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Research Article

Hematology and Serum Biochemical Indices of West African Dwarf Goats Fed Shea Nut Cake Multi-Nutrient Blocks as Supplements to Daniella oliveri Based Diet

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ABSTRACT

Goats are hardy, with small body size, broad feeding habits and short reproductive cycle. However, dry season is usually accompanied by low forage growth and reduced nutrients therefore resulting into undergrazing and reduced performance. This study is evaluating the inclusion of shea nut cake multi-nutrient blocks compacted using three different binders; cassava flour, cement, industrial starch (T1, T2 and T3 respectively), as dry season supplement. Effects of this dietary inclusion were evaluated on the hematology and serum biochemical indices of the experimental goats. Result showed that the highest Red Blood Cell (RBC) value 17.73 ± 0.23 103/ul was recorded in T2, serum low density lipoprotein (LDL), Alanine amino transferase (ALT), Urea, were significantly lower assigned to treatment 2 as compared to the control. The values recorded for Alkaline phosphatase (ALP) ranged between 41.68 to 64.02, with experimental goats in treatment 3 having the lowest value. The bilirubin of goats (2.74 mg/dl) was observed to be significantly lowest in treatment 3 compared to the control value. The highest total protein (TP) (10.50 g/dl) recorded in treatment 2 was significantly higher than the control and statistically similar to values obtained for treatment 3 (10.05 g/dl). The highest level of serum cholesterol (63.37 mg/dl) was found in T3, although all cholesterol values observed in this study falls within the normal physiological range. Industrial starch can be used as shea nut cake multi-nutrient block binder without adverse physiological effect on experimental animals.

Keywords: Shea-nut cake; Nutrient block; Goat; Hematology; Serum biochemistry

INTRODUCTION

Goat possesses multipurpose functions which range from production of meat, milk, skin and income for meeting household needs. Despite its significance, small ruminant production is hindered by inadequate feeds. Feed shortage constitutes a major constraint to ruminant production in the tropical region especially during the dry season. This is normally characterized by loss of tree leaves with notable decline in forage quality and quantity, thereby subjecting livestock to under grazing. The forage obtainable during the dry season have low quality, insufficient crude protein (as low as 2%), high lignin and fibre. The consumption of which result into low feed intake, depressed digestibility, reduced nutrient utilization, weight loss, low birth weight and low resistance to diseases. Hence, there is need to advance the productivity of goats through superior feeding and management. In a bid to obtain a sustainable solution to this problem and reduce overall cost of production, there is a necessity to use cheap and locally available by products such as Sheanut based feed blocks. The hematological and biochemical indices of animals have been known to reflect the deep perception of the level of tolerance of an animal to different diets, possible toxicity and performance potentials resulting from the feed consumption [1]. This study was designed to determine the effect of Sheanut based feed blocks on the hematology and serum biochemistry of West African Dwarf Goats (WAD).

MATERIALS AND METHODS

Experimental site

This research was carried out at the small ruminant section, Teaching and Research Farm, Faculty of Agriculture, Kwara State University, Malete, Moro Local Government Area of Kwara State, located within the middle belt, Guinea Savannah region of Nigeria. The rainy season starts from March and ends in October while the dry season begins in November and ends in early March. The total annual rainfall ranges from 800 mm to 1,200 mm and 1,000 mm to 1,500 mm. With a minimum temperature range of the state is 24°C-32°C.

Sources and preparation of experimental samples

The Shea nut cake was obtained from local Shea butter processing factory in Idofian Fufu, Ilorin, Kwara State. Molasses was acquired from Ilorin; Urea was sourced from Agricultural Development Project in Ilorin. Cement and salt obtained from Dangote Company, Gliricidia powder was prepared from Gliricidia leaves and other

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ingredients used were purchased from reputable source in Ilorin, Kwara State, Nigeria.

Methods of feed block production

Production of feed blocks: Cold process was used for the production of feed blocks. The blocks were made manually in batches with no sophisticated equipment. The molasses, mineral, protein, energy and binders and water were mixed. The lumps were broken into smaller particles to ensure proper mixing when fed to animals. Additional water was added to make a 20 litre capacity bowl, followed by a simultaneous addition of urea and salt of known quantities to completely dissolve for about 20 minutes. The mixture was stirred for 10 minutes to obtain a homogeneous mix. The energy sources and the binder were added. Water was added to the mixture to further obtain a homogeneous mix. The homogenous mixture was made up to 100 kg each for feed blocks, carefully placed in moulds, compressed and left in a well-ventilated room till ready for use.

Moulding and drying of feed blocks: The mixed material was placed into a 16 cm x 15 cm x 16 cm (Length x Width x Height) wooden mould. The mould was lined with plastic sheets to prevent the block from sticking to the walls and allow easy removal from the moulds. Water was smeared inside the plastic liner of the mould for easy removal of the cast blocks. The moulds were removed from the blocks after setting. It was air dried and covered with net under room temperature at 28°C for 21 days till use.

Experimental animals and management

Twelve growing West African dwarf goats weighing between 6.5-10 kg with good body condition were purchased from local market (Alapa) in Ilorin. The experimental animals were tagged, divided into three groups, replicated four times and randomly assigned to the experimental diets. A standardized open pen for small ruminants was used. At the beginning of the study, all animals were dewormed with Albendazole, orally administered. Multinor (Injectable multi-vitamin) and Oxytetracycline injection 20% L.A (Tremadox L.A) were administered intramuscularly, each at 1 ml/10 kg body weight (BW). Ivermectin (2% L.A) was administered sub-cutaneously through the jugular vein at 0.25 ml/10 kg (BW). Experimental animals were weighed at the beginning of the experiment in the morning before feeding, using suspended spring balance. Animals were provided with feed block every day before eating forages for two hours. Animal were given ad-libitum access to fresh water throughout the period of the experiment.

Experimental feed block

Three different binders (cassava flour, cement, industrial starch) were used for the production of the feed (Sheanut based cake) block to produce the best binder, using the hardness and the compactness of the binder for evaluation. The experimental diets are as shown below;

Assessment of block for hardness, compactness and physical characteristics

The blocks were assessed for hardness compactness and physical characteristics over 7 and 21 days respectively by 3 persons independently using a subjective scale: Soft (+) Medium (++) and Good (+++). Hardness was tested by pressing with the thumb in the middle of the block. Compactness was tested by the ease to break the block by hand [2]. The physical characteristics such as colour, taste, texture and smell/aroma were determined according to Hadjipanayiotou method.

Acceptability study

The WAD goats were fed for 14 days to ascertain the relative preference and acceptability, with a 7 day adjustment (acclimatization) and 7 day data collection periods respectively, using the cafeteria method reported by Ogunbosoye et al. [3]. Feed blocks were provided twice daily between 08:00 and 12:00 hour and in the evening between 4:00 and 6:00 hour in an individual opened paddock for 14 days. 5 kg of forage was placed in each compartment and left over of the feed block was weighed and recorded daily. Variation between quantity of feed offered and the left over was recorded as intake. The position of the feed was changed to prevent responsive adjustment of the animal to particular feed block. The feed resources preferred was accessed from coefficient of preference (COP) value, calculated from the ratio between the intake of individual feed resource and the average intake of the feed resources. Therefore, feed block was inferred to be relatively acceptable provided the COP was greater than unity.

COP=intake of individual feed offered/Mean intake of all the feed offered

Blood collection and analysis

5 ml of blood was collected through the jugular vein of the research goats into heparinized bottles, to investigate hematological parameters, using one animal per replicate and four animals per treatment, while 5 ml of the sampled blood was collected into plain bottles, centrifuged to separate the serum for analysis. The blood samples were temporarily stored using iced cubes and transported to a reputable laboratory for analysis.

Chemical analysis

Feed ingredients used and samples collected from the blocks were analysed for proximate, using Association of Official Analytical Chemists methods, neutral detergent fibre (NDF) were assessed using the method proposed by Van Soest.

Statistical analysis and design

The experimental design is a completely randomized type. Data obtained were subjected to one way analysis of variance (ANOVA), using SAS package (2010).

RESULTS AND DISCUSSION

Chemical composition

The chemical composition of the feed block is as shown in Tables 1 and 2. The moisture content of the feed block ranged between (3.67%) in diet 3 and (5.76%) in T2. The value obtained were higher than the reported mean for Gliricidia-based multi-nutrient blocks by Aye et al., while they were comparable to the levels reported for urea-molasses blocks by Onwuka. The crude protein contents varied between (5.17%) and (7.70%), the CP is higher in T2 (7.706%) and the least value was obtained from T1. The crude protein level of T2 and T3 were at the range of 7.00 to 8.00% opined for the efficient functioning of rumen microorganisms, which means the feed block in this study can be employed as a protein supplement to low quality feed. The crude fibre content was within the range of 1.41% to 4.50%. The variations may be due to varietal difference in the binder. The T3 feed block had low to moderate content of fibre, since the voluntary DM intake and digestibility are dependent on cell wall constituent (fibre), especially NDF.

Table 2 also shows that T2 had the highest NDF value of 28.32%, while the least 23.42% was recorded for T1. The feed block contain a group of substance, insoluble in water and organic compounds like ether, referred to as crude lipids (CL) which act as stores of energy. Highest value of CL was obtained in T2 (4.63%) which is an indication of high energy feed. CL is the lipid component and energy derived from it is utilized by animal body maintenance and production and least value was obtained in T3 (4.26%). The ash contents of feed block ranged from (6.78% to 8.82%). Ash content of any feed is a measure of mineral level, value of ash was lowest in T1 (6.78%) and highest value was obtained in T2.

Table 1: Composition of experimental diets using different binders (%).

Ingredients	T1 (Cement %)	T2 (Cassava flour %)	T3 (Industrial starch %)
Shea-nut cake	30	30	30
Rice bran	5	5	5
Soybean waste	10	10	10
Gliricidia leave	15	15	15
Urea	10	10	10
Binder	10	10	10
Molasses	15	15	15
Bone meal	3	3	3
Premix	1	1	1
Salt	1	1	1
Total	100	100	100

Table 2: Proximate composition (%DM) of feed block (SNC) with different binders.

Parameters	T1(cement)	T2 (cassava flour)	T3 (industrial starch)	Proximate
				Proximate
Moisture	3.75b	5.77a	3.68c	Proximate
Ash	6.79c	8.82a	8.45b	Proximate
Crude protein	5.18b	7.71a	7.56a	Proximate
Crude lipid	4.35a	4.64a	4.26a	Proximate
Crude fibre	4.26a	4.50a	4.42a	Proximate
CHO	56.51a	44.73c	51.53b	Proximate
NDF	23.43c	28.33a	27.52b	Proximate
Calorific v	1530.23b	1510.71b	1605.31a	Proximate

Note: abc=Means (average of 3 analyses each) in different columns with the same superscript are not significantly different (p>0.05).

Hardness and compactness

Result of measurement of hardness and compactness is presented in Table 3. Hardness and compactness of feed blocks has been described by Asaolu as an important aspect to be considered during block manufacturing. All formulated feed block, resulted in blocks of comparable hardness and compactness (7 to 21 days) after casting. This is different from what was reported by Chenost, who suggested that blocks will be readily dried for use after 10 days of exposure, when dried preferably in the shade, as high sun intensity causes cracking in blocks. However, T2 was adjudged to be of optimum hardness (+++) and compactness (+++) which might be too hard for the animals, while hardness and compactness of the T1 blocks was soft and would be too easy for animals to consume, T3 hardness was observed to be moderately compacted and to have correct hardness for the animals. This has the advantage of ensuring gradual release of urea when fed such feed blocks, to prevent urea toxicity. Block hardness expressed as penetration resistance by Herrera et al. and Birbe et al., is a factor that markedly determines animal intake. Asaolu observed that as block hardness increases, animal intake diminished. Onwuka stated that optimum hardness and compactness of feed blocks depicted that the ingredients used were held together reasonably well by the industrial binder, with the attendant advantage of ensuring gradual release of the urea component. In this study, ingredient composition and mixing sequence were likely to have had more influence on observed trends in block hardness and compactness than the binder, which was used at a constant level (10%) as supported by Asaolu. The study showed that hardness of blocks increased progressively with an increase in drying period. This agrees with the findings of Sansoucy, who stated that with cement as a binder, hardening time was much longer and it increased with time. The drying period in this study was 15 to 30 days, which is in line with Mubi et al., but contrary to the report of Mohammed et al., who recorded a drying period of 7 days after compounding; this difference may be due to climatic conditions during production [2]. It is observed that T3 (industrial starch) produced a good hardness and compactness than T1 (cement) and T2 (cassava flour). This indicates that industrial starch can replace most cement as most animal welfare advocates are against the usage of cement in animal feed preparation.

Table 3: Hardness and compactness of feed blocks at 7 and 21 days after moulding.

Days of molding		Day 7			Day 21	
Treatments	T1	T2	T3	T1	T2	T3
Compactness	+	++	+++	+	++	+++
Hardness	+	+++	++	+	+++	++
Weight (kg)	2.3 kg	2.9 kg	2.7 kg	2.1 kg	2.2 kg	2.2 kg

Note: Soft (+), Medium (++) and Good (+++).

Table 4 shows the hematological indices of experimental goats given shea-nut feed block. All parameters assessed were not significantly affected except for those of RBC, MPV, PDW, MCH and the HCT. A significantly depressed RBC values 1.63 to 1.89 x 103/ul were recorded in this study, which falls below the normal physiological range obtainable for normal goats (8.0-18.0 x 106/ul) as reported by Banerjee and Aiello [4]. This condition reveals that utilization of sheanut feed block with cassava flour, cement and industrial starch might not have enhanced effective oxygen transportation within the tissues of WAD goats for oxidation of digested food. Which is necessary for energy release and proper functioning of cells, as against the report of Etim [5]? Perhaps, this was also reflected in the HCT values (15.43%-21.73%) of experimental goats across the treatments, with significantly depressed values and falls below the normal physiological range of HCT in goats (22%-38%) as reported by Samira et al. HCT also known as hematocrit is an expression of the percentage of red blood cells within a whole sample of blood. Higher HCT would reflect higher ability of blood to carry enough oxygen for breaking down of food substances to ensure adequate energy release. Results obtained in this study for RBC and HCT also corroborate HB values recorded, which is also significantly lower than the physiologically normal range (8-12 x g/dl) for goats [6]. The lowered values of HB observed in this study could have resulted into insufficient supply of oxygen to cells known as hypoxia. The normal range of MCH for goats as reported by Feldman et al. is 5.20 pg to 8.00 pg [7]. The obtained MCH values of experimental goats in this study is significantly high compared to values obtained in this study (32.13 pg-38.10 pg), perhaps, this could have resulted into macrocytic hypochromic anaemia, which is commonly observed in recovery stages in animals with acute blood loss. The cause for the increase in the MCH value is not known as at the time of this write up. The highest MPV (Mean platelet value) of the experimental goats was recorded to be 13.85 fl in treatment 3, containing industrial starch. Platelets, produced from megakaryocytes are small blood cell, essential for blood clotting, which engages in processes that enhance quick reduction in bleeding after an injury. The significance of MPV blood test is to measure average accurate size of platelets, diagnose bleeding disorders and diseases of bone marrow [8]. The values recorded in this study are higher than the range of values reported by Samira et al. for goats. A high MPV value suggests the presence of increased thrombopoiesis. Platelets with larger volume are more reactive; therefore, fewer platelets will need to be produced from megakaryocytes to maintain constant hemostatic potential if those larger platelets circulate [9]. There is an inverse relationship between platelet count and size in steady state platelet production. There is agreement that "stress" platelets are larger than steady state platelets and have greater functional capacity than smaller platelets [10]. Perhaps, the incidence of increased MPV values across all treatments occurred as a result of a sort of physiological stress from the diet, which could have enhanced an increased functional capacity of platelets in blood clotting mechanism.

Table 4: Effect of experimental treatment on the haematological parameters of goats.

Parameters	T1(cement)	T2 (cassava flour)	T3(industrial starch)
RDW	16.60 ± 0.33	17.73 ± 0.23	17.68 ± 0.57
HB (g/dl)	6.40 ± 0.51	7.28 ± 0.19	6.23 ± 0.27
RBC	1.63 ± 0.13b	1.89 ± 0.61a	1.80 ± 0.11ab
WBC	16.03 ± 0.79	15.93 ± 0.68	16.20 ± 1.74
MCH	37.00 ± 0.36a	38.10 ± 1.98ab	32.13 ± 1.27 a
MCV	100.93 ± 6.96	110.88 ± 3.42	105.24 ± 4.06
MCHC	38.13 ± 2.79	31.93 ± 0.92	31.88 ± 3.17
PDW	12.63 ± 0.94a	12.68 ± 0.51a	11.43 ± 0.46ab
MPV	13.13 ± 0.13c	13.28 ± 0.14cb	13.85 ± 0.3b
LYM	10.40 ± 0.33	11.03 ± 0.62	12.31 ± 1.18
HCT	15.43 ± 0.25b	21.73 ± 0.69a	21.20 ± 1.95a
LYMP	64.88 ± 1.22	69.33 ± 1.11	68.40 ± 0.44
PLCR	51.43 ± 1.54	51.33 ± 0.98	52.00 ± 1.92

Note: abc Means in the same row bearing different superscripts differed significantly (P<0.05). WBC, White blood cell; LYMP, lymphocytes; RBC, red blood cell; Hb, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, corpuscular hemoglobin concentration; RDW, Red cell distribution width; MPV, mean platelet Volume; PDW (Platelete Distribution width). *According to Feldman et al., from Schalm's Veterinary Hemetology. Philadelphia. Baltimore, New York, London, Buenos Aires, Hong Kong, Sidney, Tokyo: Lippincott Williams and Wilkins [7].

The result of the serum biochemical indices of WAD goats is shown in Table 5. All the values evaluated showed a significant response to the test ingredient except for AST, conjugated bilirubin and Uric acid values, which did not vary significantly across the treatments. Highest total protein (TP) value (10.50 g/dl) recorded for WAD goats in treatment 2 were significantly higher compared to control value and statistically similar to values obtained for treatment 3 (10.05 g/dl). The observed higher total protein in WAD goats under treatment 2, containing cassava flour as a binder, is an advantage since TP is an indication of protein digestion, absorption and metabolism. The TP value obtained in this study was higher than mean values documented by other researchers on WAD goats and other goat breeds (6.36 ± 0.1 gm/100 ml; 7.1 ± 0.1; 6.37 ± 0.21) [11-13]. This is in tandem with the discovery of Lukuyu et al., who reported that cattle weight gain was twice its initial weight (201 to 402 g/day) with increasing intake of dried cassava foliage for a period of 3 months and feed conversion improved with added nitrogen from the cassava hay [14]. Perhaps, the inclusion of cassava flour as a binder could have improved the protein utilization of the goats. According to Ikhimioya et al. total proteins functions adequately in regulating osmosis, enhances immunity status and transportation of several substances in the body [6]. Higher serum TP is closely related with better performance, as it is a measure of how the experimental animal utilized the protein in the diet. This is because it shows an estimation of the nutritive state of animals and helps to monitor how the diet is being utilized. It also helps to reflect the alterations in metabolism. The cholesterol values of cholesterol 58.22-63.37 mg/dL in this study is higher than 41.00-62.80 mgdL−1 reported by Oduguwa et al. [15]. The highest level of serum cholesterol (63.37 mg/dl) was found in T3 containing inclusion of industrial starch, although all cholesterol values observed in this study falls within the normal physiological range of 55-200 mg/dl as reported by Pampori [16]. According to Esonu et al. and Igwebuike et al., their reports showed that serum cholesterol is associated with the quantity and quality of protein supplied in the diet [1]. It is therefore indicated that the protein provided by the SNC combined was enough and of good quality to meet the nutritional needs of the animals. Cholesterol makes up the essential structural component of animal cell membranes. Cholesterol also serves as a precursor for the biosynthesis of steroid hormones, bile acid and vitamin D. Cholesterol is also a key regulator of membrane fluidity in animals, it's the principal sterol synthesized by all animals. Values obtained for cholesterol, Triglycerides and Urea were significantly high and comparable for WAD under treatment 3. Increased serum concentration of cholesterol is an indication of diet high in carbohydrates/sugars, while low levels indicate low fat diet, malabsorption, or carbohydrate sensitivity. However, the result showed that serum LDL, ALT, Urea, were significantly (p>0.05) lower in WAD assigned to treatment 2 as compared to the control. The AST recorded values (10.08-11.99 U/l) were not significantly influenced by dietary treatments. This is in agreement with the observations of Ogunbosoye et al., who recorded no significant difference (p>0.05) in the values obtained for AST, after feeding varying levels of sheanut butter cake rations to WAD goats [3]. Serum concentrations of ALT and AST are suitable tests to assess the level of liver damage. Therefore, the insignificant values obtained for AST, is an indication of normal functioning of liver among groups of animals fed experimental diets. However, concentration of ALT was observed to vary significantly ranging from 15.86 to 26.53 u/l with T1 having the highest concentration, although values were within range of normal animal blood indices. This shows that the diets supported the health status of the research animals. Values recorded for ALP in this study ranged between 41.68 to 64.02 UL−1 with WAD goats in treatment 3 having the lowest value. The normal physiological range of ALP for goats is 42-775 UL−1 as reported by Blood et al. [17]. Value recorded in T3 (41.68 UL−1) is lower than the lowest normal physiological range of WAD goat. This is an indication that there was a slight damage to heart muscle, liver, muscle cells and to liver functions. This could have indicated that inclusion of shea nut cake multi-nutrient feed blocks with industrial starch in diets of WAD goats had adverse effect on bone formation of the experimental goats. Abnormally high serum levels of ALP could mean incidence bone disease, liver disease or bile obstruction. Bilirubin of goats (2.74 mg/dl) was observed to be significantly lowest in treatment 3 compared to control value. Bilirubin is formed by breakdown of hemoglobin in the spleen, liver and bone marrow. It is also considered a reliable liver function test as it determines the ability of liver to take up, process and secrete bilirubin into the bile. An increased concentration of bilirubin in the serum or tissue is called jaundice, which occurs in toxic or infectious diseases of the liver. An excessive breakdown of RBC can cause permeation of large amounts of bilirubin, resulting from consumption of certain poisons. An increase in bilirubin concentration is an indication of liver cell damage, so the ability of liver to extract bilirubin from blood to form bile is reduced [18]. The bilirubin values observed in this study are within normal range obtainable for livestock. The experimental diets do not pose any health risk for animals used for this study. The highest urea value observed in this study is 12.58 nM/litre and the lowest is 11.69 nM/litre. Urea functions as a source of nitrogen for protein biosynthesis in the digestive tract. Ammonia can be absorbed in digestive system when formed in excessive quantities and enhance formation of urea, or it can be derived from urea of blood plasma when its formation from feed sources is small. An elevated level of serum urea has been explained to be related to excessive tissue protein catabolism linked with protein deficiency [19]. The recorded urea level in this study is higher than normal range of urea in goats. The relatively high values obtained for serum urea in this study as shows that the use of shea nut cake multi-nutrient feed blocks supplemented with either cement, starch or cassava flour, perhaps resulted into an increased catabolic reaction in the goats [20,21]. This fact is evident in the high values observed for serum total protein values and WAD sheep [19,20].

Table 5: Serum Biochemical parameters of experimental diets using different binders.

Parameters	T1(cement)	T2 (cassava flour)	T3 (industrial starch)	SEM
Total protein (g/dl)	8.38b	10.50a	10.02a	0.23
Cholesterol (mg/dl)	58.22b	59.02b	63.37a	0.51
Trig. (mg/dl)	94.92b	97.36b	105.08a	1.04
LDL (mg/dl)	45.10a	27.76b	28.27b	1.21
AST (u/l)	11.7	10.08	11.99	0.35
ALT (u/l)	26.53a	15.86c	16.68c	0.43
ALP (u/l)	64.02a	56.39a	41.68b	1.87
Bilirubin (mg/dl)	3.18ab	2.96ab	2.74b	0.07
Conj. Bil. (mg/dl)	1.31	1.41	1.32	0.02
Urea (nM/L)	12.42a	11.69b	12.58a	0.11
Uric acid (mM/L)	3.56	3.18	3.49	0.08

CONCLUSION

The experimental diet seems not to support maximum health status of the experimental WAD goats, however, there is a need to further research into the use of sheanut feed blocks to determine its potential in improving the performance of goats during the dry season as well as use of other breeds to ascertain the observations in this study.

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AUTHORS' CONTRIBUTIONS

Dupe Ogunbosoye conceived and designed the study, Performed research and brought her professional expertise to bear on the management of the experimental animals. Olayinka Abosede Ojo contributed new methods, did the statistical analysis, interpretation of result and writing of this manuscript.

CONFLICTS OF INTEREST/COMPETING INTERESTS

The authors have no conflict of interest

AVAILABILITY OF DATA AND MATERIAL

Data obtained in this study are transparent.

ANIMAL WELFARE STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received. The authors confirm that they have followed EU standards for the protection of animals used for scientific purposes.

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