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Effect of Dietary Supplementation of Nano Copper and Nano Zinc on Haematology and Biochemical Metabolites of Hariana Calves

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ABSTRACT

This study was conducted to determine the effect of dietary supplementation of nano copper (Cu) or nano zinc (Zn) alone or in combination on the haematology and blood metabolites in young calves. A total of 24 young Hariana calves were randomly assigned into four groups (6 calves per group) on body weight and age basis for a period of 120 days. Experimental calves were either received a basal diet devoid of supplemental Cu (control) or were supplemented with 10 ppm nano Cu ($_{nano}Cu_{10}$), 32 ppm nano Zn ($_{nano}Zn_{32}$) or combination of nano Cu and nano Zn ($_{nano}Cu_{10}$+$_{nano}Zn_{32}$). Blood was sampled at day 0, 30, 60, 90 and 120 post nano Cu and nano Zn supplementation for the determination of haematology attributes and blood metabolites. The mean plasma albumin concentrations were lower and plasma globulin concentrations was higher in $_{nano}Cu_{10}$+$_{nano}Zn_{32}$ group across 120 days study. However, there were no effect of treatments on the haematological attributes and most of the studied blood metabolites. Although, the treatment had significant effect on the plasma levels of Cu and Zn but no effect on plasma levels of other minerals was observed. Plasma Cu concentrations were higher in $_{nano}Cu_{10}$ and $_{nano}Cu_{10}$+$_{nano}Zn_{32}$ groups while plasma levels of Zn were higher in $_{nano}Zn_{32}$ and $_{nano}Cu_{10}$+$_{nano}Zn_{32}$ groups.

KEYWORDS: Blood metabolites, Haematology, Nano Cu, Nano Zn, Hariana calf

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INTRODUCTION

Minerals fulfil several important functions for the maintenance of animal growth and reproduction as well as health status (Suttle, 2010). Among the trace minerals, Cu and Zn are the most suitable trace mineral for improving immune function, health status, and growth performance of calves. For efficient production and maintenance of normal health of calves, it is necessary to provide these micronutrients in an appropriate proportion (Garg et al., 2000). Different sources of Cu and Zn, such as Cu chloride, Cu oxide, Cu citrate, Cu and Zn sulphate and Zn oxide at different concentrations have been applied in cattle and poultry feed, depending on Cu and Zn bioavailability (Pang et al., 2009). The bioavailability of Cu and Zn from inorganic sources is less. Therefore, these minerals are added more than the normal requirement of the animal. Also, the utilization of inorganic salts is very low; approximately 70-80% of them are excreted in the faeces (McDowell, 1992). Less bioavailability and more excretion is the major environmental issue related to the inorganic mineral sources. Therefore, alternatives to inorganic minerals need to be identified. There are various alternatives to inorganic sources with higher bioavailability, like chelated minerals, hydroxy minerals and nanominerals. Among them, nanominerals have the maximum bioavailability (Zhao et al., 2014).

Nanotechnology has modernized the commercial application of nano-sized minerals, which have been used recently as a tool in the fields of mineral nutrition, physiology, biology, biotechnology, reproduction and pharmacology in animals (Peters et al., 2016). It has been demonstrated that nanomaterials have novel properties which changes from normal sized particles, such as increasing the surface area available to interact with biological support, prolong compound residence time in GIT, decreasing the influence of intestinal clearance mechanism, infiltrating deeply into tissues through fine capillaries, cross epithelial lining fenestration, enabling efficient uptake by cells and efficiently delivery of the active compounds to target sites in the body (Chen et al., 2020). The use of Cu and Zn nanoparticles has recently received much attention due to their high electrical, melting points, low electrochemical migration behaviour, high bioavailability, high catalytic activity, less toxic and relatively lower cost of production (Tamilvanan et al., 2014). Even though the basic information regarding the effects of nano Cu and Zn has been shown in ruminants, confirmation of the effect of nano Cu and Zn supplementation on blood metabolism in dairy animals is still lacking. Considering these facts, the present study is therefore designed to investigate the effect of nano Cu and Zn supplementation on the haematology and blood metabolites in young Hariana calves.

MATERIALS AND METHODS

Experimental design

A total of 24 young dairy calves were randomly assigned to four dietary treatments on a body weight (27.52±3.43 kg) and age (25.33±8 days) basis for a period of 120 days. The experimental calves either received a basal diet devoid of supplemental nano Cu and nano Zn (Control group) or were supplemented with 10 ppm of nano Cu ($_{nano}Cu_{10}$) as cupric oxide nano powder (CuO, actual particle size 50 nm, molecular weight 79.54, minimum assay purity 99%, Sisco Research Laboratories Pvt. Ltd. India), 32 ppm of nano Zn ($_{nano}Zn_{32}$) as zinc oxide nano powder type 1 (ZnO, actual particle size 50 nm, molecular weight 81.38, minimum assay purity 99.9%, Sisco Research Laboratories Pvt. Ltd. India) or a combination of nano Cu and nano Zn i.e. 10 ppm nano Cu+32 ppm nano Zn ($_{nano}Cu_{10}+_{nano}Zn_{32}$). The nutrient requirements of calves were met by feeding milk, calf starter, available green fodder, and wheat straw (NRC 2001). Milk and calf starter were offered at the rates of 10% and 1% of the body weight, respectively. Berseem fodder and wheat straw were available *ad libitum*. Calves were housed in a well-ventilated shed having the proper arrangement for feeding and watering. Deworming of all the experimental animals was done before the start of the experiment by the oral administration of Fentas bolus (Intas Pharmaceuticals Pvt. Ltd., India) at a dose level of 10 mg/kg body weight. The nutrient composition of feedstuffs and milk fed during the entire experimental period is presented in Table 1.

Table 1. Percent composition of diet fed during the study

Nutrient	Calf starter	Wheat straw	Berseem fodder	Milk composition
DM (%)	89.4	88.5	12.6	-
CP (%)	23.6	3.04	19.5	3.74
EE (%)	4.37	1.41	3.96	4.39
CF (%)	12.9	35.12	21.5	-
Total Ash (%)	9.56	13.3	12.8	0.65
AIA (%)	1.84	4.93	3.75	-
NFE (%)	49.5	47.1	42.2	-
NDF (%)	35.9	74.31	45.3	-
ADF (%)	17.9	52.7	28.2	-
ADL (%)	3.28	7.59	2.68	-
Lactose	-	-	-	4.89
Total solids	-	-	-	13.8
Ca[β]	1.22	0.48	1.98	972
P[β]	0.63	0.08	0.47	984
Cu[γ]	13.7	2.71	6.46	82
Zn[γ]	46.4	4.29	19.2	3491
Fe[γ]	356	165	287	0.63

([a]Minerals in feedstuffs are presented in % while minerals in milk are presented in mg/L, [a]minerals in feedstuffs are presented in mg/kg DM while minerals in milk are presented in g/L)

Sampling and laboratory analysis

Peripheral blood samples were collected before feeding and watering of heifers at 07:00 h in heparinised vacuutainer tubes (BD Franklin, USA) at 0, 30, 60, 90, and 120 days post-nano Cu and nano Zn supplementation. A fraction of whole blood samples were used for haematological parameters and remaining amount of blood samples were centrifuged at 3000 rpm for 30 min to separate the plasma from packed erythrocytes. Plasma samples were stored at -20°C until further analysis of metabolites. Haematological parameters such as red blood cells (RBCs), white blood cells (WBCs), haemoglobin (Hb) concentration, packed cell volume (PCV) or haematocrit (HCT), mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) were estimated by using Celltac-α auto haemoanalyser (Nihon kohden, Japan). Plasma samples were subjected for further analysis of glucose, triglyceride, cholesterol, total protein, albumin and minerals concentrations (Span Diagnostic Ltd. India). Plasma globulin content was determined by subtracting the albumin content from total protein content.

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). Mineral content in the representative samples of feeds and fodders offered, residue left and faeces was determined by using ICP-OES (5800 ICP-OES, Agilent, CA, USA).

Statistical analysis

The generated data of haematological and biochemical attributes was analyzed by using the MIXED procedure of SPSS (Version 20.0, Inc., Chicago, IL), using repeated measures. The model used was as follows:

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

Where, Yijk is the dependent variable, μ is the overall mean of the population, Ti is the mean effect of the treatment (0, 10 ppm Cu, 32 ppm Zn and 10 ppm Cu +32 ppm Zn), Dj is the mean effect of period of blood sampling (0, 30, 60, 90, and 120 days post-treatment), (T×D)ij is the effect of the interaction between the effect of treatment and the period of blood sampling, and eijk is the unexplained residual element assumed to be independent and normally distributed. The effects of treatment, period, and treatment by period interaction were considered as fixed and calf as a random effect. If the analysis revealed a significant effect, the differences between treatment, period, and treatment by period interaction were then determined by Duncan's post hoc test at $P \leq 0.05$. Results are presented as least squares means and pooled standard errors of the means (SEM).

RESULTS AND DISCUSSION

Haematological profile

In the present study, RBCs and WBCs count, haemoglobin, haematocrit value, MCV and MCHC were used as heamatological parameters (Table 2). Dietary nano Cu and nano Zn supplementation did not exert any significant effects on the studied haematological attributes. In a study conducted by Kushwaha et al. (2021), blood Hb concentration, haematocrit value (PCV %), and RBCs count in inorganic and nano-Cu supplemented groups was significantly higher ($P<0.05$) than non-supplemented group. However, WBCs count, MCHC, MCV and platelets were unaltered in growing Sahiwal heifers supplemented with inorganic and nano-Cu which was similar to our findings. Similarly, the hematological traits RBC, WBC, MCV, HGB, HCT and PLT in piglets were not affected by nano-Cu supplementation (Gonzales-Eguia et al., 2009). Similar to the findings of the present study, Datta et al. (2007) also observed no effect of dose and source of Cu on haemoglobin, PCV, TEC and TLC in goat kids throughout the experimental period. However, Dezfoulian et al. (2012) found that Cu supplementation caused no significant difference between experimental treatments in lambs; however, Cu level had a significant effect on PCV and Hb, with the 20 ppm level causing higher values. Chang et al. (2018) reported similar results on the WBC, RBC, Hb, and HCT contents at 14 days and 28 days, respectively, in both inorganic and nano Cu fed groups in pigs. Kim et al., (2021) also observed that similar results were observed for WBC, RBC, Hb, and HCT in nano Cu supplemented weaned pigs. In opposite to the findings of the present study, Mondal et al. (2007) and Shen et al. (2021) noticed higher Hb, PCV and TEC in Cu-propionate groups than with $CuSO_4$ supplementation. On the other hand, WBCs count, ESR, and H/L ratio were significantly reduced in the nano Cu group relative to the control and $CuSO_4$ groups (El-kazaz and Hafez, 2020). Higher RBC counts and haemoglobin concentration might be due the fact that Cu has a significant role in Fe metabolism, RBC production, and synthesis of haemoglobin (Samanta et al. 2011; Ognik, 2018).

Similar to the findings of the present study, no effect of Zn supplementation on WBCs, RBCs, Hb concentration, PCV and MCV in buffalo calves was observed by Ramulu et al. (2015). In a similar to the present study, supplementation of 35 ppm Zn as $ZnSO_4$ or Zn-propionate (32.5 ppm) in crossbred calves did not affect the blood Hb concentration and PCV (Mandal and Das, 2010). In contrast to the findings of the present study, Nagalakshmi et al. (2018) observed a reduced concentration of RBCs and WBCs in growing rats fed diets supplemented with Zn at a higher concentration (48 ppm) than the NRC (1995) recommended (12 ppm), it might be due to the negative impact of Zn on other trace minerals (Cu and Fe) involved in RBC and WBC proliferation and maturation. Thus, the present study clearly indicated that Zn supplementation at 32 ppm had no adverse effect on haematological constituents.

Table 2. Effect of nano Cu and nano Zn supplementation on haematological attributes

Parameters	Group				SEM	P value		
	Control	${}_{nano}Cu_{10}$	${}_{nano}Zn_{32}$	${}_{nano}Cu_{10}+{}_{nano}Zn_{32}$		Treatment (T)	Period (P)	T×P
RBCs count (10^6/µL)	7.91	8.77	8.39	9.03	0.40	0.70	0.48	0.98
WBCs count (10^3/µL)	10.9	11.4	11.4	11.5	0.12	0.69	0.64	1.00
Hb (g/dL)	9.86	11.0	10.6	11.3	0.30	0.44	0.63	0.77
Haematocrit value (%)	29.6	32.4	31.9	32.9	0.74	0.92	0.38	1.00
MCV (fL)	31.4	32.4	32.02	33.8	0.66	0.82	0.38	0.89
MCHC (g/dL)	32.7	33.8	33.4	34.4	0.27	0.61	0.72	0.95

Biochemical attributes

The feeding of a diet supplemented with 10 ppm nano Cu or 32 ppm of nano Zn or their combination did not exert any significant effect ($P>0.05$) on plasma glucose concentration (Table 3). Similar to the plasma glucose concentration, the plasma triglycerides and cholesterol concentrations were also not affected by nano Cu and nano Zn supplementation. There was no effect of nano Cu and nano Zn supplementation on the plasma total protein concentration. The mean plasma albumin concentration was lower in calves fed on the diet supplemented with 10 ppm nano Cu + 32 ppm nano Zn compared to control. In contrary to the findings of plasma albumin levels, plasma globulin concentrations were observed to be higher in the ${}_{nano}Cu_{10}+{}_{nano}Zn_{32}$ group than other groups. Dietary nano Cu and nano Zn supplementation had no effect ($P>0.05$) on plasma concentrations of studied minerals except plasma Cu and Zn levels. Dietary nano Cu and nano Zn supplementation had a significant effect ($P<0.05$) on Cu and Zn concentration. Plasma Cu concentration was higher in Cu supplemented group as well as in combination group. Dietary nano Cu and nano Zn supplementation had a significant effect ($P<0.05$) on Zn concentration and levels were reported higher in the $_{nano}Zn_{32}$ and $_{nano}Cu_{10}+_{nano}Zn_{32}$ groups.

Table 3. Effect of nano Cu and nano Zn supplementation on biochemical attributes

Parameters	Group				SEM	P value		
	Control	$_{nano}Cu_{10}$	$_{nano}Zn_{32}$	$_{nano}Cu_{10}+_{nano}Zn_{32}$		Treatment (T)	Period (P)	T×P
Glucose concentration (mg/dL)	78.6	75.49	77.60	78.2	1.99	0.90	0.38	1.00
Triglycerides concentration (mg/dL)	37.7	37.3	40.0	37.4	2.70	0.83	0.57	0.95
Cholesterol concentration (mg/dL)	99.9	103.90	99.3	98.3	3.16	0.72	0.57	0.88
Total protein concentration (g/dL)	6.96	6.93	6.98	7.30	0.14	0.15	0.49	0.73
Albumin concentration (g/dL)	3.76^{c}	3.58^{bc}	3.26^{ab}	3.12^{a}	0.07	<0.001	0.47	0.83
Globulin concentration (g/dL)	3.20^{a}	3.35^{a}	3.72^{ab}	4.18^{b}	0.07	<0.001	0.39	0.74

Similarly, Shen et al. (2021) and Kushwaha (2021) noticed that the concentration of plasma cholesterol was not affected by nano Cu in goats and heifers, respectively. The results of the present study are similar to the findings of Vaswani et al. (2018), who reported that concentrations of total cholesterol were not affected by the source of Cu in the diet of growing heifers. Cortinhas et al. (2012) also found that there were no effects of Zn, Cu and Se treatments on plasma concentrations of glucose and total cholesterol, either pre-partum or during lactation in cows. Correa et al. (2012) observed that there was no effect of Cu supplementation on the average concentrations of triglycerides and cholesterol in Nellore beef cattle. A similar trend was observed in plasma glucose and total cholesterol in Cu supplemented piglets (Liao et al., 2017). These results are in agreement with Cortinhas et al. (2012), who did not observe effects of Cu on plasma concentrations of total protein, either pre-partum or during lactation in cows. However, Gonzales-Eguia et al. (2009) reported that total globulin levels were highest in the nano Cu group and were significantly different from both the $CuSO_4$ and control groups in piglets. However, Wen et al. (2019) observed lower plasma cholesterol in broilers fed 40 mg Cu/kg diets than those in the control group. Scott et al. (2018) observed that levels of cholesterol, triglycerides and glucose were significantly lower in groups treated with nano Cu in relation to $CuSO_4$ and the control groups in broiler chicken.

The plasma glucose and cholesterol concentrations were not influenced by dietary supplementation of Zn up to 140 ppm, corroborating with findings of Malcolm-Callis et al. (2000) and Whitman et al. (2007) with Zn supplementation of 90 and 200 ppm in steers, respectively. Similar to the present findings, a comparable and similar serum total protein in the Zn supplemented Murrah buffalo calves was observed by Ramulu et al. (2015). In concurrence with the findings of this study, Mandal and Dass (2010) reported no effect on serum total protein with supplementation of 35 ppm Zn either as $ZnSO_4$ or Zn propionate in calves fed a basal diet (32.5 ppm). However, Daghash and Mousa (2002) reported a significant increase in total protein and total globulin in buffalo calves after Zn supplementation over 180 days, but albumin did not change significantly. The increased serum globulin concentrations in the combination group in the present study imply a better immune response with nano Cu + nano Zn supplementation.

The dietary supplementation of either nano Cu or nano Zn alone or their combination did not exert any significant effect on plasma concentrations of most of the studied minerals (Table 4). However, the plasma concentration of Cu was higher in the $_{nano}Cu_{10}$ and $_{nano}Cu_{10}+_{nano}Zn_{32}$ groups, while plasma Zn levels were greater in the $_{nano}Zn_{32}$ and $_{nano}Cu_{10}+_{nano}Zn_{32}$ groups. Higher plasma concentrations of Cu and Zn in the respective nano Cu and nano Zn supplemented groups were evidenced by better absorption of Cu and Zn during digestion trials. Kushwaha (2021) observed no significant difference in the mean plasma Ca, P, Zn and Fe concentrations between inorganic Cu, nano Cu and non-supplemented Sahiwal heifers. Whereas, plasma Cu level showed significant effect on dietary inorganic and nano Cu supplementation. Cu level was observed maximum in 10 ppm nano Cu supplemented heifers, followed by 5 ppm nano Cu and 10 ppm inorganic Cu. The results of the present study are similar to the observations of Mondal et al. (2007) who reported that supplementation of Cu (irrespective of organic or inorganic form) increased plasma Cu concentration in goats. A similar trend was observed by Shen et al. (2021), who found that after supplementation of nano Cu or Cu sulfate, Cu concentration in blood was significantly increased in goats, with a lower increase with inorganic Cu. Accordingly, Vaswani et al. (2018) also reported that the plasma Cu concentration increased with the Cu supplementation in growing heifers, indicating that dietary Cu levels were reflected in blood Cu concentrations. Mondal et al. (2007) also reported that supplementation of 10 ppm Cu proteinate did not alter Ca, P, Fe, Zn and Mn concentrations in goats. Recent findings of Wen et al. (2019) showed that Ca and P balance were not affected by the dose of Cu in broilers. Cu supplementation at 50, 100 and 150 ppm level in mink and reported linear effect of dose of Cu on plasma Cu concentration (Wu et al., 2015).

Table 4. Effect of nano Cu and nano Zn supplementation on plasma mineral concentration

Parameters	Group				SEM	P value		
	Control	$_{nano}Cu_{10}$	$_{nano}Zn_{32}$	$_{nano}Cu_{10}+_{nano}Zn_{32}$		Treatment (T)	Period (P)	T×P
Ca level (mg/dL)	11.2	11.3	11.4	11.4	0.27	0.69	0.86	0.99
P level (mg/dL)	4.98	5.11	5.61	5.27	0.13	0.59	0.67	0.83
K level (mEq/L)	4.30	4.69	4.93	4.76	0.13	0.693	0.72	0.94
Mg level (mg/dL)	2.40	2.50	2.61	2.60	0.05	0.99	0.58	0.77
Cu level (mg/L)	0.78^a	1.59^b	0.86^a	1.57^b	0.10	0.02	0.38	0.69
Zn level (mg/L)	1.43^a	1.55^a	2.32^b	2.24^b	0.08	0.04	0.55	0.83
Fe level (mg/L)	4.01	4.33	4.49	4.40	0.40	0.88	0.28	0.51
Cr level (µg/L)	0.30	0.31	0.32	0.32	0.01	0.69	0.70	0.82
Mn level (mg/L)	0.25	0.25	0.27	0.26	0.02	0.66	0.76	0.85

The mean value with different superscript letters in a row differs significant (P<0.05).

All calves had adequate blood plasma Zn levels, suggesting that the treatment diets provided sufficient Zn to meet animal requirements during the period of this study. Kinal et al. (2005) reported an increase in blood plasma Zn when animals were supplemented with organically chelated Zn compared with inorganic (sulfate) Zn. Cu plasma and Mo plasma levels, it is suggested that feeding Zn at the recommended or lower level in either form does not affect Cu metabolism (Cope et al., 2009). In previous studies (Spears and Kegley, 2002; Kessler et al., 2003), supplementing relatively low concentrations (10 to 25 mg/kg) of Zn to diets containing 24 to 35 mg/kg of Zn has not increased plasma Zn concentrations in cattle. However, consistent with previous studies (Cao et al., 2000), supplementation with high concentrations (500 mg/kg) of Zn resulted in elevated concentrations of Zn in plasma, liver, kidney, and bone shaft.

CONCLUSION

The findings of the present study revealed that the supplementation of either nano Cu or nano Zn alone or in combination significantly lowers plasma albumin and elevated plasma globulin levels in the combination group but treatment did not exert any effect on other studied haematological and blood metabolites. Treatment had significant effect on the plasma levels of Cu and Zn but no effect on plasma levels of other minerals was observed. Plasma Cu concentrations were higher in ${}_{nano}Cu_{10}$ and ${}_{nano}Cu_{10}+{}_{nano}Zn_{32}$ groups while plasma levels of Zn were higher in ${}_{nano}Zn_{32}$ and ${}_{nano}Cu_{10}+{}_{nano}Zn_{32}$ groups.

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