EFFECT OF GREEN SYNTHESES NANO ZINC OXIDE ON PERFORMANCE CHARACTERISTICS AND HAEMATOBIOCHEMICAL PROFILE OF WEST AFRICAN DWARF GOATS

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ABSTRACT

A study was conducted to investigate the effect of green syntheses nano zinc oxide on performance characteristics, haematobiochemical profile and serum zinc levels of West African Dwarf (WAD) goats. A total of 12 apparently healthy WAD goats (5 – 6 month of age, 7.3 ± 1.15 kg body weight) were randomly assigned to T1, T2 and T3 diets for 56 days in a complete randomised designed experiment. Result showed that dry matter intake (DMI) and average daily gain (ADG) significantly increased (p<0.05) across the groups with increasing levels of nano zinc oxide. The mean values of haemoglobin, packed cell volume and red blood cell were found not to be significantly different (p>0.05) among the different groups. However, there were significant differences (p<0.05) in white blood cell counts, neutrophil, lymphocytes, mean corpuscular volume and mean corpuscular haemoglobin values among the different groups. The mean values of serum glucose also did not differ among the groups; however significant variations were noticed in total protein and blood urea nitrogen as higher value were obtained in T1 (7.25) and control group (13.17) respectively as compared with other treatments. There was no significant variation in the activities of alanine aminotransferase among the treatments, however, significant difference (p<0.05) was observed in the activity of aspartate aminotransferase as lower value was obtained in T3 (31.00) as compared with other treatments. Result obtained on Zn concentration showed that WAD goats placed on T2 and T3 diet had significantly higher (p<0.05) levels of zinc concentration compared to control diet.

Keywords: Goat, Nano-zinc, Weight gain, Blood profile, Feed intake

INTRODUCTION

Zinc (Zn) is a component of numerous metalloenzymes and transcription factors (O'Dell, 2000), which plays significant roles in the nutrients metabolism in ruminants (Jia *et al*., 2008). The two predominant sources of Zn used by the animal feed industry are ZnO and $ZnSO_4.H_2O$ (Wedekind and Baker, 1990). Nano zinc oxide (nZnO) is a new substance that has been produced and marketed using nanotechnologies. This substance has found many applications in the pigments, food and electronics industries as well as in medicine (Song *et al*., 2010). Limited knowledge of the toxic effects of these substances on ruminants highlights the need for immediate research to identify the possible adverse effects when used as a nutritional supplement in livestock and poultry feeding. Nano-sized nutrients and supplements have been claimed to have an improved functionality or bioavailability and thereby minimize the concentrations needed in the food product (Weiss *et al*., 2006). Nano form of supplementation increases the surface area that would increase mineral absorption

(Desai *et al*., 1997) and thereby its utilization leading to reduction in the quantity of its supplementation and ultimately reduction in feed cost. Feeding minerals with higher bioavailability not only reduces its cost of supplementation it also reduces the excretion of excess minerals and thereby reduces environmental pollution.

While a number of researches have investigated the effect of zinc oxide on the growth rate when used as food supplement in livestock (Kincaid *et al*., 1997; Puchala *et al*., 1999; Phiri *et al*., 2009), similar studies on nZnO are limited. Since there is limited information on the effects of nZnO when used as a dietary supplement, this study evaluates the effects of nano zinc on performance characteristics and haematobiochemical profile of WAD goats.

MATERIALS AND METHODS

Experimental Site: The experiment was conducted at the Small Ruminant Experimental Unit, of the Teaching and Research Farms, Department of Animal Production, University of Ilorin, Ilorin, Kwara State, Nigeria between March and May 2019**.**

Animal Source and Management: Goats were purchased from villages located around the university. The pen was cleaned, washed and disinfected with Morigad solution before the arrival of animals. On arrival, the goats were given prophylactic treatments, consisting of intramuscular injection of oxytetracycline and thylosine against cold stress. Animals were quarantined for a period of 1 week. The animals were housed individually in an open sided well-ventilated pen, which had slated wooden flooring to prevent the animals coming in contact with their faeces. The animals were treated against internal and external parasites with ivermectin. The goats were allowed an adaptation period of one week during which they were fed with *Panicum maximum* and formulated diet (control diet). Clean and fresh water was given to the animals *ad libitum*.

Ethics: Experiments performed comply with current laws and written consent of the Scientific Ethics Committee and National Animal Care Authority (Retnam *et al*., 2016).

Preparation of Nano Zinc Particle: Nano zinc oxide was prepared by green synthesis method using plant extracts.

Extraction of Plant Extract: Kola pod weighing 30 g was thoroughly washed in distilled water, dried and cut into fine pieces. The fine cut kola pod was added into 100 ml distilled water and boiled to 60°C – 70°C for 15 minutes. Then the resulting crude extract was filtered through Whatman Filter Paper Number 1 having pore size of 25 µm.

Synthesis of ZnO Nano Particles: 100 ml of 100 mM Zinc Sulphate heptahydrate solution was prepared and stirred at 750 rpm on a magnetic stirrer set at 60˚C for 15 minutes. 15 ml of the kola pod extract was added in a drop wise manner and a brown colour change is observed. pH was checked and adjusted to 12 by the addition of a 1 M solution of NaOH. The solution was left for two hours in same condition. The solution was incubated overnight at room temperatures which result in a change of color of the solution to pale white which is a visual confirmation of the synthesized ZnO nanoparticles. Centrifugation of the solution was done at 5000 rpm for 20 minutes (Yedurkar *et al.,* 2016). The pale-white pellet residue formed was collected and dried in an oven at 150˚C. White dried pellet was powdered and stored in sealed vial pending use (Agarwal *et al*., 2017).

Experimental Design: Twelve WAD goats of both sexes, 5 – 6 months of age, and 7.3 ± 1.15 kg body weight were randomly assigned in completely randomized designed of four treatments replicated thrice, with each replicate having one WAD goat. The treatments were: (Control) - non-nano zinc oxide + non-premix based diet, (T1) non-nano zinc oxide + premix based diet; (T2) 0.004 % nano zinc oxide based diet and (T3) 0.008 % nano zinc oxide based diet. Nano zinc was added to the premix using salt as a carrier.

Routine Management and Feeding: Each experimental animal received one of the experimental diets at *ad libitum* for a period of 56 days. The control group received the control ration (non-nano zinc oxide + non-premix based diet), T1 group received non-nano zinc oxide + premix based diet; T2 group received 0.004 % nano zinc oxide based diet and T3 received 0.008 % nano zinc oxide based diet. Clean and fresh water was also given to the animals *ad libitum.* Proximate compositions of experimental diets are presented in Table (1).

Data Collection Procedure

Performance: Individual goat body weights at the beginning of the experiment were recorded in kilograms. Body weight, weight gain for each animal were recorded weekly, while daily feed offerings and refusals were recorded prior to the morning feeding to obtain feed intake for each goat, feed efficiency ratio (FER), being total feed intake per unit weight (Babale *et al.,* 2018) was calculated. Parameters such as dry matter, zinc, calcium and potassium intake; final body weight, average daily gains were measured to account for growth. Dry matter intake (DMI) was obtained by correcting daily as-fed intakes for DM content of the experimental diet (Huzzey *et al.,* 2007). While mineral intake were calculated as the product of DMI with the amount of mineral content present in experimental diets. Average daily gains were calculated as the difference between final body weight and initial body weight divided by the number of feeding days (Mekuriaw and Asmare, 2018).

Haemato-Biochemical Analysis: 10 ml blood was collected from the jugular vein of each animal in the morning (before watering and feeding) at the end of experimental feeding. 5 ml of the blood samples were heparinized and centrifuged to obtain plasma and the other 5 ml was heparin free and centrifuged to obtain serum.. Blood parameters measured were red blood cell (RBC), white blood cell (WBC), packed cell volume (PCV), haemoglobin (Hb), mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), neutrophils, lymphocytes, total protein, glucose, blood urea nitrogen (BUN), aspartate aminotransferase (AST), alanine Aminotransferase (ALT) and serum zinc were determined according to the methods described by Baker and Silverton (1985) using Abacus Junior 30 Hematology Analyzer (Diatron, MJ PCC, Hungary). Serum total protein concentration was determined by Biuret colorimetric reaction, glucose (mg/dl) using glucose oxidase method described by Bauer *et al.* (1974) and serum zinc using Atomic Absorption Spectrophotometer (AAS Model Bulk Scientific Accuzy 211).

Statistical Analysis: The data obtained were analyzed using one-way analysis of variance (ANOVA) with Statistical Analysis System (SAS, 2000). Significant means were separated using Duncan's Multiple Range Test of the same package. Means were considered significant at $p<0.05$.

RESULTS

In the current study, dry matter intake (DMI), final body weight, average daily gain (ADG), feeding efficiency, and minerals intake of WAD goats in different groups are presented in Table 2. Statistical analysis of data revealed that DMI and ADG significantly increased ($p<0.05$) across the groups, however this increase was more prominent in T3 (346.30 and 36.01) as compared to other groups.

The haematological parameters of WAD goats in different nano zinc treatment groups are presented in Table 3. The mean values of blood Hb, PCV and RBC were not significantly different ($p>0.05$) across all groups. However, there were significant difference ($p<0.05$) in the mean values of WBC, neutrophil, lymphocytes, MCV and MCH values among the different groups.

The mean values of blood biochemistry, enzymatic parameters and serum minerals in WAD goats in different groups are presented in Table 4. The mean values of serum glucose did not differ among the groups, however significant variation ($p<0.05$) were noticed in total protein and BUN as higher value were obtained in T1 (7.25 g/dl) and control group (13.17 mg/dl) respectively.

Table 1: Composition of the basal diets and the experimental diets formulated for West African Dwarf goats supplemented nano zinc oxide

Feed ingredients	Control	Treatment 1	Treatment 2	Treatment 3
Cassava waste	55	55	55	55
Rice husk	33	33	33	33
Palm Kernel Cake	10	10	10	10
Salt	1	1	1	1
Premix	-	1	1	1
Experimental diets				
Nano zinc oxide (ppm)	-	-	40	80
Proximate Composition (% DM basis)				
Dry matter	93.16	93.16	93.16	93.16
Crude protein	14.81	14.81	14.81	14.81
Crude fibre	3.42	3.42	3.42	3.42
Ether extract	11.09	11.09	11.09	11.09
Total ash	3.65	3.65	3.65	3.65
Nitrogen free extract	52.14	52.14	52.14	52.14
Minerals (mg/L)				
Calcium (Ca)	0.77	0.91	0.61	1.930
Potassium (k)	23.60	24.90	24.70	26.40
Zinc(Zn)	0.46	0.66	0.86	0.60

Table 2: Effect of nano-zinc oxide supplementation on feed intake and growth performance of WAD goats

Parameters	Control	Treatment 1	Treatment 2	Treatment 3
Dry matter Intake	247.32 ± 73.35^{a}	329.11 ± 15.52^{b}	342.97 ± 40.09^{b}	346.30 ± 23.72^{b}
Crude protein Intake	36.63 ± 10.86^{a}	48.74 ± 2.30^{b}	50.79 ± 5.94^{b}	51.29 ± 3.51^{b}
Crude fibre Intake	8.46 ± 2.51^{a}	11.26 ± 0.53^{b}	11.73 ± 1.37^{b}	11.84 ± 0.81^{b}
Ether Extract Intake	27.43 ± 8.13^{a}	36.50 ± 1.72^{b}	38.04 ± 4.45^{b}	38.40 ± 2.63^{b}
Total ash Intake	9.03 ± 2.68^{a}	12.01 ± 0.57^{b}	12.52 ± 1.46^{b}	12.64 ± 0.87^{b}
Nitrogen free extract intake	128.95 ± 38.24^{a}	171.60 ± 8.09^{b}	178.83 ± 20.90^{b}	180.56 ± 12.37^{b}
Initial body weight (kg)	7.45 ± 0.29^{bc}	7.03 ± 0.18^{ab}	6.63 ± 0.37^{a}	7.88 ± 0.63^{c}
Final body weight (kg)	8.67 ± 0.24^{a}	8.5000 ± 0.00^{a}	$8.33 \pm .58^{a}$	9.90 ± 0.70^{b}
Average daily gain (g/day)	21.73 ± 2.56^{a}	26.33 ± 3.28^{a}	30.36 ± 8.84^{ab}	36.01 ± 6.93^{b}
Feed conversion efficiency	0.09 ± 0.04	0.07 ± 0.00	0.08 ± 0.03	0.10 ± 0.02
Zinc intake (%)	0.0001 ± 0.00001^{a}	0.0002 ± 0.00003^{b}	0.0003 ± 0.00001^{c}	0.0002 ± 0.00003^{b}
Calcium intake (%)	0.0002 ± 0.00001^{a}	0.0003 ± 0.00002^{b}	0.0002 ± 0.00005^{a}	0.0007 ± 0.00006^{c}
Potassium (%)	0.0058 ± 0.0004^{a}	0.0082 ± 0.0010^{b}	0.0085 ± 0.0006^{b}	0.0091 ± 0.0017^{b}

Means bearing different superscript in a row differ significantly. Control: non nano zinc oxide + non premix based diet, T1: non nano zinc oxide + premix based diet, T2: 0.004% nano zinc oxide based diet, T3: 0.008% nano zinc oxide based diet.

The results on the effect of zinc nanoparticle supplementation on serum enzymes showed that there was no significant variation ($p>0.05$) in the activities of ALT among the treatments, however, significant difference ($p<0.05$) was observed in the activity of AST as lower value was obtained in T3 (31.00 IU/L) as compared with other treatments. Result obtained on Zn concentration showed that WAD goats placed on T2 and T3 diets had significantly higher ($p<0.05$) levels of zinc concentration compared to the control diet.

DISCUSSION

Growth Performance and Feed Efficiency: Zinc nanoparticles showed a great potential as mineral feed supplements in animals than the conventional sources (Sindhura *et al.*, 2014). Higher dry matter intake, final body weight and

Table 3: Effect of nano-zinc oxide supplementation on haematological parameters of WAD goats

Parameter	Control	Treatment 1	Treatment 2	Treatment 3
PCV (%)	30.38 ± 7.95	28.38 ± 1.94	27.88 ± 1.59	21.88 ± 0.18
WBC (x10^3 /µL)	10.85 ± 2.71^{ab}	9.16 ± 0.65^{a}	13.86 ± 0.30^{b}	11.27 ± 0.23^{ab}
RBC (x10^{12}/L)	13.48 ± 3.56	13.86 ± 0.86	14.06 ± 0.81	10.58 ± 0.20
Hbg (g/dl)	10.44 ± 2.22	10.05 ± 0.87	9.80 ± 0.42	7.93 ± 0.05
Neutrophil (%)	34.50 ± 5.66^{ab}	35.50 ± 0.00^{b}	25.75 ± 3.18^{a}	34.00 ± 1.41^{ab}
Lymphocyte (%)	61.75 ± 3.89^{a}	60.75 ± 1.06^{a}	70.75 ± 2.47^{b}	61.00 ± 1.41^{a}
MCV (fl)	22.54 ± 0.05^{c}	20.38 ± 0.01^{b}	19.83 ± 0.01^{a}	20.68 ± 0.22^{b}
MCH (pg)	7.79 ± 0.43^{b}	7.25 ± 0.18^{ab}	6.97 ± 0.10^{a}	7.50 ± 0.19^{ab}

Means bearing different superscript in a row differ significantly at p<0.05. Control: non-nano zinc oxide + non-premix based diet, T1: non nano zinc oxide + premix based diet, T2: 0.004% nano zinc oxide based diet, T3: 0.008% nano zinc oxide based diet.

Table 4: Effect of nano-zinc oxide supplementation on blood biochemistry, enzymatic activity and serum mineral of WAD goats

Parameter	Control	Treatment 1	Treatment 2	Treatment 3
Glucose (mg/dl)	72.00 ± 23.40	68.40 ± 7.20	53.10 ± 0.90	85.50 ± 35.10
Total Protein (g/dl)	5.75 ± 0.15^{a}	7.25 ± 1.05^{b}	5.70 ± 0.10^{a}	5.35 ± 0.55^{a}
BUN (mg/dl)	13.17 ± 2.25^{b}	7.42 ± 1.82^{a}	4.34 ± 1.54^{a}	7.42 ± 1.82^{a}
AST (IU/L)	60.50 ± 28.50^{ab}	89.00 ± 30.00^{b}	73.00 ± 29.00^{ab}	31.00 ± 16.00^{a}
ALT (IU/L)	13.00 ± 7.00	9.00 ± 1.00	6.50 ± 2.50	7.00 ± 3.00
Zinc (ppm)	27.25 ± 0.78^{a}	28.55 ± 0.21^{b}	31.10 ± 0.28^{c}	29.35 ± 0.21^{b}

Means bearing different superscript in a row differ significantly. Control: non nano zinc oxide + non premix based diet, T1: non nano zinc oxide + premix based diet, T2: 0.004% nano zinc oxide based diet, T3: 0.008% nano zinc oxide based diet. BUN: Blood Urea Nitrogen, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase

average daily gain in T3 as compared to other groups indicated that supplementation of Zn nano particles as zinc oxide at 0.008% level in the diet improved the growth performance of WAD goats. This was consistent with the report of Li *et al*. (2016) who observed similar growth rate of weanling piglets supplemented with 120 mg/kg of nano-Zn, organic-Zn or ZnO in the diet. Contrary to this study, Zaboli *et al.* (2013) did not find any effect of 20 or 40 ppm nZnO on average daily gain in Iranian Angora goat kids fed control diet containing 22 ppm nano zinc. It is noteworthy that, data obtained from this study suggested that the supplementation of Zn nano particles at 0.004 and 0.008 % levels in Diets T2 and T3 had significant effect on dry matter intake and average daily gain of WAD goats suggesting a better absorption and higher bioavailability of nano-zinc, owning to their particle size and faster diffusion through villia to the cells of intestinal cavity.

Haematology: The values of blood Hb, PCV and RBC had no significant difference (p<0.05) across all groups, indicating that supplementation of Zn nano particles as zinc oxide at (0.004 and 0.008) % in T2 and T3 group diets had no significant effect on these parameters. Similar to the observations of this study, Najafzadeh *et al.* (2013) who fed 20 mg zinc nano particles per kg body weight daily for 25 days in lambs reported no significant change in any of the blood parameters except for creatinine. Further, mean values of WBC, neutrophil, lymphocytes, MCV and MCH were significantly different among the treatments. In contrast with the observations of this study Raje *et al.* (2018) observed that haematological parameters of Wister rats were not affected by source and different levels of nano Zn supplementation.

Blood Biochemistry: Comparable values of serum glucose among experimental groups of WAD goats in the present study was in concurrence with findings of Uniyal *et al.* (2017) who observed no significant difference in blood biochemical parameters of guinea pig fed 20 ppm level nano zinc from different sources. In contrast to the observations made in this study, Mohamed *et al*. (2017) indicated that

serum glucose of Ossimi ewes and their lambs fed NP-Zn was significantly increased compared with control group. The present study revealed variation in the mean values of total protein among the groups as higher value was obtained in T1 (7.25 g/dl). This result is in contrast with Mohamed *et al*. (2015) who indicated that serum total protein with NP-Zn was increased compared with control group. The blood urea nitrogen is a good indicator for renal function. If kidney function falls the BUN levels will rise. Thus, the significantly decrease of BUN level in the nano zinc oxide supplemented groups in this study suggested that there was no indications of renal dysfunction and also no sign of nano zinc oxide toxicity in WAD goats. Due probably to dosage and period of exposure of experimental animals to nano zinc oxide as reported by Swain *et al.* (2016) that the toxicity of nano zinc oxide is associated with dose and duration of exposure to the nano particles.

Serum Enzyme Profile: The blood biochemical tests are frequently used in diagnosis diseases of liver and kidney (Najafzadeh *et al*., 2013). They are also widely used in monitoring the response to the exogenous toxic exposure. In ruminants, AST is often tested along with ALT, ALP and other serum enzymes to evaluate whether the liver is damaged or diseased. When the liver is dysfunction, the levels of the above enzymes will rise. There was no significant variation in the activities of ALT in this study. However, there was decrease in its activities among the groups. Therefore, a decreased level of this enzyme indicates no destruction of liver cells. The result of the current study was in contrast with Fazilati (2013) who observed that zinc oxide nanoparticles (25 – 200 mg) significantly increased ALT activity in serum male rats. Also, Jung *et al.* (2010) showed increasing ALT in mice fed ZnO nano particle compared with the control. The increment may be due probably to dosage and time of animal exposed, as ZnO nano particles have dose and time dependent cytotoxicity and its mechanism may be through oxidative stress, lipid peroxidation, cell membrane and oxidative DNA damage (Najafzadeh *et al*., 2013). Significant difference observed in the activity of AST in this study with T3 group having lower serum AST levels may have indicated that 0.008 % nano zinc oxide treated group had no liver cells damage. This result was in agreement with the report of Mansouri *et al.* (2015) who reported lower serum AST values in ZNP-3 group rats supplemented with 300 mg/kg of ZNP as compared to ZNP-1 group (5 mg/kg) and ZNP-2 group (50 mg/kg). However, there was no significant difference between ZNP-3 group and control.

Serum Mineral: Zinc concentrations in blood serum or plasma are the most widely used indicator of Zn status, as low values are to be expected as an early change during Zn deficiency (Underwood and Suttle, 1999), but it did not give certainty and sensitivity as a diagnostic tool. The significant increase in the serum zinc level in this study might be due to the greater absorption of nano zinc oxide. It has been reported that nano particles are absorbed in duodenum by active transport and nano-elemental forms can cross the small intestine and further distribute into the blood (Hillyer and Albrecht, 2001) and also indicating higher availability of Zn for various metabolic functions. Findings in this study were in agreement with those of Najafzadeh *et al*. (2013) who also observed increased serum zinc levels after oral administration of nano zinc oxide in lambs.

Conclusion: In this study, the administration of nano zinc oxide at levels of 0.004 and 0.008 % in diets fed to WAD goats in groups T2 and T3 had favorable influences on growth performance, haematology, serum parameters, and equally significantly improved the serum Zn levels. Thus, nano zinc oxide opens a window for better bio-available zinc source. Nano minerals are having a great potential as mineral feed supplements in animals even at very lower doses than the conventional sources by increasing their bioavailability in biological system due to the increase in the surface area and surface activity of nano minerals. Present study thus recommends the supplementation of nano zinc oxide at 0.004 and 0.008 % in diets

fed to WAD goats to improve the growth performance and serum Zn levels.

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