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Effects of inorganic and nano copper supplementation on haematology, blood biochemical and plasma mineral status in growing cattle

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ABSTRACT

Effect of inorganic and nano copper supplementation on growth performance, haematology, blood biochemical and plasma mineral status in growing Sahiwal heifers was studied. Animals (24) were randomly allocated into four groups having six animals in each and fed for 120 days. Feeding regimen was similar in all the groups except that treatment groups were supplemented with 0 mg copper, 10 mg inorganic copper, 5 and 10 mg of nano copper per kg DM in four respective groups. Nutrient requirements were met by feeding concentrate mixture, berseem and wheat straw. Feed intake and growth performance were similar in all groups. In Cu supplemented groups, Hb content, PCV and RBCs count was higher than non-supplemented group. Dietary supplementation of Cu did not exert any effect on biomarkers of energy and lipid mobilization, i.e. plasma glucose, cholesterol, triglycerides, non-esterified fatty acids and beta hydroxyl butyrate concentration. There were no significant effect of treatment on plasma total protein, albumin, globulin, urea nitrogen and creatinine levels in all four groups. Alkaline phosphate was lower in Cu supplemented groups but AST, ALT and bilirubin values were similar in all the groups. Plasma Cu levels were higher in Cu supplemented groups than non-supplemented group. There were no changes in plasma levels of Ca, P, Zn and Fe in all the groups. Finally, it was concluded that nano Cu @ 5 ppm can be selected for feeding in growing cattle as it exerts similar effects as showed by 10 ppm inorganic Cu.

Keywords: Blood biochemicals, Growing cattle, Haematology, Nano copper, Plasma mineral

Minerals are essential for growth and reproduction, and are involved in a large number of digestive, physiological and biosynthetic processes within the body (Close 1998). They therefore fulfill several important functions for the maintenance of animal growth and reproduction (Underwood 1981). Copper (Cu) 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). Prior to absorption in the small intestine, dietary Cu in the form of sulphate gets dissociated into ionic form in digestive tract (Nose *et al.* 2006). Due to its ability to easily accept and donate electrons, copper is involved in numerous biochemical processes (Maltais *et al.* 2013). Feed ingredients are commonly deficient in Cu; hence, the commercial livestock diet contains copper sulphate as the main Cu source; which shows poor bioavailability. Traditionally, inorganic Cu salts have been used in feed formulation (Aksu *et al.* 2010), causing significant pollution to the soil and water (Jackson *et al.* 2003).

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Recent advancement in nanotechnology has enabled Cu to be engineered at the nano size scale (1-100 nm), exhibiting ultrahigh physical activity and chemical neutrality. It has been documented that copper nanoparticle has beneficial effects on the animal performance and could be used to replace copper sulphate (El Basuini *et al.* 2016). Nano Cu has several advantages over copper sulphate, including improved efficacy, lower dosage with improved results, no interference with other ingredients, and less environmental excretion (Ognik *et al.* 2016). Although, high Cu has benefits such as antibacterial, promoting the secretion of growth hormone, increasing feed intake, and production performance, but it can also damage the vital organs like liver and kidney as intoxicant in food and water due to environmental pollution. This study investigated the effect of dietary nano-copper supplementation on haematology, blood biochemicals and plasma mineral status of growing cattle besides determining the optimal level of nano-copper for growing cattle.

MATERIALS AND METHODS

Animals, diets and experimental design: Growing Sahiwal heifers (24) were selected from Livestock Farm Complex (LFC), DUVASU, Mathura and randomly assigned into four dietary treatments on the basis of body weight (100.20±4.84) and age (12-15 months). Animals in control group (Cu_0) were fed basal TMR without Cu supplementation; whereas, basal TMR was supplemented with 10 mg/kg DM as $CuSO_4$ ($inCu_{10}$) in inorganic group and 5 ($nanoCu_5$) and 10 ($nanoCu_{10}$) mg Cu/kg DM as nano Cu oxide in nano groups (CuO, molecular weight 79.54, APS: 40 nm, SSA: 80 m^2/g, minimum assay purity 99%, SRL Pvt. Ltd. Maharashtra, India). The nutrient requirements of experimental heifers were met by feeding TMR consisting 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 calculated amount of Cu, the calculated amount of $CuSO_4.5H_2O$ and CuO nanopowder was mixed with barley flour and the premix prepared (@ 2 ppm Cu/g of barley flour) and offered prior to providing the ration. TMR 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 proper arrangement for individual feeding and watering without having access to the other animal's diet.

Observation recorded and analytical procedures: Dry matter intake (DMI) was recorded daily. Body weight of the experimental animals was recorded at fortnightly interval post-Cu supplementation by using computerized weight management system (Leotronic Scales Pvt. Ltd., India). 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) (AOAC 2005). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined according to the procedures described by Van Soest *et al.* (1991). Ingredients and chemical composition of the basal diet fed during the experimental period is given in Table 1. Peripheral blood samples were collected before feeding and watering of heifers at 07:00 h in heparinized vacutainer tubes (BD Franklin, USA) at 0, 30, 60, 90 and 120 day. A fraction of blood samples was used for analysis of hematological attributes (Hb, PCV, RBCs, WBCs, MCV, MCHC and Platelets). Remaining quantity of blood was centrifuged at 3,000 rpm for 30 min to separate the plasma. Plasma samples were stored at - 20°C until further analysis of biomarkers of liver and kidney function (ALP, AST, ALT, AST/ALT, bilirubin and creatinine), protein metabolism (total protein, albumin, globulin, and PUN), mineral (Ca, P, Cu, Zn and Fe) concentrations and energy and lipid metabolism (Glucose, NEFA, BHBA, Triacylglycerides, Cholesterol).

Haematological attributes in blood were analyzed by Celltac-α (MEK-6500K) auto haemoanalyser made by Nihon Kohden, Surat, India. The plasma concentration of AST, ALT, ALP, bilirubin, creatinine, total protein, albumin and PUN, tiacylglycerides, cholesterol and glucose was determined by using automated biochemical analyzer (BS-120 Chemistry Analyzer, Shenzhen Mindray Biochemical Electronics Co. Ltd., China) using commercial kits (Span Diagnostic Ltd. Surat, India). Plasma globulin concentration was determined by subtracting the albumin content from total protein content. NEFA was determined in plasma of heifers by using 'Bovine Non-esterified Fatty Acid ELISA Test Kit' (Bioassay Technologies, China). BHBA was determined in plasma of heifers by using 'Bovine Beta-Hydroxybutyric Acid ELISA Test Kit' (Bioassay Technologies, China). Plasma Ca, P, Cu, Fe and Zn were analyzed by following AAS Manual Guidelines (Perkins, USA).

Table 1. Ingredients and chemical composition of total mixed ration

Ingredient 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
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 (per kg): vitamin A, 700000 IU; vitamin D3, 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]Cu_{10} group, 10 mg/kg Cu as $CuSO_4.5H_2O$; $nanoCu_5$ and $nanoCu_{10}$ groups, 5 and 10 mg/kg Cu as nano copper oxide, respectively.

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

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

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

Parameter	Supplemental Cu (mg/kg DM)				SEM	P value		
	Cu_0	$inCu_{10}$	$nanoCu_5$	$nanoCu_{10}$		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

where Yijk, dependent variable; μ, overall mean of the population; Ti, mean effect of the Cu; Dj, mean effect of days of sampling (j = 0, 30, 60, 90 and 120 days of dietary treatment); (T × D)ij, effect of the interaction between treatment and days of trial and eijk is unexplained residual element assumed to be independent and normally distributed. The statistical difference between the means was determined by using 'Tukey's honest significant difference (HSD) test'.

RESULTS AND DISCUSSION

Growth performance: Effect of inorganic and nano Cu supplementation on DM intake, body weight gain and feed conversion ratio (FCE) in control, $inCu_{10}$, $nanoCu_5$ and $nanoCu_{10}$ groups was similar (Table 2). DMI increased with the increase in the body weight of the experimental heifers. Similarly, earlier findings also reported non-significant effect on DMI and weight gain in lamb (Cheng *et al.* 2008), Simmental steers (Engle and Spears 2001) and heifers (Mullis *et al.* 2003, Vaswani *et al.* 2018) or goat kids (Waghmare *et al.* 2014) on Cu supplementation. However, an improvement in weight gain on Cu supplementation was reported in *Black Bengal* kids (Mondal and Biswas 2007) and Cashmere goats (Zhang *et al.* 2008). This might be due to its role in metabolism of nutrients.

Haematology: In the present study, blood Hb concentration, PCV and RBC count in inorganic and nano Cu supplemented groups was significantly higher ($P<0.05$) than non-supplemented group (Table 3). Whereas, WBC count, MCHC, MCV and platelets were unaltered in heifers supplemented with inorganic and nano Cu. Similar observations on Hb, PCV and RBC were reported in goats (Mondal and Biswas 2007, Shen *et al.* 2020). On the contrary, no effect on the concentration of Hb, PCV, TEC and TLC was reported in goat kids (Datta *et al.* 2007) and lamb (Dezfoulian *et al.* 2012) regardless of source and level of Cu. However, a higher dose fed to lamb (20 ppm) leads to significantly higher PCV and Hb value in this study.

Plasma mineral status: Mineral nutrition plays an important role in the evolution, growth, and reproduction of animals (Huma *et al.* 2017). There was no significant difference in the mean plasma Ca, P, Zn and Fe concentrations ($P>0.05$) between inorganic Cu, nano Cu and non-supplemented groups (Table 3). However, plasma Cu level was significantly higher ($P<0.05$) in dietary inorganic and nano Cu supplementation groups. Cu level was observed maximum in 10 ppm nano Cu supplemented heifers followed by $nanoCu_5$ and $inCu_{10}$ groups. Similarly, increase in plasma Cu concentration in goats (Mondal *et al.* 2017, Shen *et al.* 2020), lambs (Hosienpour *et al.* 2014) and growing heifers (Vaswani *et al.* 2018) was reported on supplementation of Cu (irrespective of organic or inorganic form). On the contrary, a study on dairy cows reported no effect on plasma Cu levels by supplementation of different Cu sources (Sinclair *et al.* 2013). No effect of 10 ppm Cu supplementation on plasma concentration of other minerals is similar to report on heifers (Rabiansky *et al.* 1999) and goats (Mondal *et al.* 2007, Zhang *et al.* 2008).

Table 3. Effect of inorganic and nano copper supplementation on haematology and plasma mineral status

Parameter	Supplemental Cu (mg/kg DM)				SEM	P value		
	Cu_0	$inCu_{10}$	$nanoCu_5$	$nanoCu_{10}$		Treatment (T)	Period (P)	T×P
Haematological parameter								
Hb (g/dl)	8.13^a	9.10^b	9.16^b	9.42^b	0.22	<0.001	0.994	0.003
RBCs (10^6/μl)	7.96^a	9.07^b	9.00^b	9.21^b	0.49	<0.001	0.781	0.007
WBCs (10^3/μl)	9.93	9.67	9.66	9.23	0.36	0.170	0.982	0.969
PCV (%)	29.38^a	31.48^{ab}	31.34^{ab}	32.50^b	0.50	<0.001	0.196	<0.001
MCV (fl)	38.20	39.68	40.31	38.58	0.71	0.543	0.991	0.996
MCHC (%)	29.63	30.11	29.80	29.48	0.19	0.336	0.946	0.982
PLT (10^3/μl)	255.17	261.73	258.20	256.60	12.13	0.988	0.996	0.999
Plasma mineral status								
Ca (mg/dl)	10.01	10.13	10.15	10.03	0.41	0.884	0.446	0.581
P (mg/dl)	5.91	6.23	6.18	6.20	0.16	0.205	0.844	0.863
Cu (mg/l)	0.72^a	0.83^b	0.85^b	0.89^b	0.017	<0.001	0.652	<0.001
Zn (mg/l)	1.55	1.60	1.59	1.56	0.05	0.950	0.934	0.979
Fe (mg/l)	3.31	3.35	3.32	3.34	0.10	0.904	0.989	0.995

Table 4. Effect of inorganic and nano copper supplementation on blood biochemicals

Parameter	Supplemental Cu (mg/kg DM)				SEM	P value		
	Cu_0	$inCu_{10}$	$nanoCu_5$	$nanoCu_{10}$		Treatment (T)	Period (P)	T×P
Glucose (mg/dl)	71.21	73.27	72.04	74.02	3.40	0.607	1.000	0.999
Cholesterol (mg/dl)	154.42	151.43	150.71	153.07	4.96	0.247	0.001	0.133
Triglyceride (mg/dl)	51.97	54.32	51.18	53.33	2.54	0.816	0.900	0.930
ALP (IU/l)	166.74[b]	158.67[ab]	153.72[ab]	145.07[a]	4.26	<0.001	0.945	0.095
AST (IU/l)	48.43	46.73	45.04	43.15	1.65	0.104	0.954	0.438
ALT (IU/l)	14.68	13.97	13.82	13.46	0.46	0.227	0.863	0.350
Bilirubin (mg/dl)	0.53	0.54	0.51	0.55	0.02	0.803	0.827	0.744
Creatinine (mg/dl)	0.98	1.00	0.97	0.99	0.02	0.963	<0.001	0.001
Total protein (g/dl)	8.73	9.06	9.12	9.14	0.14	0.582	0.033	0.056
Albumin (g/dl)	3.80	3.93	3.90	3.83	0.06	0.825	0.422	0.803
Globulin (g/dl)	4.88	5.27	4.88	5.06	0.16	0.796	0.183	0.061
PUN (mg/dl)	22.22	20.34	21.68	20.42	1.01	0.324	0.894	0.837
NEFA (µmol/l)	162.18	152.71	160.24	155.58	4.52	0.504	0.744	0.773
BHBA (µg/ml)	39.59	37.42	36.85	37.79	0.96	0.126	0.147	0.275

On the contrary, Dezfoulian *et al.* (2012) reported that Cu supplementation had negative effect on plasma Fe concentrations in Cu-fed lambs. This might be due to higher dose used in this study in lambs (20 ppm).

Blood biochemicals: Present study revealed a non-significant (P>0.05) effect of inorganic and nano Cu supplementation on plasma glucose, cholesterol, triglycerides, NEFA and BHBA concentration in four different groups across 120 days study (Table 4). Similarly, no effect on plasma cholesterol levels in goat (Shen *et al.* 2020), heifers (Vaswani *et al.* 2018) and triglycerides and cholesterol concentration in Nellore beef cattle (Correa *et al.* 2012) was reported. Similarly, no effect of Zn, Cu and Se treatment on plasma concentrations of glucose, NEFA, BHBA and total cholesterol was reported either pre-partum or during lactation in cows (Cortinhas *et al.* 2012). However, some studies on higher dosage fed 20 or 40 mg Cu/kg DM reported increased serum triglyceride and NEFA concentrations and decreased total cholesterol concentrations in Cu supplemented lambs (Cheng *et al.* 2008) and goats (Datta *et al.* 2007).

Plasma concentrations of the protein metabolism biomarkers, i.e. plasma total protein, albumin, globulin and PUN showed non-significant effect (P>0.05) of inorganic and nano Cu supplementation. These results are similar to reports on lactating cows (Cortinhas *et al.* 2012) and goat kids (Datta *et al.* 2007). Shen *et al.* (2020) reported similar levels of blood urea nitrogen in goats.

Studied biomarkers of liver function test in present study were ALP, ALT, AST and bilirubin whereas; plasma creatinine was used as biomarker of kidney function test. Plasma ALP concentration was significantly lower (P<0.05) in 10 ppm nano Cu group than non-supplemented group. Plasma ALT, AST and bilirubin and plasma creatinine were found unaffected by supplementing inorganic and nano Cu in heifers, which indicates that the animals were apparently healthy throughout the experimental duration. Any increase in serum ALP and other enzyme activities suggest that muscle and kidney cells may also be damaged (Shen *et al.* 2020). Similar findings were reported in growing heifers (Vaswani *et al.* 2018) and goats (Mondal and Biswas 2007). However, significantly lower serum AST, ALT, ALP, and creatinine were reported in copper-deprived *Guizhou black* goats, fed on nano-copper and copper sulfate (Shen *et al.* 2020). This was due to pre damaged liver cells as reported in this study.

Supplementation of Cu either from inorganic or by nano source did not exert significant effect on growth performance. Dietary supplementation of Cu significantly increased plasma Cu levels in both inorganic and nano Cu supplemented group. However, plasma Ca, P, Zn and Fe concentrations were unaffected. Although, there were significant improvement in haematological parameters in Cu supplemented groups but other blood biomarkers were unaffected. Findings of the present study revealed that nano Cu @ 5 ppm can be selected for feeding in growing cattle as it exerts similar effects as showed by 10 ppm inorganic Cu.

The present work is on an emerging source of copper (nano copper) for livestock feeding in current scenario. By using copper in nano form, quantity of copper applied to animal diets and its consequent contamination of the environment can be notably reduced. Hence this study recommends the usage of nano copper in livestock, but more studies are required to understand the full potential of mineral nanoparticles.

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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. *Journal of Animal Sciences* **23**: 1066–72.

AOAC. 2005. *Official Methods of Analysis*. Association of Analytical Communities International. 18th ed. AOAC International, Washington, DC, USA.

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 Australasian Journal of Animal Sciences* **21**(5): 685–91.

Close W H. 1998. The role of organic trace mineral proteinates in pig nutrition. *Biotechnology in the Feed Industry*. *Proceedings of Alltech's 14th Annual Symposium* 469–83.

Correa L B, Zanetti M A, Claro G R D, de Melo M P, Rosa A F and Netto A S. 2012. Effect of supplementation of two sources and two levels of copper on lipid metabolism in Nellore beef cattle. *Meat Science* **91**: 466–71.

Cortinhas C S, Junior J E de F, Naves J de R, Porcionato M A de F, Silva L F P, Renno P F and Santos M V. 2012. Organic and inorganic sources of zinc, copper and selenium in diets for dairy cows: Intake, blood metabolic profile, milk yield and composition. *Revista Brasileira de Zootecnia* **41**(6): 1477–83.

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 Science and Technology* **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. *Livestock Science* **147**: 9–19.

El Basuini M F, El-Hais A M, Dawood M A O, Abou-Zeid A E S, EL-Damrawy S Z, Khalafalla M M E S, Koshio S, Ishikawa M and Dossou S. 2016. Effect of different levels of dietary copper nanoparticles and copper sulfate on growth performance, blood biochemical profiles, antioxidant status and immune response of red sea bream (*Pagrus major*). *Aquaculture* **455**: 32–40.

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. *Journal of Animal Sciences* **79**: 2920–25.

Hosienpour N, Norouzian M A, Afzalzadeh A, Khadem A and Alamouti A A. 2014. Source of copper may have regressive effects on serum cholesterol and urea nitrogen among male fattening lambs. *Biological Trace Element Research* **159**: 147–51.

Huma V, Singh V K and Singh M P. 2017. Heavy metal pollution due to coal washery effluent and its decontamination using a macrofungus, *Pleurotus ostreatus*. *Ecotoxicology and Environmental Saftey* **145**(4): 42–49.

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. *Journal of Environmental Quality* **32**: 535–40.

Maltais D, Desroches D, Aouffen M, Mateescu M A, Wang R and Paquin J. 2013. The blue copper ceruloplasmin induces aggregation of newly differentiated neurons: A potential modulator of nervous system organization. *Journal of Neuroscience* **121**: 73–82.

Mondal M K, Biswas P and Mazumdar D. 2007. Effect of supplementation of two sources of copper on plasma lipid profile and mineral balance of Black Bengal goats. *Animal Nutrition and Feed Technology* **7**: 37–46.

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 Australasian Journal of Animal Sciences* **20**: 1067–75.

Mullis L A, Spears J W and McCraw R L. 2003. Estimated copper requirements of Angus and Simmental heifers. *Journal of Animal Sciences* **81**(4): 865–73.

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

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 Metabolism* **4**: 235–44.

Ognik K, Stępniowska A, Cholewińska E and Kozłowski 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**: 2045–51.

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. *Journal of Dairy Science* **82**: 2642–50.

Shen X, Song C and Wu T. 2020. Effects of nano-copper on antioxidant function in copper-deprived Guizhou black goats. *Biological Trace Element Research.*

Sinclair L A, Hart K J, Johnson D and Mackenzie A M. 2013. Effect of inorganic or organic copper fed without or with added sulfur and molybdenum on the performance, indicators of copper status, and hepatic mRNA in dairy cows. *Journal of Dairy Science* **96**: 4355–67.

Underwood E J. 1981. *The mineral nutrition of livestock.* CAB, London, England, pp. 88-94.

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. *Journal of Dairy Science* **74**(10): 3583–97.

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 Journal of Animal Sciences* **88**(7): 812–18.

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 Journal of Animal Nutrition* **31**: 44–48.

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 Ruminant Research* **74**: 188–93.