



# Effect of nano zinc supplementation on serum biochemical and mineral levels in Malabari kids fed on complete rations with varying energy levels<sup>#</sup>

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## Abstract

A study was conducted to assess the effect of dietary supplementation of nano zinc oxide (Zn) on mineral retention and serum mineral levels of Malabari kids. Fifteen Malabari kids of 2-3 months of age were randomly allotted to three dietary treatments with five replicates in each. The dietary treatments were: T1 (Control)- Complete feed containing CP- 14%, TDN- 70%, T2- Complete feed containing CP-14%, TDN- 65% with supplementation of 20 ppm nano zinc, T3 – Complete feed containing CP- 14%, TDN- 60% with supplementation of 20 ppm nano zinc for a period of 90 days. Blood was collected subjected to assess serum mineral status and serum biochemical analysis at end of trial. Serum zinc levels were significantly higher ( $p<0.05$ ) in group T2 ( $1.65\pm0.23$  mg/L), T3 ( $1.77\pm0.31$  mg/L) compared to T1 ( $0.82\pm0.07$  mg/L) group. Supplementation of nano zinc had no effect on mineral levels of calcium, phosphorous, copper and manganese. Supplementation of nano zinc at 20 ppm had no effect ( $P>0.05$ ) on haemoglobin (g/dL), serum glucose, total protein, globulin and serum enzymes like ALT (IU/L), AST (IU/L), ALP (IU/L) and creatinine (mg/dL) levels. The study concluded that nano zinc supplementation to complete ration having lower energy (60 % TDN or 65 % TDN) in kids did not alter the serum calcium, phosphorous, copper, manganese and serum biochemical parameters while significantly ( $p<0.05$ ) higher serum zinc levels was observed showing improved bioavailability.

**Key words:** Nanozinc, Malabari goats, Mineral status, Biochemical parameters

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Zinc is the second most prevalent trace element in the body and is essential for normal physical, physiological and disease-free status of an animal (Swain *et al.*, 2016). Zinc is also an important co-factor for more than 200 zinc- metalloenzymes like alcohol dehydrogenase, carbonic anhydrase, glutamic dehydrogenase, carboxypeptidase, glyceraldehyde-3-phosphate dehydrogenase, alkaline phosphatase, lactic dehydrogenase and many others enzymes (Riordan, 1976) which have a vital role in carbohydrate, protein and lipid metabolism. Several studies have shown that zinc supplementation in the feed has increased nutrient digestion because of improved secretion and activation of digestive enzymes (Hu *et al.*, 2012; Brugger and Windisch, 2016; Lee *et al.*, 2021). It also helps in improving osteoblastic activity, calcium deposition, cell division, bone growth and proper functioning of endocrine system and hormonal regulation.

The prevalence of zinc deficiency among livestock is twenty-five per cent (Goklaney *et al.*, 2019) in India. Poor bioavailability, interaction with minerals and poor storage makes it more critical and a continuous supply through feed is required. Zinc absorption is also affected by excess calcium (Adham *et al.*, 1980), copper (Prasad, 1993) or cadmium (Ahokas *et al.*, 1980).

Through nanotechnology, the particle size could be reduced and their quantum properties could be changed and hence converting zinc to nano form improves its bio availability (Li *et al.*, 2016; Swain *et al.*, 2019; Kumar *et al.*, 2020). Objective of this study was to assess the zinc status in Malabari kids fed on complete ration having different energy levels on supplementation with nano zinc. The aim was also to evaluate the effect of prolonged supplementation of nano zinc on mineral status, blood biochemistry and serum enzyme levels in growing Malabari kids.

## Material and Methods

### Experimental animals

Fifteen Malabari weaned kids of 2-3 months of age were procured from University Goat and Sheep Farm, Mannuthy. They were dewormed and maintained at standard

condition in an experimental shed for a period of 90 days. The kids were randomly allotted into three groups of five animals each as uniformly as possible with regard to age, sex and body weight.

### Experimental treatments

The kids in all three experimental groups were fed *ad libitum* with iso-nitrogenous complete feed with varying energy levels. The dietary composition and chemical composition of three complete ration are given in Table 1 and 2 respectively. Three experimental rations were

#### Treatment I /

Control: - Complete feed with 14% CP and 70% TDN

Treatment II: - Complete feed with 14% CP and 65% TDN+ 20 ppm nano zinc supplementation.

Treatment III: - Complete feed with 14% CP 60% TDN + 20 ppm nano zinc supplementation.

Blood was collected at the end of experiment period and analyzed for haemoglobin (Cyanmethaemoglobin method), blood glucose (GOD-POD Method), serum total protein (Biurette method), serum albumin (Bromo cresol green method), serum creatinine (modified Jaffe's method) and serum enzymes like aspartate aminotransferase (AST), serum alanine aminotransferase (ALT) (Modified IFCC method) and creatinine by using test kits.

Serum calcium concentration (Modified Arsenazo III method) and serum phosphorous level (Phosphomolybdate method) were estimated with semi- automatic blood analyzer. Micro minerals like zinc, copper, manganese in serum were estimated by atomic absorption spectrophotometer (Perkin Elmer pinAAcle 500).

### Statistical analysis

The experimental data were analysed using one-way ANOVA (SPSS 24.0) and means were compared using Duncan's multiple range test, by adopting standard statistical procedures (Snedecor and Cochran, 1994).

**Table 1.** Dietary composition of three different experimental rations ( per cent )

Ingredients	Treatment 1 Control	Treatment 2	Treatment 3
Maize	35	23	10
Corn gluten fibre	20	8	2
Coconut oil cake	8	7	2
Rice polish	7	10	5
Black gram husk	8	20	36
De-oiled rice barn	15	19	28
Alpha hay	4	10	14
Calcite	1.5	1.5	1.5
Salt	1	1	1
Mineral mixture	0.5	0.5	0.5
Total	100	100	100

Vitamin AD2EK was supplemented @ 20g/ 100 kg.

T2 and T3 supplemented with 20 ppm of nano zinc oxide.

**Table 2.** Chemical composition of complete feed offered to experimental kids ( % ) on dry matter basis

Parameters	T1	T2	T3
Dry matter	93.17	92.47	91.58
Crude protein	14.18	13.98	14.15
Total digestible nutrient (calculated)	69.56	64.52	61.28
Calcium (%)	1.01	1.08	1.1
Phosphorous (%)	0.72	0.68	0.66
Zinc, mg/kg	46.38	64.29	61.86
Copper, mg/kg	22.26	23.42	24.04
Manganese, mg/kg	45.66	56.82	63.76

## Results and discussion

### Haemoglobin

Haemoglobin values of kids at the end of the experiment were  $9.20 \pm 0.49$ ,  $8.89 \pm 0.15$  and  $9.15 \pm 0.19$  g/dL respectively and is shown in Table 3. The values were in normal physiological range (Kaneko *et al.*, 2008). Similar observations were also reported by Sethy *et al.* (2016) and Swain *et al.* (2019) who observed no significant effect on haemoglobin levels with zinc supplementation either inorganic or organic or nano form. However, Sobhanirad *et al.* (2014) reported supplementation of zinc had significantly increased the haemoglobin concentration in Baluchi goats. Chavan *et al.* (2016) and Ulutas *et al.* (2020) suggested that, increased haemoglobin levels with zinc supplementation could be due to its role in erythropoiesis along with iron, folate and cyanocobalamin.

### Blood Glucose

The blood glucose levels of kids estimated at end of experiment were  $59.69 \pm 2.95$ ,  $56.20 \pm 1.81$  and  $57.35 \pm 2.65$  mg/dl for the three treatments T1, T2 and T3 respectively as shown in Table 3. The values obtained were in accordance with Kaneko *et al.* (2008). Comparable values of serum glucose levels in the experimental animals could be due to insulin-like activity of zinc which helps in regulating serum glucose levels (Attia *et al.*, 2015). The values were in concurrence with findings of Anil *et al.*, (2020) who observed no difference in serum glucose levels in calves supplemented with inorganic or nano zinc (5 or 10 ppm respectively). The improved blood glucose concentration were also reported in Nubian goat (Elamin *et al.*, 2013), Black Bengal goat (Sethy *et al.*, 2016) and Osmanabadi goat (Govardhan, 2019) suggestive of its important role in carbohydrate metabolism.

### Serum biochemical profile

The serum albumin, globulin and albumin to globulin ratio were found to be normal among the treatment groups and was in concurrence with values reported by Jasmine *et al.* (2017) in growing Malabari kids and Singh *et al.* (2018) in Jalauni kids. Serum enzymes, Alanine transaminase (ALT) levels were  $18.40 \pm 0.95$  IU/L,  $19.01 \pm 0.54$  IU/L and  $17.65 \pm 0.47$  IU/L and Aspartate aminotransferase (AST) levels were  $79.30 \pm 1.54$ ,  $82.14 \pm 3.11$  and  $75.98 \pm 1.59$  IU/L in T1, T2 and T3, respectively and creatinine levels were  $0.45 \pm 0.07$ ,  $0.61 \pm 0.03$  and  $0.51 \pm 0.08$  IU/L in T1, T2 and T3, respectively. The ALT, AST and creatinine levels were in normal range and did not differ significantly ( $p > 0.05$ ) among treatment groups showing that long term supplementation of nano zinc had no significant effect on serum biochemical parameters of Malabari kids. Similar reports were also observed by Dhruw (2017), Swain *et al.* (2019) and Govardhan (2019) showing the supplementation of nano zinc at various levels in goats had no significance effect on serum enzymes such as serum ALT, AST and creatinine levels. In contrary, supplementation

of nano zinc had increased AST levels in rats (Jung *et al.*, 2010; Sharma *et al.*, 2012), rabbits (Ismail and El-Araby, 2017) and lambs (Najafzadeh *et al.*, 2013) and lowered AST in Mehraban lambs (Alimohamady *et al.*, 2018). Studies also showed a higher ALT, ALP and creatinine levels in rats (Jung *et al.*, 2010; Sharma *et al.*, 2012), and in lamb (Najafzadeh *et al.*, 2013) with higher levels of nano zinc supplementation.

### Serum mineral profile

Serum calcium, phosphorous, copper and manganese were found to be similar in all groups and are shown in Table 4 and Fig. 1 and Fig 2. Serum zinc levels in T1, T2 and T3 were  $0.826 \pm 0.07$ ,  $1.651 \pm 0.23$  and  $1.736 \pm 0.31$  ppm respectively found to be significantly ( $p < 0.05$ ) higher in nano zinc supplemented groups (T2 and T3) over T1. Serum calcium levels in T1 ( $9.34 \pm 0.18$ ), T2 ( $9.84 \pm 0.25$ ) and T3 ( $10.06 \pm 0.21$ ) and Phosphorous level in T1 ( $5.82 \pm 0.21$ ), T2 ( $5.97 \pm 0.45$ ) and T3 ( $5.45 \pm 0.48$ ) did not differ significantly among treatments and were similar to values reported by Jasmine *et al.*, (2017) and Roshma *et al.* (2020) for

**Table 3.** Haematological and serum biochemical parameters of the experimental animals maintained on three experimental rations at end of the trial

Parameters	T1	T2	T3	P value	F value
Haemoglobin, g/dl	$9.20 \pm 0.49$	$8.89 \pm 0.15$	$9.15 \pm 0.19$	0.794 <sup>ns</sup>	0.234
Glucose, mg/dl	$59.69 \pm 2.95$	$56.20 \pm 1.81$	$57.35 \pm 2.65$	0.60 <sup>ns</sup>	0.53
Total protein, g/dl	$7.04 \pm 0.16$	$7.22 \pm 0.13$	$7.44 \pm 0.11$	0.187 <sup>ns</sup>	1.04
Albumin, g/dl	$4.85 \pm 0.10$	$4.92 \pm 0.07$	$4.7 \pm 0.11$	0.303 <sup>ns</sup>	0.54
Globulin, g/dl	$2.18 \pm 0.16$	$2.30 \pm 0.18$	$2.74 \pm 0.23$	0.137 <sup>ns</sup>	2.296
Albumin/Globulin	$2.29 \pm 0.19$	$2.2 \pm 0.17$	$1.76 \pm 0.16$	0.133 <sup>ns</sup>	2.337
AST (IU/L)	$79.30 \pm 1.54$	$82.14 \pm 3.11$	$75.98 \pm 1.59$	0.332 <sup>ns</sup>	0.123
ALT (IU/L)	$18.40 \pm 0.95$	$19.01 \pm 0.54$	$17.65 \pm 0.47$	0.545 <sup>ns</sup>	0.633
Creatinine (IU/L)	$0.45 \pm 0.079$	$0.61 \pm 0.037$	$0.51 \pm 0.085$	0.241 <sup>ns</sup>	1.578

**Table 4.** Serum calcium, phosphorous, zinc, copper and manganese levels of the experimental animals maintained in three experimental rations

	Ca (mg/dL)	P (mg/dL)	Zn (ppm)	Cu (ppm)	Mn (ppm)
T1	$9.34 \pm 0.18$	$5.82 \pm 0.21$	$0.826^a \pm 0.07$	$0.69 \pm 0.08$	$1.40 \pm 0.04$
T2	$9.84 \pm 0.25$	$5.97 \pm 0.45$	$1.651^b \pm 0.23$	$0.721 \pm 0.05$	$1.44 \pm 0.04$
T3	$10.06 \pm 0.21$	$5.45 \pm 0.48$	$1.736^b \pm 0.31$	$0.760 \pm 0.09$	$1.48 \pm 0.04$
P value	0.105 <sup>ns</sup>	0.657 <sup>ns</sup>	0.016 <sup>**</sup>	0.41 <sup>ns</sup>	0.415 <sup>ns</sup>
F value	2.665	0.433	5.591	0.176	0.937

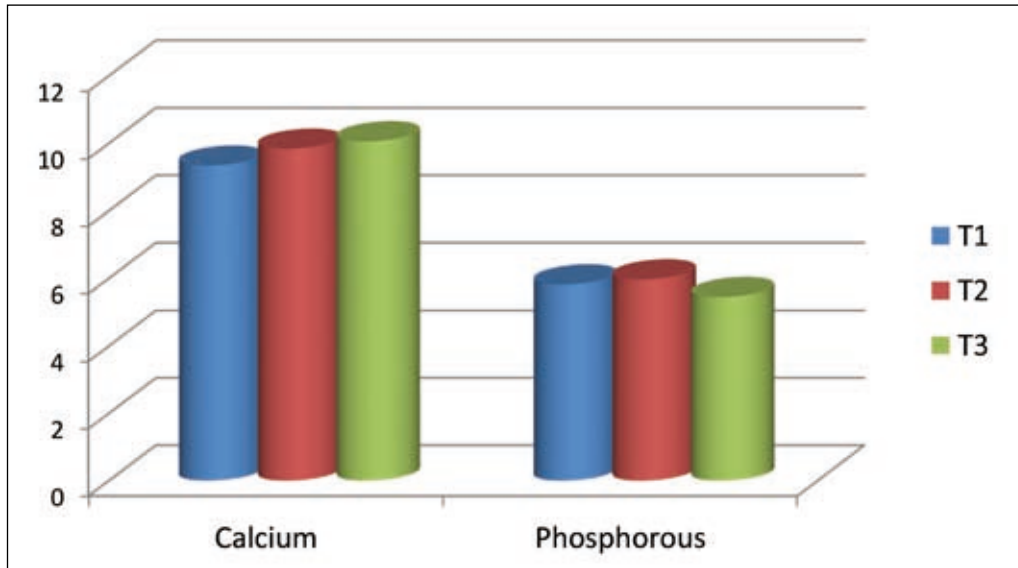
a,b- Means with different superscripts within the same column differ significantly\* ( $p < 0.05$ )

ns- non significant,  $p > 0.05$

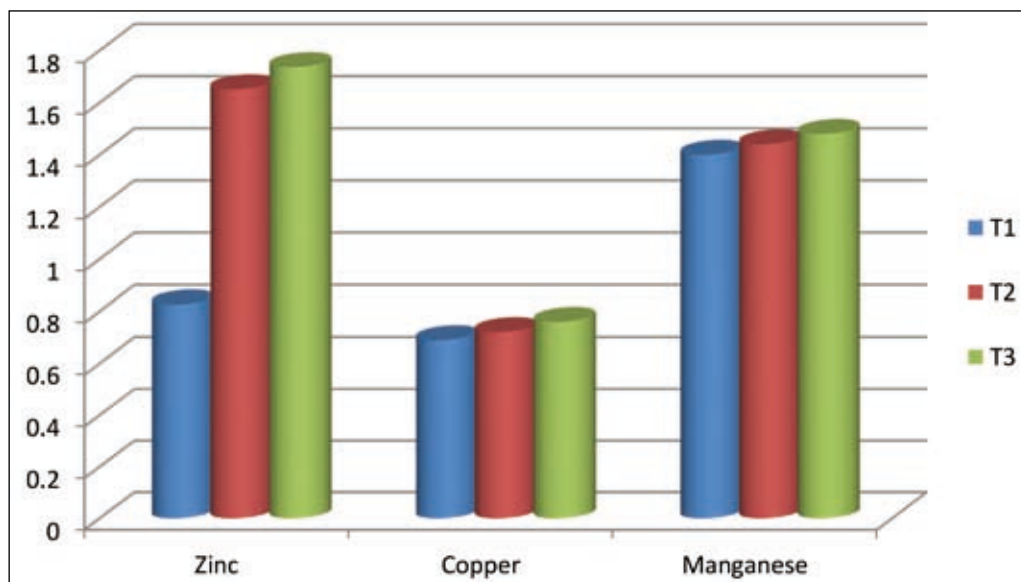
Malabari kids. Serum copper and manganese ranged from 0.69 to 0.78 ppm and 1.44 to 1.48 ppm respectively and values were found to be within normal physiological range in growing Malabari kids. Lower copper levels in serum (Jia *et al.*, 2009) and plasma (Salama *et al.*, 2013) in goats were reported on zinc supplementation suggestive of their interaction.

In present study, serum zinc levels were

higher ( $p < 0.05$ ) in nano zinc supplemented groups (T2 and T3) compared to T1 could be due to higher zinc retention and increased serum zinc levels did not affect the serum mineral status of calcium, phosphorous, copper and manganese. Similarly, reports on supplementation of inorganic zinc to buffalo (Jadhav *et al.*, 2005), organic zinc to calves (Mandal *et al.*, 2007), Muzzafanagari lambs (Garg *et al.*, 2008) and Mehraban lambs (Aliarabi *et al.*, 2015) and



**Fig. 1.** Concentration of serum calcium and phosphorous at the end of kids maintained on three experimental rations, mg/ dl



**Fig. 2.** Concentration of serum zinc, copper and manganese at end of kids maintained on three experimental ration, ppm

nano zinc supplementation in non-descriptive goats (Dhruw, 2017) and Osmanabadi goats (Govardhan, 2019) were observed to have improved serum zinc levels without affecting serum mineral status of calcium, phosphorous, copper or iron levels. In contrast to our findings, serum calcium levels were increased significantly in growing calves (Khan, 1978; Bedi, 1979) and decreased in buffaloes (Daghash and Mousa, 1999) and in West African goats (Phiri *et al.*, 2009). Serum zinc levels were not improved by supplementation of inorganic zinc in Markhoz goat kids (Zaboli *et al.*, 2013) and Nubian goats (Elamin *et al.*, 2013).

## Conclusion

The results of the study showed kids maintained on complete feed with lower energy levels (65 or 60 TDN) with supplementation of nano zinc at 20 ppm were at par with unsupplemented growing kids fed on higher energy level (70 TDN) without any change in haematological and serum biochemical profile. Serum glucose levels were in normal range and comparable between treatment groups fed on different energy levels. The mineral status of all groups were similar except serum zinc levels were higher in nano zinc supplemented groups showing higher zinc bio availability. Thus, it can be concluded that nano zinc oxide at 20 ppm could be incorporated safely in complete feed having low energy (60 % TDN) to growing Malabari kids.

## Conflict of interest

The authors declare that they have no conflict of interest.

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