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Rumen Fermentation Characteristic of West African Dwarf Does Fed Microbial Treated Bambara Nutshell

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Abstract **Original Research Article**

The high cost and seasonal scarcity of conventional feed ingredients remain major constraints to sustainable small ruminant production in Nigeria, necessitating the exploration of agro-industrial by-products such as bambara nut shell. However, the high fibre content and low digestibility of untreated bambara nut shell limit its efficient utilisation by West African Dwarf (WAD) does. This study therefore evaluated the effects of microbial treatment of bambara nut shell using Pleurotus pulmonarius and Aspergillus niger on rumen fermentation characteristics and microbial ecology of WAD does. Twenty-one does were assigned to seven experimental diets containing untreated or treated bambara nut shell at 25, 50 and 75 mL inclusion levels over a 56-day feeding trial. Results showed that diets treated with P. pulmonarius significantly ($p < 0.05$) improved rumen fermentation compared with A. niger, producing higher acetic (7.31 vs 5.50 mmol/100 mL), propionic (6.97 vs 4.86 mmol/100 mL), butyric (6.67 vs 4.67 mmol/100 mL) and total volatile fatty acids (112.48 vs 67.86 mmol/100 mL). Increasing treatment level enhanced fermentation, with the 75 mL inclusion yielding the highest acetic acid (9.89 mmol/100 mL), propionic acid (9.42 mmol/100 mL), butyric acid (8.98 mmol/100 mL), lactic acid (14.28 mmol/100 mL) and total volatile fatty acids (140.78 mmol/100 mL), alongside a favourable rumen pH (6.52). The interaction of 75 mL P. pulmonarius produced the highest total volatile fatty acids (142.11 mmol/100 mL) and optimal ammonia-nitrogen concentration (0.57%), indicating enhanced microbial efficiency. Overall, microbial treatment of bambara nut shell, particularly with P. pulmonarius, improved rumen fermentation, energy availability and microbial stability in WAD does, highlighting its potential as a cost-effective and sustainable alternative feed resource.

Keywords: Bambara nut shell, Pleurotus pulmonarius, Aspergillus niger, Rumen fermentation, West African Dwarf goats.

1. Introduction

Ruminant production systems in the tropics are increasingly challenged by the rising cost and inconsistent availability of conventional feed ingredients, prompting renewed interest in the use of locally available agro-industrial residues as alternative feed resources (Alimi *et al*., 2024). Among these residues, Bambara nut (*Vigna subterranea* (L.) Verdc) shell is widely generated in West Africa but remains underutilised in ruminant feeding systems. Its limited use is largely attributed to its high fibre content and

poor digestibility, which restrict voluntary intake and nutrient release in the rumen (Afolayan, 2022). The Bambara nutshell contains approximately 6.7% crude protein, 3.9% ash, 47.6% neutral detergent fibre, and 29.8% acid detergent fibre, reflecting a predominance of structural carbohydrates that constrain its nutritional value (Ibrahim *et al*., 2022). Improving the utilisation of such fibrous materials is therefore essential for enhancing their contribution to ruminant diets. Microbial treatment has gained attention as a practical approach for modifying lignocellulosic residues through partial degradation of cell wall components and enrichment of fermentable substrates (Khan *et al*., 2025). In particular, lignocellulolytic microorganisms, including *Aspergillus niger* and *Pleurotus pulmonarius*, have demonstrated potential in reducing fibre fractions and improving ruminal degradability of crop by-products (Elghandour *et al*., 2023). Changes induced by such treatments are often reflected in rumen fermentation characteristics, which serve as reliable indicators of dietary utilisation. Parameters such as rumen pH, volatile fatty acid profile, ammonia-nitrogen concentration, and microbial activity provide insight into the efficiency of ruminal fermentation and nutrient metabolism (McDonald *et al*., 2021). West African Dwarf does are particularly suited to low-quality diets and represent a vital component of small ruminant production in humid tropical regions (Adewuyi, 2020). Nevertheless, suboptimal rumen fermentation can limit their productive potential even under adaptive conditions. Previous research has shown that biologically treated fibrous feeds can favourably alter rumen fermentation by enhancing propionate production and improving nitrogen utilisation (Joch *et al*., 2018). Such improvements may also contribute to reduced methane emission and better environmental efficiency of ruminant systems (Joch *et al*., 2018, Khan *et al*., 2025). Despite these advantages, information on the fermentation response of West African Dwarf does to microbial-treated Bambara nut shell remains limited. In particular, there is a lack of data describing key rumen fermentation indices associated with its utilisation. This study therefore evaluates the rumen fermentation characteristics of West African Dwarf does fed microbial-treated Bambara nut shell to assess its suitability as a sustainable feed resource.

2. Materials and method

Experiment site: The experiment was carried out at the Sheep and Goat Unit of the Teaching and Research Farm of the Federal University of Technology, Akure, Ondo State, Nigeria. Akure is located on longitude 4.944055o E and 5.82864o E, and latitude 7.491780oN with annual rainfall ranging between 1300 and 1650 mm and annual daily temperature ranging between 270 and 38o C (Daniel, 2015).

Collection and preparation of experimental diets: Bambara nutshell was collected from famers in Okene in Kogi State. One thousand (1000) grams of sun-dried bambara nutshell was moistened with one litre of water and was sterilized in the autoclave at a temperature of 121^{o}C for15 minutes to eliminate microbial contamination of the bambara nu shell. The bambara nutshell was allowed to cool at room temperature (25^{o}c) and was inoculated with 25mls, 50mls and 75mls (*Pleurotus pulmonaris a*nd *aspergillus niger)*. Thereafter, they were sun-dried for some days based on the intensity of the sun. The concentrate was formulated with crushed cassava-peel, wheat offal, urea, palm kernel cake (PKC), microbial treated bambara nutshell, bone meal, premix and salt were mixed manually. Seven experimental diets with varying level of microbial treated bambara nutshell were formulated as shown in Table 1.

Experimental layout and animal management: Twenty-one WAD goats (does) of about of 1-1$^{1/2}$ years were bought at open market in Akure and Itaogbolu with average weight of 10.50 ± 0.36 kg were randomly assigned to seven dietary treatments of three replicate per treatment in a 2 × 4 factorial Completely Randomized Design. Animals were housed in individual pens and offered fresh clean water. Prior to the commencement of the experiment, the WAD goats were vaccinated against PPR disease and treated against ecto-parasites. The goats were given daily ration at 5 % of their body weight. The feeding trial lasted for 56 days excluding the 2 weeks of adaptation. All animals were cared

for and managed according to the ethical approval and guidelines of NENT (2016).

Table 1 Gross composition (g/kg) of the experimental diets

Ingredient	A	B	C	D	E	F	G
Cassava peel	50	50	50	50	50	50	50
UBNS	6	-	-	-	-	-	-
TBNS	-	6	6	6	6	6	6
Wheat offal	24	24	24	24	24	24	24
Palm kernel cake	15	15	15	15	15	15	15
Bone meal	1	1	1	1	1	1	1
Urea	1	1	1	1	1	1	1
Salt	2	2	2	2	2	2	2
Premixes	1	1	1	1	1	1	1
Total	100	100	100	100	100	100	100

A= Untreated bambara nut shell; B = 25ml *Pleurotuspulmonarius*; C= 50ml *Pleurotuspulmonarius;* D = 75ml *Pleurotuspulmonarius;* E = 25ml *Aspergillus niger*; F = 50ml *Aspergillus niger;* G = 75ml *Aspergillus niger*; UBNS= untreated bambara nut shell TBNS = treated bambara nut shell

Data collection and laboratory analysis

Chemical composition of diets

Chemical analyses (proximate, fibre fractions) were carried out on the experimental diets, according to AOAC (2002), AOAC (2012), Van Soest *et al.* (1991) and Makkar *et al.* (1996) procedures. The fiber fractions measured were Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF) and Acid Detergent Lignin (ADL) and were determined according to Van Soest *et al.* (1991). Hemicellulose was calculated as the difference between NDF and ADF and cellulose as the differences between ADF and ADL.While the metabolizable energy (ME) will be calculated as follows:ME (kcal/kg DM) = (37 × %CP) + (81.8 × %NFE) (Pauzenga, 1985)

Ruminal fermentation characteristics trial

Approximately 30-40 mL of rumen liquor were collected from each goat using a suction tube, 3 hours after morning feeding, at the end of the digestibility trial. The rumen liquor samples were filtered through a single layer of cheesecloth to remove sediments, then 2 mL of the filtered rumen liquor was diluted in 4 mL of methyl green-formalin-saline solution, stored in glass bottles at room temperature in a dark place, and used to count protozoa (Elghandour *et al*., 2017).

For volatile fatty acids (VFA) analysis, a portion of the filtered rumen samples was thawed at 4°C and analyzed using high-performance liquid chromatography (HPLC). The filtrate was acidified with 5% sulfuric acid (H_2SO_4) solution and allowed to rest for 30 minutes. After centrifugation, the liquid was decanted and titrated with sodium hydroxide (NaOH) using phenolphthalein as an indicator. Standards for acetic, propionic, and butyric acids were prepared, and a wavelength of 210 nm was used for analysis. The titre values were used to determine the VFA concentrations (AOAC, 2011). Another portion of the filtered rumen samples was centrifuged, and the supernatant was collected for ammonia content analysis using the AOAC (2011) method, following Parsons, (2013) for rumen ammonia nitrogen (NH_3-N) determination.

Determination of rumen microbiota

The plate count method was used to determine the total bacterial and anaerobic bacterial counts. A sample of each goat's rumen liquor was examined for the presence of bacteria, fungi, and coliforms. The pour plate technique was

employed to measure colony-forming units per milliliter (cfu mL^{-1}) for bacteria and fungi, using nutrient agar and potato dextrose agar as culture media. Most ruminal bacterial species were cultivated on simple media containing carbohydrates (e.g., cellulose, starch, glucose), ammonia, trypticase, B-vitamins, heme, vitamin K derivatives, mineral salts, and reducing agents like sodium sulfide and L-cysteine. The plates were incubated at 37°C for 24 hours, and all visible colonies were counted at the end of incubation.

Data analysis

Data collected were subjected to two-way analysis of variance (ANOVA) procedure of SPSS (2017) version 21.0 using general linear model. Significant means were separated using the Duncan's multiple range test of the same software.

3. Result

Nutrient composition (%) of the experimental diets containing bambara nut shell treated with varied levels of *Pleurotus pulmonarius* and *Aspergillus niger*

The nutrient composition of experimental diets containing bambara nut shell treated with *Aspergillus niger* and *Pleurotus pulmonarius* is shown in Table 2a. Most parameters were significantly ($p < 0.05$) influenced by the organism, except dry matter, crude protein, neutral detergent fibre, acid detergent fibre, acid detergent lignin and hemicellulose. Diets treated with *Pleurotus pulmonarius* recorded higher crude fibre (18.40%), ether extract (9.20%) and hemicellulose (20.08%), whereas *Aspergillus niger* treatments had lower values (15.95%, 7.66% and 13.83%) but higher ash (11.26%) and nitrogen-free extract (35.34%). Treatment level also significantly affected nutrient composition, with 50 mL giving the highest crude fibre (20.43%), neutral detergent fibre (48.00%) and cellulose (40.50%), while 75 mL produced the lowest values (13.93%, 35.50% and 28.50%). Ash (11.17%) and nitrogen-free extract (39.50%) were highest at 75 mL, whereas the lowest ash (9.29%) and nitrogen-free extract (33.90%) occurred at 0 mL and 50 mL, respectively. Metabolizable energy ranged from 2625.44 kcal/kg at 50 mL to 2994.06 kcal/kg in the untreated diet. The interaction effect showed significant differences, with crude fibre ranging from 21.19% (50 mL *Pleurotus pulmonarius*) to 11.86% (75 mL *Aspergillus niger*), and metabolizable energy varying between 2505.40 kcal/kg (50 mL *Aspergillus niger*) and 3061.82 kcal/kg (untreated).

Volatile fatty acids production of WAD does fed diets containing bambara nut shell treated with varied levels of *Pleurotus pulmonarius* and *Aspergillus niger*

Table 3 shows the volatile fatty acid (VFA) production of WAD does fed bambara nut shell diets treated with *Pleurotus pulmonarius* and *Aspergillus niger*. All parameters were significantly ($p < 0.05$) influenced by the organism, with *Pleurotus pulmonarius* producing higher concentrations of acetic acid (7.31 mmol/100 mL), propionic acid (6.97 mmol/100 mL), butyric acid (6.67 mmol/100 mL), valeric acid (6.62 mmol/100 mL), total VFA (112.48 mmol/100 mL) and lactic acid (10.95 mmol/100 mL), compared with *Aspergillus niger* which recorded lower values (5.50, 4.86, 4.67, 4.63, 67.86 and 7.25 mmol/100 mL, respectively). Rumen pH was lower in *Pleurotus pulmonarius* (6.92) but higher in *Aspergillus niger* (7.13), while ammonia nitrogen was highest in *Pleurotus pulmonarius* (0.59%) and lowest in *Aspergillus niger* (0.49%). Treatment level also significantly affected VFA production, with 75 mL yielding the highest acetic (9.89 mmol/100 mL), propionic (9.42 mmol/100 mL), butyric (8.98 mmol/100 mL), valeric (8.95 mmol/100 mL), total VFA (140.78 mmol/100 mL) and lactic acid (14.28 mmol/100 mL), whereas 0 mL recorded the lowest values, including acetic acid (5.03 mmol/100 mL), propionic acid (4.81 mmol/100 mL), total VFA (69.21 mmol/100 mL) and lactic acid (7.03 mmol/100 mL). Ammonia nitrogen was highest at 50 mL (0.59%) and 25 mL (0.58%), while pH was lowest at 75 mL (6.52). The interaction effect showed that 75 mL *Pleurotus pulmonarius* produced the highest acetic acid (10.02 mmol/100 mL), propionic acid (9.56 mmol/100 mL), butyric acid (9.13 mmol/100 mL), valeric acid (9.09 mmol/100 mL), total VFA (142.11

mmol/100 mL) and lactic acid (14.42 mmol/100 mL). In contrast, the lowest values were observed in 50 mL *Aspergillus niger* diets, with total VFA of 57.43 mmol/100 mL, lactic acid of 5.83 mmol/100 mL, pH as low as 6.50 in 75 mL *Pleurotus pulmonarius*, and ammonia nitrogen ranging from 0.44 to 0.60 mmol/100 mL across treatments.

Microbial count of WAD does fed diets containing bambara nut shell treated with varied levels of*Pleurotus pulmonarius* and *Aspergillus niger*

Figure 1 shows the microbial count of WAD Does fed diets containing bambara nut shell treated with varied levels of*Pleurotus pulmonarius* and *Aspergillus niger*The least total bacteria count (5.24 cfu/mL) was obtained in does fed diet D while the highest (5.77 cfu/mL) was obtained in does fed diet E. The does fed diet E had the highest value (2.43 cfu/ml) total coliform count while the least value (2.30 cfu/mL) was obtained in does fed diet A. The least total protozoa value (2.58 cfu/mL) was observed in does fed diet G while the highest value (2.78 cfu/mL) was obtained in does fed diet C. (1.53 cfu/mL). The total fungi count with the highest value (1.56 cfu/mL) was observed in does fed diet F while the least value (1.48 cfu/ml) was obtained in does fed diet A.

Rumen microbial isolates of WAD does fed diets containing bambara nut shell treated with varied levels of *Pleurotus pulmonarius* and *Aspergillus niger*

The result of the total microbial isolates of WAD does fed diets containing bambara shell treated with varied levels of *Pleurotus pulmonarius* and *Aspergillus niger* is presented in Table 4. *Escherichia coli* was coliform isolated in all does fed diets containing Bambara shell treated with *Pleurotus pulmonarius* and *Aspergillus niger*. The total bacteria isolated in does fed diet A was (*Clostridiumwelchii, B. cereus*) diet B (*Clostridium welchii, Bacteria ruminicola*) diet C (*B. cereus, streptococcus lactis, Pseudomonas aureginosa*) diet D (*Clostridium welchii, Bacteriodes amylophilus*) diet E (*B. cereus, Klebsiella aerogens, Micrococcus luteus*) diet F (*Micrococcus acidophilus, Streptococcus faecium*) and diet G (*Staphloccocus aureus, Micrococcus luteus*). The total fungi isolated in all the does fed diets A, B and F include, (*Aspergillusniger* and *Fusariumoxysporum)*. *Penicilliumoxysporium* and *Fusariumoxysporum* was the isolate obtained in does fed diet C while *Aspergillus niger, Penicillium oxysporium* was the isolate observed in does fed diet D . The total fungi isolated in all the does fed diets E were, (*Aspergillus niger, Fusarium oxysporum)*.

Table 2: Nutrient composition (%) of the experimental diets containing bambara nut shell treated with varied levels of *Pleurotus pulmonarius* and *Aspergillus niger*

Source of variation	DM (%)	CP (%)	CF (%)	EE (%)	Ash (%)	NFE (%)	NDF (%)	ADF (%)	ADL (%)	HEM(%)	CEL (%)	ME (Kcal/kg)
Organism effect												
PP	93.28	19.50	18.40[a]	9.20[a]	10.85[b]	34.31[b]	43.33	37.33	17.25	6.00	20.08[a]	2687.76
ASP	93.58	19.56	15.95[b]	7.66[b]	11.26[a]	35.34[a]	39.33	32.00	18.17	7.33	13.83[b]	2606.43
±SEM	0.3	0.26	0.21	0.30	0.10	0.32	1.36	1.50	0.37	0.66	1.50	27.52
P-value	0.443	0.835	0.001	0.003	0.012	0.001	0.060	0.250	0.103	0.172	0.011	0.060

Treatment effect

0 mL		94.13	19.65	16.76^{b}	14.14^{a}	9.29^{b}	34.31^{b}	43.00^{ab}	34.00^{ab}	18.50^{ab}	9.00^{a}	15.50^{b}	2994.06^{a}
25 mL		93.31	19.20	17.18^{b}	7.75^{c}	10.85^{a}	38.34^{a}	40.50^{bc}	35.00^{ab}	19.50^{a}	5.50^{b}	15.50^{b}	2630.79^{b}
50 mL		93.55	19.93	20.43^{a}	8.15^{bc}	11.15^{a}	33.90^{b}	48.00^{a}	40.50^{a}	17.38^{bc}	7.50^{ab}	23.13^{a}	2625.44^{b}
75 mL		93.44	19.47	13.93^{c}	9.38^{b}	11.17^{a}	39.50^{a}	35.50^{c}	28.50^{b}	16.25^{c}	7.00^{ab}	12.25^{b}	2685.06^{b}
	±SEM	0.32	0.25	0.30	0.37	0.12	0.39	1.67	1.84	0.46	0.80	1.83	33.71
	P-value	0.876	0.156	0.001	0.020	0.167	0.001	0.001	0.002	0.001	0.221	0.003	0.408
PP	0 mL	94.13^{a}	19.65	16.76^{cd}	14.14^{a}	9.29^{c}	34.31^{c}	43.00	34.00	18.50^{b}	9.00^{a}	15.50^{b}	3061.82^{a}
	25 mL	94.07^{a}	19.19	17.93^{c}	11.54^{b}	11.53^{a}	38.69^{b}	41.00	38.00	18.25^{b}	3.00^{b}	19.75^{b}	2596.07^{cd}
	50 mL	92.33^{b}	19.53	21.29^{a}	10.60^{b}	10.55^{b}	30.37^{d}	51.00	44.00	15.00^{c}	7.00^{a}	29.00^{a}	2628.59^{cd}
	75 mL	93.47^{ab}	19.78	16.00^{d}	10.25^{bc}	10.47^{b}	36.98^{b}	38.00	30.00	18.50^{b}	8.00^{a}	11.50^{b}	2843.19^{b}
ASP	0 mL	94.13^{a}	19.65	16.76^{cd}	14.14^{a}	9.29^{c}	34.31^{c}	43.00	34.00	18.50^{b}	9.00^{a}	15.50^{b}	3061.82^{a}
	25 mL	92.56^{b}	19.2	16.43^{d}	12.77^{b}	10.17^{b}	37.99^{b}	40.00	32.00	20.75^{a}	8.00^{a}	11.25^{b}	2738.03^{bc}
	50 mL	94.77^{a}	20.33	19.58^{b}	14.59^{a}	11.75^{a}	37.43^{b}	45.00	37.00	19.75^{ab}	8.00^{a}	17.25^{b}	2505.40^{d}
	75 mL	93.41^{ab}	19.16	11.86^{e}	9.52^{d}	11.86^{a}	42.03^{a}	33.00	27.00	14.00^{c}	6.00^{ab}	13.00^{b}	2858.84^{b}
	±SEM	0.45	0.36	0.43	0.45	0.25	0.52	1.90	2.14	0.56	0.91	2.25	42.04
	P-value	0.002	0.179	0.014	0.014	0.001	0.001	0.731	0.548	0.001	0.026	0.057	0.021

^{abc} = means within the same row with different superscripts are significantly different (P<0.05). DM = dry matter; CP = crude protein; CF = Crude fibre; EE = Ether extract; ADF= Acid detergent fibre; NDF= Neutral detergent fibre; ADL= Acid detergent lignin; NFE= Nitrogen free extract; HEM = Hemicellulose; CEL = Cellulose; ME = Metabolisable energy; PP = *Pleurotus pulmonaris*; ASP = *Aspergillusniger* SEM = Standard error mean

Table 3 Volatile fatty acids productionof WAD does fed diets containing bambara nut shell treated with varied levels of *Pleurotus pulmonarius* and *Aspergillus niger*

Source of variation		Acetic acid (mmole /100ml)	Propionic acid (mmole/100ml)	Butyric acid (mmole/100ml)	Valeric acid (mmole/100ml)	TVFA (mmole/100ml)	Lactic acid (mmole/100ml)	pH	NH3-N (%)
Organism effect									
PP		7.31^{a}	6.97^{a}	6.67^{a}	6.62^{a}	112.48^{a}	10.95^{a}	6.92^{b}	0.59^{a}
ASP		5.50^{b}	4.86^{b}	4.67^{b}	4.63^{b}	67.86^{b}	7.25^{b}	7.13^{a}	0.49^{b}
	±SEM	0.42	0.44	0.4	0.41	6.45	0.65	0.13	0.04
	P-value	0.001	0.003	0.001	0.001	0.021	0.001	0.056	0.001
Treatment effect									
0 mL		5.03^{c}	4.81^{c}	4.53^{c}	4.49^{c}	69.21^{c}	7.03^{c}	7.28^{a}	0.50^{b}

25 mL		7.27[b]	6.85[b]	6.57[b]	6.55[b]	101.89[b]	10.35[b]	6.92[ab]	0.58[a]
50 mL		7.18[b]	6.77[b]	6.48[b]	6.46[b]	100.95[b]	10.26[b]	7.01[a]	0.59[a]
75 mL		9.89[a]	9.42[a]	8.98[a]	8.95[a]	140.78[a]	14.28[a]	6.52[b]	0.56[a]
	±SEM	0.41	0.42	0.39	0.40	6.42	0.63	0.12	0.03
	P-value	0.001	0.001	0.001	0.001	0.001	0.001	0.019	0.001
Organism *Treatment									
PP	0 mL	4.81	4.58	4.38	4.36	68.13	6.91	7.30	0.49
	25 mL	7.24	6.91	6.60	6.57	102.69	10.42	6.90	0.59
	50 mL	7.17	6.84	6.54	6.50	101.71	10.32	7.00	0.60
	75 mL	10.02	9.56	9.13	9.09	142.11	14.42	6.50	0.57
ASP	0 mL	4.81	4.58	4.38	4.36	68.13	6.91	7.30	0.49
	25 mL	5.08	4.85	4.63	4.61	72.03	7.31	7.30	0.48
	50 mL	4.05	3.86	3.69	3.67	57.43	5.83	7.20	0.44
	75 mL	7.38	7.04	6.72	6.69	104.63	10.62	6.90	0.56
	±SEM	0.45	0.47	0.43	0.44	6.60	0.67	0.14	0.04
	P-value	0.001	0.001	0.001	0.001	0.001	0.001	0.019	0.001

[abc]: Means with different superscripts within a row are significantly different (P<0.05). TVFA = Total volatile fatty acid, NH_3-N = Ammonia-nitrogen, PP = *Pleurotus pulmonarius* ASP = *Aspergillus niger*

Table 4 Rumen microbial isolates of WAD does fed diets containing bambara nut shell treated with varied levels of *Pleurotus pulmonarius* and *Aspergillus niger*.

Parameters	A	B	C	D	E	F	G
Total bacteria	*Clostridium welchii, B. cereus*	*Clostridium welchii, Bacteria ruminicola*	*B. cereus, streptococcus lactis, Pseudomonas aureginosa*	*Clostridium welchii, Bacteriodes amylophilus*	*B. cereus, Klebsiella aerogens, Micrococcus luteus*	*Micrococcus acidophilus, Streptococcus faecium*	*Staphloccocus aureus, Micrococcus luteus*
Total coliform	*E. coli*	*E. coli*	*E. coli*	*E. coli*	*E. coli*	*E. coli*	*E. coli*
Total fungi	*Aspergillus niger, Fusarium oxysporum*	*Aspergillus niger, Fusarium oxysporum*	*Penicillium oxysporium, Fusarium oxysporum*	*Aspergillus niger, Penicillium oxysporium*	*Fusarium oxysporum, Aspergillus flavus*	*Aspergillus niger, Fusarium oxysporum*	*Aspergillus niger*

A = untreated B = 25 ml *P. pulmonarius* inclusion, C = 50 ml *P. pulmonarius* inclusion, D = 75 ml *P. pulmonarius* inclusion, E = 25 ml *A. niger* inclusion, F = 50 ml *A. niger* inclusion, G = 75 ml *A. niger* inclusion.

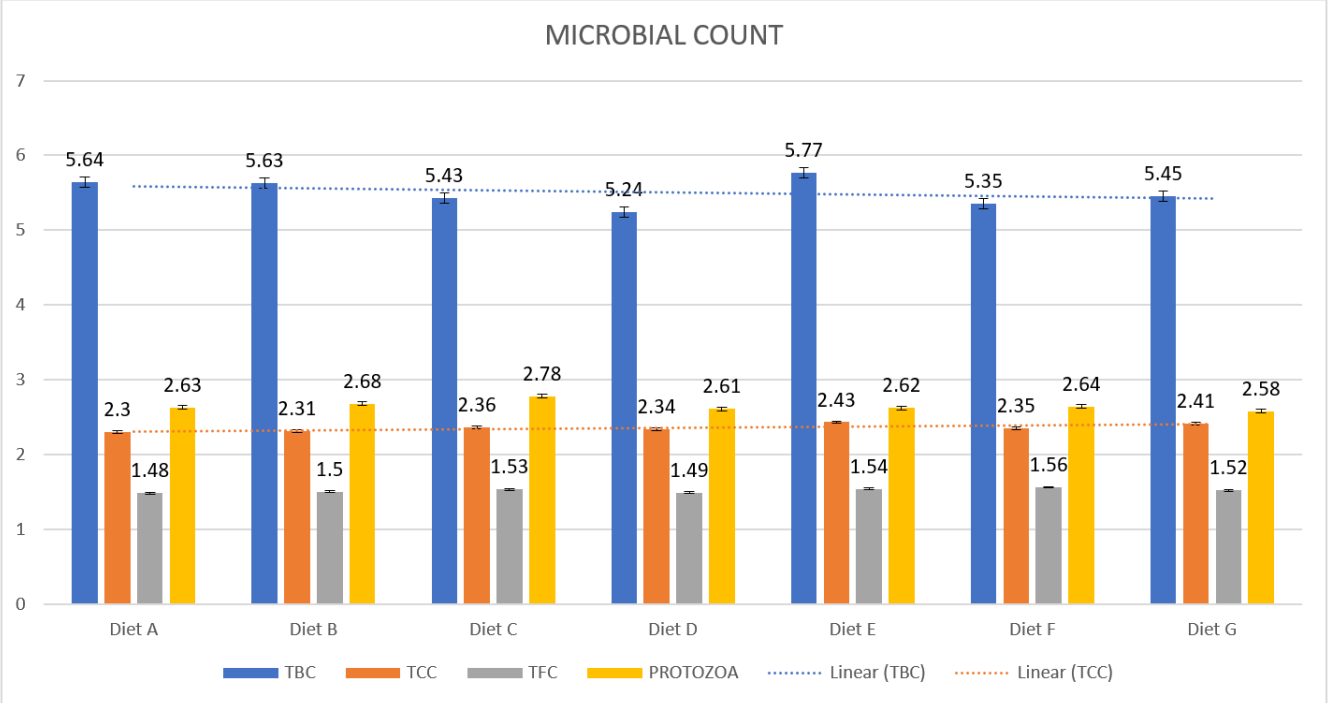

Figure 1 **Microbial count of WAD Does fed diets containing bambara nut shell treated with varied levels of Pleurotus pulmonarius and Aspergillus niger**

4. Discussion

Nutrient composition (%) of the experimental diets containing bambara nut shell treated with varied levels of *Pleurotus pulmonarius* and *Aspergillus niger*

The chemical composition of bambara nut shell treated with *Pleurotus pulmonarius* and *Aspergillus niger* showed high dry matter contents (92.40–93.00%), reflecting low inherent moisture and effective fungal fermentation, and exceeding values reported for *P. pulmonarius*-treated cocoa bean shell meal (Fajemisin *et al*., 2018; Borreani *et al*., 2018). Crude protein values were comparable to earlier reports (19.73–28.88%) and met the minimum 8% requirement for ruminants, with the highest value recorded in the 50 mL *Aspergillus niger* treatment (20.33%), consistent with Norton (2003). The increase in crude protein and reduction in crude fibre at higher inclusion levels, particularly at 75 mL *Pleurotus pulmonarius*, were attributed to enhanced fungal biomass and enzymatic activity, as reported by Hassan *et al*. (2013). Neutral detergent fibre values compared favourably with earlier findings, while acid detergent fibre and lignin contents were lower than those reported for *Aspergillus*-treated cassava waste diets (Belewu and Fagbcmi, 2007). Although cellulose and hemicellulose values were lower than those reported by Fajemisin *et al*. (2018), they remained adequate to support rumen function and fibre utilisation in line with Owens and Basalan (2016). Nitrogen-free extract, ash and metabolizable energy values aligned with previous reports and exceeded earlier energy values for similar substrates, indicating improved dietary energy availability and potential for enhanced ruminant performance (Omotoso *et al*., 2019; Zuidhof, 2019).

Volatile fatty acids production of WAD does fed diets containing bambara nut shell treated with varied levels of *Pleurotus pulmonarius* and *Aspergillus niger*

Volatile fatty acids (VFAs) are major ruminal fermentation products that supply up to 70% of the energy requirements of West African Dwarf (WAD) does and are essential for growth,

reproduction and milk synthesis (Oni *et al*., 2012; Aluwong *et al*., 2010). In this study, total VFA, acetic, propionic, butyric and lactic acid concentrations followed similar trends across diets, indicating effective ruminal degradation of bambara nut shell treated with *Pleurotus pulmonarius* and *Aspergillus niger*. Higher acetate values compared with propionate across treatments reflected the high-fibre nature of the diets, which favours acetate production and supports milk fat synthesis, in agreement with Okoruwa and Agbede (2019). The observed rumen pH values (6.57–7.30) were within the optimal range for microbial activity, confirming a stable rumen environment conducive to efficient fermentation (Jallow and Hsia, 2011; Nagaraja and Titgemeyer, 2007). Rumen ammonia-nitrogen concentration was highest in the 50 mL *Pleurotus pulmonarius* treatment but remained within the optimal range (0.5–2.0%) required for microbial growth and protein synthesis (Russell and Rychlik, 2001). Overall, the enhanced VFA production, stable rumen pH and adequate NH_3-N levels suggest improved nutrient utilisation and energy availability in WAD does fed microbial-treated bambara nut shell diets (Adeoye *et al*., 2021).

Rumen microbial isolates of WAD does fed diets containing bambara nut shell treated with varied levels of *Pleurotus pulmonarius* and *Aspergillus niger*

Rumen microbial isolates play a crucial role in fibre degradation, fermentation efficiency and overall productivity of West African Dwarf (WAD) does, particularly through the activities of cellulolytic and fermentative microorganisms (Ogunbosoye and Babayemi, 2010; Salami *et al*., 2018). In this study, total bacteria, fungi and coliform counts varied with dietary treatments containing bambara nut shell treated with *Pleurotus pulmonarius* and *Aspergillus niger*, indicating a clear dietary influence on rumen microbial ecology. The bacterial isolates identified included *Bacteroides*, *Clostridium*, *Klebsiella aerogenes*, *Staphylococcus aureus*, *Micrococcus* spp. and *Streptococcus* spp., reflecting a diverse microbial community involved in carbohydrate and protein metabolism (Zhou *et al*., 2015). Notably, *Clostridium welchii* was absent in does fed diets treated with 25, 50 and 75 mL *Aspergillus niger*, likely due to the antifungal and antimicrobial metabolites produced by *A. niger* that suppress pathogenic bacteria (Sharma *et al*., 2022). The presence of fibre-degrading genera such as *Bacteroides* and *Streptococcus* spp. supports effective polysaccharide breakdown and nutrient availability in the rumen (Russell and Rychlik, 2001; Bayer *et al*., 2008). Overall, the observed microbial profile suggests that microbial-treated bambara nut shell diets promote a favourable rumen environment that supports efficient fermentation, fibre utilisation and improved animal performance in WAD does (Weimer, 2022).

Microbial count of WAD does fed diets containing bambara nut shell treated with varied levels of *Pleurotus pulmonarius* and *Aspergillus niger*

Microbial count in the rumen is an important indicator of rumen health, fermentation efficiency and nutrient utilisation in West African Dwarf (WAD) does (Ogunbosoye and Babayemi, 2010). In this study, the inclusion of bambara nut shell treated with *Pleurotus pulmonarius* and *Aspergillus niger* increased bacterial, fungal, coliform and protozoal counts, although values varied across treatments, with the 75 mL *P. pulmonarius* diet recording the lowest counts. The highest total bacterial count was observed in does fed diet F and exceeded the 2.44×10^{-6} cfu/mL previously reported by Omotoso *et al*. (2022), suggesting enhanced fibre degradation and microbial proliferation. Increased bacterial population implies improved fermentation of plant cell wall carbohydrates and greater microbial protein supply to the host animal (Russell *et al*., 2009). The rumen environment remained stable, as microbial counts are closely linked to rumen ammonia concentration and pH, both of which are influenced by diet composition (Choudhury *et al*., 2015). Compared with earlier reports, protozoal counts were lower than the 200 protozoa/mL reported by Adebayo *et al*. (2017), whereas fungal and coliform counts were higher than those reported by Omotoso *et al*. (2022),

indicating dietary modulation of rumen microbial ecology.

5. Conclusion

Microbial treatment of bambara nut shell significantly improved rumen fermentation characteristics, volatile fatty acid production and microbial activity in West African Dwarf does. Diet containing 75 mL *Pleurotus pulmonarius* consistently produced the highest total volatile fatty acids, favourable rumen pH and optimal ammonia-nitrogen levels. Therefore, the 75 mL *Pleurotus pulmonarius*-treated bambara nut shell diet is recommended as the best option for improving rumen efficiency and nutrient utilisation in WAD does.

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