RESEARCH ARTICLE Indian Journal of Animal Research, Volume 56 Issue 1: 58-64 (January 2022)

Effect of Reduced Dietary Copper Levels Sourced from Organic and Nanoparticles Forms on Performance and Nutrient Utilization in Giriraja Birds

Noor Aminullah[1], T.M. Prabhu[1], Jaya Naik[2], B.N. Suresh[3], N. Suma[1]

10.18805/IJAR.B-4538

ABSTRACT

Background: Copper (Cu) is an essential trace mineral for growth, production and health of poultry. Due to its poor absorption from inorganic source which is common supplemental form, large amount of Cu is excreted in the excreta. This experiment was conducted to assess the bioavailability of Cu from organic and nano sources at lower level of supplementation to reduce the excretion level without compromising the growth performance of chicken.

Methods: 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 both chick (0-6 weeks) and grower phases (7-10 weeks) were supplemented with 20 and 30 ppm of Cu from inorganic source as control (T_1), the groups T_2, T_3 and T_4 supplemented with 100, 75 and 50% of control from organic Cu, T_5, T_6 and T_7 treatment diets supplemented with 75, 50 and 25% of control from Cu nanoparticles, respectively.

Result: The grower phase and cumulative body weight gain was significantly ($p<0.05$) higher in T_2 compared to T_1, T_6 and T_7 groups, while the feed intake in chick phase was lower in T_2, T_5 and T_7 groups as compared to T_1, T_3, T_4 and T_6. The chick phase feed conversion ratio was significantly ($p<0.05$) better in T_2 and T_5 groups compared to T_1, T_3, T_4, T_6 and T_7, whereas the cumulative FCR was better in T_2 and T_5 compared to T_6 and T_7 groups, while it was not affected during grower phase. The dry matter and crude protein metabolizability were significantly ($p<0.05$) higher in T_2 and T_5, the organic matter and ether extract metabolizability was higher only in T_5. The crude fiber and nitrogen free extract was not affected by the treatment diets. Nitrogen excretion was significantly ($p<0.05$) lower and its retention was higher in T_2 and T_5 treatment groups but was comparable with control. The Cu excretion through excreta was significantly ($p<0.05$) higher and its retention was lower in control as compared to all dietary treatments. Hence, it was concluded that the Cu supplementation from organic or nanoparticles form can be reduced by 50 and 75% of standard, respectively without compromising the growth performance of birds and nutrient digestibility of diets.

Key words: Copper, Giriraja, Nanoparticles, Organic mineral.

INTRODUCTION

Copper (Cu) is an essential inorganic trace element required for growth, production, reproduction and health of animals and birds (Collins *et al*., 2010). It serves as a cofactor for more than 200 enzymes such as cytochrome oxidase, lysyl oxidase, ceruloplasmin and superoxide dismutase in animal body (Wu *et al.,* 2015). However, the Cu content in major feed ingredients included in poultry diet are unable to meet Cu requirement of birds hence, its supplementation is necessary to maintain the productivity and health of the birds. Further, the absorption and utilization of Cu from conventionally used inorganic sources is poor (Scott *et al*., 2018). Hence, a large amount of Cu is excreted and its accumulation in the environment causes toxicity in plants and grazing animals (Ferket *et al.,* 2002). Therefore, biotechnological interventions are essential to increase the Cu bioavailability and absorption and reduce its environmental pollution.

It is reported that the organic source of Cu can improve its utilization and minimize the level in the excreta and environmental pollution (Nollet *et al*., 2007) due to its better absorption and metabolism properties. Nollet *et al*. (2007)

[1]Department of Animal Nutrition, Veterinary College, Karnataka Veterinary, Animal and Fisheries Sciences University, Bengaluru-560 024, Karnataka, India.
[2]Department of Poultry Science, Veterinary College, Karnataka Veterinary, Animal and Fisheries Sciences University, Bengaluru-560 024, Karnataka, India.
[3]Department of Livestock Farm Complex, Veterinary College, Karnataka Veterinary, Animal and Fisheries Sciences University, Bengaluru-560 024, Karnataka, India.

Corresponding Author: Aminullah Noor, Department of Animal Nutrition, Veterinary College, Karnataka Veterinary, Animal and Fisheries Sciences University, Bengaluru-560 024, Karnataka, India. Email: aminullah.noor18@gmail.com

How to cite this article: Aminullah, N., Prabhu, T.M., Naik, J., Suresh, B.N. and Suma, N. (2022). Effect of Reduced Dietary Copper Levels Sourced from Organic and Nanoparticles Forms on Performance and Nutrient Utilization in Giriraja Birds. Indian Journal of Animal Research. 56(1): 58-64. DOI: 10.18805/IJAR.B-4538.

Submitted: 22-05-2021 **Accepted:** 03-08-2021 **Online:** 31-08-2021

also reported no differences in performance of broilers fed diet contained reduced levels of organic chelated Cu but significantly ($p<0.05$) lower fecal Cu excretion rates. Similarly, nano particles (NP) of Cu having large surface-to-volume ratio, higher bioavailability and less antagonism with other minerals are also better alternatives. As per the current literature available, the Cu-NP is better than its bulk form in enhancing performance of animals (Al-Bairuty *et al.,* 2016). The organic and NP of Cu enhance growth performance, nutrient utilization, reduce pathogen load and improves health of breeder hens (Raje *et al*., 2018). The dietary Cu was reduced to 50 and 25% of control from organic and NP sources, respectively in Swarnadhara breeder hens without affecting egg production, its quality and hatchability (Aminullah *et al.,* 2021). However, such studies in other improved breeds of birds on growth performance and nutrient utilization are lacking. Hence, the present study was planned to find out the most biologically effective form of Cu with higher efficiency and to reduce its supplementation and excretion level without compromising growth performance and nutrients utilization in Giriraja chicken.

MATERIALS AND METHODS

All the procedures with regards to the management and care of the birds followed during the trial were approved by the Institutional Animal Ethics Committee of the University having approval number VCH/IAEC/2020/01, dated 03.03.2020.

A genotype akin to country fowl named Giriraja, a multi-colored dual purpose cross breed chicken developed and released by Veterinary College, KVAFSU, Bengaluru for rural scavenging conditions were used. A total of 420-a day-old Giriraja chicks were wing banded and randomly assigned to seven treatment groups of four replicates with 15 chicks in each replicate maintained for 10 weeks of study period under deep litter system with all standard managemental practices. All treatment groups received respective iso-nitrogenous and iso-caloric diets according to their growth stage *viz*., chick' diet from 0 to 6 weeks and grower diet from 7 to 10 weeks of age. The daily required amount of feed was weighed and offered replicate wise and recorded. Water was provided *ad libitum* during the trial period. The experiment was conducted at Veterinary College, Hebbal, Bengaluru, Karnataka, India, located at 13.030N and 77.600E in 2020 for 10 weeks of time period.

The Cu nano particles were procured from M/s Matrix Nano Pvt Ltd., New Delhi and inorganic copper sulphate and organic copper as copper proteinate from M/s Zeus-Biotech Pvt. Ltd., Mysuru, Karnataka. The particle size of Cu-NPs was 50-80 nm with 98% purity. The basal diets were formulated as per ICAR (2013) nutrient specifications except Cu. The basal diet was supplemented with 20 ppm Cu in chick phase and 30 ppm in grower phase from inorganic $CuSO_4$ as control. The treatment diets for chick and grower phases were supplemented with different levels of Cu from organic and NP sources as described in Table 1. The ingredient and chemical composition of basal diets is presented in Table 2. The concentration of Cu in experimental diets (Table 1) was estimated using ICP-OES (Perkin Elmer Optima 8000).

After an adaptation period of 3 days during terminal week of the trial, a total excreta collection method involving two birds in each replicate housed in cage for 3 days was employed to study the bioavailability of different sources of Cu and other nutrients utilization. The samples of feed offered, residue left and excreta voided were analyzed for proximate composition. The nitrogen retained in the body was calculated as the difference between nitrogen intake and nitrogen excreted and expressed as gram per day per kg metabolic body weight ($W^{0.75}$) or per cent of intake nitrogen.

RESULTS AND DISCUSSION

The cumulative body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) of birds under different treatments is presented in Table 3. In the study, the BW values of the chick's stage (0-6th week) indicated no effect of source and inclusion levels of Cu on chicks BWG. In contrast, during grower phase (7-10th week) and cumulatively *i.e.* 1-10th week, the BWG was significantly ($p<0.05$) higher in T_2 as compared T_6 and T_7 and control groups. The improved BWG in T_2 treatment group was due to higher (100%) dose and better bioavailability of copper proteinate as compared to inorganic $CuSO_4$ (Das *et al*., 2010). Cu plays important role in poultry growth performance which is attributed to its antimicrobial properties that can improve

Table 1: Description of experimental groups and dietary total Cu content.

Tr. no.	Copper source and % standard recommendation	Supplemented Cu (ppm)		Analyzed dietary Cu content (ppm)	
		Chick diet	Grower diet	Chick diet	Grower diet
T_1	Inorganic Cu (100)	20	30	27.94	38.35
T_2	Organic Cu (100)	20	30	27.62	36.56
T_3	Organic Cu (75)	15	22.5	22.65	29.37
T_4	Organic Cu (50)	10	15	17.70	23.52
T_5	Cu-NP (75)	15	22.5	22.73	28.41
T_6	Cu-NP (50)	10	15	18.06	24.41
T_7	Cu-NP (25)	5	7.5	12.77	16.43

Organic Cu = Copper proteinate, Cu-NP = Copper nanoparticles.

the intestinal health (Usman *et al*., 2013). In previous reports, the effect of Cu on growth rate was attributed to its function on improved growth hormone axis (Yang *et al*., 2011) and hypothalamic appetite regulation expression in weanling pigs (Zhu *et al*., 2011).

It is hypothesized that Cu-NP due to its novel properties such as large surface area, higher reactivity and better utilization can improve the growth performance and reduce dietary supplementation and excretion rate as well. In the present study, however there is no improved BWG due to Cu-NP, but reduction even up to 75% level had no adverse effect on growth performance as the body weight was comparable to control. The findings are in accordance with those of Sawosz *et al.* (2018) and Kozlowski *et al.* (2018) who supplemented Cu-NP reduced by 75 and 80% in broilers and turkey, respectively without observing any reduction in growth performance. The reason is that Cu-NP can more eûectively penetrate cell membrane barriers and can be rapidly distributed independent of the blood circulation (Anjum *et al*., 2016).

Table 2: Ingredient and chemical composition of basal diets.

Ingredients (kg)	Chick diet (0-6 week)	Grower diet (7-10 week)
Yellow maize	61.30	57.00
Soybean meal	34.50	24.36
De-oiled rice bran	-	15.00
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**	0.025	0.03
Vitamin B complex***	0.035	0.04
DL-Methionine	0.10	0.08
Hepatocare	0.10	0.10
Common salt	0.31	0.31
Total	100.00	100.00
Chemical composition		
ME (kcal/kg)[a]	2881	2795
Crude protein	21.20	18.34
Calcium	1.09	1.10
Total phosphorus	0.86	0.65
Lysine	1.17	0.97
Methionine[a]	0.46	0.41
Selenium (ppm)[a]	0.25	0.27
Zinc (ppm)	105	124
Fe (ppm)	114	127.09
Mn (ppm)	94	127
Cu (ppm)	7.97	8.27

*Contains: Ca-32%, P-9%, Fe-2000 ppm, 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 and K-10 mg.***Each gram contains: Vitamin B_1-4 mg, B_6-8 mg, B_{12}-40 mg, E-40 mg, Pantothenate-40 mg, Niacin-60 mg. [a]Calculated value.

No effect of dietary treatments was observed on feed intake during grower phase (7-10 weeks) as well as cumulative period *i.e.* 1-10 week. In the chick's stage, the feed intake was significantly lower in T_2 and T_5 as compared to all other treatment groups except of T_7. Despite of reduced feed intake in T_2 and T_5 treatment groups, the BWG was not affected resulting in significant (p<0.05) improvement in feed conversion ratio. During grower phase there was no sighnificant difference in feed consumption among the treatment groups.

Cu plays important role in cytochrome C oxidase that contributes for efficient production of adenosine triphosphate and energy metabolism (Wu *et al.,* 2015). Therefore, the better feed conversion ratio at higher Cu level receiving groups (T_2 and T_5) reflects improved energy and nutrients utilization of the diet.

The improved gut health due to antimicrobial properties of Cu (Usman *et al*., 2013) also might have contributed for the better feed conversion ratio. Ramesh (2014) reported that dietary inclusion of Cu-NP at 25 to 50% of requirement can reduce feed intake without compromising the feed conversion ratio in laying hens. However, Wen *et al.* (2019) reported no significant effect of 20 and 40 ppm $CuSO_4$ or Cu-methionine on feed intake and feed conversion ratio in broilers.

The metabolizability of various nutrients presented in Table 4 indicates significant (p<0.05) increase of DM and CP metabolizability due to Cu supplementation at 30 ppm (organic) and 22.5 ppm (NP). The metabolizability of OM and EE was higher (p<0.05) at 22.5 ppm Cu-NP inclusion as compared to all other groups except for T_2 while CF and NFE metabolizability remained unaffected. The results revealed that the reduced dietary Cu levels sourced from organic or NP has no adverse effect on nutrient utilization. Meanwhile, the highest Cu levels *i.e.* 100% from organic and 75% from NP sources as compared to standard $CuSO_4$ were also optimum for nutrient utilization of the diet.

The reports suggesting effective utilization of nutrients due to Cu antimicrobial properties (Usman *et al*., 2013), better digestibility of DM, OM and NFE (Gonzales *et al*., 2009) and greater bioavailability of Cu organic (Nollet *et al*., 2008) and NP forms (Tamilvanan *et al*., 2014) are supportive of the finding of the present study. The improved fat utilization can be attributed to the enhanced lipase and phospholipase activities in small intestine with Cu supplementation (Das *et al*., 2010). The improved nutrient utilization at 22.5 ppm Cu-NP inclusion level confirms its better efficiency as compared to 30 ppm inorganic $CuSO_4$. However, the results are in contrast to the findings of Sarvestani *et al.* (2016) who reported no significant effect of 100 ppm Cu-NP dietary inclusion on nutrients digestibility in broilers.

Nitrogen balance of experimental birds is presented in Table 5. The source and level of Cu had no effect on total nitrogen intake (g/kg $W^{0.75}$/day), while significantly (p<0.05) lower excretion and higher (p<0.05) retention (g/kg $W^{0.75}$/day or per cent of N intake) of nitrogen observed at 100%

Table 3: Phase wise growth performance of birds as influenced by different treatments.

Particulars		Cumulative body weight gain (g/bird)				Feed intake (g/bird)			Feed conversion ratio		
Treatment group	Copper source and level (%) of requirement	Initial	Week 0-6	Week 7-10	Cumulative	Week 0-6	Week 7-10	Cumulative	Week 0-6	Week 7-10	Cumulative
T_1	Inorganic Cu (100)	44.24± 0.59	712.68± 14.23	975.04^{b} ±6.45	1643.5^{b} ±10.24	1640.7^{b} ±8.50	2707.4 ± 4.34	4348.1 ± 15.39	2.31^{b} ±0.05	2.77± 0.04	2.64^{ab} ±0.008
T_2	Organic Cu (100)	44.42± 0.46	699.08± 3.37	1065.5^{a}± 12.48	1720.1^{a}± 7.23	1465.6^{a}± 8.50	2853.5± 9.45	4318.7± 9.68	2.09^{a}± 0.01	2.67± 0.02	2.51^{a}± 0.02
T_3	Organic Cu (75)	43.29± 0.41	708.52± 11.34	1006.0^{ab}± 7.23	1671.2^{ab}± 12.45	1606.8^{b}± 9.60	2780.7± 10.45	4387.5± 8.98	2.27^{bc}± 0.03	2.76± 0.03	2.62^{ab}± 0.01
T_4	Organic Cu (50)	43.10± 0.66	704.21± 7.56	1017.2^{ab}± 7.87	1678.3^{ab}± 7.34	1603.2^{b}± 6.50	2821.2± 10.34	4424.4± 7.45	2.27^{bc}± 0.29	2.77± 0.04	2.63^{ab}± 0.03
T_5	Cu-NP (75)	45.13± 0.96	717.87± 11.65	1028.6^{ab}± 8.34	1701.3^{ab}± 12.08	1487.6^{a}± 9.23	2872.2± 7.23	4359.7± 80	2.07^{a}± 0.03	2.79± 0.07	2.55^{a}± 0.02
T_6	Cu-NP (50)	44.21± 0.66	700.40± 13.12	969.60^{b}± 14.34	1625.8^{b}± 14.23	1632.8^{b}± 7.56	2778.1± 9.34	4410.9± 1.06	2.33^{b}± 0.04	2.86± 1.3	2.71^{b}± 0.25
T_7	Cu-NP (25)	43.91± 0.55	694.05± 8.56	1002.1^{b}± 13.34	1652.3^{b}± 14.91	1571.5^{a}± 11.23	2803.4± 6.45	4374.9± 8.07	2.27^{bc}± 0.05	2.80± 0.02	2.64^{b}± 0.05
	SEM	0.681	8.724	5.044	8.722	9.575	7.346	54.997	0.041	0.069	0.029
	P value	0.375	0.811	0.001	0.000	0.000	0.356	0.845	0.000	0.645	0.002

^{a,b}Mean values bearing different superscripts within the column differ significantly ($p<0.05$).

inclusion level from organic and 75% from NP form of Cu as compared to other dietary treatments, however the values were comparable to control.

In the present study, the inclusion of organic (100%) and NP (75%) form of Cu in relation to $CuSO_4$ during grower phase was optimum for nitrogen and other nutrients metabolizability which reflect its greater utilization efficiency. The improved nitrogen retention could be speculated that Cu-NP supplementation boost hormone and growth factors in chicken (Das *et al*., 2010), thereby enhancing protein synthesis and deposition in tissue. The better nitrogen retention can also be associated to the improved energy (OM and EE) metabolizability that can be attributed to better gut health of the chicken. Scott *et al*. (2016) also reported significantly improved energy and nitrogen utilization and reduction in nitrogen excretion at 20 ppm Cu-NP than $CuSO_4$ in drinking water. In the present study, however, with reduced Cu supplemental level from organic or NP source, the nitrogen retention and excretion was not adversely affected by the treatment diets as compared to control.

Cu balance in the body has been presented in Table 6; indicates that its excretion in dropping in mg per kg $W^{0.75}$ per

Table 4: Metabolizability of various nutrients (%) of experimental diets.

Treatment group	Copper source (% of requirement)	Dry matter	Organic matter	Crude protein	Ether extract	Crude fiber	Nitrogen free extract
T_1	Inorganic Cu (100)	$64.37^{b}\pm0.70$	$66.93^{b}\pm1.02$	$64.74^{ab}\pm4.17$	$76.56^{b}\pm2.62$	22.91 ± 3.03	70.29 ± 0.12
T_2	Organic Cu (100)	$69.46^{a}\pm1.16$	$70.44^{ab}\pm1.46$	$72.56^{a}\pm3.12$	$83.38^{ab}\pm2.37$	21.13 ± 2.21	72.55 ± 2.27
T_3	Organic Cu (75)	$66.97^{b}\pm1.33$	$68.76^{b}\pm1.39$	$62.81^{ab}\pm1.50$	$74.48^{b}\pm3.48$	22.78 ± 1.91	73.51 ± 2.21
T_4	Organic Cu (50)	$65.87^{b}\pm0.47$	$68.34^{b}\pm0.45$	$62.22^{ab}\pm2.70$	$77.65^{b}\pm3.20$	22.54 ± 1.96	72.99 ± 1.08
T_5	Cu-NP (75)	$70.30^{a}\pm1.00$	$73.62^{a}\pm0.44$	$74.21^{a}\pm2.19$	$89.72^{a}\pm2.74$	23.06 ± 1.86	74.18 ± 1.77
T_6	Cu-NP (50)	$67.99^{b}\pm1.37$	$68.59^{b}\pm1.44$	$61.19^{b}\pm3.23$	$76.63^{b}\pm0.77$	20.57 ± 1.68	73.83 ± 1.89
T_7	Cu-NP (25)	$66.40^{b}\pm0.39$	$70.19^{b}\pm0.36$	$65.53^{ab}\pm1.93$	$75.25^{b}\pm2.54$	19.85 ± 1.45	72.11 ± 0.89
	SEM	1.060	1.123	3.039	2.844	2.082	1.753
	P value	0.006	0.006	0.019	0.006	0.841	0.697

^{a,b}Mean values bearing different superscripts within the column differ significantly ($p<0.05$).

Table 5: Intake, excretion and retention of nitrogen under different treatments.

Treatment group	Copper source (% of requirement)	Intake (g/kg $W^{0.75}$/d)	Excretion (g/kg $W^{0.75}$/d)	Excretion (%)	Retention (g/kg $W^{0.75}$/d)	Retention (%)
T_1	Inorganic Cu (100)	2.69 ± 0.13	$0.93^{ab}\pm0.07$	$35.25^{ab}\pm4.19$	$1.76^{b}\pm0.18$	$64.74^{ab}\pm4.17$
T_2	Organic Cu (100)	2.63 ± 0.08	$0.72^{a}\pm0.07$	$27.66^{ab}\pm3.35$	$1.91^{a}\pm0.14$	$72.34^{ab}\pm3.35$
T_3	Organic Cu (75)	2.71 ± 0.11	$1.01^{b}\pm0.07$	$37.18^{ab}\pm1.50$	$1.69^{b}\pm0.05$	$62.81^{ab}\pm1.50$
T_4	Organic Cu (50)	2.73 ± 0.09	$1.03^{b}\pm0.10$	$37.78^{ab}\pm2.70$	$1.68^{b}\pm0.02$	$62.22^{ab}\pm2.71$
T_5	Cu-NP (75)	2.71 ± 0.09	$0.69^{a}\pm0.63$	$25.78^{a}\pm2.19$	$2.02^{a}\pm0.11$	$74.21^{a}\pm2.19$
T_6	Cu-NP (50)	2.49 ± 0.16	$0.95^{ab}\pm0.02$	$38.32^{b}\pm2.14$	$1.54^{b}\pm0.17$	$61.68^{b}\pm3.27$
T_7	Cu-NP (25)	2.58 ± 0.08	$0.88^{ab}\pm0.03$	$34.46^{ab}\pm1.92$	$1.69^{b}\pm0.09$	$65.53^{ab}\pm1.93$
	SEM	0.108	0.134	2.978	3.063	0.071
	P value	0.731	0.009	0.020	0.018	0.020

^{a,b}Mean values bearing different superscripts within the column differ significantly ($p<0.05$).

Table 6: Intake, excretion and retention of copper under different treatments.

Treatment group	Copper source with requirement level %	Intake (mg/kg $W^{0.75}$/d)	Excretion (mg/kg $W^{0.75}$/d)	Excretion (%)	Retention (mg/kg $W^{0.75}$/d)	Retention (%)
T_1	Inorganic (100% as standard)	$2.79^{a}\pm0.08$	$1.62^{a}\pm0.05$	$58.39^{a}\pm2.59$	$1.17^{ab}\pm0.06$	$41.62^{c}\pm2.45$
T_2	Organic (100)	$2.61^{a}\pm0.05$	$1.22^{b}\pm0.06$	$46.53^{b}\pm4.35$	$1.39^{a}\pm0.13$	$53.47^{b}\pm0.82$
T_3	Organic (75)	$2.14^{b}\pm0.05$	$1.04^{c}\pm0.07$	$48.59^{b}\pm2.71$	$1.10^{ab}\pm0.04$	$51.40^{b}\pm2.65$
T_4	Organic (50)	$1.73^{c}\pm0.03$	$0.68^{d}\pm0.03$	$39.18^{bc}\pm3.45$	$1.05^{bc}\pm0.13$	$60.81^{ab}\pm1.35$
T_5	Cu-Np (75)	$2.08^{b}\pm0.04$	$0.83^{cd}\pm0.02$	$40.05^{bc}\pm2.56$	$1.24^{ab}\pm0.04$	$59.92^{ab}\pm1.75$
T_6	Cu-Np (50)	$1.64^{c}\pm0.06$	$0.62^{d}\pm0.19$	$38.50^{bc}\pm2.34$	$1.02^{bc}\pm0.05$	$61.49^{ab}\pm2.09$
T_7	Cu-Np (25)	$1.14^{d}\pm0.02$	$0.38^{d}\pm0.18$	$33.34^{c}\pm4.36$	$0.76^{c}\pm0.02$	$66.66^{a}\pm2.30$
	SEM	0.056	0.033	2.126	0.037	2.141
	P value	0.000	0.000	0.000	0.000	0.000

^{a,b,c,d}Mean values bearing different superscripts within the column differ significantly ($p<0.05$).

day as well on per cent is significantly ($p<0.05$) higher in control and linearly reduced in all the treatment groups. Parallel to the excretion, the Cu retention expressed in mg per kg $W^{0.75}$ as well on per cent was also found significantly ($p<0.05$) lower in control and linearly increased in all the treatment groups except for T_3. The excretion was further reduced with reduction of the supplementation either from organic or nano particles of Cu that may be due to minimum Cu supplemental level in the diet.

The results are closed to our hypothesis that reduced Cu dietary supplementation level sourced from organic and NP can reduce its excretion rate in dropping to the environment without compromising the chicken performance.

These findings do confirm the better efficiency and bioavailability of organic (Zafar and Fatima, 2018) and nano particles (Patra and Lalhriatpuii, 2019) forms of Cu as compared to inorganic $CuSO_4$ in the diet resulted by reduction in Cu excretion level of droppings to the environment. Similar results were also reported by many studies (Nollet *et al*., 2007; Gonzales *et al.,* 2009 and Sawosz *et al.,* 2018).

CONCLUSION

It was concluded that the organic and nanoparticle forms of Cu has dose-independent effect on growth, feed intake, feed conversion ratio, nutrient metabolizability, nitrogen and Cu balance. The dietary inclusion level of Cu can be reduced to 50 and 75% and reduce its excretion level when sourced from organic and nano forms, respectively compared to conventional inorganic $CuSO_4$ without compromising the bird's performance.

ACKNOWLEDGEMENT

The facilities extended by the Department of Poultry Science, Veterinary College, KVAFSU, Bangalore is duly acknowledged. The first author thanks India-Afghan Fellowship Program, Indian Council of Agricultural Research (ICAR), New Delhi for sponsoring his Ph.D. program.

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