



Nutrient utilization and rumen fluid characteristics of West African Dwarf goat fed diets containing varied levels of sodium bicarbonate

[Utilização de nutrientes e características do fluido ruminal de cabras anãs da África Ocidental alimentadas com dietas contendo níveis variados de bicarbonato de sódio]

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ABSTRACT

A 63 day study was conducted to investigate the effects of sodium bicarbonate (NaHCO_3) on nutrient utilization and rumen fermentation characteristics of West African Dwarf goats. A total of fifteen (15) West African Dwarf (WAD) goats were randomly assigned to the five dietary treatments with each of the treatments replicated three times such that each of the replicate had one goat in a completely randomized design. A basal diet was formulated and divided into five equal portions with sodium bicarbonate supplemented at 0.00kg, 0.50kg, 1.00kg, 1.50kg and 2.00kg to 100.00kg to represent Diet 1, 2, 3, 4, and 5 respectively. All data collected were subjected to one-way analysis of variance with the aid of SPSS and the means were compared using Duncan's Multiple Range Test at 5% significant level. Observed results showed that the chemical composition of the diets was not statistically ($P > 0.05$) significant for most of the parameters measured except for Ash content ($P < 0.05$). The nutrient digestibility profile was statistically ($P < 0.05$) significant in DM, CP, CF, EE, NDF and ADF respectively. Volatile fatty acid profile and total microbial count profiles were also statistically ($P < 0.05$) significant in most of the parameters measured except for $\text{NH}_3\text{-N}$ and protozoa count ($P > 0.05$), respectively. The major bacteria isolates identified were *Streptococcus faecalis* and *Bacteroides rumenicola*. It was concluded that the supplementation of sodium bicarbonate up to 2kg/100g in the diets of WAD bucks had no detrimental effect but significantly enhanced the microbial environment with an increase in the population of cellulolytic and proteolytic bacteria necessary for fiber digestion.

Keywords: additives, nutrient intake, rumen microbes, small ruminant, volatile fatty acid.

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Received: May 25, 2025
Accepted: Oct 10, 2025

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RESUMO

Um estudo com duração de 63 dias foi conduzido com o objetivo de investigar os efeitos do bicarbonato de sódio (NaHCO_3) sobre a utilização de nutrientes e as características da fermentação ruminal em bodes da raça Anã da África Ocidental (West African Dwarf). Um total de quinze (15) bodes West African Dwarf (WAD) foi distribuído aleatoriamente em cinco tratamentos dietéticos, com três repetições por tratamento, de modo que cada repetição continha um animal, em um delineamento inteiramente casualizado. Uma dieta basal foi formulada e dividida em cinco porções iguais, suplementadas com bicarbonato de sódio nas proporções de 0,00 kg, 0,50 kg, 1,00 kg, 1,50 kg e 2,00 kg por 100,00 kg de ração, correspondendo, respectivamente, às Dietas 1, 2, 3, 4 e 5. Todos os dados coletados foram submetidos à análise de variância unidirecional (ANOVA) com o auxílio do software SPSS, e as médias foram comparadas pelo teste de Duncan a 5% de significância. Os resultados indicaram que a composição química das dietas não apresentou diferenças estatisticamente significativas ($P>0,05$) para a maioria dos parâmetros avaliados, exceto para o teor de cinzas ($P<0,05$). O perfil de digestibilidade dos nutrientes foi estatisticamente significativo ($P<0,05$) para matéria seca (MS), proteína bruta (PB), fibra bruta (FB), extrato etéreo (EE), fibra em detergente neutro (FDN) e fibra em detergente ácido (FDA). O perfil de ácidos graxos voláteis e a contagem total de microrganismos também apresentaram diferenças significativas ($P<0,05$) na maioria dos parâmetros, exceto para nitrogênio amoniacal ($\text{NH}_3\text{-N}$) e contagem de protozoários ($P>0,05$). As principais bactérias isoladas foram *Streptococcus faecalis* e *Bacteroides ruminicola*. Concluiu-se que a suplementação de até 2 kg de bicarbonato de sódio por 100 kg de dieta em bodes West African Dwarf não apresentou efeitos deletérios, mas melhorou significativamente o ambiente microbiano ruminal, promovendo o aumento da população de bactérias celulolíticas e proteolíticas essenciais para a digestão de fibras.

Palavras-chave: aditivos, ácidos graxos voláteis, ingestão de nutrientes, microrganismos ruminais, pequenos ruminantes.

INTRODUCTION

Animal production is a very important sector of the Nigerian economy and is crucial in ensuring food security. Small ruminants (especially goats and sheep) form an integral and important component of the pattern of animal production in most rural communities (Ajayi et al. 2015) and it remains popular among the rural populace and resource-poor people. According to (Santa et al. 2003), high carbohydrate diets decrease rumen pH and fiber digestibility thereby reducing the buffering capacity of the rumen contents and increasing the risk of ruminal acidosis also, high-energy, low-fiber rations increased acid levels in the rumen, which could be detrimental to the rumen papillae. Ruminal papillae damage usually results in poor absorption of feed nutrients

in the rumen which may reduce feed intake there by reducing weight gain and performance of the animal (Kawas et al. 2007).

To avoid this incidence, several nutritional therapies have been tried, including the use of dietary buffers. Sodium bicarbonate (NaHCO_3) is a natural buffer (Erdman 1988), and one of the dietary buffers commonly used to prevent ruminal pH reduction and enhance ruminal fermentation of low roughage diets. The addition of sodium bicarbonate helps in the stabilization of ruminal pH that can potentially prevent ruminal acidosis, maintaining blood pH of the animal which is necessary for proper metabolic function (Meschy et al. 2004).

Although, there is several literature on the effects of NaHCO_3 supplementation in large ruminants, particularly in dairy cattle and fattening ewes fed a high concentrate diet. However, there are still limited information on the effect of sodium bicarbonate on goat especially on nutrient utilization. This study hypothesized that the supplementation of sodium bicarbonate up to 2kg in 100kg of ruminant diet will help to improve the nutrient intake, nutrient digestibility and also serves as a rumen modulator in goats. Therefore, the aim of the present study is to investigate the effect of different levels of dietary sodium bicarbonate on intake, nutrient utilization and rumen fermentation profile of West African Dwarf goats for better and efficient productivity.

MATERIAL AND METHODS

Experimental Site

This experiment was carried out at the Small Ruminant Unit of the Teaching and Research Farm, and the chemical analysis of the feed, faecal and urine were carried out at the Nutrition Laboratory of the Department Animal Production and Health, Federal University of Technology, Akure, Ondo State.

Experimental materials and feed formulation

The cassava peels were collected at cassava processing industries in Akure Ondo State. The peels were sun dried for 2-4days thereafter milled into 2mm particle size. Other

Conventional feed ingredients (wheat offal, palm kernel cake salt, premix, urea sodium bicarbonate and di-calcium phosphate) were bought from a reputable feed mill industry in Akure.

Feed Formulation

Table 1. Present the Gross composition of the basal diet. The diet was divided into five equal portions and each was supplemented with graded levels of NaHCO₃ (sodium bicarbonate) as shown below.

Table 1: Gross Composition of basal Diet*(%)

Ingredient	Quantity(%)
Cassava peel meal	49.70
Wheat offal	34.00
Palm Kernel Cake	13.00
Premix	0.60
Di-calcium Phosphate	1.00
Salt	0.70
Urea	1.00
Total	100.00

*Diet A = Basal diet; Diet B = Basal diet + 0.5kg NaHCO₃ /100kg; Diet C = Basal diet + 1kg NaHCO₃ /100kg; Diet D = Basal diet + 1.5kg NaHCO₃/100kg; Diet E = Basal diet + 2kg NaHCO₃/100kg.

Experimental Animals and Management

Fifteen (15) West African Dwarf bucks were used for this study. The goats were selected randomly from the already existing flocks at the goat unit between the ages of 12 to 13 months, with an average weight of 9kg and were acclimatized for 2 weeks. The goats were given prophylaxis treatment against peste des petits Ruminants (PPR vaccine) and parasite control (Ivermectin injection) to ensure good health conditions after which they were tagged for proper identification. The goats were weighed and randomized into five treatment groups of 3 bucks per treatment replicated three times in a completely randomized design. The experimental diet was given to the animals at 5 % of their body weight. The animals were fed twice daily.

Data Collection

Digestibility trial and nitrogen retention

Total faeces were collected in the morning before feeding and watering during the last 7 days of the experiment. The faeces were weighed fresh and 10% of faeces collected from each animal were oven-dried to determine dry matter of the faeces. The faecal samples for each goat were thoroughly mixed and milled and then put in sealed polythene bags and were stored at room temperature until it was required for analysis. Urine was also collected into a plugue bucket placed under each cage to which a few drops of H₂SO₄ was added to prevent volatilization of the ammonia from the urine (Ahamefule et al. 2006). The daily volume of urine output per animal for a period of 7 days was recorded and 10% of daily output was stored in stopper plastic bottles, labeled and stored in a deep refrigerator at -5°C. At the end of the day's collection, the samples were bulked for each goat and sub-samples were taken for analysis. Nitrogen balance was calculated as nitrogen output (nitrogen in urine and faeces) deducted from nitrogen intake. The unit is expressed in grams per day. Percentage apparent nutrient digestibility coefficient and nitrogen retention of the goats was calculated as follows.

$$\% \text{ Apparent nutrient digestibility} = \frac{\text{Nutrient intake} - \text{Nutrient output}}{\text{Nutrient intake}} \times 100$$

$$\% \text{ Nitrogen retention} = \frac{\text{Nutrient intake} - \text{nutrient output}}{\text{Nutrient intake}} \times 100$$

Rumen collection and fermentation characteristics

At the end of the feeding trial, rumen fluids were collected from the goats using a suction tube of 0.8cm in diameter and 150cm long into a 1000 mL airtight container attached to the tube, connected to a vacuum pump for fluid collection three hours before their morning feeding to assess the rumen fluid fermentation characteristics. The collected fluid was filtered through sterilized cheesecloth, and the pH of the rumen content was promptly measured using a portable digital pH meter. For the determination of volatile fatty acids (VFAs), a portion of the filtered rumen samples was thawed at 4°C before analysis by high performance liquid chromatography (HPLC). Approximately 5 ml of the filtrate was mixed with 1 ml of 5% (v/v) sulfuric acid (H₂SO₄)

solution in a test tube and allowed to stand for 30 minutes. The tubes were then centrifuged at 3000 rpm for 10 minutes, and the supernatant was decanted into a beaker. Phenolphthalein was added to the solution, and it was titrated with sodium hydroxide (NaOH) to determine acidity. Standards of acetic acid, propionic acid, and butyric acid were prepared, and their concentrations were measured at a wavelength of 210 nm using spectrophotometry. The titration values provided the concentration of volatile fatty acids (AOAC, 2007). Another portion of the filtered rumen samples underwent centrifugation at 12000rpm for 20 minutes. Five milliliters of the supernatant were collected and used for the determination of ammonia nitrogen content (Persons et al. 1976).

Rumen microbial evaluation

The total bacteria count, total anaerobic bacteria count, and fungi count were assessed using the plate count method. Portions of rumen fluid from each goat were utilized to determine the presence of bacteria, fungi, and coliforms. Colony-forming units per milliliter (CFU/ml) of both bacteria and fungi were obtained via pour plate techniques using nutrient agar and potato dextrose agar, respectively. Most ruminal bacterial species were cultured on straightforward media containing carbohydrates such as cellulose, starch, and glucose. The plates were then incubated for 24 hours at 37°C. All colonies observed at the conclusion of the incubation period were enumerated using a digital colony counter.

Chemical analysis of feed, faeces and urine

The air-dried samples of feed and feces collected from all experimental animals were analyzed for dry matter, crude protein, crude fiber, ash, ether extract, anti-nutrients, and minerals. The urine samples were analyzed for nitrogen and minerals following AOAC (2002) procedures.

Experimental Design/Statistical Analysis

All data collected were subjected to one-way analysis of variance (ANOVA) where significant difference was observed, the means were compared using Duncan Multiple Range Test (DMRT) at $p < 0.05$ with the aid of SPSS.

RESULTS AND DISCUSSION

The nutrient composition of the experimental diet is presented in table 2 below. The value for dry matter and ash among diets shows a progressive increase as the inclusion of sodium bicarbonate increases in the diet. This was similar to the observation of (Abbas et al. 2019) who reported an increase in ash content of experimental diets as the inclusion of sodium bicarbonate increased. Presented in table 3 below is the nutrient digestibility, result obtained shows that the addition of sodium bicarbonate increases the digestibility of all nutrients across the diets. The result obtained showed significant differences ($p < 0.05$) in the dry matter, crude protein, crude fibre, ether extract, neutral detergent fibre and acid detergent fibre digestibility. The improvement in the protein digestibility is likely due to the enhanced microbial activities in the rumen as sodium bicarbonate helps to maintain a more favourable pH level which also supports the growth of the microbes that breaks down protein into amino acids which are then absorbed by the animal. The results observed in these studies were similar to the observation of (Bodas et al. 2009) who reported a significant increase in NDF digestibility as the inclusion of sodium bicarbonate increased. Improvements in dietary fiber digestibility have been occasionally noted and have been attributed to the maintenance of a pH level in the rumen that is more favorable for cellulolytic microbes.

Table 4 shows the volatile fatty acid production of the rumen liquor. The different ratio of VFAs produced depends on ruminal conditions, rumen modulating agents, substrate composition, and microbial population in the anaerobic digestion system (Lukitawesa et al. 2020). In this study it was observed that the acetic acid, total volatile acid and lactic acid were decreased as the inclusion of sodium bicarbonate increased as a result of the increase in ruminal pH.

Castillo-Gonzalez et al. (2014) reported that diets rich insoluble carbohydrates produce a higher portion of acetic acid than propionic acid. Contrarily, in this study lactic acid decreased while propionic acid increased as the level of sodium bicarbonate increased with a significant increase in the rumen pH. The pH values obtained in this study ranged from 5.09 to 6.42 and show a progressive increase as the dietary levels of sodium bicarbonate increased. This observation corroborates the findings of Alzahal et al. (2009) who reported that adding NaHCO_3 significantly improved the pH level in goat rumen.

Fiber-digesting microbes, such as cellulolytic bacteria and protozoa, thrive optimally at a ruminal pH range of 6.0-6.4 (Antanaitis et al. 2020). These microbes are responsible for breaking down complex fiber components like cellulose and hemicellulose into simpler compounds that can be further fermented and utilized by the animal.

Presented in table 5 is the microbial count isolated and it was observed that the higher total protozoa count in the present study is similar to the report of Santra et al. (2003) who recorded higher protozoa count as the inclusion of sodium bicarbonate increased in the diet. The increased population of cellulolytic bacteria and protozoa as sodium bicarbonate supplementation increased enhance the breakdown and digestion of complex carbohydrates, such as cellulose and hemicellulose. These results support the use of sodium bicarbonate as a potential strategy to improve fibre digestion and enhance ruminant productivity.

Table 6 presents the microbial isolates of the rumen of the goats. It was observed that most of the microbes isolated from these goats were cellulolytic and proteolytic bacteria (*Enterococcus faecalis*, *Streptococcus faecium*, *Lactobacillus acidophilus*, *Staphylococcus aureus*, *Lactococcus lactis*, *Bacteria ruminicola*, *Micrococcus luteus*, and *Enterobacter species*.) Which helps in fibre degradation, protein hydrolysis processes, degradation of peptides and amino acid deamination (Castillo-Gonzalez et al. 2014 Russell et al. 2009). It was observed from this study that the three major fungi in the rumen were *Aspergillus niger*, *Penicillium oxysporium*, and *Fusarium oxysporum* which had been reported to produce enzymes that hydrolyze cellulose and xylans.

Table 2: Chemical composition (%) of diets containing graded levels of sodium bicarbonate

Nutrient	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	SEM	P-Value
Dry matter	88.80	89.20	89.24	89.33	89.54	0.26	0.370
Crude protein	11.40	11.40	11.43	11.63	11.67	0.57	0.968
Crude fibre	9.23	9.22	9.20	9.20	9.17	0.23	0.621
Ether extract	3.15	3.15	3.14	3.16	3.16	0.99	0.725
Ash	4.60 ^c	4.78 ^b	5.15 ^{ab}	5.23 ^a	5.33 ^a	0.33	0.002
Neutral detergent fibre	30.87	30.48	29.97	29.95	29.45	0.19	0.360
Acid detergent fibre	27.45	27.33	26.67	26.49	26.45	0.25	0.111
Acid detergent lignin	3.08	2.94	2.86	2.80	2.99	0.04	0.180
Metabolizable Energy (MJ/Kg)	11.83	11.84	11.84	11.86	11.89	2.38	0.892

^{abcde}: Mean with different superscript along the same row are significantly (P<0.05) different.

Diet 1 = Basal diet; Diet 2 = Basal diet + 0.5kg NaHCO₃/100kg; Diet 3 = Basal diet + 1kg NaHCO₃/100kg; Diet 4 = Basal diet + 1.5kg NaHCO₃/100kg; Diet 5 = Basal diet + 2kg NaHCO₃/100kg.

Table 3: Nutrient digestibility (%) of West African Dwarf goats fed diets containing graded levels of sodium bicarbonate

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	SEM	P-value
Dry matter	56.36 ^c	59.41 ^{bc}	63.51 ^b	72.49 ^a	76.98 ^a	1.93	0.035
Crude protein	74.71 ^b	74.93 ^b	77.23 ^{ab}	78.43 ^{ab}	82.90 ^a	1.56	0.004
Crude fibre	59.58 ^{bc}	57.58 ^c	62.03 ^{abc}	67.04 ^{ab}	68.44 ^a	1.44	0.038
Ether extract	59.96 ^b	65.93 ^b	65.93 ^b	74.53 ^a	77.90 ^a	1.86	0.005
Nitrogen free extract	63.44	59.31	67.02	66.78	69.19	1.41	0.191
Neutral detergent fiber	56.91 ^c	61.68 ^b	63.29 ^b	72.66 ^{ab}	71.33 ^a	2.84	0.023
Acid detergent fiber	62.96 ^b	68.90 ^b	68.94 ^{ab}	71.03 ^{ab}	74.86 ^a	1.92	0.015
Acid detergent lignin	65.00	65.07	65.10	65.25	65.97	1.84	0.943
Hemicellulose	72.81	68.31	63.25	61.98	59.05	1.78	0.076
Cellulose	68.38	68.76	68.81	63.88	66.92	1.78	0.286
Metabolizable energy	66.34	67.09	72.67	73.08	77.28	2.37	0.305

^{abcde}: Mean with different superscript along the same row are significantly (P<0.05) different.

Diet 1 = Basal diet; Diet 2 = Basal diet + 0.5kg NaHCO₃/100kg; Diet 3 = Basal diet + 1kg NaHCO₃/100kg; Diet 4 = Basal diet + 1.5kg NaHCO₃/100kg; Diet 5 = Basal diet + 2kg NaHCO₃/100kg.

Table 4: Volatile fatty acid production by West African Dwarf goats fed diets containing graded levels of sodium bicarbonate

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	SEM	P-Value
Acetic acid (mmole/100ml)	11.03 ^a	10.87 ^a	10.66 ^a	9.12 ^b	8.89 ^b	1.89	<0.001
Propionic acid (mmole/100ml)	19.63 ^c	20.43 ^c	23.37 ^b	23.49 ^b	25.37 ^a	1.80	<0.001
Butyric acid (mmole/100ml)	17.70 ^c	17.89 ^c	18.29 ^b	22.27 ^a	24.36 ^a	1.72	<0.001
Valeric acid (mmole/100ml)	15.30	18.13	20.06	22.16	25.06	1.70	0.561
Total volatile acid (mmole/100ml)	159.94 ^c	154.94 ^b	154.60 ^b	149.73 ^a	145.23 ^a	26.65	<0.001
Lactic acid (mmole/100ml)	14.06 ^a	13.49 ^b	13.30 ^b	13.20 ^b	13.00 ^c	2.37	<0.001
Ph	5.09 ^b	6.17 ^a	6.18 ^b	6.25 ^a	6.42 ^a	0.15	0.024
NH ₃ -N (%)	0.31	0.34	0.36	0.32	0.34	0.11	0.095

^{abcde}: Mean with different superscript along the same row are significantly (P<0.05) different.

Diet 1 = Basal diet; Diet 2 = Basal diet + 0.5kg NaHCO₃/100kg; Diet 3 = Basal diet + 1kg NaHCO₃/100kg; Diet 4 = Basal diet + 1.5kg NaHCO₃/100kg; Diet 5 = Basal diet + 2kg NaHCO₃/100kg.

Table 5: Total microbial count in the rumen of West African Dwarf goats fed diets containing graded levels of sodium bicarbonate

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	SEM	P-Value
Total bacterial count (x 10 ⁻⁷ cfu/ml)	3.95 ^c	4.20 ^b	4.50 ^b	4.77 ^b	5.13 ^a	0.16	0.045
Total coliform count (x 10 ⁻³ cfu/ml)	1.23 ^c	1.33 ^b	1.33 ^b	1.50 ^a	1.67 ^a	0.13	0.004
Total fungi count (x 10 ⁻³ cfu/ml)	2.00 ^c	2.67 ^b	2.83 ^b	3.13 ^{ab}	3.67 ^a	0.22	0.002
Total protozoa count (x 10 ⁻³ cfu/ml)	3.00	3.10	3.30	3.33	3.67	0.14	0.863

^{abcde}: Mean with different superscript along the same row are significantly (P<0.05) different.

Diet 1 = Basal diet; Diet 2 = Basal diet + 0.5kg NaHCO₃/100kg; Diet 3 = Basal diet + 1kg NaHCO₃/100kg; Diet 4 = Basal diet + 1.5kg NaHCO₃/100kg; Diet 5 = Basal diet + 2kg NaHCO₃/100kg.

Table 6: Microbial isolate identified in the rumen of West African Dwarf goats fed diets containing graded levels of sodium bicarbonate

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Total bacteria isolate	<i>Pseudomonas aureginosa</i> , <i>Proteus morganii</i> , <i>Bacteria ruminicola</i> , <i>Enterococcus faecalis</i>	<i>Lactococcus lactis</i> , <i>Lactobacillus acidophilus</i> , <i>Staphylococcus aureus</i> , <i>Selenomonas ruminatum</i>	<i>Lactococcus lactis</i> , <i>Lactobacillus acidophilus</i> , <i>Micrococcus luteus</i> , <i>Enterobacter species</i>	<i>Micrococcus acidophilus</i> , <i>Enterococcus faecalis</i> , <i>Streptococcus faecium</i>	<i>Bacillus cereus</i> , <i>Klebsiella aerogens</i> , <i>Pseudomonas aureginosa</i> , <i>Micrococcus luteus</i>
Total fungi count	<i>Aspergillus niger</i> , <i>Penicillium oxysporium</i>	<i>Penicillium oxysporium</i> , <i>Fusarium oxysporium</i>	<i>Aspergillus niger</i>	<i>Fusarium oxysporium</i> , <i>Penicillium oxysporium</i>	<i>Aspergillus niger</i> , <i>Fusarium oxysporium</i>

Diet 1 = Basal diet; Diet 2 = Basal diet + 0.5kg NaHCO₃/100kg; Diet 3 = Basal diet + 1kg NaHCO₃/100kg; Diet 4 = Basal diet + 1.5kg NaHCO₃/100kg; Diet 5 = Basal diet + 2kg NaHCO₃/100kg.

CONCLUSIONS

This study showed that sodium bicarbonate as an additive has a positive impact on nutrient digestibility of the animals. It also enhances ruminal environment, modulating acidity, and pH levels, creating an optimal condition for fiber digestion and microbial growth and improving the production of volatile fatty acid. Ruminal pH increased significantly with increase in the level of sodium bicarbonate and within the optimal range (6.0-6.4) for fiber digestion and microbial growth. However, based on the result of this study, diet 5 with NaHCO₃ supplementation level of 2.0kg/100kg of feed showed the highest nutrient intake and rumen pH. Further studies on the performance, biochemical and antioxidants profile of goats diets fed dietary supplementation of sodium bicarbonate is also recommended.

Conflict of interest

The authors have no conflict of interest to declare.

Ethical Approval

Principles of laboratory animal care" (NIH publication No. 85- 23, revised 1985) were followed, as well as specific national laws where applicable as the animals used in research were cared for and experimental protocols were followed as approved by the Institutional Animal Ethical and Use Committee (FUTA/ETH/2023)

Authors' contributions

Author Toyin Victoria Abegunde wrote the original draft of the manuscript and also performed the field work. Author Micheal Adinoyi Joseph performed the data curation and statistical analysis Author Oluwatosin Bode Omotoso and Author Adebowale Noah Fajemisin conceptualized and designed the study.

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