

Original Research Article

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Effect of Nanozinc Supplementation on Haematological and Blood Biochemical Profiles in Goats

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A B S T R A C T

A study was conducted to evaluate the efficiency of nano zinc (NZn) as feed supplementation on haematological and blood biochemical profiles in goats (*Capra hircus*). NZn was synthesized by from 0.45 M aqueous solution of zinc nitrate [Zn(NO₃)₂.6H₂O] and 0.9 M aqueous solution of sodium hydroxide (NaOH). The particle size thus obtained was 74 nm and later it was confirmed to be zinc by using TEM-EDAX. Twenty four male goats were divided into 4 groups on the basis of body weight and were supplemented with either basal diet i.e. Concentrate mixture and finger millet (*Eleusine corocana*) straw @ 50: 50 ratio (BD) which was considered as Negative control (NC), BD with 50 mg/kg zinc from inorganic ZnO (IZn-50), BD with 50 mg/kg zinc from NZnO (NZn-50) or BD with 25 mg/kg zinc from NZnO (NZn-25) for about 4 months. Supplementation of zinc from either inorganic or nano Zn had no effect (P>0.05) on RBC (10⁶ / μl), WBC(10³ / μl), PCV (%), neutrophil (%), lymphocytes (%), eosinophil (%), monocyte (%), haemoglobin (g/dL), ALT (IU/L), AST (IU/L), ALP (IU/L) and creatinine (mg/dL) levels of goat blood. However, globulin (g/dL) and total protein (g/dL) varied significantly among the treatment groups (P<0.01) without affecting blood albumin (g/dL) and A/G ratio levels (P>0.05). The globulin level was more (P<0.01) in NZn-50 compared to both NC and IZn-50. Total protein (g/dL) was more (P<0.001) in NZn-50 which varied significantly with NZn-25 and NC, but non-significantly with IZn-50 (6.87±0.01). Hence, zinc supplementation in form of nano zinc improved globulin and total protein significantly without affecting other haematological and blood biochemical parameters in goats, which may be attributed to its better bioavailability than its inorganic counterpart.

Keywords

Blood biochemistry;
Goats;
Haematology; Nano
zinc; Zinc Oxide.

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Introduction

Zinc (Zn) is the second most abundant trace element in the animal body, but it can't be stored (Zalewski *et al.*, 2005), so regular

dietary intake is necessary to meet the normal physiology of the animals. Zinc, as a component of multiple enzymes of the animal, plays a pivotal role in the animal physiology (Swain *et al.*, 2016). Rats and humans are

susceptible to even marginal Zn deficiency which reduces immune responses (Fraker *et al.*, 1984). Someya *et al.*, (2007) observed that dietary zinc deficiency increased the number of basophils, eosinophils and neutrophils and decreased the number of lymphocytes, suggesting the change in white blood cell distribution. Miller *et al.*, (1965) reported that, serum ALP decreases in Zn deficiency which is used as an indicator of animal Zn status.

The Zn can be supplemented through feed, either from inorganic, organic or nano source. The Zn of nanometer dimension is called as nanoZn (NZn). At this scale the physical, chemical and biological properties of material differ fundamentally and often unexpectedly. The applications of nano materials in agriculture and animal husbandry are very important as Indian economy predominantly depends on agriculture (Sri Sindhura *et al.*, 2014). These NP are having higher potential than their conventional sources and thus reduce the quantity required (Sri Sindhura *et al.*, 2014). Zinc Oxide (ZnO) NP can efficiently be synthesized by using any of physical, chemical or biological methods (Swain *et al.*, 2015) which are cheap and easy (Swain *et al.*, 2016). Swain *et al.*, (2018a) reported that supplementation of NZn affects rumen fermentation in goats without affecting rumen VFA profile, rumen soluble Zn content in goats. The experimental results pertaining to haematological and biochemical profiles of goats receiving two levels of Nano Zinc (25 and 50 ppm) compared to Inorganic Zinc (50 ppm) and no added zinc (NC) were discussed in this research paper.

Materials and Methods

Synthesis and characterization of NZn particle

The nano zinc (NZn) particles were synthesized and characterized (Swain *et al.*,

2018a,b) at Department of Nano Science and Technology, TNAU, Coimbatore. The particle size was found to be 74 nm by XRD.

Animal management

Twenty four non-descriptive local breed goats (18.7 ± 0.33 kg) were divided into four groups of six animals and maintained under uniform management conditions throughout the experimental period. Goats were housed in a well ventilated with individual feeding and watering facilities. All the goats were dewormed and vaccinated against enterotoxemia and *peste des petites ruminants* (PPR).

Animals were fed with a concentrate mixture [having ingredient composition of Maize (*Zea mays*), 40 %; soybean (*Glycine max*) meal, 35 %; rice (*Oryza sativa*) bran, 22 %; mineral Mixture, 2 % and salt, 1%] and finger millet straw (*Eleusine corocana*) at 50:50 ratio as per ICAR (2013). All the animals were fed at 3% of their body weight throughout the experiment period, which was adjusted every fortnight. A mineral mixture was prepared as per the ICAR (2013) recommendations except that of the zinc. All the animals under different treatment group were provided with the same basal diet comprising of concentrate and straw at 50:50 ratio, quantified as per their body weight, only variable being the source and quantity of zinc which was fed orally as a paper capsule (cellulose paper, 75 GSM), daily.

Collection and processing of the samples

The blood was collected by jugular vein puncture before feeding on 90th day of experimental feeding and 2 mL was transferred to a heparinised vacutainer tubes and 5 mL was transferred into a 10 mL vacutainer tube for separation of serum to assess the haematological parameters. Then

the blood samples kept for serum collection were kept undisturbed for 2 h to facilitate clotting, and then centrifuged at 3000 rpm at 4°C for 20 min. A clear supernatant (sera) was separated and stored in deep freeze (-20°C) for blood biochemical analyses.

Estimation of haematology and blood biochemical profiles

Heparinised blood samples were analysed for its haematological parameters by using auto-analyser (Erba chem 5 plus, Germany).

The serum samples collected after experimental feeding were analysed to determine the different blood biochemical constituents like ALP, blood urea nitrogen were done by following the protocols of Erba diagnostic Mannheim GmbH (Germany) by using Alere (AM 2100) Micropate reader by following respective kit protocols (ERBA diagnostics Mannheim GmbH, Germany) and albumin, globulin, total protein, creatinine, AST, ALT done by using M/s. Span Diagnostics Limited, Surat, India. The serum biochemical estimations were carried out using Semiauto analyzer, Biosystems (BTS 320). Serum total protein (TP) and albumin were estimated by Biuret and BCG dye binding method (Dumas *et al.*, 1971). Globulin was calculated by subtracting serum albumin from TP and expressed as g/dl blood serum. *Albumin to globulin ratio* is mere the ratio of albumin and globulin in the blood of individual animal. *Blood urea nitrogen (BUN)* level in the serum samples were determined by following the methodology of Talke and Schubert (1965) and Tiffany *et al.*, (1972) and expressed as mg/dL. Creatinine content in the serum, expressed as mg/dL, was determined by the alkaline picrate method of Benses and Taussky (1945), where the creatinine in the protein-free solution was allowed to react with alkaline picrate to produce a red colour complex, which was subsequently measured

colorimetrically at 520 nm. *Alanine aminotransferase (ALT)* was estimated by the method described by Reitman and Frankel (1957) using diagnostic kit (manufactured by Span Diagnostic Limited, Surat, India). *Aspartate aminotransferase (AST)* in blood serum was determined as per the method given by Reitman and Frankel (1957) using diagnostic kits manufactured by Span Diagnostic Limited, Surat, India. *Alkaline phosphatase (ALP, U/L)* was estimated in the serum samples by using Wilkinson *et al.*, (1969) which is a modification of Bessey *et al.*, (1946) method.

Statistical Analysis

Data obtained on various parameters were subjected to one way analysis of variance (Snedecor and Cochran, 1994). The statistical software SPSS (SPSS Inc., Chicago, IL, USA) was used for analysis of data and analysis of variance assuming for independent constant variance structure with post-hoc Duncan to find the pair wise significance between treatments. Results were expressed as mean \pm S.E. A P-value of less than or equal to 0.05 was accepted to indicate statistical significance.

Results and Discussion

Haematological profiles

Effect of supplementation of graded doses of NZn on haematological profiles of goats is depicted in Table 1. It was observed that RBC ($10^6/\mu\text{l}$), WBC($10^3/\mu\text{l}$), PCV (%), neutrophil (%), lymphocytes (%), eisonophil (%), monocyte (%) and haemoglobin (g/dL) level in the goat blood did not differ statistically ($P>0.05$) by IZn and NZn supplementation in goats. The RBC ($10^6/\mu\text{l}$) was ranging from 17.4 ± 0.55 (NC) and 17.4 ± 0.60 (IZn-50) to 18.9 ± 0.76 NZn-50. The WBC ($10^3/\mu\text{l}$) count was 16.5 ± 1.93 , 15.0 ± 1.98 , 16.5 ± 1.34 and

13.9 \pm 2.12, respectively in NC, IZn-50, NZn-50 and NZn-25 groups. The PCV (%) was found in the range of 27.2 \pm 0.64 (NC) to 28.1 \pm 0.81 (NZn-50). The differential count of WBC was also found to be same across the treatment groups ($P>0.05$). Neutrophils (%) were found in the range of 38.0 \pm 3.49 (NZn-25) to 52.4 \pm 8.45 (NC). Proportion of lymphocytes (%) ranged from 44.2 \pm 9.00 in NC to 58.2 \pm 3.48 in NZn-25. Eosinophil (%) ranged from 1.20 \pm 0.20 in NZn-50 to 3.80 \pm 1.59 in IZn-50. Monocytes (%) ranged from 0.80 \pm 0.37 in NZn-50 to 1.40 \pm 0.24 in IZn-50. The haemoglobin (g/dL) was found to be similar among the treatment groups within a range of 8.50 \pm 0.19 in NC to 8.90 \pm 0.21 in NZn-50.

Results indicated that supplementation of zinc did not affect ($P>0.05$) the haematological profiles of the goats compared to NC. PCV (%), eosinophil (%), monocyte (%) and haemoglobin (g/dL) were found in the normal reference range given by Feldman *et al.*, (2002), whereas RBC ($10^6/\mu\text{L}$) was within the normal range in NC, IZn-50 and NZn-25, but NZn-50 showed marginally higher RBC than the reference values by Feldman *et al.*, (2002). WBC ($10^3/\mu\text{L}$) was found to be marginally higher than the reported values by Feldman *et al.*, (2002). Lymphocytes (%) in NC, IZn-50 and NZn-50 were lower than reported values of Feldman *et al.*, (2002), whereas NZn-25 was within the range. Nagalakshmi *et al.*, (2015) reported similar WBC, RBC, haemoglobin concentration, PCV, mean corpuscular volume, lymphocyte, monocyte, and granulocyte concentration among the rats fed inorganic (ZnCO_3) and organic (Zn-nic; 6, 9, and 12 ppm) sources. Kegley *et al.*, (2001) also reported similar total WBC by supplementing 360 mg Zn/d either as ZnSO_4 or Zn-amino acid complex along with either Bermuda grass hay (21 mg Zn/kg DM) or control diet (38 mg Zn/kg DM) in beef calves and heifers. Mandal and Das (2010) reported

similar haemoglobin concentration and packed cell volume (PCV) in crossbred calves after supplementing 35 mg/kg of Zn as zinc sulphate or zinc propionate to the basal diet (32.5 mg Zn/kg DM). Donmez *et al.*, (2002) also reported that supplementation of 0, 125, 500 and 1000 mg Zn per kg of drinking water in broiler chicks had no effect on erythrocyte count (RBC), hemoglobin, hematocrit, total leucocytes and differential leucocyte count (DLC), which is in accordance with the present findings in goats.

On the contrary, Sobhanirad and Naserian (2012) reported higher number of RBC, haemoglobin concentration, packed cell volume, and mean corpuscular hemoglobin concentration in the Zn-Met than control and ZnSO_4 supplemented group after supplementing 500 mg Zn/kg DM from either $\text{ZnSO}_4\cdot\text{H}_2\text{O}$ or ZnMet in Holstein cows. Akbari *et al.*, (2008) observed that addition of 60 mg Zn/kg basal diet from ZnO significantly ($P<0.05$) increased WBC and lymphocyte count with no effect on RBC count and haemoglobin in broiler chicken (21 days). It has been reported that dietary zinc deficiency increased the number of basophils, eosinophils and neutrophils and decreased the number of lymphocytes, suggesting the change in white blood cell distribution (Someya *et al.*, 2007), which was not observed in the NC which suggests that the Zinc level (17.8 ppm) in BD was sufficient for minimum requirement of the goats under trial. Haematological profiles recorded in different treatment groups were similar found to be in normal ranges.

Blood biochemical profiles

The effect of supplementation of graded doses of NZn on blood biochemical profiles of goats is shown in Table 2. ALT (IU/L) was found similar ($P>0.05$) in all the groups (16.0 \pm 3.76 in IZn-50 to 21.9 \pm 1.64 in NC). AST (IU/L) level in goats was also similar (($P>0.05$)

which varied from 197 ± 5.14 (NC) to 229 ± 17.6 (IZn-50). Similarly, ALP (IU/L) and creatinine (mg/dL) levels were also similar ($P < 0.05$) among the treatment groups. ALP was more in NZn-50 (378 ± 45.7) and minimum in NC (285 ± 61.3). Creatinine level varied from 1.10 ± 0.14 (NC) to 1.37 ± 0.14 (NZn-25) among different treatment groups.

The blood albumin (g/dL) was found to be similar ($P > 0.05$) among the treatment groups whereas, globulin (g/dL) and total protein (g/dL) varied significantly ($P < 0.01$). Albumin level varied between 3.66 ± 0.03 (NC) to 3.72 ± 0.02 (IZn-50). The globulin level was more in NZn-50 (3.20 ± 0.02) which varied significantly ($P < 0.01$) with both NC and IZn-50. The NZn-25 (3.17 ± 0.02) remained intermediate in globulin level. Similar to globulin, total protein (g/dL) was more ($P < 0.001$) in NZn-50 (6.90 ± 0.01) which varied significantly with NZn-25 (6.85 ± 0.01) and NC (6.78 ± 0.03), but non-significantly with IZn-50 (6.87 ± 0.01). Albumin: globulin ratio was similar ($P > 0.05$) in all the groups which ranged from 1.16 ± 0.01 (NZn-50 and NZn-25) to 1.18 ± 0.01 (IZn-50).

In the present study, blood enzymes such as ALT, AST and ALP (IU/L) were similar in all the groups. The values obtained in the present study were in physiological ranges suggested by Kaneko *et al.*, (2008). Results obtained in the present study are in concordance with Mandal *et al.*, (2008) in cross bred calves, Hassan *et al.*, (2011) in adult Bakri sheep and Kwiecien *et al.*, (2017) in broiler chicken with supplementation of zinc.

Contrary to the present findings obtained in the study, Spears (1989) in heifers, Jia *et al.*, (2009) in Cashmere goats, Nagalakshmi *et al.*, (2009) in Nellore lambs suggested increase in ALP whereas, Gaafer *et al.*, (2011) reported decrease of ALP due to supplementation of graded levels of zinc from organic or

inorganic sources. Serum ALP is a Zn metalloenzyme that decreases in Zn deficiency and serum ALP activity is used as an indicator of animal Zn status (Miller *et al.*, 1965), which was not observed in the present study which is an indication that the zinc level of the basal diet (NC) was not deficient enough to bring the changes in serum ALP level in the present study. Creatinine (mg/dL), total protein, albumin (g/dL) obtained in the present study are in physiological ranges suggested by Kaneko *et al.*, (2008).

There is no effect of treatment on creatinine levels obtained in the study. Total Protein and globulin (g/dL) levels were found to be more in NZn supplemented at 50 mg/kg feed group. Similar to the present study, Daghash and Mousa (1999) in buffalo calves observed increased protein levels due to zinc supplementation.

However, Nagalakshmi *et al.*, (2009) observed similar protein levels and increased globulin levels in lambs fed inorganic or organic zinc sources at 30 ppm. Huerta *et al.*, (2002) did not find any change in plasma protein and blood urea-N concentration in beef steers with zinc supplementation even at 200 ppm.

Similarly, Hassan *et al.*, (2011) in adult Bakri sheep found similar serum total protein, albumin and creatinine. Very scanty literature is available on effects of feeding NZn as feed supplement. At higher doses, serum ALT, AST and ALP contents were elevated in mice with NZnO treated groups than control (Jung *et al.*, 2010; Sharma *et al.*, 2012). The proposed mechanism may be due to the fact that, the NZn is much more active and can be rapidly transformed into respective ions in gastric juice. So large amounts of metal ions are generated and subsequently brought to liver and kidney for metabolism and excretion, which might cause damage to hepatic and renal tissues (Chen *et al.*, 2007).

Table.1 Effect of supplementation of two levels of NZn (50 and 25 mg/kg) on haematology of goats

Attributes	NC	I ^{Zn} -50	N ^{Zn} -50	N ^{Zn} -25	Reference values*	SEM	P
RBC (10⁶/ µl)	17.4 ±0.55	17.4 ±0.60	18.9 ±0.76	17.7 ±0.71	8-18	0.34	0.714
WBC(10³/ µl)	16.5 ±1.93	15.0 ±1.98	16.5 ±1.34	13.9 ±2.12	4-13	0.88	0.344
PCV (%)	27.2 ±0.64	28.0 ±0.98	28.1 ±0.81	27.6 ±0.89	22-38	0.39	0.875
Neutrophil (%)	52.4 ±8.45	48.8 ±6.41	50.0 ±5.28	38.0 ±3.49	30-48	3.11	0.391
Lymphocytes (%)	44.2 ±9.00	46.0 ±5.81	48.0 ±5.62	58.2 ±3.48	50-70	3.15	0.423
Eisonophil (%)	2.40 ±0.98	3.80 ±1.59	1.20 ±0.20	2.60 ±0.51	1-8	0.49	0.344
Monocyte (%)	1.00 ±0.00	1.40 ±0.24	0.80 ±0.37	1.20 ±0.20	0-4	0.12	0.374
Haemoglobin (g/dL)	8.50 ±0.19	8.68 ±0.28	8.90 ±0.21	8.50 ±0.11	4-13	0.10	0.493

Each value is an average of six observations. *Feldman *et al.*, (2002).

Table.2 Effect of supplementation of graded doses of NZn (50 and 25 mg/kg) on blood biochemical profiles in goats

Attributes	NC	I ^{Zn} -50	N ^{Zn} -50	N ^{Zn} -25	SEM	P
ALT (IU/L)	21.9 ±1.64	16.0 ±3.76	19.9 ±0.66	17.3 ±1.89	1.17	0.298
AST (IU/L)	197 ±5.14	229 ±17.6	207 ±20.3	198 ±6.19	7.07	0.374
ALP (IU/L)	285 ±61.3	353 ±61.9	378 ±45.7	356 ±44.9	26.1	0.657
Creatinine (mg/dL)	1.10 ±0.14	1.16 ±0.10	1.21 ±0.09	1.37 ±0.14	0.06	0.413
Albumin (g/dL)	3.66 ±0.03	3.72 ±0.02	3.69 ±0.02	3.67 ±0.02	0.01	0.207
Globulin (g/dL)	3.12 ^b ±0.02	3.14 ^b ±0.02	3.20 ^a ±0.02	3.17 ^{ab} ±0.02	0.01	0.009
Total protein (g/dL)	6.78 ^c ±0.03	6.87 ^{ab} ±0.01	6.90 ^a ±0.01	6.85 ^b ±0.01	0.01	0.000
Albumin: Globulin	1.17 ±0.01	1.18 ±0.01	1.16 ±0.01	1.16 ±0.01	0.01	0.318

^{a,b,c}Means with different superscripts in a row differs (P<0.05) significantly. Each value is an average of six observations.

Thus, results indicated that supplementation of zinc especially NZn caused improvement in total protein and globulin concentrations without affecting albumin level in goats.

Zinc supplementation in form of nano zinc improved globulin and total protein significantly without affecting other haematological parameters like RBC ($10^6/\mu\text{l}$), WBC($10^3/\mu\text{l}$), PCV (%), neutrophil (%), lymphocytes (%), eosinophil (%), monocyte (%), haemoglobin (g/dL) as well as blood biochemical parameters like ALT (IU/L), AST (IU/L), ALP (IU/L) and creatinine in goats, which may be attributed to its better bioavailability than its inorganic counterpart.

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