Indian J. Anim. Nutr. 2021. 38 (3): 278-285
doi: 10.5958/2231-6744.2021.00041.4

Indian Journal of
Animal Nutrition

Effects of Inorganic and Nano Copper Supplementation on Growth Performance, Nutrient Utilization and Mineral Availability in Growing Sahiwal Heifers

Raju Kushwaha, Vinod Kumar*, Muneendra Kumar, Shalini Vaswani and Avinash Kumar
Department of Animal Nutrition, College of Veterinary Science and Animal Husbandry,
Pt. Deen Dayal Upadhyaya Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go Anusandhan Sansthan,
Mathura-281 001, Uttar Pradesh, India

ABSTRACT

This study was conducted to evaluate the effect of inorganic and nano copper supplementation on feed intake, nutrient utilization, growth performance and mineral availability in growing Sahiwal heifers. Twenty four growing Sahiwal heifers were randomly allocated into four groups having six heifers in each groups and fed for 120 days. Feeding regimen was similar in all the groups except that treatment groups were supplemented with 0.0 mg copper, 10.0 mg inorganic copper, 5.0 and 10.0 mg of nano copper per kg dry matter (DM; ppm) in four respective groups. Nutrients requirement of experimental heifers were met by feeding concentrate mixture, berseem and wheat straw as basal diet. Group fed on basal diet supplemented with 0.0 mg copper/kg DM served as a control. Experimental heifers were monitored daily for DM intake (DMI) and fortnightly body weight change. At the end of the study, a digestion trial of 7 days was conducted to study the effect of inorganic and nano copper supplementation on nutrients utilization. Feed intake, nutrient digestibility and growth performance were similar in all experimental groups fed on basal diet with or without supplemental Cu. Dietary supplementation of inorganic Cu and nano Cu did not exert any effect on absorption of Ca, P, Zn and Fe in different groups. However, absorption of Cu in nano Cu supplemented groups were higher ($P<0.05$) than other groups. It was concluded nano Cu can be used in the diet at 5 ppm for feeding in growing cattle as it exerts similar effects as showed by 10 ppm of inorganic Cu.

Key words: Growth Performance, Nutrient Utilization, Mineral Availability, Nano copper

INTRODUCTION

Copper is an essential trace element of animals. It can effectively maintain the stability of the internal environment, and is closely related with haematopoiesis, metabolism, growth, reproduction, and other important life activities (Ognik *et al.,* 2016). Copper entered to the body in the form of sulphate is dissociated in the digestive tract to ion form, which is then absorbed in the small intestine (Nose *et al.,* 2006). Feed ingredients are commonly deficient in Cu; hence, the commercial diet should provide the essential amount of Cu in a biologically dynamic form, which depends on the physical and chemical properties of the form of the supplement in which the Cu is given in the diet. It can ensure the growth and decline in the homeostasis and peroxides damage to the body (Song *et al.,* 2014). Cu deficiency has been linked to a variety of clinical signs, including pale coat, poor fleece, anemia, spontaneous fractures, poor capillary integrity, myocardial degeneration, hypomyelinization of the spinal cord, impaired reproductive performance, decreased resistance to infectious disease, diarrhoea and generalized ill-health (Tessman *et al.,* 2001). Copper sulphate is the main Cu source in the diet of livestock; which shows poor bioavailability caused by the presence of ingredients that can inhibit absorption. Thus, inorganic mineral administration in animal feed poses a risk to the environment, as excretion of high mineral levels contaminates soil and water. Traditionally, inorganic copper salts have been used in feed formulation (Aksu *et al.,* 2010), causing significant pollution to the soil and water (Jackson *et al.,* 2003). The bioavailability of Cu salts is very low, and approximately 80% of Cu is excreted in the faeces (McDowell, 1992) and consequently, its effect on soil microorganisms, plants and aquatic species is currently

*Corresponding author: E-mail: vinodsidhu@rediffmail.com; Department of Animal Nutrition, C.V.Sc., DUVASU, Mathura-281 001, Uttar Pradesh, India, M-9837636535

one of the crucial environmental concerns causing the environmental pollution (Leeson, 2009).

Copper nano particle (Cu-NP) is one of the nano metal which is prepared for application in various fields, so if the copper absorption is enhanced, the copper supplementation and excretion amount may be reduced. It has been documented that Cu-NP has beneficial effects on the animal performance and could be used to replace copper sulphate (Miroshnikov *et al.,* 2015). The unique bioactivity of Nano Cu is mainly due to the particle size and the large surface area. The size is important for penetration through for which a pore structure of a cellular membrane is required, thus affecting the number of proliferating cells (Salata, 2004). Nano particles can bypass conventional physiological ways of nutrient distribution and transport across tis-sue and cell membranes, as well as protect compounds against destruction before reaching their targets. Nano copper has several advantages over copper sulphate, including improved efficacy, lower dosage with improved results, no interference with other ingredients, and last but not least it reduces environmental excretion (Ognik *et al.,* 2016). This study was aims to investigate the effect of dietary nano-copper supplementation on growth performance, nutrient utilization and mineral availability of indigenous heifers.

MATERIALS AND METHODS

Animal care procedures were approved (approval number, IAEC/18/14) and conducted under the established standard of the Institutional Animal Ethics Committee (IAEC), constituted as per the article number 13 of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) rules laid down by the Government of India.

A total of 24 growing Sahiwal heifers were selected from the cattle herd maintained at Livestock Farm Complex (LFC), DUVASU, Mathura. Experimental heifers were randomly assigned into four groups (N=6) on body weight (100.20±4.84) and age (12-15 months) basis, and the duration of the experiment lasted 120 days. Heifers either received a basal diet devoid of supplemental Cu (control) or were supplemented with 10 ppm of inorganic Cu as copper sulphate pentahydrate ($CuSO_4.5H_2O$, molecular weight 249.68, minimum assay purity 99%, Central Drug House (CDH) Pvt. Ltd. New Delhi), 5 ppm and 10 ppm of nano Cu as cupric Oxide Nano-powder (CuO, molecular weight 79.54, APS: 40 nm, SSA: 80 m^2/g, minimum assay purity 99%, Sisco Research Laboratories (SRL) Pvt. Ltd. Taloja, Maharashtra). The nutrient requirements of experimental heifers were met by feeding total mixed ration (TMR) consisted of concentrate: green berseem fodder: wheat straw in the proportion of 50:35:15 following NRC (2001) guidelines. Concentrate mixture used for feeding of experimental heifers consisted of 26 parts barley grains, 20 parts wheat bran, 20 parts gram chuni, 32 parts mustard oil cake and 2 parts mineral mixture.

To ensure that each animal consumed the requisite amount of Cu, the calculated amount of $CuSO_4.5H_2O$ and CuO nano powder was mixed with barley flour and prepared the premix (at 2ppm Cu/g of barley flour) and offered prior to providing the ration. TMR was prepared daily by hand mixing and was offered at 09:00 h and 18:00 h. The animals were provided with fresh and clean drinking water free of choice twice daily at 08:00 h and 17:00 h. Experimental heifers were housed in a well-ventilated shed having the proper arrangement for individual feeding and watering without having access to the other animal's diet.

Feeds and fodders consumption was recorded daily by weighing TMR offered and orts left, and dry matter intake (DMI) was calculated and recorded daily according to the DM content of the diet. Body weight of the experimental animals was recorded at the onset of experiment, and subsequently at 15, 30, 45, 60, 75, 90, 105 and 120 days post-Cu supplementation by using computerized weight management system (Leotronic Scales Pvt. Ltd., India). Heifers were weighed for 2 consecutive days in the morning at 06:00 h before offering feeds, fodders, and water. The average of two days was considered as body weight for that fortnight and considered for average daily gain (ADG), feed-to-gain ratio (FCR), and feed conversion efficiency (FCE). The representative samples of feeds and fodders offered and orts left were dried in a hot air oven at 60°C till a constant weight was attained and then ground in a Wiley mill to pass a 1-mm sieve. The samples were analyzed for DM (method 973.18c), CP (method 4.2.08), ether extract (EE; method 920.85), and total ash (TA; method 923.03) following standard procedures (AOAC, 2005). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to the procedures described by Van Soest *et al.* 1991. Ca and P in feeds, fodders, orts left, and fecal samples were analyzed by Talapatra *et al.* 1940 and volumetric method, respectively. Cu, Zn and Fe content in samples of feeds and fodders, and orts left were analyzed by Atomic Absorption Spectrophotometer (AAS; Perkins, USA). Ingredients and chemical composition of the basal diet fed during the experimental period is depicted in Table 1. The Cu content of basal diet without supplemental Cu was estimated at 8.75 mg Cu/kg DM.

Table 1. Ingredients and chemical composition of total mixed ration

Ingredients composition	Content (g/kg DM or as depicted)
Berseem fodder	350
Wheat straw	150
Barley grains	130
Wheat bran	100
Gram chunni	100
Mustard oil cake	160
Mineral mixture and vitamin premix[α]	10
Copper supplement[β]	Variable
Chemical composition	
Dry matter, g/kg of diet	620.9
on dry matter basis	
Crude protein	161.6
Ether extract	33
Neutral detergent fiber	534.5
Acid detergent fiber	274.3
Calcium	11.6
Phosphorus	6.4
Copper, mg/kg DM	8.75
Zinc, mg/kg DM	39.40
Iron, mg/kg DM	296.49

[α]Mineral and vitamin premix contained (kg^{-1}): vitamin A, 700000 IU; vitamin D_3, 70,000 IU; vitamin E, 250 mg; nicotinamide, 3.0 g; Ca, 190 g; P, 90 g; Na, 50 g; Zn, 9.6 g; Fe, 1.5 g; Mn, 6.0 g; I, 325 mg; Co, 150 mg; Se, 10 mg; Mg, 19.0 g; [β]In $inCu_{10}$ group- 10.0 mg/kg Cu as $CuSO_4.5H_2O$, $nanoCu_5$ and $nanoCu_{10}$ group 5 and 10.0 mg/kg Cu as nano copper oxide, respectively

The data for measured variables were subjected to analysis of variance (ANOVA) using the mixed model repeated measure procedure of the Statistical Software Package (SPSS for windows, V21.0; Inc., Chicago, IL, USA). The effect of treatments, days in trial and their interaction on performance variable like weight gain was analyzed by using following model:

$$Yijk = \mu + Ti + Dj + (T \times D)ij + eijk$$

where Yijk is the dependent variable, μ is the overall mean of the population, Ti is the mean effect of the Cu, Dj is the mean effect of days of sampling (j = 0, 30, 60, 90 and 120 days of dietary treatment), (T × D)ij is the effect of the interaction between treatment and days of trial and eijk is unexplained residual element assumed to be independent and normally distributed. The effect of treatments on nutrients digestibility and mineral metabolism was analyzed by using one-way ANOVA with significance defined at $P<0.05$. Individual animal was used as the experimental unit for all data. The statistical difference between the means was determined by using ‘‘Tukey’s honest significant difference (HSD) test.’’

RESULTS AND DISCUSSION

Effect of inorganic and nano Cu supplementation on dry matter intake and growth performance are depicted in Table 2. Dietary supplementation of inCu and nanoCu in heifers had no significant effect on DMI. DMI increased with the increase in the body weight of the experimental heifers. Percent DMI (kg/100 kg BW) in different groups showed similar effect of inorganic and nano Cu supplementation. The variation between the groups for mean ADG (g) were not significantly different ($P>0.05$). FCR and FCE were used as feed efficiency measures which were not significantly different ($P>0.05$). Similar to present findings Cu supplementation had no effects on average daily gain and daily feed intake in lambs (Cheng *et al.,* 2008; Dezfoulian *et al.,* 2012)), heifers (Mullis *et al.,* 2003; Vaswani *et al.,* 2018), Simmental steers (Engle and Spears, 2001) and goat kids (Waghmare *et al.,* 2014).

Table 2. Effect of inorganic and nano copper supplementation on dry matter intake and growth performance

Parameters	Supplemental Cu (mg/kg DM)				SEM	P value		
	Cu_0	in Cu_{10}	nano Cu_5	nano Cu_{10}		Treatment (T)	Period (P)	T×P
DMI (kg/day)	4.08	4.31	4.29	4.30	0.20	0.303	0.198	0.998
DMI (% BW)	3.17	3.23	3.18	3.24	0.14	0.383	0.574	0.998
ADG (g/day)	517.86	528.27	535.71	546.13	40.53	0.487	0.698	0.887
FCR	9.12	9.01	8.77	8.72	0.72	0.559	0.147	0.928
FCE	0.124	0.127	0.131	0.132	0.009	0.337	0.451	0.922

DMI, dry matter intake; ADG, average daily gain; FCR, feed conversion ratio; FCE, feed conversion efficiency

In contrary to the present findings, a growth improvement response has been reported in castrated *Black Bengal* kids (Mondal and Biswas, 2007) and Cashmere goats (Zhang *et al.,* 2008). Some studies compared the inorganic forms of Cu with Cu-NP and the latter showed an improvement in the growth performance of piglets (Gonzales-Eguia *et al.,* 2009; Chang *et al.* (2018). Results obtained in this study also demonstrate that intake of DM, CP, DCP, TDN and ME in control, $inCu_{10}$, $nanoCu_5$ and $nanoCu_{10}$ groups of

Table 3. Effect of inorganic and nano Cu supplementation on nutrient intake during digestion trial

Nutrient	Supplemental Cu (mg/kg DM)				SEM	P value
	Cu_0	$inCu_{10}$	$nanoCu_5$	$nanoCu_{10}$		
Dry matter intake						
kg/day	4.93	5.01	5.06	5.09	0.18	0.585
kg/100 kg BW	3.28	3.31	3.26	3.27	0.04	0.364
g/kg BW	32.56	33.11	32.59	32.65	0.37	0.362
g/kg $W^{0.75}$	114.08	115.68	114.87	114.79	1.21	0.816
Crude protein intake						
g/day	769.41	767.09	775.75	801.24	31.38	0.598
g/100 kg BW	506.01	506.14	497.69	511.62	3.04	0.300
g/kg BW	5.06	5.06	4.98	5.12	0.03	0.297
g/kg $W^{0.75}$	17.67	17.69	17.56	18.01	0.19	0.943
Digestible crude protein intake						
g/day	567.54	562.21	563.44	594.89	27.53	0.643
g/100 kg BW	369.15	367.87	360.49	375.88	3.96	0.954
g/kg BW	3.69	3.68	3.61	3.76	0.04	0.954
g/kg $W^{0.75}$	12.93	12.89	12.73	13.27	0.25	0.729
Total digestible nutrient intake						
kg/day	3.24	3.32	3.31	3.47	0.15	0.746
kg/100 kg BW	2.14	2.18	2.11	2.20	0.03	0.591
g/kg BW	21.37	21.80	21.14	21.10	0.30	0.592
g/kg $W^{0.75}$	74.59	76.32	74.64	77.58	1.15	0.799
Metabolizable energy intake						
Mcal/day	11.72	12.02	11.95	12.55	0.54	0.746
Mcal/100 kg BW	7.73	7.88	7.64	7.95	0.11	0.593
kcal/kg BW	77.28	78.82	76.44	79.53	1.09	0.592
kcal/kg $W^{0.75}$	269.71	275.95	269.88	280.52	5.23	0.799

heifers were statistically non significant ($P>0.05$) which is depicted in Table 3. Mudgal *et al.* (2007) did not observe any effect on CP and DCP intakes in the buffalo calves supplemented with 10 ppm of Cu and 0.3 ppm of Se.

Data on the apparent nutrient digestibility across 7 days digestion trial is shown in Table 4. Use of nanoparticles, including Cu nanoparticles, as feed additives can improve the digestion and absorption of nutrients in livestock (Hill and Li., 2017). The present study showed that, digestibility of DM, OM, EE, CF, NFE and fibre fractions (NDF and ADF) was similar and there was no effect on digestibility of nutrients ($P>0.05$) during digestion trial. In agreement with present findings, earlier finding reported no effect on digestibility of nutrients in kids (Waghmare *et al.,* 2014), Chokla rams (Shinde *et al.,* 2013), Cashmere goats (Zhang *et al.,* 2008) and in heifers (Vaswani *et al.,* 2018) by dietary supplementation of Cu.

Mondal and Biswas (2007) reported that supplemental copper sources had no effect on DM, OM, NFE digestibility whereas, supplementation of Cu-proteinate significantly improved digestibility of EE and CF as compared to $CuSO_4$.Similarly, an increasing trend in digestibility of DM and OM in Cu-proteinate supplemented kids (Datta *et al.,* 2007) and digestibility of crude fat and energy in pigs (Zhang *et al.,* 2008; Gonzales-Eguia *et al.,* 2009) was reported. Dezfoulian *et al.* (2012) found that copper source had a significant effect on digestibility of nutrients except EE, as proteinate source resulted in higher digestibility of DM, CP, NDF, ADF, NFC and OM in lambs.

The data regarding Ca, P, Cu, Zn and Fe absorption observed during digestion trial has been presented in Table 5. Mean values for Cu absorption in inorganic Cu supplemented group was significantly higher ($P<0.05$) than control group and lower than nano Cu supplemented groups whereas; mean values for Cu absorption in nano Cu supplemented group was significantly higher ($P<0.05$) than both control and inorganic Cu supplemented group. Cu has been reported to interact or influence the metabolism of a number of other elements. In present study, Cu absorption showed positive correlation with supplemented inorganic and nano Cu levels and was found highest in 10 ppm nano Cu supplemented group whereas, no effect of inorganic and nano Cu supplementation was observed on absorption of Ca, P, Zn and Fe. It has been found that changes in essential trace mineral digestibility in the gastrointestinal tract are primary mechanisms for maintaining trace mineral homeostasis (King *et al.,* 2000). Copper absorbed by animals is mainly stored in liver (Zatulovskaia *et al.,* 2015). Different forms of Cu differ in their solubility, availability and effects on

Table 4. Effect of inorganic and nano Cu supplementation on nutrient digestibility (% or as mentioned)

Nutrient	Supplemental Cu (mg/kg DM)				SEM	P value
	Cu_0	$inCu_{10}$	$nanoCu_5$	$nanoCu_{10}$		
Initial body weight (kg)	151.33	149.83	153.83	155.00	6.46	0.603
Final body weight (kg)	154.83	153.50	157.67	158.67	6.49	0.618
Body weight gain (kg)	3.50	3.67	3.83	3.67	0.29	0.470
Average daily gain (g)	500.00	523.81	547.62	523.81	41.83	0.470
Dry matter	59.77	59.61	60.03	59.70	0.85	0.495
Organic matter	65.97	65.80	66.06	65.81	0.74	0.544
Crude protein	73.04	72.72	72.41	73.48	0.74	0.483
Ether extract	82.10	83.29	81.54	84.07	0.53	0.836
Crude fibre	44.70	45.73	46.37	44.79	1.16	0.940
Nitrogen free extract	72.04	70.55	71.41	72.48	1.43	0.447
Neutral detergent fibre	51.78	51.07	52.44	51.09	1.13	0.174
Acid detergent fibre	37.78	39.41	39.49	39.12	1.26	0.417

animal per-formance. The increased Cu bioavailability likely results in an increase in the activities of enzymes involved in nutrient metabolism, resulting in better energy digestibility (Scott *et al.,* 2018b). The results of the present study are similar to the observations of Shen *et al.* (2020) who reported that nano-Cu supplementation for up to 10 days, the Cu content in blood significantly increased in goats and the concentration of Fe significantly decreased (P<0.01) and the effect of $CuSO_4$ group was significantly lower than that in nano-Cu group (P<0.01). Similar pattern of Cu retention observed in organic Cu supplemented animals (Shinde *et al.,* 2013). In agreement to present study, Gonzales-Eguia *et al.* (2009) reported that Cu availability was significantly improved and the faecal copper level was reduced in nano Cu fed group as compared to $CuSO_4$ fed group in piglets. Copper supplementation at 30 mg/kg significantly increased liver Cu concentration in heifers compared with Cu supplementation at 15 mg/kg (Yost *et al.,* 2002). The lowest amount of additional Cu in the form of nanoparticles increased the apparent digestibility of Cu and Zn in turkeys. Therefore, the environmental burden of excreted Cu was substantially reduced along with decreasing dietary Cu levels but to a lesser extent when copper sulfate was replaced with Cu nanoparticles (Jankowski *et al.,* 2020).

In contrary to the present study, Dezfoulian *et al.* (2012) reported that Cu supplemented groups had a lower percentage of Cu absorption compared to the control group. Moreover, liver and plasma Cu

Table 5. Effect of inorganic and nano Cu supplementation on mineral absorption

Mineral	Supplemental Cu (mg/kg DM)				SEM	P value
	Cu_0	$inCu_{10}$	$nanoCu_5$	$nanoCu_{10}$		
Calcium						
Intake (g/day)	54.10	55.33	54.57	57.20	2.17	0.766
Voided in faeces (g/day)	37.63	37.53	36.44	38.82	1.95	0.577
Absorption (g/day)	16.47	17.80	18.13	18.39	1.17	0.093
Absorption (%)	31.47	32.14	31.29	32.04	2.43	0.219
Phosphorus						
Intake (g/day)	29.80	30.48	30.06	31.51	1.19	0.766
Voided in faeces (g/day)	12.73	12.56	12.61	13.22	0.53	0.520
Absorption (g/day)	17.07	17.92	17.45	18.29	1.07	0.954
Absorption (%)	56.89	58.07	56.63	57.50	1.70	0.253
Copper						
Intake (mg/day)	40.83	98.82	70.48	102.20	6.11	0.109
Voided in faeces (mg/day)	36.49	86.89	59.16	85.09	5.19	0.150
Absorption (mg/day)	4.34^a	11.93^b	11.32^b	17.11^c	1.04	0.008
Absorption (%)	10.68^a	12.32^b	16.11^c	16.81^c	0.57	0.014
Zinc						
Intake (mg/day)	524.07	535.97	528.57	554.10	20.98	0.766
Voided in faeces (mg/day)	411.64	413.68	415.68	422.65	15.36	0.690
Absorption (mg/day)	112.43	122.29	112.89	131.45	12.94	0.571
Absorption (%)	20.94	21.73	20.94	22.04	2.20	0.778
Iron						
Intake (mg/day)	1542.34	1577.36	1555.60	1561.26	60.46	0.823
Voided in faeces (mg/day)	1109.99	1086.18	1078.82	1054.65	38.28	0.138
Absorption (mg/day)	432.35	491.19	476.78	506.62	36.62	0.220
Absorption (%)	28.11	30.60	29.79	31.13	1.53	0.906

concentrations in heifers were not greatly influenced by different supplemental Cu sources (Rabiansky *et al.,* 1999). The *in vitro* study suggests that copper accumulated in the intestines reduces absorption of calcium and zinc, but does not affect iron absorption (Ognik *et al.,* 2016). Cu excretion was lower in chickens treated with Cu-NP as compared to the $CuSO_4$ treated groups (Scott *et al.,* 2018). Our results are suggesting that the nano form of Cu (CuO) was more efficient than inorganic Cu (Cu sulphate) in influencing the Cu metabolism in Sahiwal heifers. Study contributes to further understanding the application of nano Cu and inorganic Cu mineral diets as a nutritional strategy for heifers to reduce dietary faecal excretion and thus Cu pollution in soil and water.

CONCLUSION

It was concluded that supplementation of Cu either from inorganic or by nano source did not exert any adverse effect on growth performance, nutrient utilization and absorption of most of the minerals except Cu. Cu absorption was better in nano Cu supplemented animals than non supplemented or inorganic Cu supplemented group. Findings of the present study revealed that level of 5 ppm nano Cu can be used for feeding in growing cattle as it exerts similar effects as showed by 10 ppm of inorganic Cu.

ACKNOWLEDGMENTS

Financial assistance for the research was provided under University Grant supported by Indian Council of Agricultural Research, New Delhi, India. Thanks are due to the Faculty and Staff of the Department of Animal Nutrition and Instructional Livestock Farm Complex, College of Veterinary Science and Animal Husbandry, DUVASU, Mathura, Uttar Pradesh, India.

REFERENCES

Aksu, D.S., Aksu, T., Özsoy, B. and Baytok, E. 2010. The effects of replacing inorganic with a lower level of organically complexed minerals (Cu, Zn and Mn) in broiler diets on lipid peroxidation and antioxidant defense systems. *J. Anim. Sci.* 23:1066-1072.

AOAC. 2005. *Official Methods of Analysis*, 18th edn. Association of Official Analytical Chemists, Arlington.

Chang, Z., Zhang, H., Dong, H., Mehmood, K., Ijaz, M., Ahmad, H.I., Naeem, M.A., Wu, Q., Nabi, F. and Zhu, H. 2018. Effect of $CuSO_4$ and nano copper on serum antioxidant capacity in weaned piglets. *J. Biol. Regul. Homeost.* 32: 99-104.

Cheng, J., Fan, C., Zhang, W., Zhu, X., Yan, X., Wang, R. and Jia, Z. 2008. Effects of dietary copper source and level on performance, carcass characteristics and lipid metabolism in lambs. *Asian Australas. J. Anim. Sci.* 21: 685-91.

Datta, C., Mondal, M.K. and Biswas, P. 2007. Influence of dietary inorganic and organic form of copper salt on performance, plasma lipids and nutrient utilization of Black Bengal (*Capra hircus*) goat kids. *Animal Feed Sci. Technol.* 135: 191-209.

Dezfoulian, A.H., Aliarabi, H., Tabatabaei, M.M., Zamani, P., Alipour, D., Bahari, A. and Fadayifar, A. 2012. Influence of different levels and sources of copper supplementation on performance, some blood parameters, nutrient digestibility and mineral balance in lambs. *Livest. Sci.* 147: 9-19.

Engle, T.E. and Spears, J.W. 2001. Performance, carcass characteristics, and lipid metabolism in growing and finishing Simmental steers fed varying concentrations of copper. *J. Anim. Sci.* 79: 2920-25.

Gonzales-Eguia, A., Fu, C.M., Lu, F.Y. and Lien, T.F. 2009. Effects of nanocopper on copper availability and nutrients digestibility, growth performance and serum traits of piglets. *Livest. Sci.* 126: 122-129.

Hill, E.K. and Li, J. 2017. Current and future prospects for nanotechnology in animal production. *J. Anim. Sci. Biotechnol.* 8: 26.

Jackson, B.P., Bertsch, P.M., Cabrera, M.L., Camberato, J.J., Seaman, J.C. and Wood, C.W. 2003. Trace element speciation in poultry litter. *J. Environ. Qual.* 32: 535-540.

Jankowski, J., Otowski, K., Kozlowski, K., Pietrzak, P., Ferenc, K., Ognik, K., Juœkiewicz, J., Sawosz, E. and Zduñczyk, Z. 2020. Effect of different levels of copper nanoparticles and copper sulfate on morphometric indices, antioxidant status and mineral digestibility in the small intestine of turkeys. *Ann. Anim. Sci.* 20: 975-990.

King, J.C., Shames, D.M. and Woodhouse, L.R. 2000. Zinc homeostasis in humans. *J. Nutr.* 130: 1360S-1366S.

Leeson, S. 2009. Copper metabolism and dietary needs. *World Poult. Sci.* 65:353-366.

McDowell, L.R. 1992. *Mineral in Animal and Human Nutrition.* Academic. Press. New York Inc. pp. 176-200.

Miroshnikov, S., Yausheva, E., Sizova, E. and Miroshnikova, E. 2015. Comparative assessment of effect of copper nano-and microparticles in chicken. *Orient. J. Chem.* 31: 2327-2336.

Mondal, M.K. and Biswas, P. 2007. Different sources and levels of copper supplementation on performance and nutrient utilization of castrated black Bengal (*Capra hircus*) kids diet. *Asian Australas. J. Anim. Sci.* 20: 1067-75.

Mudgal, V., Garg, A.K. and Dass, R.S. 2007. Effect of dietary selenium and copper supplementation on growth and nutrient utilization in buffalo (*Bubalus bubalis*) calves. *Anim. Nutr. Feed Technol.* 7: 79-88.

Mullis, L.A., Spears, J.W. and McCraw, R.L. 2003. Estimated copper requirements of Angus and Simmental heifers. *J. Anim. Sci.* 81): 865-873.

Nose, Y., Kim, B.E. and Thiele, D.J. 2006. Ctr1 drives intestinal copper absorption and is essential for growth, iron metabolism, and neonatal cardiac function. *Cell Metab.* 4:235–244.

NRC. 2001. *Nutrient Requirements of Dairy Cattle*, 2nd revised edn. National Research Council, National Academy of Sciences, Washington, DC.

Ognik, K., Stêpniowska, A., Cholewiñska, E. and Kozlowski, K. 2016. The effect of administration of copper nanoparticles to chickens in drinking water on estimated intestinal absorption of iron, zinc, and calcium. *Poult. Sci*. 95:2045–2051.

Rabiansky, P.A., McDowell, L.R., Velasquez-Pereira, J., Wilkinson, N.S., Percival, S.S., Martin, F.G., Bates, D.B., Johnson, A.B., Batra, T.R. and Salgado-Madriz, E. 1999. Evaluating copper lysine and copper sulfate sources for heifers. *J. Dairy Sci.* 82: 2642-50.

Salata, O.V. 2004. Applications of nanoparticles in biology and medicine. *J. Nanobiotechnol.* 2: 3.

Scott, A., Vadalasetty, K.P., £ukasiewicz, M., Jaworski, S., Wierzbicki, M., Chwalibog, A. and Sawosz, E. 2018. Effect of different levels of copper nanoparticles and copper sulphate on performance, metabolism and blood biochemical profiles in broiler chicken. *J. Anim. Physiol. Anim. Nutr.* 102: e364-e373.

Shen, X., Song, C. and Wu, T. 2020. Effects of nano-copper on antioxidant function in copper-deprived Guizhou black goats. *Biol. Trace Elem. Res.* 199: 2201-2207.

Shinde, A.K., Sankhyan, S.K. and Regar, R.K. 2013. Effect of chelated mineral supplementation on copper and zinc absorption, retention and wool yield in Chokla rams. *Indian J. Small Rumin.* 19: 50-55.

Song, L., Connolly, M., Fernández-Cruz, M.L., Vijver, M.G., Fernández, M., Conde, E., de Snoo, G.R., Peijnenburg, W.J, and Navas, J.M. 2014. Species-specific toxicity of copper nanoparticles among mammalian and piscine cell lines. *Nanotoxicology.* 8: 383-393.

Talapatra, S.K., Roy, S.C. and Sen, K.C. 1940. The analysis of mineral constituents in biological materials. Estimation of phosphorus, chlorine, calcium, manganese, sodium and potassium in foodstuffs. *Indian J. Vet. Sci. Anim. Husb.* 10: 243-258.

Tessman, R.K., Lakritz, J., Tyler, J.W., Casteel, S.W., Williams, J.E. and Dew, R.K. 2001. Sensitivity and specificity of serum copper determination for detection of copper deficiency in feeder calves. *J. Am. Vet. Med. Assoc.* 218: 756-760.

Van Soest, P.J., Robertson, J.B. and Lewis, B.A. 1991. Symposium: carbohydrate methodology, metabolism and nutritional implications in dairy cattle. Methods for dietary fiber, neutral detergent fiber and non starch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74: 3583-3597.

Vaswani, S., Kumar, V., Roy, D., Kumar, M. and Kushwaha, R. 2018. Effect of different sources of copper supplementation on performance, nutrient utilization, blood-biochemicals and plasma mineral status of growing Hariana heifers. *Indian J. Anim. Sci.* 88 : 812-818.

Waghmare, S., Dass, R.S., Garg, A.K., Mohanta, R.K. and Dhayagude, R.S. 2014. Effect of copper methionine supplementation on growth rate and nutrient utilization in male goat kids. *Indian J. Anim. Nutr.* 31: 44-48.

Yost, G.P., Arthington, J.D., McDowell, L.R., Martin, F.G., Wilkinson, N.S. and Swenson, C.K. 2002. Effect of Copper Source and Level on the Rate and Extent of Copper Repletion in Holstein Heifers. *J. Dairy Sci.* 85: 3297-3303.

Zatulovskaia, Y.A., Ilyechova, E.Y. and Puchkova, L.V. 2015. The features of copper metabolism in the rat liver during development. *PLoS One.* 10: e0140797.

Zhang, W., Wang, R., Kleemann, D.O., Lu, D., Zhu, X., Zhang, C. and Jia, Z. 2008. Effects of dietary copper on nutrient digestibility, growth performance and plasma copper status in cashmere goats. *Small Rumin. Res.* 74: 188-93.

Received on 14.12.2021 and accepted on 20.12.2021