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Dietary Supplementation of Copper and Zinc Nanoparticles in Young Dairy Calves: Effects on Growth Performance and Nutrients Utilization

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ABSTRACT

The use of nanotechnology to produce nano-sized minerals is a potential alternative to both organic and inorganic sources. The aim of this study was to determine the effect of dietary supplementation of nano copper (Cu) or nano zinc (Zn) alone or in combination on the growth performance and nutrients utilization in young calves. A total of twenty-four young cattle 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 ($_{nano}Cu_{10}+_{nano}Zn_{32}$). Experimental calves were monitored fortnightly for body weight change. A digestion cum metabolism trial was conducted to determine the bioavailability of minerals. Dietary supplementation of either nano Cu or nano Zn alone or in combination did not exert any effect on growth performance, apparent nutrient digestibility. Eventhough, the nano Cu and nano Zn supplementation exerts a positive effect on its absorption in respective groups but there were no effect on the absorption of other studied minerals. Absorption and bioavailability of Cu is higher in $_{nano}Cu_{10}$ as well as in combination group ($_{nano}Cu_{10}+_{nano}Zn_{32}$). However, Absorption and bioavailability of Zn was higher in $_{nano}Zn_{32}$ and combination group ($_{nano}Cu_{10}+_{nano}Zn_{32}$). In conclusion, dietary supplementation of nano Cu and Zn improve bioavailability of Cu and Zn without having any adverse effect on the nutrient digestibility and growth performance. Similarly, dietary supplementation of nano Cu and Zn did not exert any interaction with other minerals.

KEYWORDS: Calf, Growth performance, Nano Cu, Nano Zn, Nutrients utilization

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INTRODUCTION

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). A number of trace elements have been shown to be important for adequate functioning of the immune system, among which copper (Cu) and zinc (Zn) play a major role. Cu effectively maintains the stability of the internal environment and is closely related to growth, health status, haematopoiesis, metabolism and reproduction (Ognik et al., 2016). Due to its ability to easily receive and donate electrons, Cu is involved in numerous biochemical processes (Maltais et al., 2013). Cu is part of the active sites of many enzymes, including superoxide dismutase (SOD), ceruloplasmin, cytochrome oxidase, L-lysine oxidase, ascorbate oxidase, tyrosinase and dopamine beta-hydroxylase (Gaetke and Chow, 2003). These enzymes play an important role in antioxidant defence mechanisms, haemoglobin and melanin synthesis, the formation of connective tissue and mitochondrial respiration (Maltais et al., 2013). Involvement of Cu in antioxidant defence protects cell membranes from damage caused by free radicals or oxidative stress. Cu actively participates in immune processes and, by contributing to the transformation of arachidonic acid and prostaglandin synthesis; it plays a vital role in reducing the severity of inflammatory processes.

Zn is the second most abundant trace element in the animal body, but it cannot be stored in the animal's body (Zalewski et al., 2005) and requires regular dietary intake to meet its physiological needs. Zn influences various biological functions and is also a cofactor for more than 300 metalloenzymes (Chasapis et al., 2012). Zn is essential for the body's proper physiological functions like growth (Case and Carlson, 2002), health status, reproduction (Uchida et al., 2001), DNA synthesis, cell division and gene expression, wound healing (Zhao et al., 2014), ossification (Roughead and Kunkel, 1991), augmenting the immune system of the body (Parashuramulu et al., 2015), lymphocyte replication and proliferation and protection of cell membranes from bacterial endotoxins and antibody production (Nockels, 1994).

The bioavailability of minerals from their inorganic sources is quite low due to minerals being inert and having more interactions with other minerals, so absorption of other minerals is decreased. As a result, these minerals are added 20-30 times more than an animal's normal requirement. Higher levels of Cu and Zn excreted from the faeces of the supplemented animals have raised concerns pertaining to environmental pollution (Feng et al., 2010). Thus, this problem opens a window for better bioavailable Cu and Zn sources that will be very useful in reducing the supplemental dose of Cu and Zn to the animals as well as reducing the risk of environmental pollution. To overcome the limitation of inorganic sources of minerals, various other alternatives have been tried and found that nano minerals can be the best alternative. In the animal body, nanominerals interact more effectively and efficiently with organic and inorganic substances due to their larger surface area (Zaboli et al., 2013). The nano-sized minerals have higher potential than their conventional sources and thus reduce the quantity required (Sindhura et al., 2013). Therefore, the use of nanotechnology to produce nano-sized minerals is a potential alternative to both organic and inorganic sources. In the past, numerous studies in farm animals have been conducted with organic and inorganic sources of mineral supplementation. Studies with nano Cu and nano Zn are restricted until separate use of these minerals. None of the studies has been conducted to see the effect of nano Cu and nano Zn in combination. Considering these facts, this study was designed to study the effects of either nano Cu or nano Zn alone or in combination on the growth performance and nutrients utilization in young dairy calves.

MATERIALS AND METHODS

Animals, diets and experimental design

For the experiment a total of 24 young dairy calves were approved (approval number IAEC/21/15) under the established standard of the Institutional Animal Ethics Committee (IAEC). The 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 duration 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 nanopowder (CuO, actual particle size 50 nm, zeta potential of +41 mV, 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 nanopowder type 1 (ZnO, actual particle size 50 nm, zeta potentials of 28.8 mV, 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. Green fodder and straw were fed *ad libitum*. Calves were housed in a well-ventilated shed and deworming was done at beginning of research by oral administration of Fentas bolus (Intas Pharmaceuticals Pvt. Ltd., India) at a dose level of 10 mg/kg body weight. The calculated amount of nano Cu as CuO nano powder and nano Zn as ZnO, was supplemented in encapsulated form. Amount of supplemental nano Cu and nano Zn calculated as per dry matter intake (DMI), weighed (ATX224 Shimadzu weighing balance) and filled inside the gelatinized capsule having capacity of 500 mg each. The calves were provided with fresh and clean *ad libitum* water round the clock. The nutrient composition of feedstuffs and milk fed during the experimental period is presented in Table 1.

Table 1. Nutrient composition (%) of diet fed during the experimental period

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	35.1	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	74.3	45.3	-
ADF (%)	17.9	52.7	28.2	-
ADL (%)	3.28	7.59	2.68	-
Lactose	-	-	-	4.89
Total solids	-	-	-	13.8
Ca*	1.22	0.48	1.98	972
P*	0.63	0.08	0.47	984
Cu**	13.8	2.71	6.46	82
Zn**	46.39	4.29	19.2	3491
Fe**	356.19	165.29	287.49	0.63

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

Observation recorded and laboratory analysis

The body weight of the experimental calves was recorded at the start of experiment and then at a fortnightly interval by using a computerized weighing machine (Leotronic Scales Pvt. Ltd., India). Calves were weighed for 2 consecutive days in the morning at 06:00 h before offering feeds, fodders and water. The average of consecutive two days was considered as BW for that fortnight and was considered for average daily gain (ADG). To compare the efficiency of nutrient utilization and mineral absorption in the experimental calves, a digestion trial with a collection period of 7 days was conducted at the end of the study. Samples of feeds and fodders offered, residue left and faeces voided were dried in a hot air oven at 60 °C until a constant weight was achieved and then ground to pass through a 1-mm sieve in a Wiley mill. 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 ADG was analyzed by using the MIXED procedure of SPSS (Version 20.0, Inc., Chicago, IL), using repeated measures. 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 PdH0.05. Data regarding digestibility trial was analysed by using one way ANOVA. Results are presented as least squares means and pooled standard errors of the means (SEM).

RESULTS AND DISCUSSION

Growth performance and nutrients utilization

The actual particle size and zeta potential of nano Zn were 50 nm and 28.8 mV, respectively. However, actual particle size and zeta potential of nano Cu were

50 nm and 28.8 mV, respectively. The mean ADG, apparent nutrient digestibility and mineral absorption across 7 days metabolism trial is shown in Table 2. The ADG during 120 days study period in different groups ranges in between 201.22-327.56 g/day. Dietary supplementation of either nano Cu or nano Zn alone or their combination did not exert any impact on the growth performance of the experimental calves. There were no study has between conducted to see the effect of combination of NANO Cu and nano Zn in dairy animals. Therefore, findings of this study has been discussed with the previous research work conducted either with Cu or with Zn. Kushwaha et al. (2021) found no effects of 10 ppm inorganic Cu, 5.0 and 10.0 ppm nano Cu on the growth performance in growing Sahiwal heifers, which was similar to the findings of our study. Vaswani et al., (2018) reported a similar observation that supplementing 8.0 mg Cu/kg DM either in the form of Cu-proteinate, Cu-propionate and Cu sulphate did not affect ADG in growing heifers. Kim et al. (2021) also found a similar effect on growth performance in nano Cu supplemented pigs compared with inorganic and organic Cu. The results of the present study are similar to the observations of Dezfoulian et al. (2012) who reported that there was no significant effect of Cu supplementation on ADG in lambs. Waghmare et al. (2014) observed that supplementation of Cu as $CuSO_4$ and Cu-methionine did not alter ADG and feed: gain ratio in kids. Similar to the present findings, Cu supplementation had no effect on growth performance in heifers (Mullis et al., 2003) and Simmental steers (Engle and Spears, 2001). However, in contrast to the findings of the present study, some studies compared the inorganic forms of Cu with nano Cu and the latter showed an improvement in the growth performance of piglets (Gonzales-Eguia et al., 2009; Chang et al., 2018).

No significant difference in body weight observed on supplementation of different levels and different sources of Zn has been observed in previous research, though overall body weight increases as the age of experimental animal advances. Spears (1995) observed that Zn supplementation above NRC (2001) recommended requirements did not consistently affect growth rate in cattle. The results of the present study are similar to the observations of Zaboli et al. (2013) who reported that ADG in goat kids was not affected due to the supplementation of Zn from different sources at different levels. However, contrary to the findings of present study, El-Nour et al. (2010) observed the growth promoting effect of organic Zn has than that of an inorganic Zn source. In another study, Chang et al. (2020) showed that supplementation with Zn-methionine but not ZnO, significantly increased the ADG of newborn calves in the first 2 wk after birth. Similarly, Seifdavati et al., (2018) observed a significant effect on final weight and weight gain in the calves supplemented with nano Zn oxide (30 and 60 mg/kg DMI in their diet) than without Zn supplemented group. Anil et al. (2019) observed significantly higher body weight gain and ADG in the 20 ppm nano Zn supplemented calves group followed by 10 ppm nano Zn, 5 ppm nano Zn supplemented groups and 25 ppm $ZnSO_4$ group. The discrepancy in growth performance in the findings of different studies may be a consequence of different sources and levels of Cu and Zn used, differing ages of animals used in the study, differences in study period, different genetics in various breeds, etc.

Table 2. Effect of nano Cu and nano Zn supplementation on growth performance and nutrient digestibility

Parameters	Group				SEM	P value
	Control	$_{nano}Cu_{10}$	$_{nano}Zn_{32}$	$_{nano}Cu_{10}+_{nano}Zn_{32}$		
ADG (g/day)	256.4	267.9	262.8	273.4	3.62	0.74
Nutrient digestibility (%)						
DM	66.4	68.6	70.2	69.7	1.83	1.00
CP	70.2	71.8	73.2	71.9	1.63	0.99
EE	81.3	82.4	83.3	81.3	2.47	0.52
CF	55.2	56.2	58.9	57.2	1.79	0.68
NFE	72.9	74.3	75.6	75.7	1.65	0.49
NDF	51.5	54.1	56.3	55.03	1.01	082
ADF	32.4	35.8	39.1	37.4	1.43	1.00

The apparent digestibility of different nutrients also shows a non-significant effect of treatment. The results obtained in this study demonstrated that the effect of nano Cu and nano Zn supplementation had no impact on the apparent nutrient digestibility which was comparable to the findings of Waghmare et al. (2014) in kids and Vaswani et al. (2018) in heifers supplemented with Cu. Mondal et al. (2007) reported that supplemental Cu sources had no effect on the apparent digestibility of DM, OM and NFE. Accordingly, there was no effect of organic Cu supplementation on the digestibility of DM, OM, CP, EE, CF and NFE in Chokla rams was observed by Shinde et al. (2013). However, in contrast to present findings, Gonzales-Eguia et al. (2009) reported that Cu-nano particles improved the digestibility of EE and energy in pigs. Dezfoulian et al. (2012) found that Cu sources had a significant effect on digestibility of nutrients except EE, as proteinate sources resulted in higher digestibility for DM, CP, NDF, ADF, NFC and OM.

The digestibility coefficient of DM, CP, EE, CF, NFE, NDF and ADF were not impacted by supplementation of different levels of nano Zn in the diet of the growing calves. Mandal et al. (2007) observed no significant difference in digestibility of DM, CP, EE, NDF and ADF among the calves with a basal diet containing 32.5 mg Zn/kg diet and supplementation of 35 mg Zn/kg diet from $ZnSO_4$ and Zn-propionate in the treatment groups, respectively. Jadhav et al. (2008) observed similar digestibility of organic nutrients among the buffalo calves that were supplemented with 0, 35 and 70 ppm Zn-sulphate. Garg et al. (2008) recorded no significant difference in the digestibility of DM, OM, CP, EE, NDF and hemicelluloses between inorganic (20 mg/kg diet from $ZnSO_4$) and organic Zn (20 mg/kg diet from Zn-methionine complex) supplemented lamb groups. In other studies, Zn supplementation ranging from 20 to 135 ppm did not influence the digestibility of DM and CP (Kumar et al., 2002), NDF and ADF (Salama et al., 2003) in ruminants. Singh et al. (2018) concluded that there was no difference in digestibility coefficient of the nutrients between micro-ZnO (60 ppm) and nano-ZnO (60 ppm) supplemented lamb. On the other hand, Shakweer et al. (2010) and Mallaki et al. (2015) reported an improvement in nutrient digestibility.

Mineral utilization

Dietary supplementation of Cu or Zn alone or in combination showed a non-significant effect on the intake, faecal excretion and absorption of the most of the studied minerals (Table 3). However, treatment showed a significant ($P<0.05$) effect on the intake, faecal excretion and absorption of Cu and Zn. The intake, faecal excretion and absorption Cu were significantly ($P<0.05$) higher in the ${}_{nano}Cu_{10}$ and ${}_{nano}Cu_{10}+{}_{nano}Zn_{32}$ groups compared to the control and ${}_{nano}Zn_{32}$ groups. Accordingly, the intake, faecal excretion and absorption of Zn were significantly ($P<0.05$) higher in ${}_{nano}Zn_{32}$ and ${}_{nano}Cu_{10}+{}_{nano}Zn_{32}$ groups compared to the control and ${}_{nano}Zn_{32}$ groups. The results of the present study are similar to the observations of Shen et al. (2021), who reported that after nano-Cu supplementation for up to 10 days, the Cu content in blood significantly increased in goats due to an increase of its absorption. In agreement with present study, Gonzales-Eguia et al. (2009) reported that Cu availability and absorption were significantly improved and the faecal Cu level was reduced in the nano Cu fed group as compared to the $CuSO_4$ in piglets. The lowest amount of additional Cu in the form of nanoparticles increased the absorption of Cu and Zn in turkeys (Jankowski et al., 2020). In contrast 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 concentrations in heifers were not greatly influenced by different supplemental Cu sources (Rabiansky et al., 1999). Our results are suggesting that the nano form of Cu was more efficient than inorganic Cu in influencing the Cu absorption in growing calves.

Table 3. Effect of nano Cu and nano Zn supplementation on mineral utilization

Parameters	Group				SEM	P value
	Control	$_{nano}Cu_{10}$	$_{nano}Zn_{32}$	$_{nano}Cu_{10}+_{nano}Zn_{32}$		
Ca utilization						
Intake (g/day)	9.91	9.35	9.61	9.90	0.73	0.49
Voided in faeces (g/day)	6.30	5.78	5.85	6.09	0.52	0.72
Absorption (g/day)	3.61	3.57	3.75	3.81	0.06	1.00
Absorption (%)	36.4	38.2	39.09	38.4	0.57	0.73
P utilization						
Intake (g/day)	3.43	3.34	3.54	3.54	0.05	0.99
Voided in faeces (g/day)	1.77	1.71	1.75	1.76	0.05	0.68
Absorption (g/day)	1.66	1.64	1.78	1.78	0.04	0.41
Absorption (%)	48.3	48.9	50.4	50.3	0.50	0.79
Na utilization						
Intake (g/day)	6.96	7.16	7.38	7.37	0.46	0.55
Voided in faeces (g/day)	0.88	0.82	0.71	0.79	0.03	1.00
Absorption (g/day)	6.08	6.33	6.67	6.58	0.33	0.39
Absorption (%)	87.3	88.5	90.3	89.2	2.62	0.41
K utilization						
Intake (g/day)	109.8	112.8	116.2	116.4	6.58	0.33
Voided in faeces (g/day)	18.2	17.5	15.9	17.1	1.47	0.99
Absorption (g/day)	91.5	95.3	100.30	99.3	4.80	0.58
Absorption (%)	83.3	84.5	86.2	85.3	2.61	0.98
Mg utilization						
Intake (g/day)	7.35	7.36	7.13	6.94	0.42	1.00
Voided in faeces (g/day)	5.30	5.26	4.99	4.91	0.28	0.57
Absorption (g/day)	2.05	2.10	2.15	2.03	0.13	0.66
Absorption (%)	27.8	28.5	30.09	29.3	2.48	0.84
Cu utilization						
Intake (g/day)	6.50^a	16.9^b	6.68^a	16.8^b	1.07	<0.001
Voided in faeces (g/day)	5.88^a	13.5^b	5.99^a	13.7^b	0.29	0.03
Absorption (g/day)	0.62^a	3.41^b	0.69^a	3.10^b	0.25	0.02
Absorption (%)	9.48^a	20.17^b	10.3^a	18.3^b	0.73	0.02
Zn utilization						
Intake (g/day)	22.0^a	20.8^a	53.3^b	54.0^b	1.22	0.01
Voided in faeces (g/day)	17.5^a	16.3^a	34.8^b	35.7^b	1.3	0.05
Absorption (g/day)	4.51^a	4.43^a	18.5^b	18.3^b	1.03	0.04
Absorption (%)	20.4^a	21.3^a	34.7^b	33.9^b	1.09	0.03
Fe utilization						
Intake (g/day)	206.3	212.0	218.4	218.7	2.97	0.49
Voided in faeces (g/day)	147.5	150.5	150.6	152.9	1.10	1.00
Absorption (g/day)	58.8	61.4	67.8	65.8	2.06	0.89
Absorption (%)	28.5	28.9	31.05	30.1	0.57	0.53
Mn utilization						
Intake (g/day)	36.8	38.0	35.9	38.06	0.52	0.72
Voided in faeces (g/day)	35.7	36.7	34.6	36.5	0.48	0.35
Absorption (g/day)	1.18	1.25	1.25	1.53	0.08	0.99
Absorption (%)	3.19	3.29	3.48	4.02	0.19	0.48
Cr utilization						
Intake (g/day)	0.23	0.23	0.22	0.21	0.004	1.00
Voided in faeces (g/day)	0.22	0.22	0.21	0.21	0.003	0.49
Absorption (g/day)	0.01	0.01	0.01	0.01	0.002	1.00
Absorption (%)	2.51	2.66	2.71	2.69	0.045	0.08

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

Similar to the findings of the present study, effect of nano Zn supplementation was observed on the absorption of other studied minerals. Mondal et al. (2007) observed no significant difference in Ca and P absorption among the calf groups with basal diet containing 32.5 mg Zn/kg DM and supplemented with a 35 mg/kg DM from $ZnSO_4$ and Zn-propionate. Garg et al. (2008) also noticed no significant difference in mineral (N, Ca and P) balance and retention among the lamb groups supplemented with Zn-methionine complexes, $ZnSO_4$ and control groups. The nano minerals have novel properties differed 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 increased Cu and Zn bioavailability in nano-Cu supplemented groups might be due to these properties of nano particles. Furthermore, the absence of an effect of nano Cu and nano Zn supplementation on the absorption of the other studied minerals is indicative of no interaction among nano Cu and nano Zn with dietary minerals.

CONCLUSION

The findings of the present study revealed that supplementation of either nano Cu or nano Zn alone or in combination did not exert any adverse effect on growth performance, nutrient utilization and absorption of most of the minerals except Cu and Zn. Absorption of Cu and Zn was higher in the respective nano Cu, nano Zn and combination groups.

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