**Effect of nano zinc oxide supplementation on the blood profile, mineral metabolites and oxidative stress biomarkers of maradi bucks**

Abstract

This study investigated the blood profile, mineral metabolites and blood oxidative stress biomarkers of maradi bucks fed nano zinc oxide supplementation diets. A total of sixteen (16) bucks aged between 7-9 months with average weight of 9.15±1.64kg were used for this experiment. Sixteen (16) maradi bucks with similar average weight were randomly allotted after weight equalization to four treatment groups (0mg/kg nZnO, 650mg/kg nZnO, 700mg/kg nZnO, 750mg/kg nZnO respectively) in a completely randomized design. Blood was collected at the end of 84days feeding trial and data collected includes; haematological and serum biochemical indices, blood minerals and oxidative stress biomarker indices. Data collected were subjected to a one-way analysis of variance. Results revealed there were significant differences (P<0.05) in Packed Cell Volume (PCV), Haemoglobin (Hb) and Mean Corpuscular Volume (MCV) values across experimental treatment. Bucks on 0mg/kg nZnO, 650mg/kg nZnO, and 700mg/kg nZnO were significantly higher than bucks on 750mg/kg nZnO. Biochemical indices such as total protein, albumin and aspartate aminotransferase (AST) also differ across the treatment groups. Potassium and chloride values were similar across the supplementation groups while sodium and calcium differ significantly (P<0.05). The activity of the oxidative stress indicator (catalase) was greatly influenced by nano zinc oxide. The use of nZnO is environmentally friendly as it helped to reduce the levels of environmental pollution that could have happen using conventional Zinc

Keywords: oxidative stress, buck, blood mineral, haematological and serum biochemical indices

**Introduction**

Feed supplements containing trace minerals in nanoparticles have recently been used to improve production and reproduction in livestock (Egwurugwu *et al.,* 2013, Tsai *et al.*, 2016). The use of these trace minerals with nanoparticle technology has necessitated the use of nano zinc oxide. Zinc (Zn) is part of a number of metalloenzymes and transcription factors (O'Dell, 2000) that play important roles in the metabolism of nutrients in ruminants (Jia *et al.*, 2008). According to Wedekind and Baker (1990), there are essentially two sources of zinc used in the feed industry, namely zinc oxide and zinc sulphate. Nano zinc oxide (nZnO) is a new substance that has been developed and commercialized using nanotechnology. However, Song *et al*. (2010) noted that nZnO has many applications in the pigments, food and electronics sectors as well as in medicine. The limited knowledge of the toxic effects of these substances on ruminants has highlighted the need for urgent research into possible adverse effects of their use as a feed additive in livestock. Novels of nutrients and supplements are said to have increased functionality or bioavailability, thus minimizing the concentration required in the foodstuff (Weiss *et al*., 2006). The nano form of supplementation increases the surface area, which would increase mineral absorption (Desai *et al*., 1997) and therefore use, which would lead to a reduction in the amount of supplementation and ultimately a reduction in the cost of feed. However, feeding minerals with higher bioavailability not only reduces the cost of supplementation, but also reduces the removal of excess minerals from the bloodstream, thus reducing environmental pollution**Materials and methods**

All the experimental procedures including animal care, management and sampling were performed in accordance with the guidelines for livestock experiment in the ethics of keeping animals, Joseph Sarwuan Tarka University, Makurdi, Benue State (CAS/ANP/2017/2018/10). The experiment was conducted in the livestock teaching and research farm of the University. Sixteen (16) red Sokoto bucks of similar age were used for the experiment. The animals were obtained from shinge market in lafia, Nasarawa state.

Nano zinc oxide was obtained from a reputable firm in China. Other feed ingredients (maize offal, rice offal, bone ash and table salt) were bought from North Bank market within Makurdi metropolis. Four (4) experimental diets were formulated and compounded to contain 0 mg, 650 mg, 700 mg and 750 mg nano zinc oxide, designated T1, T2, T3 and T4 respectively (Table 1).

**Experimental house and animal management**

The experimental house was a high walled building with adequate windows as well as high roof for proper ventilation. The house was divided into pens and each pen was divided into individual compartments. The concrete floor was covered with wood shavings to act as litter materials as well as beddings. Each compartment was equipped with feeding troughs and drinkers.

Two weeks before the arrival of the animals, the animal house was thoroughly washed using disinfectant (Izal) and allowed to dry after which wood shavings were spread on the floor. The drinkers and feeders were properly washed and allowed to dry, and then arranged. On arrival, the animals were weighed and kept in a quarantine pen where they were kept and necessary medication were administered. Long-acting antibiotic (LA) was administered at 1ml/10 kg body weight; multivitamins was also administered at the same dosage. Ivermectin was administered at 1ml/20kg body weight for both endo and ectoparasites and randomly distributed into four treatment groups of four animals each. The concentrate diets were fed daily at the rate of 2.5% of the buck’s weight at 8:00hr and 10:00hr, daily and a period of one hour was allowed before feeding the forage *ad libitum.* Feeding of the forage was divided into two i.e. the first feeding was at about 10:00hr while the second feeding was at 14:00 hr, this was to help reduce feed wastage and encourage intake. Mineral supplements were also provided for each animal. All the experimental animals were provided with fresh clean water daily.

**Chemical analysis**:

Feed samples were ground to pass a 1-mm sieve screen using laboratory blender and were analyzed for crude protein, ether extract, and ash contents as enunciated by AOAC, (2005). Neutral detergent fibre (NDF) was analyzed by a method of Van Soest et al. (1991). Acid detergent fibre (ADF) was analysed sequentially on the same sample by a method of AOAC (2005).

**Blood analysis**

On the last day of the feeding trials blood was collected through the jugular vein for blood profile analysis. Set of bottles with EDTA was used for haematological collection while those without EDTA were used for serum and mineral analysis. Blood samples (5ml each) were collected through the jugular vein-puncture of the goats using purple top and red top vacutainer tubes for haematological and blood biochemical analysis, respectively.

The packed cell volume was measured from each ethyl diamine tetra acetic acid (EDTA) anticoagulant samples within 24hr of collection using the micro haematocrit method. Haemoglobin concentration was also measured in fresh EDTA anticoagulant samples using the Sahl’s (acid haematin) method (c, 1978). RBC was measured in fresh EDTA with the aid of Neubauer counting chamber (haemocytometer). Blood smear was for total WBC counts and WBC differential relative and absolute counts (TevaresDias *et al*., 2008). Differential relative and absolute counts were classified as lymphocyte, neutrophils, eosinophils, basophils and monocytes and were determined by Giemsa’s stain method (Coles, 1986). Mean corpuscular haemoglobin (MCH) and Mean corpuscular haemoglobin concentration (MCHC) values were calculated from PCV, Hb and RBC values (Jain, 1986).

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Total serum protein was measured in Serum for individual goat using biuret method. Serum Alanine aminotransferase and Serum Aspartate aminotransferase were analyzed spectrophotometrically by using commercially available diagnostic kits (Randox Test kits). Serum globulin was measured using bromocresol purple method of Varley *et al.* (1980). Serum total cholesterol was determined spectrophotometrically by Randox kit according to the method of Allain *et al*. (1974). Serum glucose was determined spectrophotometrically by using Randox kit following the method of Barham and Trinder (1972).

**Statistical analysis**

Data obtained were subjected to one way analysis of variance in a completely randomized design. Duncan multiple range test was used to separate significantly different means (SPSS, 23). The model for the experimental design is Yij= µ + Ti + eij

Where; Yij= Observed value;

µ =The overall mean;

Ti =Nano zinc effect;

eij =Random residual error

Table 1: Dietary composition table of experimental diets containing varying levels of nano zinc oxide (nZnO) supplementation

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Ingredients**  **Experimental diets** | | | | |
|  | T1 (0mg) | T2 (650mg) | T3 (700mg) | T4 (750mg) |
| Maize offal | 35 | 35 | 35 | 35 |
| Soybean meal | 20 | 20 | 20 | 20 |
| Rice offal | 25.5 | 25.5 | 25.5 | 25.5 |
| Palm kernel cake | 15 | 15 | 15 | 15 |
| Bone ash | 4 | 4 | 4 | 4 |
| Salt | 0.5 | 0.5 | 0.5 | 0.5 |
| Total | 100 | 100 | 100 | 100 |
| ***Determined analysis*** |  |  |  |  |
| Dry matter | 88.80 | 90.30 | 88.58 | 89.64 |
| Crude protein | 16.10 | 16.17 | 16.27 | 16.39 |
| Crude fibre | 33.32 | 22.34 | 27.09 | 25.25 |
| Ether extract | 11.50 | 12.99 | 9.03 | 9.76 |
| Ash | 8.50 | 8.63 | 8.75 | 8.88 |
| Neutral detergent fibre | 65.99 | 70.81 | 68.55 | 67.50 |
| Acid detergent fibre | 41.08 | 39.53 | 43.28 | 36.95 |

**Result and Discussion**

The result for the haematological indices of the experimental bucks is presented in Table 2. All the parameters measured were similar across the experimental diets, except the PCV values, Hb, MCV and Eosinophil values. The PCV value for 0 mg nZnO (28.33 %) was significantly higher (P<0.05) than 750mg nZnO (25.67 %). However, there was no significant difference between PCV values for 650mg nZnO and 700mg nZnO. The PCV values were comparable with 22.00-38.00% PCV reported by Plumb (1999) but lower than 38.20-40.20% and 34.40-36.05% reported by Oloche *et al*., (2018) and Odoemalam *et* *al*. (2014) respectively. The PCV values were lower than some researchers but still within the reference range reported by Merck (2011) who reported 22-38% for goats. This could mean that the goats had enough non-iron deficient and enough oxygen carrying capacity of the blood. Hb values were within 8-12 g/dl and 7.00-15 g/dl reported Merck (2011) and Daramola *et al*. (2005) for WAD goats. Hb values was however lower than 12.72-13.36 g/dl observed by Oloche *et al*. (2014) for WAD goats fed treated orange peels. Although the bucks responded differently to the nano zinc oxide supplementation, the Hb were still within the reported reference range. This is an indication that the supplementation of nZnO does not have an adverse effect on the Hb production. Mean corpuscular volume (MCV) values of experimental bucks was similar for 0mg nZnO, 650 mg nZnO, and 700 mg nZnO respectively. However, MCV value for bucks on 700 mg nZnO (18.20fl) differs significantly from those on 750 mg nZnO (16.53fl). There was no significant difference (P>0.05) in all white blood cell differential count except eosinophils. Eosinophils values for 0 mg nZnO (2.00%) differs significantly from 700 mg nZnO (0.67 %) and 750 mg nZnO (0.00%). This result shows the animals were not infected with any disease. The result obtained were similar with Oloche *et al*. (2018) and Merck (2011).

Table 2: haematological indices of Maradi Bucks fed varying levels of nano zinc oxide supplementation

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Parameters** | **T1 (0mg)** | **T2 (650mg)** | **T3 (700mg)** | **T4 (750mg)** | **SEM** |
| Packed cell volume (%) | 28.33a | 27.33ab | 28.67a | 25.67b | 0.45 |
| Red blood cell (×1012/l) | 15.90 | 16.50 | 15.77 | 15.47 | 0.37 |
| Haemoglobin (g/dl) | 9.43a | 9.13ab | 9.57a | 8.67b | 0.15 |
| MCV (fl) | 17.73ab | 17.20ab | 18.20a | 16.53b | 0.25 |
| MCH (pg) | 59.07 | 44.00 | 60.90 | 55.67 | 3.65 |
| MCHC (g/dl) | 33.27 | 33.40 | 33.33 | 33.33 | 0.03 |
| White blood cell (×109/l) | 8.70 | 9.03 | 9.90 | 10.13 | 0.31 |
| Lymphocytes (%) | 33.27 | 33.40 | 33.33 | 33.33 | 0.03 |
| Basophil (%) | 0.00 | 0.67 | 0.67 | 0.00 | 0.19 |
| Neutrophil (%) | 29.67 | 25.33 | 29.00 | 30.33 | 1.05 |
| Eosinophil (%) | 2.00a | 1.00ab | 0.67b | 0.00b | 0.26 |

a,bMeans in the same row with different superscripts are highly significantly different (p<0.05),

MCV- mean corpuscular volume, MCH- mean corpuscular haemoglobin, MCHC- mean corpuscular haemoglobin concentration

The result for serum biochemical indices of the experimental bucks is presented in Table 3. The globulin, urea, cholesterol, alanine aminotransferase and alkaline phosphate were not significant across the experimental treatment. The total protein values were between 2.90 and 5.60 g/dl. The total protein value of 750mg nZnO (5.60 g/dl) was significantly higher (P<0.05) than that of 650 mg nZnO (2.90 g/dl). However, there was no significant difference (P>0.05) between total protein values for 0 mg nZnO (4.37 g/dl) and 700 mg nZnO (5.37 g/dl). However, Merck (2011) reported 6.4- 7.0 g/dl for goats which as higher than the current research values. Differences in values could be as a result of age of animal, the diets composition being fed. Total protein values obtained were at variance with the observed values for some researchers; such as 6.30-8.50 g/dl, 7.54-8.24g/dl and 6.62-8.02 g/dl for Daramola *et al.* (2005); Oloche *et al.* (2015) and Oloche *et al*. (2018) respectively. The increase total protein could also be attributed to the bioavailability of some nutrients.

Albumin values were 2.13, 3.30, 3.33 and 3.43 g/dl for 0 mg, 650mg nZnO, 700mg nZnO and 750 mg nZnO respectively. These was comparable with Saka *et al.* (2016) who reported lower albumin values (2.63-3.53 g/dl) for healthy WAD goats. However, there were no significant difference in urea, cholesterol, creatinine, alanine amino transferase and alkaline phosphate. This is an indication that the test material was not injurious to the liver and kidney function of the bucks.

Table 3: Serum biochemical indices of Maradi bucks fed varying levels of nano zinc oxide supplementation

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Parameters** | **T1 (0mg)** | **T2 (650mg)** | **T3 (700mg)** | **T4 (750mg)** | **SEM** |
| Total protein (g/dl) | 4.37ab | 2.90b | 5.37a | 5.60a | 0.37 |
| Albumin (g/dl) | 3.33a | 2.13b | 3.30a | 3.43a | 0.17 |
| Globulin (g/dl) | 29.87 | 44.20 | 45.67 | 47.43 | 3.50 |
| Urea | 44.30 | 37.97 | 40.23 | 28.60 | 2.67 |
| Cholesterol (mg/dl) | 96.73 | 93.93 | 95.83 | 92.83 | 0.95 |
| Creatinine (mg/dl) | 0.91 | 0.89 | 0.68 | 1.04 | 0.08 |
| Aspartate aminotransferase (IU) | 72.57ab | 73.77a | 66.53ab | 61.07b | 2.12 |
| Alanine aminotransferase (IU) | 23.07 | 23.33 | 23.50 | 28.20 | 1.66 |
| Alkaline phosphate (IU) | 26.80 | 26.80 | 24.67 | 25.57 | 1.30 |

a,bMeans in the same row with different superscripts are highly significantly different (p<0.05),

The result for blood mineral metabolites and oxidative stress biomarkers of maradi bucks is presented in Table 4. There was no treatment effect on mineral metabolites and oxidative stress biomarkers such as potassium (K), chloride (Cl-), MDA and glutathione peroxidase. The potassium value shows no significant variation which is an indication that the experimental diets was able to support the normal functioning of the cells with good muscle contractions as well as the adequate electrolyte function. Potassium values were comparable with 3.5-6.7mmol reported by Mereck, (2025) for goats and slightly higher than 3.85- 4.34 mmol reported by Shittu *et al.* (2025) for pregnant red Sokoto does. The slight difference could be attributed to contraction and electrolyte activities during pregnancy.

Sodium values varied from 98.80 – 108.17 mmol across the experimental treatments. However, Sodium values for 0mg (101.30mmol), 700mg nZnO (103.03mmol), and 750mg nZnO (108.17mmol) differs significantly from 650 mg nZnO (98.80mmol). Merck, (2025) and Shittu *et al*., (2025) reported higher value (142-155mmol) for goats and 108.92-134.56 mmol for pregnant red Sokoto does. The experimental diets do not have negative effect on the mineral metabolism as they are with the reference values as reported by Merck, (2025). Calcium values differ significantly (P<0.05) across the experimental treatment, however, 0mg nZnO (9.47 mmol) differs from 650mg nZnO (7.97 mmol) and 700mg nZnO (7.97 mmol). The calcium value is in tandem with Merck, (2025) and Shittu *et al*. (2025) who reported 8.9-11mmol and 7.10-8.00mmol respectively. The relative similarities in calcium values across different experiment could suggest the importance of calcium in muscle relaxation and releasing of hormones that help the normal functions of the body as a cofactor. Catalase values varied from 88.55 – 109.30 mm/mg across various experimental treatments. Catalase as an antioxidant enzyme helps to destroy the cellular hydrogen peroxide to form oxygen and water which are less harmful to the body. Inclusion of nZnO supplementation help reduce the level of catalase in the blood which is an indication of the antioxidant properties of nZnO. Catalase value of 0mg nZnO (109.30 mm/mg) and 650mg nZnO (102.65 mm/mg) differ significantly from 700 mg nZnO and 750 mg nZnO supplementation.

Table 4: Blood mineral metabolites and oxidative stress biomarkers of maradi bucks fed varying levels of nano zinc oxide supplementation

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Parameters** | **T1 (0mg)** | **T2 (650mg)** | **T3 (700mg)** | **T4 (750mg)** | **SEM** |
| Potassium (mmol) | 5.80 | 6.00 | 4.67 | 5.80 | 0.26 |
| Sodium (mmol) | 101.30ab | 98.80b | 103.03ab | 108.17a | 1.59 |
| Chloride (mmol) | 155.00 | 102.23 | 92.33 | 83.60 | 10.19 |
| Calcium (mmol) | 9.47a | 7.97b | 7.97b | 8.33ab | 0.29 |
| Catalase (mm/mg) | 109.30a | 102.65ab | 98.80b | 88.55c | 2.96 |
| MDA (mm/l) | 7.30 | 6.55 | 7.20 | 10.50 | 0.96 |
| GPx (mg) | 93.35 | 93.25 | 94.00 | 86.95 | 1.76 |

a,bMeans in the same row with different superscripts are highly significantly different (p<0.05), MDA: malondialdehde; GPx: glutathione peroxidase; SOD: superoxide dismutase

**Conclusion and recommendation**

Based on the findings of this experiment, it can be concluded that:

Inclusion of nano zinc oxide supplementation up to 750 mg in the diets of maradi bucks did not have adverse effects on the haematological parameters, serum biochemical indices, mineral metabolites and oxidative stress biomarker. It can therefore be recommended that farmers can use nano zinc oxide up to 750 mg supplementation in the diet of maradi goat.

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