



## Effect of different levels and sources of dietary copper on the growth and immunity of broiler chicken

Sagar Dukare<sup>1</sup>, AB. Mandal<sup>1</sup>, Nasir Akbar Mir<sup>1\*</sup>, Kapil Dev<sup>2</sup>, Praveen K. Tyagi<sup>1</sup>, Avishek Biswas<sup>1</sup>, JJ. Rokade<sup>1</sup>, Pramod K. Tyagi<sup>1</sup>

<sup>1</sup> ICAR- Central Avian Research Institute, Izatnagar, Bareilly, Uttar Pradesh, India, PIN: 243122

<sup>2</sup> Thomas J. Long School of Pharmacy, University of the Pacific Stockton - CA 95211, United States

### Article info

Received: 26 October 2021

Received in revised form: 09 December 2021

Accepted: 11 December 2021

Published online: 13 December 2021

### Keyword

Chicken  
Growth  
Immunity  
Green nano copper  
Sources  
Levels

\* Corresponding author:

Nasir Akbar Mir

Email: [nasirakbar129@gmail.com](mailto:nasirakbar129@gmail.com)

### Reviewed by:

Aswathy PB

Department of Poultry Science, College of Veterinary and Animal Sciences, Pookode, Wayanad - 673576, Kerala

### Abstract

A study was conducted to evaluate the effects of different levels and sources of dietary copper (Cu) in broiler chicken. The experimental design was 3x4 factorial with three levels of Cu (8, 12, 16 ppm) and four sources (inorganic – IC, organic – OC, green nano – GNC, and market nano – MNC) resulting in 12 dietary treatments. The GNC was synthesised in the laboratory. A total of 480 day old chicken of same hatch and uniform body weight were procured and divided randomly in 12 treatment groups having 5 replicates with 8 birds in each (40 birds/treatment). The results revealed significantly higher body weight gain and feed intake in birds supplemented with GNC or MNC at 16 ppm Cu level. The better feed efficiency was observed at 16 ppm level of GNC or MNC. However, no significant effects of Cu levels or sources were observed on the carcass characteristics and cutup parts of broiler chicken. Similarly, enhanced cell mediated immune response measured as response to PHAP was observed in birds supplemented with GNC followed by MNC and at 16 ppm level. The humoral immune response, measure as response against sheep red blood cells, was higher in birds supplemented with MNC or GNC at 16 ppm level. Thus, this study concludes that supplementation of Cu in broiler chicken at 16 ppm level of nano form improves the growth and feed efficiency along with an enhanced immunity. The green nano Cu synthesised in this study was equally better in improving growth performance and immunity of birds as market nano Cu. However, green nano Cu was superior to market nano Cu in improving the cell mediated immunity of broiler chicken.

This is an open access article under the CC Attribution-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>)

## 1. Introduction

In tropical countries like India high temperature and humidity conditions constitute a major stress in birds which result in reduced growth performance, immune suppression, and increased mortality (Mujahid et al. 2005; Mandal 2010). Since there are financial consequences associated with heat stress, much attention has been given to the role that nutritional modulation can play in reducing its effects. In order to manipulate their diets, antioxidants, vitamins, and minerals are frequently added to the diet of chicken raised in hot and humid environments (Sahin and Kucuk 2003). Among these dietary manipulations for stress alleviation copper (cu) supplementation constitutes an important dietary manipulation. Along with its function in iron metabolism, copper also has a

significant impact on the immune system and antioxidant activity (Kim et al. 2008; Ognik et al. 2016). As a result, it is a crucial trace element for regulating the essential bodily systems to promote appropriate growth performance (Banks et al. 2004). It has been shown in rats that Cu deficiency impairs the functioning of antioxidant system (Kim et al. 1992) which may potentially reduce the growth performance. The dietary requirement of Cu for broiler chicken is 12 ppm (BIS 2007). However, the assessment of nutrient requirement of boiler chicken is a continuous process because of the continuous genetic improvement of birds for better growth performance. Therefore, this study was conducted to evaluate the effect of different levels and sources of Cu on the growth performance, carcass characteristics, and immune response of broiler chicken.

## 2. Materials and methods

### 2.1. Experimental setup

The day old CARIBRO Vishal broiler chicken (480) from same hatch were procured from the experimental hatchery of the institute and reared in battery cages for 42 days experimental period. In each battery 8 birds were housed providing the space of 1.25 ft<sup>2</sup> per bird. The experiment was conducted during the hot and humid environmental conditions having temperature humidity index (THI) equal to 84.93. The diets were formulated as pre-starter (0-14 d), starter (14-24 d), and finisher (24-42 d) (BIS 2007) (Table 1). In this study four sources of copper (Cu) (inorganic - IC, organic - OC, green nano - GNC, and market nano - MNC) were used at three levels (8, 12, and 16 ppm) resulting in 12 experimental dietary

**Table 1 Ingredients and nutrient composition of basal diet**

Ingredients (%)	Pre-starter (0-14 day)	Grower (14-24 day)	Finisher (24-42 day)
Maize	51.94	55.11	60.82
Soybean	41.10	37.00	31.00
Guar korma	3.00	4.00	4.00
Oil	0.50	0.60	1.00
Limestone	0.90	0.90	0.90
DCP	1.70	1.60	1.50
Salt	0.30	0.30	0.30
DL-Meth	0.20	0.13	0.12
TM. Premix1	0.10	0.10	0.10
Vit Premix2	0.15	0.15	0.15
Vitamin B complex	0.02	0.02	0.02
Ch. Chloride	0.05	0.05	0.05
Toxin binder	0.05	0.05	0.05
Total	100.00	100.00	100.00
<i>Nutrient composition (calculated based on the analysed values of ingredients)</i>			
Crude Protein	22.50	21.50	19.53
M Energy (kcal/kg diet)	2856	2894	2973
Lysine	1.36	1.28	1.13
Methionine	0.60	0.52	0.48
Threonine	0.97	0.91	0.81
Calcium	1.02	0.98	0.94
Available P	0.45	0.43	0.40
Crude Fiber	4.85	4.91	5.02
1 Trace mineral mixture (1 kg): FeSO <sub>4</sub> .7H <sub>2</sub> O – 80 g, MnSO <sub>4</sub> .H <sub>2</sub> O - 100 g, KI - 300 g, Zn - 80 ppm			
2 Vitamin premix (1 g): Vitamin A - 82.5 IU, Vitamin B2 - 50 mg, Vitamin D3 -12000 unit, Vitamin K -10 mg			
3 Vitamin B complex (1 g): Vitamin B1- 8 mg, Vitamin B6-16 mg, Vitamin B12-80 mcg, Niacin -120 mg, Calcium panthotheonate-80 mg , Vitamin E 50% -160 mg, L-lysine-10 mg and DL-Methionine- 10 mg			
ME: Metabolizable energy			

treatments. The inorganic form of copper used was copper sulphate and organic for used was copper-methionine chelate. The green nano copper was synthesised in the laboratory and market nano copper was purchased from the market. Five replicate of birds containing 8 birds in each were assigned to each experimental dietary treatment group (40 birds/treatment).

### 2.2 Synthesis of Cu nano particles

For the Cu nano particle synthesis healthy *Eucalyptus sp.* leaves were washed with distilled water, dried in shade, and cut into small pieces. About 20 g of dried eucalyptus leaves were boiled in 100 ml of distilled water for 20 minutes at 80 °C to get the aqueous extract. The brown extract was filtered through Whatman filter No. 1 and refrigerated at 5 °C after cooling. In a 250 ml Erlenmeyer flask, 100 ml of a 1 mM aqueous copper sulphate solution and 10 ml of the produced leaf extract were mixed. Continuous stirring was done which caused the blue colour of the solution turn pale yellow. Cu nanoparticles separated out and settled at the bottom of the flask after being left at room temperature during the course of the night. By repeated centrifugation at 12,000 rpm for 15 minutes and redispersing the pellet in deionized water, the resulting Cu nanoparticles were cleaned. In an oven set at 80 °C, the purified Cu nanoparticles were dried. The particle size of synthesised nano particles was measured by transmission electron microscope (JEOL JEM -1400) at Anatomy department of Govind Ballabh Pant University of Agriculture and Technology, Pantnagar. The concentration of Cu in the synthesised product was measured using atomic absorption spectrophotometer (ECIL, Model 4141). The Cu nano particle size was observed in the range of 20-37 nm and the average concentration of Cu in the product was 39.94%.

### 2.3 Growth performance

Weekly body weight of birds was recorded. To determine the weekly feed intake of birds, weighed quantity of the respective diets were daily offered to the birds *ad libitum*. Based on the feed consumption and weight gain of the birds, the feed conversion ratio was computed.

### 2.4 Carcass characteristics

After 12-hours fasting, ten birds from each treatment (two birds per replication) were randomly chosen to assess the carcass traits. The cut-up parts and carcass traits were expressed as a percentage of the live body weight of the birds.

### 2.5 Immune response of birds

The immunological response in birds was examined in terms of humoral immunity against sheep red blood cells (SRBC) and cell-mediated immunity (CMI) against the phyto-haemagglutinin-P (PHA-P) mitogen. The CMI was measured in 10 birds per treatment at 4 weeks of age and humoral immunity against SRBC was also measured in 10 birds per treatment (other than those used for CMI) at 5 weeks of age (Dukare et al. 2020).

## 2.6 Statistical analysis

Each replicate served as an experimental unit for the growth performance analysis, and each sampled bird served as an experimental unit for the carcass features and immune response parameters. The IBM SPSS software-20 was used to conduct a two-way ANOVA analysis of the data using the general linear model technique. At a significance threshold of  $P < 0.05$ , the Tukey post-hoc test was employed to distinguish the significant mean differences.

## 3. Results

### 3.1 Growth performance

The results of the growth performance of broiler chicken in response to different levels and sources of Cu are given in Table 2. Birds supplemented with 16 ppm Cu gained

**Table 2 Effect of different levels and sources of copper on the growth performance of broiler chicken**

Cu source	Cu level (ppm)	Body weight gain	Feed intake	FCR
IC	8	1467.8 <sup>a</sup>	2752.0 <sup>a</sup>	1.98 <sup>g</sup>
OC		1523.5 <sup>c</sup>	2779.1 <sup>b</sup>	1.88 <sup>e</sup>
GNC		1732.3 <sup>e</sup>	2852.6 <sup>cd</sup>	1.75 <sup>bc</sup>
MNC		1740.9 <sup>e</sup>	2874.6 <sup>de</sup>	1.79 <sup>bc</sup>
IC	12	1516.5 <sup>b</sup>	2785.2 <sup>b</sup>	1.97 <sup>g</sup>
OC		1563.0 <sup>d</sup>	2788.6 <sup>b</sup>	1.85 <sup>d</sup>
GNC		1795.1 <sup>f</sup>	2894.9 <sup>ef</sup>	1.75 <sup>b</sup>
MNC		1812.3 <sup>f</sup>	2914.0 <sup>f</sup>	1.78 <sup>b</sup>
IC	16	1542.3 <sup>b</sup>	2799.2 <sup>b</sup>	1.94 <sup>f</sup>
OC		1603.2 <sup>d</sup>	2817.4 <sup>bc</sup>	1.81 <sup>c</sup>
GNC		1813.0 <sup>g</sup>	2892.6 <sup>ef</sup>	1.70 <sup>a</sup>
MNC		1838.5 <sup>g</sup>	2904.8 <sup>f</sup>	1.68 <sup>a</sup>
SEM		6.83	7.93	0.02
<b>Main effects</b>				
Copper levels (ppm)				
	8	1613.4 <sup>a</sup>	2813.6 <sup>a</sup>	1.86 <sup>c</sup>
	12	1668.0 <sup>b</sup>	2842.7 <sup>b</sup>	1.84 <sup>b</sup>
	16	1693.5 <sup>c</sup>	2852.5 <sup>c</sup>	1.81 <sup>a</sup>
Copper sources				
	IC	1508.8 <sup>a</sup>	2778.8 <sup>a</sup>	1.96 <sup>c</sup>
	OC	1563.2 <sup>b</sup>	2795.0 <sup>b</sup>	1.84 <sup>b</sup>
	GNC	1779.8 <sup>c</sup>	2880.02 <sup>c</sup>	1.77 <sup>a</sup>
	MNC	1808.2 <sup>c</sup>	2895.1 <sup>c</sup>	1.72 <sup>a</sup>
<b>Probability</b>				
	Interaction	$P < 0.01$	$P < 0.05$	$P < 0.05$
	Copper Levels	$P < 0.01$	$P < 0.01$	$P < 0.01$
	Copper Source	$P < 0.01$	$P < 0.01$	$P < 0.01$

Values bearing different superscripts within the row differ significantly  
 NS: Non significant; FCR: Feed conversion ratio; SEM: Standard error of mean  
 IC: Inorganic Copper, OC: Organic Copper, GNC: Green Nano Copper, MNC: Market Nano Copper

significantly more body weight ( $P < 0.01$ ) than those supplemented with 12 ppm Cu, whereas lower gain was observed in birds supplemented with 8 ppm Cu. The body weight gain of birds supplemented with IC was significantly ( $P < 0.01$ ) lower than that of the birds supplemented with OC source, however the birds supplemented with GNC and MNC source of Cu exhibited larger body weight gains. But the body weight gain of birds supplemented with GNC or MNC did not differ significantly from each other. The interaction effect of Cu levels and sources on bird body weight gain was statistically significant ( $P < 0.01$ ). In general, birds supplemented with 16 ppm Cu of either a GNC or MNC source gained more body weight than those on an 8 ppm Cu diet of IC source. The other combinations yielded intermediate results.

At 8 ppm Cu level a significantly ( $P < 0.01$ ) reduced feed intake of birds was observed followed by 12 ppm level. Similarly, birds supplemented with IC exhibited significantly ( $P < 0.01$ ) decreased feed intake followed by OC supplemented birds compared to GNC or MNC, which did not vary statistically from one another. The interaction between Cu levels and sources had a significant ( $P < 0.05$ ) impact on the feed intake of birds. In general, birds supplemented with 12 or 16 ppm Cu of either a GNC or MNC source had higher feed intake than birds supplemented with 8 ppm Cu of an IC source, and other Cu level and source combinations led to intermediate feed intake values.

Significant impact of copper levels, copper sources, and their interaction have been observed in the FCR of birds. Birds supplemented with 16 ppm Cu had significantly ( $P < 0.01$ ) better feed efficiency than those supplemented with 12 ppm Cu, while 8 ppm Cu supplemented birds had significantly ( $P < 0.01$ ) poor feed efficiency. GNC or MNC sources of Cu significantly ( $P < 0.01$ ) outperformed birds fed with IC source in terms of feed efficiency. The OC supplemented birds exhibited average feed efficiency. The birds supplemented with 16 ppm Cu of a GNC or MNC source exhibited superior feed efficiency than those supplemented with 8 or 12 ppm Cu from an IC source, whereas intermediate feed efficiency of birds was observed in other combinations.

### 3.2 Carcass characteristics

There was no significant ( $P > 0.05$ ) differences in the carcass trait (Table 3) and cut-up part (Table 4) of broiler chicken due to different copper levels, sources, and their interaction. However, the live weight of birds showed significant effect of Cu levels and sources and revealed the trend shown by the body weight gain of birds.

### 3.3 Immune response

Table 5 provides the findings of the immunological response to feeding broiler chicken different levels and sources of Cu. At 8 ppm level the PHAP and SRBC values were significantly ( $P < 0.01$ ) lower, followed by values at 12 ppm level, compared to the 16 ppm level. The PHAP values of the birds supplemented

**Table 3 Effect of different levels and sources of copper on the carcass characteristics of broiler chicken (% live weight)**

Cu source	Cu level (ppm)	Live weight (g)	Blood loss	Eviscerated weight	Heart	Liver	Gizzard	Abdominal fat
IC	8	1437.8 <sup>a</sup>	2.53	63.73	0.49	3.18	2.34	1.09
OC		1503.5 <sup>c</sup>	2.76	65.17	0.50	3.13	2.44	1.08
GNC		1702.2 <sup>e</sup>	3.13	63.94	0.53	3.26	2.68	1.19
MNC		1710.8 <sup>e</sup>	3.53	64.84	0.54	3.04	2.57	0.93
IC	12	1506.6 <sup>b</sup>	2.55	66.33	0.51	2.76	2.38	1.08
OC		1523.0 <sup>d</sup>	3.03	65.55	0.48	2.81	2.57	0.75
GNC		1775.1 <sup>f</sup>	2.61	64.66	0.51	3.17	2.68	1.52
MNC		1802.3 <sup>f</sup>	2.26	66.09	0.46	2.88	2.46	0.93
IC	16	1522.2 <sup>b</sup>	3.70	62.58	0.48	3.27	2.59	0.90
OC		1600.2 <sup>d</sup>	2.77	65.64	0.47	2.76	2.36	0.93
GNC		1801.9 <sup>g</sup>	2.00	67.25	0.52	2.70	2.55	1.18
MNC		1818.5 <sup>g</sup>	3.24	66.72	0.47	2.75	2.43	0.89
SEM		9.59	0.17	0.31	0.01	0.06	0.04	0.06
<b>Main effects</b>								
Copper levels (ppm)								
8		1593.4 <sup>a</sup>	2.99	64.42	0.51	3.15	2.51	1.07
12		1638.0 <sup>b</sup>	2.61	65.50	0.49	2.91	2.52	1.07
16		1663.5 <sup>c</sup>	2.93	65.66	0.48	2.87	2.48	0.97
Copper sources								
IC		1498.8 <sup>a</sup>	2.92	64.21	0.49	3.07	2.43	1.02
OC		1543.2 <sup>b</sup>	2.85	65.45	0.48	2.90	2.45	0.92
GNC		1749.8 <sup>c</sup>	2.58	65.28	0.52	3.04	2.64	1.29
MNC		1788.2 <sup>c</sup>	3.01	65.88	0.49	2.89	2.49	0.92
<b>Probability</b>								
Interaction		P<0.01	NS	NS	NS	NS	NS	NS
Copper Levels		P<0.01	NS	NS	NS	NS	NS	NS
Copper Source		P<0.01	NS	NS	NS	NS	NS	NS

Values bearing different superscripts within the row differ significantly  
NS: Non significant; FCR: Feed conversion ratio; SEM: Standard error of mean  
IC: Inorganic Copper, OC: Organic Copper, GNC: Green Nano Copper, MNC: Market Nano Copper

with Cu from GNC source were ( $P < 0.01$ ) greater than those of the birds fed Cu from MNC source and lower values were obtained in birds supplemented with IC source followed by OC. However, the SRBC values of birds fed Cu of GNC and MNC source were statistically similar but were significantly higher compared to OC source and least value was observed due to IC supplementation of birds. The interaction between levels and sources of copper revealed higher ( $P < 0.01$ ) SRBC values in birds supplemented with 16 ppm Cu of GNC or MNC source compared to 8 ppm Cu of IC source. Intermediate SRBC values were observed at other combinations. No significant interaction effect was observed on PHAP values.

#### 4. Discussion

In this study, weight gain, feed intake, as well as feed efficiency improved at higher copper levels or nano particle

size. However, it cannot be established clearly whether the improved weight gain was due to the increase of feed intake of birds or the direct effect of the Cu on the gastrointestinal tract of the broiler chicken. Similar to the results of this study the supplementation of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  at 150 mg/kg improved the live weight gain of broiler chicken and this improvement was supposed to be due the reduction of gut pathogen load under the influence of Cu (Xia et al. 2004). Similar results by Choi and Paik (1989) showed that supplementation of 25-50 ppm Cu improved the growth and feed conversion efficiency of broiler chicken. Also, in swine the intravenous injection of Cu stimulated the growth of weaning piglets (Zhou et al. 1994). However, contrary to the results of this study no effect of nano Cu supplementation was observed on the growth performance of the chicken (Ognik et al. 2018).

In this investigation, no discernible relationship between

Table 4 Effect of different levels and sources of copper on the cutup parts of broiler chicken (% live weight)

Cu source	Cu level (ppm)	Neck	Wing	Breast	Back	Thigh	Drum stick
IC	8	4.14	8.23	16.02	15.54	10.51	9.44
OC		3.71	8.08	15.91	17.53	11.20	9.03
GNC		3.98	7.67	15.32	17.21	10.21	9.64
MNC		4.29	8.08	13.97	16.95	11.04	10.56
IC	12	4.00	7.91	15.28	16.46	10.31	9.52
OC		4.12	7.93	16.31	17.00	11.04	9.38
GNC		4.01	7.88	14.73	17.22	10.93	9.08
MNC		3.94	8.32	15.16	17.17	12.65	9.63
IC	16	4.80	7.74	15.25	14.89	9.20	9.41
OC		5.26	8.23	15.65	15.40	11.02	10.10
GNC		4.29	8.69	16.02	17.07	11.02	9.50
MNC		3.88	8.42	15.66	17.66	11.11	9.87
SEM		0.10	0.06	0.14	0.18	0.20	0.12
<b>Main effects</b>							
Copper levels (ppm)							
8		4.03	8.02	15.30	16.81	10.74	9.67
12		4.02	8.01	15.37	16.96	11.23	9.40
16		4.56	8.27	15.64	16.26	10.59	9.72
Copper sources							
IC		4.31	7.96	15.52	15.63	10.01	9.46
OC		4.36	8.08	15.96	16.64	11.08	9.50
GNC		4.09	8.08	15.36	17.17	10.72	9.41
MNC		4.04	8.27	14.93	17.26	11.60	10.02
<b>Probability</b>							
Interaction		NS	NS	NS	NS	NS	NS
Copper Levels		NS	NS	NS	NS	NS	NS
Copper Source		NS	NS	NS	NS	NS	NS

NS: Non significant; FCR: Feed conversion ratio; SEM: Standard error of mean

IC: Inorganic Copper, OC: Organic Copper, GNC: Green Nano Copper, MNC: Market Nano Copper

Cu levels and sources and broiler chicken carcass traits was found. Similar to the findings of this study, blood loss, feather loss, eviscerated weight, and ready to cook yield did not show any significant effects of Cu supplementation broiler chicken (Shamsudeen 2007). Additionally, varied dietary Cu sources and levels showed no discernible impact on the shrinkage loss due to fasting, blood loss, eviscerated yield, and dressed weight of broiler chicken (Kulkarni 2009).

Cu has been associated with specific as well as non-specific immune mechanism and the specific immune mechanism includes humoral and cell mediated immunity which have been studied here. The inadequacy or deficiency of dietary Cu negatively affects CMI by increasing mast cells (non-specific immune cells) in muscles (Schusehke et al. 1994) and decreasing the T cell population (specific immune cells) (Muihern and Koller 1988). Increase in the levels of immunoglobulins and cytokine IL-6 with decreased lysozyme

activity has been observed in the blood of broiler chicken in a dose dependent manner of nano Cu supplementation (Ognik et al. 2018). However, contrary to the results of this study no significant effect of Cu source was observed on the CMI of broiler chicken (Shamsunder 2007). Even the supplementation of dietary Cu at higher levels (400 mg/kg diet) have been reported to suppress the immunoglobulin synthesis and hence negatively affect the humoral immune function of broiler chicken (Yang et al 2008).

## 5. Conclusions

The dietary supplementation of Cu in broiler chicken at 16 ppm level of nano form improves the growth and feed efficiency along with an enhanced immunity. The green nano Cu synthesised in this study was equally better in improving growth performance and immunity of birds as market nano Cu. However, green nano Cu was superior to market nano Cu in



improving the cell mediated immunity of broiler chicken. Therefore, this study recommends the 16 ppm green nano Cu in broiler chicken for better growth performance and immunity

**Table 5 Effect of different levels and sources of copper on the cell mediated and humeral immunity of broiler chicken**

Cu source	Cu level (ppm)	PHAP (mm)	SRBC (log2)
IC	8	0.23	7.60 <sup>a</sup>
OC		0.29	8.38 <sup>d</sup>
GNC		0.38	8.61 <sup>f</sup>
MNC		0.35	8.57 <sup>f</sup>
IC	12	0.23	7.66 <sup>b</sup>
OC		0.31	8.41 <sup>e</sup>
GNC		0.39	8.76 <sup>g</sup>
MNC		0.38	8.64 <sup>g</sup>
IC	16	0.25	7.70 <sup>c</sup>
OC		0.32	8.44 <sup>e</sup>
GNC		0.40	8.62 <sup>h</sup>
MNC		0.39	8.68 <sup>h</sup>
SEM		0.03	0.08
<b>Main effects</b>			
Copper levels (ppm)			
	8	0.31 <sup>a</sup>	8.28 <sup>a</sup>
	12	0.33 <sup>b</sup>	8.34 <sup>b</sup>
	16	0.34 <sup>c</sup>	8.37 <sup>c</sup>
Copper sources			
	IC	0.24 <sup>a</sup>	7.65 <sup>a</sup>
	OC	0.30 <sup>b</sup>	8.41 <sup>b</sup>
	GNC	0.39 <sup>d</sup>	8.63 <sup>c</sup>
	MNC	0.36 <sup>c</sup>	8.73 <sup>c</sup>
<b>Probability</b>			
	Interaction	NS	P<0.01
	Copper Levels	P<0.01	P<0.01
	Copper Source	P<0.01	P<0.01
Values bearing different superscripts within the row differ significantly NS: Non significant; FCR: Feed conversion ratio; SEM: Standard error of mean IC: Inorganic Copper, OC: Organic Copper, GNC: Green Nano Copper, MNC: Market Nano Copper			

of broiler chicken.

## Declarations

**Funding:** Not received any funding

**Conflict of interest:** None declared

**Ethics approval:** The study was approved by the Institutional Animal Ethics Committee of Indian veterinary Research

Institute, Izzatnagar, Bareilly

**Availability of data and materials:** Available on reasonable request from corresponding

**Acknowledgements:** None to acknowledge

## References

- Banks KM, Thompson KL, Rush JK, Applegate TJ. (2004). Effects of copper source on phosphorus retention in broiler chicks and laying hens. *Poultry Science* 83: 990-996.
- BIS (2007). Nutrient requirements for poultry 1S: 9863, Bureau of Indian Standards, New Delhi, India.
- Choi YJ, Paik IK. (1989). The Effect of Supplementing copper sulfate on the performance of broiler chicken. *Korean Journal of Animal Nutrition and Feeding* 13: 193-200.
- Dukare S, Mir NA, Mandal AB, Dev K, Begum J, Tyagi PK, Rokade JJ, Biswas A, Tyagi PK, Bhanja SK (2020). Comparative study on the responses of broiler chicken to hot and humid environment supplemented with different dietary levels and sources of selenium. *Journal of Thermal Biology* 88: 102515. <https://doi.org/10.1016/j.jtherbio.2020.102515>
- Kim BE, Nevitt T, Thiele DJ. (2008). Mechanisms for copper acquisition, distribution and regulation. *Nature Chemical Biology* 4(3): 176-185.
- Kim S, Chao PY, Allen GD. (1992). Inhibition of elevated hepatic glutathione abolishes copper deficiency cholesterolaemia. *The FASEB Journal* 6: 2467-2471.
- Kulkarni RC. (2009). Comparative efficacies of chelated and organic copper and iron supplementation in broiler diet. M.V.Sc thesis, IVRI Deemed University, Izzatnagar, Bareilly, India.
- Mandal AB. (2010). Dietary modulation to curb impact of changing climate on livestock and poultry. In: International conference on physiological capacity building in livestock under changing climate scenario. 1: 127-136.
- Muihern SA, Koller LD. (1988). Severe or marginal copper deficiency result in a graded reduction of the immune status in mice. *Journal of Nutrition* 118: 1041-1047.
- Mujahid A, Yoshiki Y, Akiba Y, Toyomizu M. (2005). Superoxide radical production in chicken skeletal muscle induced by acute heat stress. *Poultry Science* 84: 307-314.
- Ognik K, Stepniowska A, Cholewinska E, Kozlowski K. (2016). The effect of administration of copper nanoparticles to chickens in drinking water on estimated intestinal absorption of iron, zinc, and calcium. *Poultry Science* 95: 2045-2051.
- Ognik K, Iwona S, Ewelina CN, Jan J, Krzysztof K, Jerzy J, Zenon Z. (2018). The effect of administration of copper nanoparticles to chickens in their drinking water on the immune and antioxidant status of the blood. *Animal Science Journal* 89: 579-588.
- Sahin K, Kucuk O. (2003). Heat stress and dietary vitamin supplementation of poultry diets. *Nutrition Abstracts and Reviews Series B: Livestock Feeds and Feeding* 73: 41R-50R.
- Schuschke DA, Saari JT, West CA, Miller FN. (1994). Dietary copper deficiency increases the mast cell population of rat. *Proc. Soc. Experimental Biology and Medicine* 207: 274-277.
- Shamsudeen P. (2007). In vitro and bio-interaction of chelated and inorganic trace minerals with aflatoxine in broiler chicken. PhD thesis, Deemed University, IVRI, Izzatnagar, Bareilly, India.

- Xia MS, Hu CH, Xu ZR. (2004). Effects of copper-bearing montmorillonite on growth performance, digestive enzyme activities, and intestinal microflora and morphology of male broilers. *Poultry Science* 83: 1868-1875.
- Yang X, Min C, Xi P. (2008). Effect of dietary high copper on serum immunoglobulin content in chicken. *Chinese Journal of Veterinary Science* 28(11): 1332-1355.
- Zhou W, Kornegay ET, Lindemann MD, Swinkels JWGM, Welten MK, Wong EA. (1994). Stimulation of growth by intravenous injection of copper in weaning pigs. *Journal of Animal Science* 72: 2395-2403.

**Citation**

Dukare S, Mandal AB, Mir NA, Dev K, Tyagi PK, Biswas A, Rokade JJ, Tyagi PK. (2021). Effect of different levels and sources of dietary copper on the growth and immunity of broiler chicken. *Letters in Animal Biology* 01(2): 26 – 32.

---