary copper reduce the PCV in blood. McNanghton and Day (1979) reported maximum haemoglobin levels and packed cell volume (PCV) of 21 day old chicks were found by feeding 80 ppm of dietary Fe and 8 ppm of dietary Cu from 1-21 days of age. Xin et al. (1991) reported that cattle which were marginally deficient in copper had reduced super oxide dismutase activity and decreased neutrophil. They also observed that dairy herds which are marginal in their copper status often seem to have higher incidence of mastitis. Dove and Haydon (1991) observed that hemoglobin levels were increased by the addition of Fe to the diet (containing 250 ppm of Cu). They indicated that levels of added Fe up to 300 ppm may help to improve the hematological status of weanling pigs fed growth promoting levels of Cu but that it has little effect on performance. Ozcelik et al. (2002) reported that the effect of excessive copper intake on hematological and haemorheological parameters. They indicate that drinking water containing 250 μg/ml copper for a period of 9 wks. Wister albino rats showed increased erythrocyte count, blood viscosity value and lower haemoglobin than control fed at a normal diet.

The average TEC in 3rd and 6th weeks of age were 3.04±0.25, 3.30±0.26, 4.19±0.19, 3.96±0.33 (10^6 cu/mm) and 3.19±0.31, 3.28±0.27, 4.25±0.21, 4.00±0.20 (10^6 cu/mm) in groups C, T_1, T_2 and T_3 respectively. Statistical analysis showed significant ($p<0.05$) difference in TEC among the group. Statistical analysis showed that significant ($p<0.05$) difference in TEC among the group. From the above findings it was observed that copper supplementation increase the TEC as copper involve in erythropoiesis. The average TLC in 3rd and 6th week of age were 30.0±0.81, 26.42±1.35, 28.65±1.10, 29.13±0.89 (10^3 cu/mm) and 29.55±1.16, 28.54±0.84, 28.02±0.88 and 27.92±1.13 (10^3 cu/mm) in group C, T_1, T_2 and T_3 respectively. Statistical analysis revealed no significant difference in DLC in different ages after the supplementation of copper.

The average plasma Cu concentration in 3rd and 6th week of age were 0.25±0.00, 0.29±0.00, 0.31±0.01, 0.30±0.01 (ppm) and 0.26±0.01, 0.29±0.01, 0.32±0.00, 0.33±0.01 (ppm) in groups C, T_1, T_2, T_3 respectively. Statistical analysis revealed significant ($p<0.01$) difference among the group. T_2, T_3 and T_1 showed high plasma Cu concentration compared to control bird. This is agreement with Cromwell et al. (1989) in poultry and Roof and Mahan (1982) in pigs.

The plasma Zn concentration in 3rd week and 6th week of age were 1.40±0.05, 1.60±0.04, 1.57±0.06, 1.35±0.03 (ppm) and 1.54±0.05, 2.04±0.04, 2.13±0.06, 2.20±0.03 (ppm) in groups C, T_1, T_2 and T_3 respectively. Statistical analysis showed significant difference ($p<0.01$) among the groups. T_2 and T_3 group showed significant ($p<0.05$) difference compared to C and T_1 group. Due to very few literatures in this aspect, the results cannot be compared. The Fe concentration in 3rd weeks and 6th week of age were of age were 1.92±0.07, 2.14±0.10, 2.32±0.07 and 2.02±0.08 (ppm) and 2.09±0.07, 2.23±0.05, 2.12±0.06 and 2.34±0.03 (ppm) in groups C, T_1, T_2 and T_3 respectively. At 3 wks of age T_2 revealed the highest concentration of plasma Fe followed by T_1, T_3 and C and at 6th week of age T_3 revealed the highest concentration of plasma Fe followed by T_1,T_2 and C. Statistical analysis showed significant ($p<0.05$) different among the groups.

On 21st and 42nd day of the present experiment the plasma cholesterol level decreased significantly ($p<0.01$) among all groups (Table 5). This decrease in plasma cholesterol after supplementation of excess dietary Cu might be due to high degradation of cholesterol which is esterifies by transfusing long chain fatty acid moiety from lecithin. Present study revealed that dietary Cu at pharmacological dose level (150, 250 mg/kg) significantly decreased the plasma cholesterol level. These observations were in conformity with the finding of Engle et al. (2000) in steers, Elliot and Bowland (1968) in porcine, Ward and Spears (1997) in cattle, Skrivanova et al. (2001) in rabbit, Sinnet-Smith and Woolliams (1987) in sheep, Thompson et al. (1973) in pig. High dietary Cu supplementation might lead to lower tissue accumulation of cholesterol by reducing cholesterol synthesis or high degradation on due to decreased hepatic glutathione formation (Kim et al., 1992; Bakalli and Pesti, 1995). Glutathione is known to regulate cholesterol biosynthesis through the stimulation of HMG-CoA reductase (Vaisala and Kurup, 1987; Konjufca et al., 1997).

CONCLUSION

From the above discussion it can be concluded that excess copper have some beneficial effect on growth performance and also haematological parameters in broiler chicken. It is advisable to poultry farmer for the use of copper as a growth promoter moreover to reduce the plasma cholesterol level to improve the quality of meat for human consumption.

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