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Influence of Nano Zinc Supplementation on the Haematological Properties, Serum Biochemical Constituents, Rumen Microbes and Minerals of West African Dwarf Sheep

Research Article

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ABSTRACT

This study is set to evaluate the influence of nano zinc oxide (nZnO) supplementation on the haematological properties, serum biochemical constituents, rumen microbial assay and minerals of eighteen (18) healthy West African Dwarf sheep with average weight of 13 ± 1.52 kg. The sheep were divided into three (3) treatment groups of six (6) animals per group and were randomly allotted to three (0 mg/kg, 300 mg/kg and 600 mg/kg nZnO) experimental diets. Data collected on collected on haematological properties, serum biochemical constituents, rumen microbial assay and minerals were subjected to one way analysis of variance in a completely randomized design (CRD) and significant means were separated using Tukey's test. The results revealed that there were no significant ($P<0.05$) differences in the haematological parameters considered. However, significant ($P<0.05$) differences were observed in the blood serum cholesterol and aspartate aminotransferase levels with the lowest values observed in the groups fed nZnO supplemented diet. Nano zinc oxide supplementation had significant effect ($P<0.05$) on rumen pH, temperature, total volatile fatty acids (VFA), acetic acid, butyric acid, and propionic acid. Sheep offered 600 mg/kg of nZnO had the highest values for pH (6.70), total VFA (5.45), acetic acid (3.63), butyric acid (2.36) and propionic acid (0.55). Meanwhile, those offered 300mg/kg had the highest rumen temperature (36.87) and control had the highest value for NH_3-N (71.16). The dietary supplement had similar effect ($P>0.05$) on all minerals measured except potassium. Nano zinc oxide supplementation, however, has no deleterious effect on the haematology, serum biochemical parameters with improved rumen environment of West African Dwarf sheep.

KEY WORDS health, nano-zinc, nutrition, rumen ecology, sheep.

INTRODUCTION

Ruminants' lifespan and the health of the herd economy depend on efficient nutrients conversion. This requires trace minerals such as zinc which is essential for genetic stability, performance, metabolism and antioxidant defence (Wu, 2017), and also takes part in degradation and synthesis of proteins and nucleic acids (McDowell, 2003). Usually, zinc is fed as inorganic salts, however, increasing zinc bioavailability can help ruminants perform better (Abedini *et al.* 2018; Alimohamady *et al.* 2018). Currently, there are several applications of nano zinc oxide (nZnO) including the areas of animal nutrition (Swain *et al.* 2016). Due to their unique characteristics such as nanoscale size, high surface activity, surface to volume ration, potent adsorbing ability and excellent catalytic efficiency, nano minerals may have a high bioavailability (Abedini *et al.* 2018; Ojha *et al.* 2018). Because of the high bioavailability of trace minerals

nanoparticles, environmental pollution from mineral excretion can be reduced (Ojha *et al*. 2018).

The blood haematological and biochemical parameters is essential in evaluating the nutritional, physiological and the health status of ruminants (Al-Eissa *et al*. 2012). Among other factors, stress, nutrition, reproductive status, management, and genetics reportedly have strong effects on the biochemical and haematological profiles of ruminants (Mohammed *et al*. 2016).

One of the aims in ruminant nutrition is to reduce the release of methane from the rumen, without adverse effects on digestibility, animal health, and productivity. This requires the best supply of nutrients and supplementary minerals in diets. The increased excretion of zinc in animals treated with zinc-supplemented diets has raised environmental pollution concerns (Musharraf and Khan, 2019). Therefore, researchers try to find a source of zinc with a higher bioavailability to reduce zinc levels in food supplements for animals. Using zinc nanoparticles compared to conventional Zn sources are reportedly more efficient and less toxic (Taheri *et al*. 2017). Although, some studies reported toxic influences on nano zinc oxide on lambs (Najafzadeh *et al*. 2013), further investigations are required to understand the possible positive influence on the effects of feeding nano zinc oxide on the performance, and health of ruminants (Swain *et al*. 2016; El Sabry *et al*. 2018). It is therefore hypothesized that the health, and rumen ecology of the experimental sheep fed nano zinc supplemented diet will be improved. Hence, this study attempts to evaluate the possible effects of nZnO supplementation on the health and rumen ecology of West African Dwarf sheep.

MATERIALS AND METHODS

Experimental site

The experiment was conducted at the ruminant unit of the Directorate of University Farms, Federal University of Agriculture, Abeokuta, Ogun State, Southwestern Nigeria with latitude 7° 13′ 48N, longitude 3° 26′ 14E and 348 m above sea level. It lies within the humid lowland rain forest region with two different seasons (Google Earth 7.1 http://www.google.com/earth).

Experimental animals and management

Eighteen (18) West African Dwarf Sheep with average weight of 13 ± 1.52 kg were used for the study. The experimental animals were sourced from small farm owners within Alabata, Ogun State and housed accordingly in an open- sided pen. The sheep were fed *Megathyrsus maximus* and dried cassava peel while in quarantine for 28 days. Prior to the arrival of the experimental sheep, pens were cleaned and disinfected with Izar solution. The sheep were given prophylactic treatments consisting of intra-muscular injection of oxytetracycline (1 mL/10 kg BW) and vitamin B complex to ensure good health of the animals. Levamisole and Ivomec were administered at 1 mL/50 kg body weight to get rid of internal and external parasites, respectively.

The animals were randomly allotted to the three treatment diets with six (6) replicates per treatment group in a Completely Randomized Design. The sheep were fed twice a day i.e. experimental diets at 4% body weight in the morning and *Megathyrsus maximus*, and dried cassava peel at noon. Clean water was given *ad libitum*. After adaptation, data was collected for 90 days. The experiment commenced after approval by the ethical committee OF College of Animal Science and Livestock Production with the number COLANIM/PG/0080

Experimental diet

Nano zinc was purchased from a reputable veterinary outlet in Abeokuta. Concentrate diets was formulated with ingredients such as maize bran, wheat offal, palm kernel cake, zinc free premix, bone meal, and salt. Nano zinc oxide was added to compounded diet at the rate of 0mg/kg, 300 mg/kg, and 600 mg/kg of feed (Table 1).

Experimental design

The eighteen (18) West African Dwarf sheep was divided into three (3) treatment groups containing six (6) animals per group and randomly allocated to the three experimental diets in a Completely Randomized Design. Each sheep served as replicate.

Data collection

Blood analysis

Blood samples were collected from each animal before the commencement of the feeding trial and on the 90th day of the experiment. Approximately five millilitres (5 mL) blood sample was collected from each animal before feeding in the morning through the jugular vein using new hypodermic syringe and needles. 3 mls of the blood sample collected were released into tubes containing Ethylene Diamond Tetra Acetic Acid (EDTA) as anticoagulant for haematological analysis.

The other 2 mL blood samples were released into sterile anticoagulant-free sample bottles for serum biochemical analysis. Packed cell volume (PCV), haemoglobin concentration (HB) were determined according to the method of Jain (1986), white blood cell (WBC) and red blood cell (RBC) were determined using the Neubauer hemocytometer after appropriate dilution. Lymphocyte, neutrophils, eosinophils, basophils, and monocytes were also determined from the sample in the EDTA bottle.

Table 1 Gross composition of the experimental diets

Ingredients (%)	Nano zinc oxide level		
	0 mg/kg	300 mg/kg	600 mg/kg
Maize bran	40.00	40.00	40.00
Wheat offal	24.50	24.50	24.50
Palm kernel cake	30.00	30.00	30.00
Bone meal	5.00	5.00	5.00
Salt (NaCl)	0.50	0.50	0.50
Total	100	100	100
Determined analysis (%)			
Dry matter	92.45	93.01	92.19
Crude protein	14.6	15.48	15.75
Ash	11.2	8.3	9.05
Crude fiber	32.8	35.2	35
Ether extract	3.9	4.4	4.1
Neutral detergent fiber	68.5	64.7	73.5
Acid detergent fiber	33.4	36.8	38.9
Acid detergent lignin	15.4	17.1	18.9
Metabolizable energy (MJ/kg DM)	26.24	27.12	26.84
Calcium	0.912	1.049	1.143
Phosphorus	0.625	0.876	0.892
Zinc (g/kg)	0.046	0.343	0.645
Iron (g/kg)	0.654	0.75	0.691
Copper (g/kg)	0.007	0.007	0.008

Mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH) and mean corpuscular volume were calculated from RBC value as described by (Jain *et al*. 1993). Serum Total protein, urea nitrogen, glucose, albumin and creatinine were determined using the method of Werner *et al.* (1987). Serum globulin was calculated by subtracting the albumin value from corresponding total serum protein value.

Rumen ecology

Ruminal fluid was collected from individual sheep at the beginning and on the 90th day of the experiment using stomach tubes. The rumen fluid was immediately measured for pH using a manual pH meter (Corning Pinnacle M530, Corning Inc., Corning, NY, USA) after being strained from a representative digesta sample. Rumen fluid was filtered through 3 layers cheesecloth and subsamples was divided into two portions. The first potion was transferred into plastic bottle to which 5 mL of IM H_2SO_4 had been added to stop microbial fermentation and then centrifuged at 3000 xg for 10 min. About 25 mL of the supernatant was then collected and analysed for NH3-N according to method of AOAC (1995). Volatile fatty acids (VFA) were determined according to Erwin *et al*. (1961) using a chromatograph (Perkin Elmer™ Claurus 500) with a FFAP Elite capillary column.

Rumen minerals

The rumen minerals (Cu, Fe, Mn. Zn, Mg. Ca) were determined using an atomic absorption spectrophotometer as described by Larrauri *et al*. (1996).

Statistical analysis

Data obtained was subjected to One Way Analysis of Variance as contained in SAS (2004). Tukey's test was used to separate significantly different means (SAS, 2004).

RESULTS AND DISCUSSION

The effect of supplementation of varying levels of nZnO on haematological profiles is presented in Table 2. All the haematological parameters considered were not affected ($P>0.05$) by supplementation of nZnO across all treatment groups. Table 3 shows the effect of supplementation of varying levels of nano zinc on serum biochemical profile of West African Dwarf Sheep. Aspartate amino transferase (IU/L) level varied significantly ($P<0.05$) across all treatment groups. Highest value was recorded in sheep fed 300mg/kg nZnO supplemented feed while those fed 600mg/kg nZnO supplemented feed recorded the lowest. The blood cholesterol (mg/dL) level was higher in the control group (75.38), which varied statistically ($P<0.05$) to those grouped on 300mg/kg nZnO (48.28) and 600 mg/kg nZnO (57.05) supplementation.

Table 2 Effect of nano zinc oxide on haematological parameters of west african dwarf sheep

Parameters	Nano zinc oxide level			SEM	*
	0 mg/kg	300 mg/kg	600 mg/kg		
PCV (%)	35.00	32.00	31.75	2.64	27-45
Haemoglobin (g/dL)	11.53	11.35	10.45	1.09	9-15
RBC (x10^6/L)	16.28	16.18	15.98	0.72	9-15
WBC (x10^3/L)	10.40	9.95	10.65	0.92	4-12
Neutrophils (%)	27.50	29.00	30.25	2.57	10-48
Lymphocytes (%)	68.75	66.50	65.25	2.82	40-75
Eosinophils (%)	1.00	1.25	1.25	0.35	0-10
Basophils (%)	1.50	1.75	2.00	0.67	0-3
Monocytes (%)	1.25	1.50	1.25	0.44	0-6
MCV (fl)	21.57	20.03	19.66	1.69	28-40
MCH (pg)	7.09	7.10	6.50	0.73	8-12
MCHC (g/dL)	32.87	35.45	33.00	2.27	31-34

PVC: packed cell volume; RBC: red blood cell; WBC: white blood cell; MCV: mean corpuscular volume; MCH: mean corpuscular haemoglobin and MCHC: mean corpuscular haemoglobin concentration.
SEM: standard error of the means.
*: reference values (Underwood and Suttle, 2004).

Table 3 Effect of nano zinc oxide supplementation on serum biochemical composition of west african dwarf sheep

Parameters	Nano zinc oxide level			SEM
	0 mg/kg	300 mg/kg	600 mg/kg	
Total protein (g/dL)	5.78	5.90	5.90	0.34
Albumin (g/dL)	3.45	3.15	3.48	0.34
Globulin (g/dL)	2.33	2.75	2.43	0.36
AST (U/L)	91.75^{ab}	99.75^{a}	78.50^{b}	14.79
ALT (IU/L)	40.50	39.75	40.00	7.61
Cholesterol (mg/dL)	75.38^{a}	48.28^{b}	57.05^{b}	16.25
Glucose (mg/dL)	70.98	65.35	75.38	7.95
Uric acid (mg/dL)	1.52	2.15	1.87	0.78
SOD	0.68	0.68	0.68	0.001
MDA	5.16	3.73	4.82	2.35

AST: aspartate Aminotransferase; ALT: alanine Aminotransferase; SOD: superoxide dismutase and MDA: malondialdehyde.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 4 revealed that nZnO supplementation had significant effect (P<0.05) on rumen pH, temperature, total volatile fatty acid, acetic acid, butyric acid, and propionic acid at both onset and at the end of the experiment, however, similar (P>0.05) results were obtained for NH_3-N at the initial and final day of the experiment. Higher final rumen pH, temperature, total VFA, acetic, butyric, and propionic values (6.27-6.70, 36.22-36.87, 5.31-5.45, 3.54-3.63, 2.36-2.36, 0.53-0.55, respectively) were obtained for sheep in 300 mg/kg and 600 mg/kg on nZnO groups while the least values were observed in the control group. Nano zinc oxide supplementation has no significant (P>0.05) effect on the microbial assay of the experimental animals as shown in Table 5 except for the initial Total bacterial count with values ranging from 0.98×10^5 in animals in the control group to 1.50×10^5 in sheep fed 600mg/kg nano zinc oxide supplemented diet. All rumen minerals measured showed similar results except in potassium with higher values observed in the control and groups fed 300mg/kg diet as shown in Table 6.

The haematological values recorded were within the recommended haematology reference values (Underwood and Suttle, 2004) for healthy sheep. This could indicate that the amount of nZnO fed is adequate for physiological activities of sheep (AJCN, 2010). Similarly, Najafzadeh *et al*. (2013) who fed 20 mg nanozinc oxide per kg body weight daily for 25 days in lambs reported no change in any of the blood parameters. In contrast to this study, Belewu and Adewumi, (2021) observed higher effects on the mean values of White blood cell, neutrophil, lymphocytes and Mean Corpuscular Haemoglobin in goats fed different levels of green synthesis nZnO.

The 8.82% reduction in blood cholesterol level observed among the treated groups may result from the regulation of lipid synthesis in the liver and degradation in the blood by regulating the biosynthesis of enzymes involved in lipids production in the blood (Zhao *et al*. 2016). This agrees with Jenkins and Kramer (1992) who observed a 10% reduction in the concentration of cholesterol when 500-1000 ppm zinc was added to the milk of the calves.

Table 4 Effect of nano zinc oxide supplementation on rumen ecology of west african dwarf sheep

Parameters	Nano zinc oxide level			SEM
	0 mg/kg	300 mg/kg	600 mg/kg	
Initial pH	8.02^{ab}	8.04^{a}	7.87^{b}	0.03
Final pH	5.77^{b}	6.27^{ab}	6.70^{a}	0.17
Initial temperature	30.30^{a}	30.00^{b}	29.95^{b}	0.06
Final temperature	35.63^{b}	36.87^{a}	36.22^{ab}	0.19
Initial NH_3-N	26.70	25.85	28.31	0.68
Final NH_3-N	71.16	53.53	59.53	3.30
Initial total VFA	6.27^{ab}	5.47	6.87^{a}	0.19
Final total VFA	4.39^{b}	5.31^{ab}	5.45^{a}	0.22
Initial acetic	4.18^{ab}	3.64^{b}	4.36^{a}	0.13
Final acetic	2.93^{b}	3.54^{ab}	3.63^{a}	0.14
Initial butyric	2.79^{ab}	2.43^{b}	2.91^{a}	0.09
Final butyric	1.96^{b}	2.36^{a}	2.36^{a}	0.10
Initial propionic	0.42^{ab}	0.36^{b}	0.44^{a}	0.01
Final propionic	0.44^{b}	0.53^{ab}	0.55^{a}	0.02

VFA: volatile fatty acids.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 5 Effects of varying level of nano zinc oxide supplementation on rumen microbial analysis of west african dwarf sheep

Parameters		Nano zinc oxide level			SEM	P-value
		0 mg/kg	300 mg/kg	600 mg/kg		
Total bacteria counts ($\times 10^5$)	Initial	0.98^{b}	1.03^{ab}	1.50^{a}	0.12	0.026
	Final	1.50	0.73	1.30	0.26	0.175
Pseudomonas spp.	Initial	-	+	+		
	Final	+	-	-		
Escherichia coli	Initial	+	+	+		
	Final	-	+	+		
Klebsiella spp.	Initial	+	-	+		
	Final	+	+	+		
Streptococcus faecalis	Initial	+	+	-		
	Final	+	+	-		
Staphylococcus aureus	Initial	+	+	+		
	Final	+	-	+		

Table 6 effect of nano zinc oxide supplementation on rumen mineral of west african dwarf sheep

Parameters	Nano zinc oxide level			SEM
	0 mg/kg	300 mg/kg	600 mg/kg	
Calcium mg/L	5.84	8.4	16.66	3.44
Potassium mg/L	37.31^{a}	33.54^{a}	23.28^{b}	2.17
Iron mg/L	1.84	1.04	1.21	0.21
Copper mg/L	0.26	0.28	0.29	0.01
Zinc mg/L	0.79	0.40	0.73	0.17

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

In contrast, Malcolm-Callis *et al.* (2000) found no effect on the serum cholesterol levels of feeder cattle fed 20 ppm, 100 ppm and 200 ppm levels of zinc sulphate. Also, Dietary zinc had no effect on blood serum cholesterol level.

In ruminants, alanine aminotransferase, aspartate aminotransferase, and other serum enzymes are tested to evaluate whether the liver is damaged or diseased. A reduction in the activity of aspartate aminotransferase observed at 600 mg/kg (T3) nano zinc oxide supplemented group recorded in this study may indicate that the West African Dwarf Sheep in this group had no liver cells damage.

This suggests that there was no disruption in liver functions and metabolic activities among nano zinc supplemented groups. The result aligned with Belewu and Adewumi, (2021) who reported lower aspartate aminotransferase (AST) levels in goats fed 0.008% nano zinc oxide treated goats. Sharma *et al.* (2012) however, reported an increase in the levels of alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase in mice fed nano zinc oxide supplemented diets.

The higher final pH obtained in both control and groups fed 300 mm/kg were still within the normal pH range for

rumen microbes and environment as stated by Olson, (1997) who stated that a ruminal pH of 5.6 to 5.8 suggests a marginal or developing problem of ruminal acidosis, and a pH greater than 5.9 is considered normal. The similar NH_3-N obtained in this study agrees with the result obtained by Bateman *et al*. (2002), where he observed that there was no effect of zinc supplementation on rumen NH_3 *in vitro* or *in vivo* studies with cows. Similar to this study, Wang *et al.* (2013) found that the addition of 10 µg/mL organic Zn source with different chelation strengths to diets did not impact ruminal NH_3 concentrations *in vitro* which might be based on the balance among NH_3-N degraded from dietary protein, biosynthesis of bacterial protein, and removal via absorption and passage to the omasum. Volatile fatty acids are considered as one of the most important parameters for ensuring anaerobic fermentation. Sarker *et al.* (2018) reported a decreased *in vitro* total VFA concentration when nano zinc oxide was included in the diet which contradicts this present result where increased total VFA was observed in nano zinc oxide supplemented groups. Therefore, nano zinc oxide supplementation in this study is not high or toxic for rumen/methanogenic bacteria which availed more converted VFA (Sarker *et al*. 2018).

There was an increase in final butyric acid which is contrary to the study of France and Siddons (1993) in steers, where there was a decrease in butyrate supplemented with ZnMet and is consistent with improved microbial efficiency of energy utilization. An increase in propionic acid recorded in this study is in tandem with the study of Adegbeye *et al.* (2019) who observed that dietary nanoparticle supplementation improved the diet fermentation and increased the propionate concentration. Also, in previous studies of Arelovich *et al*. (2000), high dietary concentrations (250–1142 mg Zn per kg) of inorganic Zn also increased molar proportion of propionate.

At the onset of the experiment, difference was observed in the total microbial count which in line with the reports of Wang *et al*. (2013) in which they found that total bacterial N were higher with an addition 10 µg/mL Zn in the form of organic source compared with the control or Zn sulfate *in vitro*.

Nano zinc oxide had no influence on copper contrasting with the study of Aksoy *et al*. (2002) where 500 mg of zinc oxide was administered orally to lambs once a week for a total of 12 weeks, it was reported that copper level significantly decreased and there was no change in iron, which correlates with this study. Pechova *et al*. (2009) further reported that daily administration of 500mg zinc per goats and the addition of 1000 ppm zinc to the feed of cattle (Miller *et al*. 1989) had no effect on copper in correlation with this study where the dietary treatment had no effect copper level across all treatment groups. Dietary treatment has effects on rumen potassium. Hubbert *et al*. (1958), in their *in vitro* studies with sheep rumen microorganisms revealed that potassium but not sodium, was essential for the rumen microbial population.

CONCLUSION

The non-significantly differed haematological parameters (within the normal reference range) indicated the absence of ill health in the experimental sheep. Aspartate Aminotransferase reduction shows that the supplemented nano zinc had no negative effect on the liver and subsequently on the health of the animal. The pH values recorded were within the normal healthy range and the overall rumen microbial assay of the sheep were not negatively affected. Hence, administering nano zinc oxide up to 600mg/kg is sufficient for West African dwarf sheep.

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