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Carcass, Bone and Meat Quality Characteristics of Giriraja Chicken Fed Reduced Levels of Organic and Nano Copper Supplemented Diets

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ABSTRACT

Copper (Cu) is an essential component of various metabolic enzymes and its inclusion in the poultry diets in the form of inorganic salts is a common practice. However, due to low bioavailability of Cu from inorganic sources, its high excretion to environment is a concern. This study was conducted to assess the reduced level of dietary organic and nano particle Cu prepared using $CuSO_4$on carcass, bone and meat quality characteristics of Giriraja chicken. A total of 420 day-old Giriraja chicks were randomly assigned to 7 treatment groups having 4 replicates each (15 chicks per replicate). The basal diets of chick (0-6 weeks) and grower phases (7-10 weeks) were supplemented with 20 and 30 ppm Cu, respectively from inorganic CuSO4 as control (T1). The test diets T2, T3, and T4 were supplemented with 100, 75 and 50% of control from organic source (Cuproteinate) while diets T5, T6 and T7were supplemented with 75, 50 and 25% of control from Cu nanoparticles, respectively. At the end of the trial, two birds per replicate were slaughtered to study carcass, bone and meat quality characteristics. The weight of breast muscle, thigh muscle and thymus was higher ($P<0.05$) at 75% Cu nanoparticles supplemental level and calcium content of thigh bone was higher at 50% Cu nanoparticles inclusion level. The weight of de-feathered and dressed carcass, liver, heart, gizzard, spleen, bursa of Fabricius, abdominal fat and intestine were similar among different treatments. The moisture, crude protein and total ash of thigh muscle and weight, length, width, total ash and phosphorous content of thigh bone were also not affected due to reduced dietary Cu level supplemented from organic or nanoparticles forms. In conclusion, the dietary $CuSO_4$ can be reduced up to 50 and 75% using organic and nanoparticle forms, respectively without compromising with the carcass, bone and meat quality characteristics.

KEYWORDS: Carcass quality, Copper sulfate, Copper proteinate, Giriraja, Nanoparticles

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INTRODUCTION

Trace minerals in sufficient amount in feeds is essential to ensure good performance and animal health. Among these, copper (Cu) is an important trace element in poultry diets and is a component of various metabolic enzymes. However, the Cu content of basic feed components does not meet the nutritional requirements of rapidly growing birds (Shen et al., 2022).Due to its metabolic importance, any deficiency in the diet can result in the emergence of metabolic disorders and deficiency symptoms in poultry. Therefore, adding Cu in the form of inorganic mineral salt to the diets is a common practice. Researchers have demonstrated that Cu and other minerals excretion increases linearly with increases in their dietary inclusion level (Bao et al., 2007). In laying hens, the Cu content per kg of excreta dry matter (DM) increased from 25.3 to 397 mg/kg when the basal diet was supplemented with $CuSO_4$ at 0 and 240 mg/kg, respectively as per the report of Skrivan et al. (2006).Due to the poor bioavailability of inorganic Cu sources, its large quantity being excreted in poultry manure, and entered into agricultural land, where its potential toxic effect is an emerging concern (Scott et al., 2018).

In many experiments, it has been demonstrated that organic trace minerals, including amino acid chelates, increases their bioavailability (Jegede et al., 2011), and decrease the faecal excretion (Mikulski et al., 2009). Hence, the organic form of Cu can be used at a reduced dietary inclusion level due to its improved absorption, utilization and metabolism properties (Nollet et al., 2007). Due to the novel properties of nano form, it is hypothesized that Cu nanoparticle (Cu-NP) is another form to substitute the conventional source of $CuSO_4$ in poultry diets (Hefnawy and El-khaiat, 2015).Our own study revealed that the Cu inclusion levels can be reduced by 50 and 75% of the conventional form of $CuSO_4$ using organic and nanoparticle sources, respectively without any adverse effects on the growth performance and nutrients utilization in Giriraja chicken (Aminullah et al., 2022a). Similar observations were noted in Swarnadhara breeder hens without affecting egg production performance, egg quality and hatchability (Aminullah et al., 2021). It was also reported that reduced dietary Cu supplementation levels using nano and organic forms can reduce the faecal Cu excretion rate in Giriraja chicken (Aminullah et al., 2022b). Copper plays important roles in the cross-linking of collagen and elastin fibers that are involved in bone (Shen et al., 2022) as well as cartilage and muscle development and as growth promoters (Miggiano and Gagliardi, 2005). Copper also has a crucial role in lysyl oxidase that is essential for calcium and phosphorus deposition in bones and its deficiency leads to decreased bone mineralization, reduced strength, and an increased risk of bone fractures (Gau et al., 2021). Therefore, the process of bone formation requires a proper supply of Cu throughout life (Palacios, 2006). Hence, the study was conducted to determine whether the dietary Cu supplemental level can be reduced using organic and nano forms without compromising the carcass quality characteristics of Giriraja chicken.

MATERIALS AND METHODS

The experiment was carried out at the Veterinary College, Karnataka Veterinary, Animal and Fisheries Science University (KVAFSU), Bengaluru, Karnataka, India, located at 13.03^0N and 77.60^0E.The Institutional Animal Ethics Committee approved all procedures and practices relating to the management and care of the birds, with approval number VCH/IAEC/2020/01, dated March 3, 2020.

Cu sources

The nano Cu source was procured from M/s Matrix Nano Pvt Ltd., New Delhi, India, and inorganic (copper sulfate)and organic copper (copper proteinate) sources were procured from M/s Zeus Biotech Pvt. Ltd., Mysuru, Karnataka, India. Copper sulfate ($CuSO_4$) was used for preparation of both organic and nano forms. The particle size of nano Cu was 50-80 nm with 98% purity as certified by the manufacturer using transmission electron imaging and particle size distribution using intensity dynamic light scattering method.

Experimental diets

The basal diets for chick (1-6 weeks) and grower phase (7-10 weeks) were prepared excluding Cu as per ICAR (2013) nutrient specifications for poultry. The ingredients and chemical composition of basal diets are presented in Table 1.

Table 1.Ingredient and nutrient composition of basal diets

Ingredients (kg)	Chick diet (1-6 week)	Grower diet (7-10 week)
Yellow maize	61.3	57.0
Soybean meal	34.5	24.3
De-oiled rice bran	-	15.0
Dicalcium phosphate	1.55	-
Mineral mixture-without copper*	2.00	3.00
Bacitracin methylene disalicylate	0.03	0.03
Salinomycin	0.05	0.05
Vitamin premix**	0.02	0.03
Vitamin B complex***	0.03	0.04
DL-Methionine	0.10	0.08
Hepatocare	0.10	0.10
Common salt	0.31	0.31
Total	100.00	100.00
Nutrient composition		
Metabolizableenergy[a] (kcal/kg)	2881	2795
Crude protein (%)	21.2	18.3
Calcium (%)	1.09	1.10
Total phosphorus (%)	0.86	0.65
Lysine[a](%)	1.17	0.97
Methionine[a](%)	0.46	0.41
Selenium[a](ppm)	0.25	0.27
Zinc (ppm)	105	124
Iron (ppm)	104	118
Manganese (ppm)	94	127
Copper (ppm)	7.97	8.27

*Contains: Ca-32%, P-9%, Fe-2000ppm, I-0.01%, Mn-0.4% and Zn-0.4%,
** Each gram contains: Vitamin A-82500 IU, B_2-50 mg, D_3-12000 IU & K-10 mg.
***Each gram contains: Vitamin B_1-4 mg, B_6-8mg, B_{12}-40mg, E-40mg, Pantothenate-40mg,Niacin-60mg.
[a] calculated value

Further, the basal diets were supplemented with 20 ppm Cu in the chick phase and 30 ppm Cu in the grower phase with the conventional form of inorganic $CuSO_4$and served as control (T1). The test diets prepared for chick and grower phases were supplemented with Cu organic form at 100 (T2), 75 (T3), and 50% (T4) or with Cu nanoparticle form at 75 (T5), 50 (T6) and 25% (T7) of recommended level used in the control (Table 2).

Table 2. Description of experimental diets with Cu levels

Treatment Group	Copper source and level (% of standard recommendation)	Supplemental Cu (ppm)		Analyzed Cu (ppm)	
		Chick diet (1-6 weeks)	Grower diet (7-10 weeks)	Chick diet (1-6 weeks)	Grower diet (7-10 weeks)
T1	Inorganic Cu (100)	20	30	27.9	38.3
T2	Organic Cu (100)	20	30	27.6	37.5
T3	Organic Cu (75)	15	22.5	22.6	29.3
T4	Organic Cu (50)	10	15	17.7	23.5
T5	Nano Cu (75)	15	22.5	22.7	28.4
T6	Nano Cu (50)	10	15	18.0	24.4
T7	Nano Cu (25)	5	7.5	12.7	16.4

OC = Organic copper (Cu proteinate); NC = Nano copper

Experimental birds

A total of 420 day-old Giriraja chicks (improved native strain) were wing banded and randomly assigned to seven treatment groups of four replicates with 15 chicks each. The birds were maintained under a deep litter system with all standard management practices for 10 weeks. All treatment groups received respective diets ad-libitum during the period 1 day to 10 weeks of age.

Organometry

On the70th day, two birds from each replicate were randomly selected and individually weighed, slaughtered as per the modified Kocher method. The de-feathered and dressed carcass weights were recorded. The liver, heart, gizzard, thymus, bursa of Fabricius, abdominal fat and intestine were carefully separated and the weights were recorded using electronic digital weighing balance. The bones were soaked in petroleum ether for 48 h, soft tissues were removed and dried at 100^{o}C until a constant weight to record the weight. To evaluate the mineral densification in bone, thigh bone index was calculated using the formula weight (mg)/length (mm) (Kocabagli, 2001).

Statistical analysis

The data were subjected to ANOVA using Statistical Package for the Social Sciences (SPSS-16). The Pd”0.05 value of traits was considered for identifying significant differences among the treatment groups.

RESULTS AND DISCUSSION

The carcass characteristics of experimental birds under different treatments are presented in Table 3. As a general trend, the inclusion levels as well as a source of Cu had no effects on carcass characteristics of Giriraja chicken. However, significantly (Pd”0.05) greater relative weight of breast and thigh muscle as well as breast muscle to bone ratio was observed at 75% Cu-NP inclusion level (T5), while the lowest (Pd”0.05) value was observed at 50% Cu-NP inclusion level (T6). The higher weight of muscle at the 75% Cu-NP inclusion level was attributed to improved tissue protein accretion and growth-stimulating effect in the respective groups. Copper is reported to be a key mineral element required for animal growth, muscle (Miggiano and Gagliardi, 2005), bones, connective tissues, heart, and several other organs development (Hefnawy and El-Khaiat, 2015). However, in the current study, the muscle protein content remained unaffected among the treatment groups (Table 5).

In the current study, except muscle weight, the rest of the organs remained similar in response to the reduced levels of dietary organic and nano Cu form. The findings were in agreement with Scott et al. (2016) and Abdullah et al. (2022) who reported increased breast muscle mass, and improved other carcass characteristics with Cu-NP inclusion in broiler diet, respectively. The results are also in corroboration with the findings of Ramesh (2014) and Sawosz et al. (2018) who reported no adverse effect of Cu-NP at75% reduction level on carcass quality traits in layer and broiler birds, respectively. Ghazanfari et al. (2021) and Yang et al. (2018) reported no effect of NP or organic source of Cu on the relative weight of various organs, as well as eviscerated carcass, breast muscle, leg muscle, and abdominal fat percentage of broiler chicken, respectively.

Hajilari et al. (2019) and Wang et al. (2014) also reported no effect (Pe”0.05) of dietary Cu supplementation on breast muscle percentage, and fat, breast and thigh muscle, drumstick, and carcass weight in broiler birds, respectively. El-Husseiny et al. (2012) reported improved dressing, liver, and breast muscle percentage but reduced heart, gizzard, inedible parts, eviscerated yield, and leg muscle percentage with 50 percent inclusion level of Zn, Mn, and Cu from organic sources in the diet as compared to the standard level of inorganic salt. This indicates that Cu plays role in tissue development, while the reduced level using an improved bioavailable form has no adverse effect.

One of the very important parameters for the immune response of birds is the lymphoid organ's index. In the present study, no effect of reduced dietary Cu levels from organic or NP sources was observed on the bursa and spleen, whereas the weight of the thymus was greater (Pd”0.05) in response to 75% Cu-NP inclusion only (Table 3). The phenomenon indicates that despite reduced Cu supplementation levels from organic or NP sources, the relative weight of the lymphoid organs can be maintained unaffected. Scott et al. (2017) also reported no effect of Cu-NP dietary inclusion on lymphoid organs. However, El-Kazaz et al. (2020) reported increased weight of lymphoid organs (spleen, thymus, and bursa of Fabricius) while Scott et al. (2016) reported decreased bursa weight due to Cu-NP inclusion in broilers diet.

Table 3. Carcass quality characteristics (per cent of pre-slaughter weight) of experimental birds under different treatments

Attribute	T1 Inorganic (100%)	T2 Organic (100%)	T3 Organic (75%)	T4 Organic (50%)	T5 Cu-NP (75%)	T6 Cu-NP (50%)	T7 Cu-NP (25%)	SEM	P value
De-feathered carcass	89.4 ±0.36	89.8 ±1.08	90.6 ±0.36	88.8 ±1.34	89.6 ±1.69	91.1 ±0.55	89.4 ±1.33	1.63	0.398
Dressed carcass	71.9 ±0.46	72.7 ±0.42	69.8 ±8.03	73.7 ±3.15	73.7 ±0.86	71.7 ±1.11	73.9 ±0.47	1.30	0.079
Breast muscle	11.24^{ab} ±0.34	11.8^{ab} ±0.47	10.5^{b} ±0.31	11.6^{ab} ±0.49	12.6^{a} ±0.76	10.1^{b} ±0.57	11.7^{ab} ±0.59	0.80	0.047
Thigh muscle	8.11^{ab} ±0.33	8.23^{ab} ±0.33	8.45^{ab} ±0.37	8.83^{ab} ±0.21	9.13^{a} ±0.32	7.66^{b} ±0.24	8.32^{ab} ±0.29	0.44	0.032
Breast bone	5.46 ±0.17	5.60 ±0.18	5.98 ±0.17	6.20 ±0.85	5.36 ±0.14	5.38 ±0.31	5.88 ±0.35	0.59	0.619
Thigh bone	0.77 ±0.03	0.71 ± 0.4	0.88 ±0.05	0.78 ±0.6	0.77 ±0.03	0.75 ±0.02	0.88 ±0.03	0.06	0.882
Breast muscle: bone ratio	2.02^{b} ±0.17	2.12^{ab} ±0.12	1.81^{b} ±0.07	2.17^{ab} ±0.27	2.26^{a} ±0.14	2.03^{b} ±0.18	1.97^{b} ±0.21	0.26	0.046
Thigh muscle: bone ratio	10.8 ±0.65	10.3 ±0.82	10.2 ±0.99	11.7 ±0.77	11.7 ±0.80	9.85 ±0.46	11.1 ±0.45	1.10	0.688
Liver	2.27 ±0.15	2.28 ±0.84	2.07 ±0.06	2.10 ±0.08	2.28 ±0.11	2.48 ±0.20	2.21 ±0.13	0.19	0.327
Heart	0.42 ±0.11	0.48 ±0.01	0.43 ±0.12	0.48 ±0.02	0.40 ±0.01	0.40 ±0.03	0.43 ±0.25	0.02	0.759
Gizzard	2.30 ±0.09	2.48 ±0.14	2.51 ±0.07	2.60 ±0.11	2.77 ±0.19	2.37 ±0.13	2.16 ±0.70	0.18	0.086
Spleen	0.22 ±0.03	0.14 ±0.11	0.26 ±0.03	0.23 ±0.03	0.24 ±0.03	0.22 ±0.03	0.21 ±0.0.03	0.04	0.436
Thymus	0.41^{ab} ±0.04	0.42^{ab} ±0.03	0.46^{ab} ±0.06	0.42^{ab} ±0.06	0.55^{a} ±0.05	0.45^{ab} ±0.07	0.38^{b} ±0.42	0.04	0.003
Bursa of Fabricius	0.01 ±0.02	0.13 ±0.03	0.15 ±0.03	0.12 ±0.04	0.13 ±0.01	0.14 ±0.02	0.15 ±0.03	0.04	0.229
Abdominal fat	0.84 ±0.10	0.93 ±0.17	1.33 ±0.15	0.83 ±0.16	1.20 ±0.16	1.01 ±0.12	0.78 ±0.14	0.21	0.096
Intestine	4.61 ±0.32	5.45 ±0.22	4.84 ±0.12	5.08 ±0.34	4.92 ±0.26	5.22 ±0.27	4.55 ±0.25	0.60	0.198

Mean values bearing different superscripts within the row differ significantly ($P \leq 0.05$)

Bone length, weight, index and mineral contents are important indicators of bone mineralization (Sahraei et al., 2012). In the present study, thigh bone characteristics such as weight, length, width and index were not affected by the treatment groups. The bones will be denser when the weight/length index is high (Kocabagli, 2001). Thigh bone total ash and phosphorus content also remained unaffected among the groups, while calcium content was higher (Pd”0.05) in the 50% Cu-NP group as compared to 100% organic Cu inclusion level (Table 4).The Cu-NP supplementation at 50% may be optimum level of Cu-NP for the improved activity of lysyl oxidase, which is Cu dependent enzyme for bone development (Nguyen et al., 2022), while there was no effect at 75% and 25% supplementation levels of Cu-NP. However, the available literature shows Cu importance in bone development (Shen et al., 2022), whereas in the current study, bone mineralization was not compromised due to reduced dietary Cu levels indicating better efficiency of Cu sourced from NP and organic forms.

In a study, the femur bone’s physical characteristics were improved through enhanced proliferating cell nuclear antigen [PCNA]-positive cells in broilers when administered Cu-NP in ovo (Mroczek et al., 2017).Banks et al. (2004) reported increased weight of tibia bone with Cu lysine supplementation in broiler diets. The bone density was improved when rats were fed diet containing Cu-NP as compared to Cu carbonate (Tomaszewska et al., 2017).

In the current study, the thigh muscle composition viz., moisture, crude protein and total ash content remained similar (Pe”0.05) among different treatment groups (Table 5). However, improved protein metabolizability and energy utilization were reported due to Cu-NP inclusion in the diet (Aminullah et al., 2022c).The results indicate that Cu sourced from organic or nanoparticle forms at reduced dietary levels can maintain muscle composition which may be due to better efficiency and utilization of the Cu sources.

Table 4. Thigh bone quality characteristics of experimental birds under different treatments

Attribute	T1	T2	T3	T4	T5	T6	T7	SEM	P value
	Inorganic (100%)	Organic (100%)	Organic (75%)	Organic (50%)	Cu-NP (75%)	Cu-NP (50%)	Cu-NP (25%)		
Weight (g)	12.6±0.82	13.3±0.83	13.4±0.78	12.9±0.87	11.7±0.83	12.6±0.52	13.9±0.68	1.16	0.552
Length (mm)	82.5±1.42	83.4±1.37	84.0±1.37	83.6±0.76	81.5±0.98	80.9±0.85	84.8±0.79	1.68	0.178
Index* (mg/mm)	153±1.07	159±1.06	159±1.12	154±0.85	143.7±0.96	156±0.56	163±0.79	1.42	0.436
Width (mm)	8.96±0.37	8.93±0.25	8.74±0.13	8.27±0.21	8.49±0.30	8.55±0.21	8.96±0.18	0.37	0.320
Total ash (%)	40.3±0.38	40.6±0.74	41.3±0.46	41.0±0.56	42.4±0.52	42.0±0.89	41.4±0.85	0.37	0.320
Calcium (%)	15.3^{ab}±0.25	14.7^{b}±0.13	15.7^{ab}±0.47	16.3^{ab}±0.44	15.7^{ab}±0.37	17.1^{a}±0.50	16.0^{ab}±0.26	0.61	0.010
Phosphorus (%)	7.23±0.35	7.27±0.37	7.18±0.25	7.62±0.34	7.19±0.37	6.95±0.42	7.41±0.14	0.50	0.888

Mean values bearing different superscripts within the row differ significantly (Pd”0.05), *Index = Weight (mg) / Length (mm)

CONCLUSION

It was concluded that the dietary Cu supplementation can be reduced up to 50 and 75% using organic and nano forms, respectively without compromising the carcass quality characteristics, bone mineralization and muscle composition.

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