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Effect of Dietary Nano Zinc Oxide Supplementation on Haematological Parameters, Serum Biochemical Parameters and Hepato-Renal Bio-Markers in Crossbred Calves

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ABSTRACT

To investigate the changes in the haematological, serum biochemical parameters and hepato-renal biomarkers upon dietary supplementation of nano zinc oxide (nZnO) and its effect on growth performance in calves, 24 weaned (6 months age) crossbred calves were randomly distributed to four dietary plans (Control, Treatment1, Treatment2 and Treatment3). The Control(C) group was fed exclusively with basal diet; T1 was supplemented with 25ppm ZnSo4, T2 and T3 with 5ppm and 10ppm nZnO respectively, along with the basal diet. Zinc content in basal was 26.24ppm. The haematological parameters and few parameters viz., serum calcium, SGOT, blood urea nitrogen and creatinine varied within the normal range upon nZnO supplementation. However, the serum zinc, total protein, serum albumin and serum globulin were significantly ($P<0.01$) higher in nZnO supplemented groups. There was a significant ($P<0.05$) difference in the blood glucose levels between C and T2 on 45th day. And, the serum phosphorus level was significantly ($P<0.05$) higher in T2 compared to T1 and T3. To conclude, nZnO has significant effect on few serum biochemical parameters which supported the growth performance of calves positively with a significant ($P<0.01$) improvement in the body weight gain and average daily gain in nZnO supplemented groups.

Introduction

Proper mineral nutrition is the key to successful dairying. Mineral deficiencies lead to disease resulting in economic loss to the farmer and Zinc is no exception. Zinc is a trace mineral which caters many of the operating metabolic functions in the body, aids in animal growth, health, reproduction and production. It conjugates with DNAbinding protein to regulate gene expression participating in the nucleic acid (Hambidge *et al.,* 1986) and protein synthesis (Hidiroglou, 1980) to affect the growth rate of animals (Chesters 1997 and MacDonald 2000). It is a nutrient involved in functioning of a variety of enzymes related to glucose, protein and lipid metabolism, in hormone production and secretion. Zinc also influences immune system function (Shinde *et al.,* 2006).

Many of the signalling pathways are zinc dependent (Beattie and Kwun, 2004; Cousins *et al.,* 2006). The normal serum Zn concentration is generally between 0.7 and 1.3 g/ml and the dietary requirements of Zn for calves is about 33mg/kg (NRC, 2001). Zn is reported to be deficient in several parts of India (Alloway, 2004) in general and Andhra Pradesh in particular (Nagalakshmi *et al.,* 2009) which reflectes in the Zn deficiency in fodder. Adding to this, the soy bean meal (contains phytates) which is a common component of concentrate mixture and unintended excess levels of Calcium in general and few other elements like Fe and Cu in the diet of animals depress the zinc absorption and bioavailability. To avoid Zn deficiency, the common practice is to add conventional Zn supplements, over and above the recommended level.

But, the conventional zinc is poorly soluble and has lower bioavailability and requires regular supplementation to maintain optimal Zn levels in blood. Zinc lack storage reserves in the body and high doses of zinc causes toxicity with degenerative changes in liver, kidney and pancreas (Allen *et al.,* 1983). In the present study nano zinc has been used which has the advantage of higher bioavailability at a lower rate of inclusion (Patil *et al.,* 2009). The haemopoetic system actively responds to the exogenous substances and to the changes of concentration and exposure duration of exogenous substances (Dimitrova *et al.,* 2010). Few studies have reported that higher Zn intake is associated with severe cytopenia (Irving *et al.,* 2003). While some reported that, the dietary Zn supplementation has no influence on few or all the haematological parameters in different species of livestock and poultry (Ramulu *et al.,* 2015; Elamin *et al.,* 2013; Milani *et al.,* 2017). However, very scanty information is available on haemoto-biochemical parameters upon supplementation of nZnO especially in crossbred calves. So this work was taken up to investigate the changes in haematological and biochemical parameters upon nZnO supplementation in crossbred calves.

Materials and Methods

24 crossbred calves of 6 months age which were randomly distributed in four groups (Control, Treatment1, Treatment2 and Treatment3) of six calves in a completely randomized block design. Calves were kept in well ventilated house with concrete flooring having provision of individual feeding and watering and also provided a 15-day adaptation period where the calves were fed with basal diet. The concentrate mixture was prepared with all the minerals except Zinc. The zinc content in the basal diet was found to be 26.24ppm which was tested using inductively coupled plasma atomic emission spectroscopy (ICP-OES).

Deworming and deticking was done before the start of the experiment. The calves were assigned to different dietary treatments viz., Control(C) group where calves were fed with basal diet only, T1, T2 and T3 were supplemented with 25ppm ZnSo4 , 5ppm and 10ppm of nZnO respectively, along with the basal diet. Two blood samples were collected aseptically at fortnight intervals via a single jugular venepuncture from each calf, one sample was collected into EDTA vaccutainer for haematological analysis and the other sample into an anti-coagulant free centrifuge tube to obtain serum. The whole blood collected into EDTA vaccutainer was kept for analysis of haematological constituents (Hb, RBC, WBC, Platelets and MCV) using automatic haematology analyser. Serum samples were obtained after centrifuging whole blood at 2000 x g for 10 min; 4º C in refrigerated centrifuge and serum was collected into eppendorf tubes and stored.

Serum stored in eppendorf tubes was analysed for serum biochemical parameters and few basic hepato-renal biomarkers (Serum Ca, P, Zn, Albumin, Globulin, Total Protein, Glucose, SGOT, BUN and Creatinine) using commercially available kits of Erba Mannheim and Genx Proton Bologicals (zinc kit). The concentrations of the parameters in serum and data analysis of results were performed using spectrophotometer and ANOVA, Post-hoc tests in SPSS version (15.01) (SPSS, 2006) respectively.

Results and Discussion

Haematological parameters

The results on the effect of dietary supplementation of nZnO on Hb concentration, RBC, WBC, Platelet count and MCV in crossbred calves are represented in the Table 1. The result revealed that there was no statistically significant difference with regard to all the haematological parameters among the groups as the parameters varied within the physiological normal range.

The results are in agreement with the findings of Ramulu *et al.,* (2015) in buffalo calves and Milani *et al.,* (2017) in weanling pigs observed no significant difference in all the haematological parameters upon supplementation of conventional zinc and nZnO respectively. Similarly, Elamin *et al.,* (2013) found no effect of supplemental zinc on Hb concentration, RBC and WBC count in goat Kids. Also, Ismail and El-Araby (2017) in rabbits found no observable variation in the Hb concentration and RBC count. In contrary, Sobhanirad *et al.,* (2014) reported a statistically significant difference in all the haematological parameters of lambs among the groups upon supplementation of Zn. Also, Ismail and El-Araby (2017) found a highly significant increase in total leukocytic count in ZnO NPs supplemented rabbits groups.

Zn at higher concentrations tends to inhibit copper and iron absorption (Salama *et al.,* 2003) which are required for RBC and WBC proliferation and maturation. Here in the present study, the haematological parameters were in normal range which could be due to the level of inclusion of Zn of the study which might not have interfered with the Cu and Fe so as to affect the haemogram.

Serum glucose level

The results of effect of dietary supplementation of nZnO on serum glucose level of crossbred calves are represented in the Table 2. The statistical analysis revealed that there was no significant difference in serum glucose level among the groups except on 45th day ($P<0.05$) where the mean serum glucose level was higher in control group compared to groups supplemented with ZnO. Similar to the results of our study, the serum glucose levels in poultry birds did not differ significantly between control and zinc supplemented groups (Sahoo *et al.,* 2014; Fathi., 2016 and Idowu *et al.,* 2011). In contrary, Sethy *et al.,* (2016) and Elamin *et al.,* (2013) reported that the serum glucose concentration in goats increased significantly ($P<0.05$) between the groups supplemented with inorganic and organic zinc in diet. Similar findings were made by Mishra *et al.,* (2014) in chicks supplemented with nano zinc.

Serum mineral parameters

The results of effect of dietary supplementation of nZnO on different serum minerals are presented in the Table 3 suggesting that the calcium remained unchanged, phosphorus was significantly (P<0.05) higher in nano zinc supplemented group on 30th day of experiment and serum zinc level was significantly higher (P<0.01) in the groups supplemented with nano zinc oxide @ 10ppm.

The results of our study are in accordance with the findings of Zaboli *et al.,* (2013) in Markhoz goat kids and Sahoo *et al.,* (2014) in broiler chicks who did not observe any significant difference in serum concentration of Ca and P among the groups upon supplementation of nano zinc. Similarly, Sahoo *et al.,* (2014) and Raya *et al.,* (2016) found that nanoparticles increased serum zinc concentration significantly in broilers and rats respectively. Garg *et al.,* (2008) also reported that the supplementation of zinc had no effect on serum inorganic P levels. Further, Dresler *et al.,* (2016) in cattle calves and Ramulu *et al.,* (2015) in buffalo calves observed a significant increase in serum zinc concentration when nano zinc is supplemented and Li *et al.,* (2016) in weanling pigs observed a significant increase in serum zinc concentration when nano zinc is supplemented.

In contrary, Al-Daraji and Amen (2011) reported a significantly (P<0.05) higher level of serum calcium and phosphorus on increasing zinc levels in the diets of broilers. But, Daghash and Mousa (1999) and Thilsing and Jorgensen (2001) noticed a significant drop in serum calcium level in buffaloes and cows respectively when supplemented with conventional zinc in their diets. Abedini *et al.,* (2017) reported that plasma zinc concentration was not significantly affected by the supplementation of layer diet with different sources of zinc. Similarly, Zaboli *et al.,* (2013) and Najafzadeh *et al.,* (2013) also reported that there were no significant differences in serum concentrations of Zn between the groups supplemented with different forms to zinc in goat kids and lambs respectively. However, Phiri *et al.,* (2009) reported that supplementation of zinc oxide to goats decreased the phosphorus concentration in the blood.

Many studies indicated that there exists an antagonistic effect between zinc and calcium. However, in the present study the dietary supplemental zinc might have been not so high as to affect the levels of calcium in the serum. From the results of the present study, it is very clear that, the level of inclusion of zinc in the study has no prominent effect on serum phosphorus levels in crossbred calves. The raise in serum zinc levels of the groups supplemented with nZnO in the present study was obvious and is attributed to the higher bioavailability of nZnO when compared to the conventional zinc source (Sahoo *et al.,* 2014).

Serum protein parameters

Serum albumin

The results on the effect of dietary supplementation of nZnO on serum protein components level in crossbred calves are represented in the Table 4. Statistical analysis revealed that the mean albumin concentration in serum of all calves varied within the normal range with serum albumin being significantly (P<0.01) lower in T1 compared to C, T2 andT3 on 15th, 30th, 75th days of experiment. Similar results were documented by Ramulu *et al.,* (2015) and Sahoo *et al.,* (2014) who observed that the serum albumin was comparable among experimental groups supplemented with different sources of zinc in calves and broiler chicks respectively.

In contrary, a significant increase in serum albumin concentration in crossbred was reported in calves fed a diet containing different sources of Zinc by Mandal (2004) and Dresler *et al.,* (2016). Similar findings were reported by Nobijari *et al.,* (2012) in young Holstein bulls. In contrary, the results of Ismail and El-Araby (2017) showed that there is a highly significant decrease in albumin levels in ZnO NPs group of rabbits.

Serum globulin

The results on the effect of dietary supplementation of nZnO on serum protein components level in crossbred calves are represented in the Table 4. Statistical analysis revealed that the serum globulin levels were significantly ($P < 0.01$) higher in group supplemented with nZnO @ 10 ppm compared to other groups.

The increased globulin concentrations in serum of crossbred calves supplemented with nZnO might be attributed to the functional role of zinc in protein synthesis and this improved globulin level implies to better immune response as serum globulins playing a role in immune response (immunoglobulins or antibodies) and provide an early line of defence.

In accordance to the present study, the reports of Ramulu *et al.,* (2015) in buffalo calves and (Gaafar *et al.,* 2011) in lactating Friesian cows witnessed a significant increase in serum globulin concentration among the experimental groups supplemented with zinc.

In contrary with our results, Ismail and El-Araby (2017) showed that there is a highly significant decrease in globulin levels of Rabbits fed with ZnO NPs. On the other hand, Sahoo *et al.,* (2014) observed no significant variation in serum globulin levels ofbroiler chicks treated with different sources of zinc.

Total protein

The results on the effect of dietary supplementation of nZnO on serum protein components level in crossbred calves are represented in the Table 4. Statistical analysis revealed that there was a significantly ($P<0.01$) higher total protein levels in the serum of groups supplemented with nZnO @ 10ppm compared to the other groups and control. The improved total protein concentration in serum of crossbred calves supplemented with nZnO might be attributed to the functional role of zinc in protein synthesis as suggested by Ibs and Rink (2003).

The results of the present study are in agreement with the reports of Nobijari *et al.*, (2012) in young Holstein bulls and Dresler *et al.*, (2016) in calves wherein the zinc supplementation increased the total protein levels significantly ($P < 0.05$). In contrary, Ismail and El-Araby (2017) observed that there is a highly significant decrease in serum total protein levels in rabbits supplemented with ZnO NPs in the diet. On the other hand, the findings of Sahoo *et al.*, (2014) and Fathi (2016) in broiler chicks and Ramulu *et al.*, (2015) in buffalo calves revealed that there was no significant variation in serum total protein levels among the groups treated with different sources of zinc.

Albumin to globulin (A/G) ratio

The results on the effect of dietary supplementation of nZnO on serum protein components level in crossbred calves are represented in the Table 4. Statistical analysis revealed that the A/G ratio varied within the normal range in all the groups with A/G being significantly ($P<0.01$) low in the nZnO supplemented groups. Similarly, Mishra *et al.*, (2014) found relatively lower A/G ratio in the groups supplemented with nZnO when compared to the control group. In contrary, Bahakaim *et al.,* (2014) found that the organic source of zinc significantly improved A/G ratio in layers compared to the inorganic source. However, the results of Sahoo *et al.,* (2014) in broiler chicks and Sethy *et al.,* (2016) in goats revealed that albumin to globulin ratio in the groups treated with different sources of zinc was comparable.

Hepatic and renal health biomarkers

The results on the effect of dietary supplementation of nZnO on hepatic and renal health biomarkers in crossbred calves are represented in the Table 5. The results revealed that the levels of SGOT, BUN and Creatinine in serum of CB calves varied within the normal range. Any abnormal increase in SGOT (Aranda-Michel and Sherman., 1998), BUN and Creatinine concentration could imply hepatic and renal dysfunction. The results of the present study imply that the rate of inclusion of nZnO has no toxic effects on hepatic and renal function.

Serum glutamic oxaloacetic transaminase (SGOT)

The results of the present study show that there was no significant difference in SGOT levels between the CB calves supplemented with nZnO and without nZnO, showing that nZnO has no significant influence on the SGOT levels in CB calves. SGOT is a relatively specific indicator of acute liver cell damage and hepatic dysfunction. The results are in accordance with Najafzadeh *et al.,* (2013) and Milani *et al.,* (2017) who found that supplementation of Zn NPs had no influence on SGOT levels in lambs and pigs respectively.

Fathi (2016) and Sahoo *et al.,* (2014) reported that there was no significant variation in serum SGOT levels of broiler chicks treated with nano zinc oxide and other sources of zinc. Wang *et al.,* (2016) also reported that long term exposure of mice to doses of nano-ZnOs did not affect the serum activities of SGOT. In contrary to the results of the current study, Ismail and El-Araby (2017) reported that there was a highly significant increase in SGOT activity in ZnO NPs supplemented rabbits.

Blood urea nitrogen (BUN)

The results of the current study show that there was no significant difference in the BUN levels between the groups. Our results are in agreement with the findings of Najafzadeh *et al.,* (2013) who found that the zinc oxide nanoparticles administration in lambs had no effect on the levels of BUN in serum. Similar results were obtained by Sahoo *et al.,* (2014) in broiler chicks. In contrary, Nobijari *et al.,* (2012) and Dresler *et al.,* (2016) observed that the zinc supplementation in calves increased the blood urea nitrogen levels. However, Dresler *et al.,* (2016) documented a drastic drop in the serum urea levels towards the end of the experiment.

Serum creatinine

Statistical analysis revealed that there was no significant difference among the groups with respect to creatinine. The results of the present study are in accordance with the findings of Atakisi *et al.,* (2009) who reported that there was no significant difference in the creatinine levels among the groups upon supplementation of zinc in Japanese quails. In contrary, Najafzadeh *et al.,* (2013) observed that the serum creatinine concentration in serum was significantly increased after oral administration of nano zinc particles in lambs. Ismail and El-Araby (2017) stated that there was a highly significant increase in serum creatinine when rabbits are supplemented with zinc oxide NPs in their diets.

Table.1 Effect of nZnO supplementation on haematological parameters in crossbred calves

Parameter	Day	Control	Treatment 1	Treatment 2	Treatment 3
Hb (g/dl)	0 Day	9.4±0.30	7.91±0.16	9.66±0.80	9.26±0.80
	90th Day	10.07±0.26	9.2±0.06	9.7±0.21	10.12±0.37
RBC ($x10^6$ /uL)	0 Day	9.36±0.44	9.3±0.40	9.61±0.77	9.21±0.83
	90th Day	9.54±0.46	9.58±0.22	9.50±0.37	9.74±0.27
WBC ($x10^3$/uL)	0 Day	10.93±0.71	11.18±0.38	11.42±0.38	11.25±1.20
	90th Day	11.32±0.48	11.27±1.1	11.58±0.43	11.33±0.81
Platelets (x 10^3 /uL)	0 Day	533.5±27.8	501.20±21.9	514.70±31.57	507.20±18.54
	90th Day	557.3±16.15	563.8±21.54	578.30±12.77	577.5±17.34
MCV (fL)	0 Day	34.02±0.65	32.67±1.20	31.82±1.50	33.32±1.06
	90th Day	36.33±0.67	32.65±0.78	33.63±1.50	34.02±1.11

Means with different superscripts in a row differ significantly (*P<0.05), (**P<0.01)

Table.2 Effect of Nano zinc oxide supplementation on serum glucose in CB calves

Glucose(mg/dL)	Control	Treatment 1	Treatment 2	Treatment 3
0 Day	72.69±2.89	83.70±1.28	76.28±3.04	89.00±8.67
45th Day*	83.77±1.55[a]	78.68±4.10[a]	60.59±9.05[b]	82.69±9.53[a]
90th Day	81.10±3.99	76.59±3.79	81.24±2.33	71.56±8.76

Means with different superscripts in a row differ significantly (*P<0.05), (**P<0.01)

Table.3 Effect of nZnO supplementation on some serum minerals in crossbred calves

Parameter	Day	Control	Treatment 1	Treatment 2	Treatment 3
Calcium(mg/dL)	0 Day	10.81±0.37	10.47±0.33	10.12±0.11	9.83±0.15
	90th Day	10.44±0.46	10.46±0.39	9.80±0.10	10.07±0.15
Phosphorus(mg/dL)	0 Day	7.50±0.41	7.23±0.20	8.22±0.41	7.25±0.35
	90th Day	8.02±0.19	7.75±0.07	7.94±0.08	7.82±0.06
Zinc(ug/dL)	0 Day	60.28±2.55	61.43±1.92	63.57±2.19	67.35±1.08
	90th Day**	86.41±0.93[d]	91.27±0.97[c]	94.83±0.59[b]	98.34±0.72[a]

Means with different superscripts in a row differ significantly (*P<0.05), (**P<0.01)

Table.4 Effect of nZnO supplementation on serum protein components in crossbred calves

Parameter	Day	Control	Treatment 1	Treatment 2	Treatment 3
Albumin (g/dL)	**0th Day**	1.88±0.08	1.76±0.13	1.87±0.14	1.46±0.12
	90th Day	2.58±0.18	2.74±0.14	2.48±0.14	2.63±0.21
Globulin (g/dL)	**0 Day**	2.59±0.10	3.02±0.06	2.60±0.18	3.235±0.21
	90th Day*	3.48±0.24^{b}	4.33±0.15^{a}	3.92±0.15^{ab}	3.84±0.21^{ab}
Total Protein (g/dL)	**0 Day**	4.47±0.11	4.79±0.13	4.48±0.09	4.70±0.18
	90th Day*	6.06±0.29^{b}	7.07±0.18^{ab}	6.40±0.21^{ab}	6.47±0.17^{a}
A/G Ratio	**0 Day**	0.73±0.05	0.58±0.04	0.75±0.10	0.47±0.65
	90th Day	0.61±0.08	0.63±0.04	0.63±0.04	0.70±0.82

Means with different superscripts in a row differ significantly (*P<0.05), (**P<0.01)

Table.5 Effect of nZnO supplementation on hepato-renal biomarkers in crossbred calves

Parameter	Day	Control	Treatment 1	Treatment 2	Treatment 3
SGOT (IU/L)	**0 Day**	53.00±2.47	52.73±1.98	55.82±2.3	54.21±1.96
	90th Day	57.83±1.84	51.99±1.73	56.23±2.60	55.63±1.89
BUN (mg/dL)	**0 Day**	13.46±0.52	13.80±0.65	13.86±0.67	14.15±041
	90th Day	14.23±0.76	13.71±0.79	14.41±0.76	13.71±0.79
Creatinine (mg/dL)	**0 Day**	1.10±0.18	0.90±0.17	0.92±0.25	1.03±0.22
	90th Day	0.77±0.22	1.19±0.28	1.04±0.22	0.99±0.24

Means with different superscripts in a row differ significantly (*P<0.05), (**P<0.01)

The inclusion of nZnO has significant effect on some serum biochemical parameters which support the growth performance of calves positively.

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