

## In Vitro Digestibility, Volatile Fatty Acids, Rumen Microbial Population and Identification of West African Dwarf does Fed Concentrate Diets Containing Graded Levels of Cassava Leaf Meal

<sup>1</sup>Kwaghfan, S., <sup>2</sup>Oni. A. O., <sup>2\*</sup>Adewumi O. O and <sup>3</sup>Adelusi O.O.

<sup>1</sup>Department of Animal Nutrition, College of Animal Science, Joseph Sarwuan Tarka University, P.M.B 2373, Makurdi, Nigeria

<sup>2\*</sup>Department of Animal Production and Health, College of Animal Science and Livestock Production, Federal University of Agriculture, P.M.B 2240, Abeokuta, Ogun State, Nigeria

<sup>2&3</sup>Department of Animal Nutrition, College of Animal Science and Livestock Production, Federal University of Agriculture, P.M.B 2240, Abeokuta, Ogun State, Nigeria

**Corresponding author:** [kwaghfan.saaondo@uam.edu.ng](mailto:kwaghfan.saaondo@uam.edu.ng); Phone: +2348159800242

**Target audience:** Ruminant Animal Scientists; Nutritionists; and Feed millers.

### Abstract

This study evaluated the *in vitro* digestibility, volatile fatty acids (VFAs), and rumen microbial population of West African Dwarf (WAD) does fed concentrate diets containing varying levels of cassava leaf (CL) meal. Fresh TME 419 cassava leaves were sun-dried, milled, and incorporated into the diets at 0, 10, 20, and 30% inclusion levels. Key parameters measured included *in vitro* gas production, total VFAs, pH, temperature, *in vitro* dry matter digestibility (IVDMD), short chain fatty acids (SCFA), carbon dioxide, and methane. Results indicated that total VFA was significantly ( $P < 0.05$ ) influenced by CL inclusion, peaking at 79.30 mM/100ml with 30% CL and lowest (73.60 mM/100ml) at 20% CL. At 0-hour post-feeding, fungi population varied significantly, with the highest count ( $1.47 \times 10^5$  cfu/ml) at 30% CL and the lowest ( $1.10 \times 10^5$  cfu/ml) at 10% CL. At 6-hour post-feeding, the highest fungi count ( $1.85 \times 10^5$  cfu/ml) occurred at 10% CL. Additionally, total VFA was highest (200 mM/100ml) at 20% CL inclusion at 6-hour post-feeding. Ammonia nitrogen levels were significantly affected at 0-hour post-feeding, with the highest value (47.63 mg/100ml) observed at 0% CL inclusion. The study concluded that incorporating up to 30% CL in concentrate diets enhances *in vitro* VFA production and total protozoa counts of WAD does.

**Keywords:** In vitro digestibility; feed evaluation; rumen microorganisms; cassava leaf.

### Description of Problem

It takes a lot of money and effort to evaluate the quality of feed using animals. Following this, Scientists have been investigating other approaches to feed evaluation. In ruminant nutrition research, *in vitro* digestibility studies are essential because they provide a

controlled and effective way to assess feed ingredients (1). These techniques spare researchers from the difficulties and moral dilemmas of *in vivo* trials, enabling them to model and examine ruminant digestive processes (2).

The capacity to replicate particular parts of

the ruminant digestive system, especially the rumen, is a major benefit of *in vitro* methods (3). Scientists can learn more about the fermentative and digestive properties of different feeds by simulating the parts of the ruminant's digestive system. In addition to lowering the need for live animals, this method also saves money and labor compared to conventional *in vivo* research (4).

Moreso, the accuracy and relevance of *in vitro* research have been significantly improved by technological developments. For instance, the creation of artificial fermenters has increased the *in vitro* processes' operational capacity and made it possible to simulate the rumen environment more accurately (5). These developments lead to more accurate data, which makes it easier to forecast how feeds will function in real ruminant digestive systems (6).

Furthermore, the "Three Rs" in animal research: reduce, refine, and replace are ethically consistent with *in vitro* techniques (7). By employing these methods, scientists can reduce the number of animals they utilize in research, improve experimental protocols to minimize harm, and, in certain situations, do away with animal models completely (8). In modern research environments, this ethical alignment is becoming more and more crucial (2).

This study, therefore, assess the *in vitro* digestibility and rumen microbial population of West African dwarf does fed concentrate diets containing graded levels of cassava leaf meal.

## Materials and Methods

### Experimental Site

The *in vitro* studies, volatile fatty acids and ammonia nitrogen determination were

carried out at the Department of Animal Nutrition, College of Animal Science and Livestock Production, Federal University of Agriculture, Abeokuta (FUNAAB). Microbial population and identification were done at Microbiology Laboratory, College of Veterinary Medicine, FUNAAB. The area falls within Latitude 7°10'N and Longitude 3°E in Odeda Local Government Area of Abeokuta, Ogun state, Nigeria. The area has a tropical climate characterized with an annual rainfall of about 1,037mm; minimum and maximum temperature of 20.66°C and 35.48°C, respectively with a relative humidity of 80%. The vegetation lies in between the tropical rainforest and derived savannah (9)

### Source and processing of cassava leaf meal.

Fresh cassava leaves together with the petiole (TME 419 variety) were obtained after root harvest from Centre for Excellence in Agricultural Development and Sustainable Environment (CEADESE) Farm, Federal University of Agriculture, Abeokuta. The leaves with petiole were sun-dried until they became very brittle. The sun-dried leaves were crushed using a hammer mill. The crushed cassava leaves were included in the formulated concentrate diets at 0, 10, 20 and 30, representing T1, T2, T3, and T4, respectively, as shown in Table 1

### Experimental animals and management

Twenty (20) West African dwarf (WAD) does of average weight of 15.20±0.61kg were collected free of monetary cost from Sheep and Goats Unit, Directorate of University Farm (DUFARM), Federal University of Agriculture, Abeokuta, for the study. The animals were immediately transferred to metabolic cages and were served four dietary

**Table 1: Gross composition (%) of the experimental concentrate diets**

Ingredients	Level of inclusion of cassava leaves			
	0%	10%	20%	30%
Yellow Maize	5.00	5.00	5.00	5.00
Dried Cassava Peel	40.00	40.00	40.00	40.00
Cassava Leaf	0.00	10.00	20.00	30.00
Brewer's Dry Grain	25.00	15.00	7.00	0.00
Palm Kernel Cake	20.00	21.00	20.00	19.00
Groundnut Cake	5.00	4.00	3.00	1.00
Bone Meal	2.00	2.00	2.00	2.00
Limestone	1.50	1.50	1.50	1.50
Salt	1.00	1.00	1.00	1.00
Premix	0.50	0.50	0.50	0.50
<b>Total</b>	<b>100.00</b>	<b>100.00</b>	<b>100.00</b>	<b>100.00</b>

Premix (each kg contains): Vitamin A: 10,000 IU; Vitamin E: 70,000 IU; Vitamin D: 1,600,000 IU; Fe: 50 g; Zn: 40 g; Mn: 40 g; Co: 0.1 g; Cu: 10 g; Se: 0.1 g; I: 0.5 g.

treatments consisting of 0, 10, 20 and 30% cassava leaf meal, respectively, in a Completely Randomized Design (CRD), with each treatment having five animals and each animal representing a replicate. The animals were fed the experimental diets for seven days before rumen fluid was collected from them.

#### ***In vitro* gas production and digestibility**

The concentrate diets comprising 0, 10, 20 and 30% cassava leaf meal-based diets were subjected to *in vitro* degradability study. Using a suction tube and the collection technique outlined by (10), rumen fluid was extracted from WAD goats. In the morning, the **rumen** fluid was gathered and placed in a thermos flask that had been preheated. Using

60ml calibrated transparent plastic syringes with a fitting silicon tube was part of the incubation process. After carefully weighing the diet samples (200 mg DM), they were placed in the incubation bags, sealed, and dropped into the syringe. Next, 30ml of inoculum (**comprising of rumen fluid and buffer solution**) was added.

To remove air from the inoculum, the syringes were inverted, tapped, and the piston forced the contents upward. To stop gas from escaping, a metal and plastic clip was used to tighten the silicon tube in the syringes. In the incubator, the syringes were positioned with care and kept at 39°C. The gas volume was measured at 3, 6, 9, 12, 15, 18, 21, 24, 30, 33, 36, 39, 42, 45, and 48 hours

after the incubation period, which was conducted at 39°C (10). To determine the amount of methane produced, 4 milliliters of NaOH were added during the post-incubation time (11).

The mean volume of gas produced from

blank syringe was deducted from the gas volume produced from samples. After estimating methane, bags containing residue were dried in the oven at 65°C for 24 hours, weighed and digestibility calculated as follows:

$$\text{IVDMD (\%)} = \frac{\text{initial dry matter incubated} - \text{final dry matter of residue}}{\text{initial dry matter incubated}} \times 100$$

The organic matter digestibility, metabolizable energy, and short chain fatty acids were calculated using the following formulae:

$$\text{OMD} = 14.88 + 0.889\text{GV} + 0.45\text{CP} + 0.651\text{XA} \quad (12)$$

$$\text{ME} = 2.20 + 0.136\text{GV} + 0.057\text{CP} + 0.0029\text{CF} \quad (12)$$

$$\text{SCFA} = 0.0239\text{GV} - 0.0601 \quad (13)$$

Where GV, CP, CF, and XA are the net gas production (ml/200mg DM), crude protein, fibre and ash of the incubated samples, respectively.

#### **Determination of *in vitro* volatile fatty acid, pH and temperature.**

Incubation fluid was collected in a bottle already rinsed with about 1ml of 5% metaphosphoric acid solution at the end of the incubation period for each of the treatment and stored-frozen at -20°C in an air-tight bottle container for the determination of volatile acid concentration and ammonia nitrogen.

Total volatile fatty acid production was determined by steam distillation process using Markham micro distillation apparatus (14). Ammonia concentration was determined according to (15) standard methods. The temperature and pH were measured using pH metre

#### **Methane gas determination**

The volume of methane gas produced by each dietary treatment was determined by dispensing 4ml of 10% sodium hydroxide into each incubated sample at the end of 48hrs of incubation period. Sodium hydroxide was added to absorb Carbon IV Oxide produced during the process of fermentation. The remaining volume of gas was recorded as methane; according to the method of (11).

#### **Microbial count and identification.**

Seven days after feeding, rumen fluid was collected at 0-hour and 6-hours post-feeding with a suction tube through the mouth of the goats. The collected fluid was sieved using a cheese layer cloth to separate the fluid from the unfermented feed particles. The sieved fluid was taken to the Laboratory for the analysis of microbial count and identification using Serial Dilutions and Pour Method.

#### **Volatile fatty acid, pH, Temperature and ammonia-nitrogen determination.**

Rumen liquor was collected from the West African Dwarf does at 0-hour and 6-hour post-feeding using a suction tube. The collected fluid was sieved using a cheese layer cloth to separate the fluid from the unfermented feed particles. The fluid was poured into a bottle already rinsed with about

1ml of 5% metaphosphoric acid solution at the end of the incubation period for each of the treatments and stored-frozen at -20°C in an air-tight bottle container for the determination of volatile acid concentration and ammonia nitrogen

Total volatile fatty acid production was determined by steam distillation process using Markham micro distillation apparatus (14). Ammonia concentration was determined according to (15) standard methods. The temperature and pH were measured using pH metre

#### Statistical analysis

The data obtained from the study was analyzed with (16) using One –way analysis of variance (ANOVA). Where significant difference was observed, treatment means were separated using Duncan Multiple Range Test.

#### Statistical model

$$Y_{ij} = \mu + T_i + \sum_{ij}$$

Where:

$Y_{ij}$  = observed value of the dependent variables

$\mu$  = population mean

$T_i$  = effect of the  $i$ th dietary treatment

$\sum_{ij}$  = random residual error

#### Results and discussion

##### ***In vitro* gas production (ml/200mgDM) of concentrate diets containing graded levels of cassava leaf meal.**

Table 2 shows the *in vitro* gas production of concentrate diets containing varying levels of cassava leaf meal. The result indicates that, throughout the incubation period, there was significant difference ( $P < 0.05$ ) in the rate of gas production at 3 and 9 hours respectively. At 3 hours, diets containing

30%, and 10% cassava leaf meal showed superior gas production, while the treatments with 0 and 20% had the least volume of gas production. At 9 hours of incubation, the diet containing 10% cassava leaf meal had the highest gas production, but was statistically similar with diets containing 0%, 20%, and 30% cassava leaf meal respectively.

The *in vitro* gas production of concentrate diet containing graded level of cassava leaves showed variation in gas production at the 3 and 9 hours of incubation respectively. This result differed from the findings of (17) who reported variation in gas production at 33, 42 and 45 hours of incubation. This difference may be as a result of the different test ingredients used. At 3 hours of incubation, diets containing 30%, 10%, 0% and 20% cassava leaves showed superior total gas production of 2.00ml, 2.00ml, 1.33ml and 1.00ml respectively, indicating digestion was taking place at higher rates. However, the total gas produced at 3 hours was superior to that reported by (18) and (17). The highest total gas production at 48 hours of incubation was similar to the highest gas production of 29.5ml/200mg DM recorded by (17).

##### ***In vitro* volatile fatty acids, temperature and pH of diets containing graded levels of cassava leaf meal.**

Table 3 shows the total *invitro* volatile fatty acid, temperature and pH of diets containing graded levels of cassava leaf meal.

The result shows that there was significant ( $P < 0.05$ ) difference in the concentration of volatile fatty acids across the treatment groups. The total volatile fatty acids (VFAs) were highest in the concentrate diets containing 30% ,0%, and 10% cassava leaf meal respectively. However, the treatments containing 0% and 10% cassava leaf meal

**Table 2: In vitro gas production (ml/200mgDM) of concentrate diets containing graded of cassava leaf meal**

Hours of incubation	Level of inclusion of cassava leaves				SEM	P
	0%	10%	20%	30%		
3	1.33 <sup>a</sup>	2.00 <sup>a</sup>	1.00 <sup>ab</sup>	2.00 <sup>a</sup>	0.67*	0.03
6	1.33	2.00	1.00	2.00	0.91 <sup>ns</sup>	0.08
9	5.33 <sup>ab</sup>	6.00 <sup>a</sup>	5.67 <sup>ab</sup>	5.33 <sup>ab</sup>	0.78*	0.01
12	7.33	9.33	8.67	8.33	1.20 <sup>ns</sup>	0.11
15	10.67	13.00	12.67	12.33	1.37 <sup>ns</sup>	0.08
18	13.67	16.67	15.67	15.33	1.96 <sup>ns</sup>	0.35
21	16.00	19.33	18.00	18.67	2.17 <sup>ns</sup>	0.29
24	19.00	21.67	21.33	20.67	2.40 <sup>ns</sup>	0.53
27	21.33	24.33	23.33	22.67	2.69 <sup>ns</sup>	0.54
30	23.00	25.67	24.67	24.00	2.80 <sup>ns</sup>	0.59
33	24.33	26.33	25.33	25.00	2.67 <sup>ns</sup>	0.67
36	25.00	27.00	26.33	25.00	2.80 <sup>ns</sup>	0.60
39	26.33	27.67	27.33	25.67	3.03 <sup>ns</sup>	0.58
42	27.33	28.33	27.67	26.33	2.92 <sup>ns</sup>	0.64
45	27.67	28.33	28.00	27.00	2.80 <sup>ns</sup>	0.58
48	28.33	29.00	29.33	27.67	2.80 <sup>ns</sup>	0.49

<sup>abc</sup>Means with different superscripts along the same row are significantly different (P<0.05)

SEM=standard error of mean ; P= probability level ;

\*=significantly different; ns= not significant

were statistically similar with the one having 20% cassava leaf meal.

The temperature varied significantly (P<0.05) across the treatment groups. The treatment with 30% and 20% cassava leaf meal had the highest temperatures. The lowest temperature was observed in the treatment with 0% cassava leaf meal. There was no significant difference (P>0.05) in the pH of the incubation fluid across the treatment groups. The values of the *in vitro* total volatile fatty acids obtained in the study were lower than the values of 120.38 ± 5.62 to 160.73 ± 15.00mM reported by (19). Volatile fatty acids are the major source of energy to ruminants (5). The pH obtained in

this study was in the normal range of 6.2 to 7.0 (neutral to slightly acidic) ideal for all rumen microbes. However, the temperature was lower than the ideal temperature (39°C) of the rumen. This low temperature may be attributed to the low ambient temperature observed post-incubation.

#### ***In vitro* post-incubation parameters of diets containing graded levels of cassava leaf meal**

Table 4 shows the *in vitro* post-incubation parameters of diets containing graded levels of cassava leaf meal. No significant (P>0.05) difference was observed on all parameters under consideration. The IVDMD ranged

**Table 3: In vitro total volatile fatty acids, temperature, and pH of concentrate diets containing graded level of cassava leaf meal**

Parameter	Level of inclusion of cassava leaves				SEM	P
	0%	10%	20%	30%		
TVFA (mM/100ml)	77.70 <sup>ab</sup>	77.20 <sup>ab</sup>	73.60 <sup>b</sup>	79.30 <sup>a</sup>	23.27*	0.05
Temperature(°C)	30.25 <sup>d</sup>	30.55 <sup>bc</sup>	30.75 <sup>ab</sup>	30.85 <sup>a</sup>	0.12*	0.00
pH	6.35	6.46	6.02	6.39	0.20 <sup>ns</sup>	0.08

<sup>abcd</sup>Means with different superscript along the same row are significantly different

SEM= standard error of mean, P= probability level, \* = significantly different; ns= not significant; TVFA= total volatile fatty acid

from 62.50 to 80.83%, while ME was in the range of 5.21 to 5.77MJ/kg DM. The organic matter digestibility (OMD) was in the range of 57.81-80.66%. The short chain fatty acids in the study were in the range of 0.38 to 0.44  $\mu$ mol, while carbon dioxide ranged from 6.33 to 8.33ml/200mg DM. Methane gas produced was in the range of 19.33 to 23.00/200mg DM.

The *in vitro* dry matter digestibility obtained in the study was higher than 46.6 to 66.9% reported by (20) and 55.1 to 60.6% recorded by (18). This points to the fact that; the inclusion of cassava leaves improved the dry matter digestibility of the feed. The metabolizable energy obtained in this study was lower than 9.05 to 9.91MJ/Kg DM reported by (17). The short chain fatty acids (SCFAs) obtained in the study was higher than the value of 0.26 to 0.37 $\mu$ mol reported by (17). Ruminants depend on SCFAs for up to 80% of their maintenance energy requirements (5). As documented by (21), in addition to contributing to global warming, methane production results to 10-12% loss of

dietary feed energy from ruminant animals. The total methane recorded in the study was higher than 13 to 15ml/200mg DM reported (17) while the carbon dioxide values were lower than 5.5 to 15 ml/200mg DM documented by the same authors.

#### **The effects of Cassava leaf meal inclusion on microbial count and identification of West African Dwarf Does**

Table 5 shows the effects of cassava leaf meal inclusion on the microbial count and identification of West African Dwarf does at zero- and six-hours post-feeding, respectively. The result shows that at zero-hour post-feeding, the total bacteria count (TBC x 10<sup>5</sup>cfu/ml) and total protozoan count (Cell/ml) were not significantly (P>0.05) varied across the treatment groups. However, the total fungi count (TFC x 10<sup>5</sup>cfu/ml) showed a significant (P<0.05) difference across the

**Table 4: In vitro post-incubation parameters of concentrate diets containing graded levels of cassava leaf meal**

Parameters	Level of inclusion of cassava leaves				SEM	P
	0%	10%	20%	30%		
IVDMD (%)	68.33	80.83	72.50	77.50	10.78 <sup>ns</sup>	0.07
OMD (%)	59.83	65.31	80.66	57.81	7.82 <sup>ns</sup>	0.08
ME (MJ/Kg DM)	5.21	5.77	5.77	5.74	0.33 <sup>ns</sup>	0.25
CO <sub>2</sub> (ml/200mg DM)	7.33	8.00	6.33	8.33	1.34 <sup>ns</sup>	0.19
CH <sub>4</sub> (ml/200mg DM)	21.00	21.00	23.00	19.33	3.01 <sup>ns</sup>	0.63
SCFA (µmol)	0.38	0.44	0.43	0.42	0.05 <sup>ns</sup>	0.49

SEM= standard error of mean; P= probability level; ns= not significant; IVDMD= in vitro dry matter digestibility; ME= metabolizable energy; MJ/kg DM = megajoule per kilogram dry matter; =; SCFA=short chain fatty acid; CO<sub>2</sub>= carbon dioxide; CH<sub>4</sub>= methane; OMD= organic matter digestibility

treatments. The treatment with 0% level of inclusion of cassava leaf meal had the least bacteria count, followed by the treatment with 10%, while 20% and 30% were similar. The total bacteria count ranged from  $1.1 \times 10^5$  cfu/ml to  $1.2 \times 10^5$  cfu/ml, while TFC was in the range of  $1.1 \times 10^5$  cfu/ml to  $1.47 \times 10^5$  cfu/ml, and 450 to 1050 TPC/ml for protozoa. A pattern of variation was observed, as the level of inclusion increases, the number of fungi count increased. At the zero-hour post-feeding, three microorganisms were isolated, namely bacteria, fungi and protozoa. At 0% level of inclusion, the bacteria isolated were the *Bacillus species*, *Lactobacillus species*, *Escherichia coli*, and the *Pseudomonas species*; 10% had the same species of bacteria except *Pseudomonas species*

which was absent and *Enterobacteria species* was present; the treatment with 20% cassava leaf meal inclusion showed the presence of *Lactobacillus species*, *Escherichia coli*, *Pseudomonas species* and *Enterobacteria species* while *Bacillus species*, *Lactobacillus species*, *Escherichia coli*, *Pseudomonas species* and *Enterobacteria species* were isolated in the rumen fluid of goats fed diets containing 30% cassava leaf meal. Across the treatment groups, *Aspergillus niger*, *Fusarium species* and *Mucor mucedo* species of fungi were isolated, while only *Holostrich specie* of protozoa was isolated in all the treatments.

At 6 hours post-feeding, the total number of bacteria count was not significantly ( $P > 0.05$ ) different across all the treatment groups and ranged from  $1.35 \times$

10<sup>5</sup>cfu/ml to 1.40 x 10<sup>5</sup>cfu/ml. The TFC was significantly (P<0.05) different across the treatments, with goats fed diets containing 10% cassava leaf meal having the highest TFC, followed by goats fed 20% and 30%. Goats fed diets containing 0% cassava leaf meal had statistically similar value as those fed 10%, 20% and 30% cassava leaf meal respectively. The TFC in the study ranged from 1.1 x 10<sup>5</sup>cfu/ml to 1.85 x 10<sup>5</sup>cfu/ml. The TPC was highly significant among the treatments. Goats fed diets containing 30% cassava leaf meal had the highest TPC of 900/ml while 10% had 250/ml. The TPC of goats offered diets containing 0%, 10% and 20% were all similar. At the 6<sup>th</sup> hour post-feeding, three microorganisms were isolated, namely: bacteria, fungi and protozoa. The bacteria species such as, *Bacillus species*, *Lactobacillus species*, *Escherichia coli*, *Pseudomonas species*, and *Enterobacteria species* were isolated in the rumen fluids of the goats fed diets containing 0%, 10%, 20% and 30% cassava leaf meal respectively. The fungi isolated were: *Aspergillusniger*, *Fusarium species* and *Mucormucedo* across the treatments. The only protozoa specie isolated across the treatment groups was *Holostrich specie*. Plant components are broken down in the rumen by rumen bacteria (22). According to (23), the rumen's nutritional availability, which promotes microbial growth, may be the cause of the microbe population density. According to (17),

when West African dwarf goats were fed diets with or without urea-treated corncob, the overall bacterial population at 0 hours after feeding was comparable to 1.02 to 1.60×10<sup>4</sup>cfu/ml. Because the times for collecting rumen fluid were comparable in both studies, the narrow range in the number of bacteria was likely caused by this. As a result, collection was carried out at the zero hour. However, (17) linked the population of rumen bacteria to the pH and ammonia concentration of rumen fluid, both of which are influenced by the kind of diet that animals are fed. The bacterial population grew numerically six hours after feeding. The results of (22), who found that dietary interventions altered microbial populations and rumen fermentation, are in line with this. The same kinds of bacteria were found and isolated at 0 and 6 hours after feeding. Anaerobically grown *Bacillus species* are among the hemicellulolytic, cell-associated bacteria that produce polysaccharidases that can liberate reducing sugars from hemicellulose, xylan, and arabinan, according to research by (24).

Lactic acid production is a well-known characteristic of the *Lactobacillus species*. In their investigation, (25) discovered the unique *E. coli*, ZH-4, which secreted these enzymes outside of cells in addition to having a full cellulase system that it contained on its own. Among other bacteria, *Pseudomonas* and *Bacillus species* were categorized as

cellulolytic by (25). It's possible that the *Enterobacterium* in the goats' rumen came from the grass, dirt, straw, and leaves they ate. This species of bacteria can break down the cellulose in the meal, according to (26).

Among the isolated bacteria, *Lactobacillus species*, *Pseudomonas species* and *Escherichia coli* were similar to those reported by (17) when goats were fed diets with or without urea treated corncob. However, (27) reported that the main rumen bacteria species are *Fibrobacter succinogens*, *Ruminococcus amylophilus*, *Prevotella ruminicola*, *Butyrivibrio fibrosolvens*, *Ruminococcus spp.*, *Selenomonas ruminantum*, *Streptococcus bovis*, *Eubacterium ruminatum*, *Lactobacillus spp.* And *Megasphaera elsdenii*. In the current study, only *Lactobacillus spp* was similar to those identified by these authors. This could be explained by differences in the isolation methods used. It has been noted that culture-based methods greatly underestimate the population of rumen bacteria (28). It has been demonstrated that rumen fungi can break down cellulose and xylans, suggesting that they could aid in the ruminant host's digestion of plant materials (29). Up to 8–12% of the microbial biomass in rumen is made up of rumen anaerobic fungi, which actively colonize plant cell walls (30). *Caecomyces cummunis*, *Piromyces cummunis*, and *Neocallismastix frontalis* have been shown to efficiently contribute to ruminant fiber degradation (31, 32).

Cellulases, hemicellulases, xylanases, avicelases, glycosidases, and other highly active fiber-degrading enzymes are secreted in high concentrations by these fungi and have been linked to rhizomycelia (33, 34). However, *Aspergillus niger* *Fusarium species*, and *Mucor mucedo* were among the fungi that were isolated at both the 0 and 6-hour marks. In their study, (35) found that the majority of *Aspergillus species* isolated from the bovine rumen are exceptional producers of phenoloxidas, xylanases, and cellulases for the breakdown of lignin. On the other hand, because *Fusarium* molds are common and can contaminate field crops in warm and temperate climates, *Fusarium species* create mycotoxins that are typically found in a variety of diets.

The toxicological effects of *Fusarium* toxins in farm animals have also been reported in other investigations (36, 37, 38). Even if *Fusarium* mycotoxins' mode of action in monogastric intestines is well understood, it's crucial to know if these mycotoxins can also enter ruminant intestines (39). The rumen flora has the ability to inactivate a number of mycotoxins, as reviewed by (40). But according to (41), *Mucor mucedo* is linked to ruminant animals' synthesis of proteases.

It is now known that protozoa generally have a detrimental impact on the rumen, especially when ruminants are fed pasture diets that are poor in real protein (42). Protozoa consume and break down

bacteria, which lowers the number of bacteria in the rumen and, as a result, the animals' protein source (43). While (44) identified two species of protozoa *Holotrich spp.* and *Trichuris spp* when West African dwarf goats were fed an enzyme-supplemented complete mixed

ration during the dry season, our study only isolated the *Holotrich species*. When soluble carbohydrates are easily accessible in the diet, the holotrichs are most prevalent. As a result, they might have a role in the metabolism of carbohydrates.

**Table 5: Effects of Cassava leaf meal inclusion levels on Microbial count and identification at 0- and 6-hours post-feeding of WAD does**

Parameter	Level of inclusion of cassava leaves				SEM	P
	0%	10%	20%	30%		
TBC(x 10 <sup>5</sup> cfu/ml)						
0 hour	1.10	1.15	1.12	1.20	0.11 <sup>ns</sup>	0.71
6 hours	1.40	1.35	1.35	1.35	0.23 <sup>ns</sup>	0.99
TFC(x 10 <sup>5</sup> cfu/ml)						
0 hour	1.10 <sup>b</sup>	1.10 <sup>b</sup>	1.37 <sup>a</sup>	1.47 <sup>a</sup>	0.09*	0.00
6 hours	1.35 <sup>ab</sup>	1.85 <sup>a</sup>	1.15 <sup>b</sup>	1.10 <sup>b</sup>	0.28*	0.04
TPC(Cell/ml)						
0 hour	1000.0				473.68 <sup>b</sup>	
	0	550.00	450.00	1050.00	<sup>a</sup>	0.35
6 hours	450.00 <sup>b</sup>	250.00 <sup>b</sup>	300.00 <sup>b</sup>	900.00 <sup>a</sup>	*	0.00
<b>Microorganisms isolated:</b>						
<b>(a) Bacteria</b>						
<b>0-hour</b>	<b>post-</b>					
<b>feeding</b>						
<i>Bacillus species</i>		+	+	-	+	
<i>Lactobacillus species</i>		+	+	+	+	
<i>Escherichia species</i>		+	+	+	+	
<i>Pseudomonas species</i>		+	-	+	+	
<i>Enterobacteria species</i>		-	+	+	+	
<b>6-hour</b>	<b>post-</b>					
<b>feeding</b>						
<i>Bacillus species</i>		+	+	+	+	
<i>Lactobacillus species</i>		+	+	+	+	
<i>Escherichia species</i>		+	+	+	+	
<i>Pseudomonas species</i>		+	+	+	+	
<i>Enterobacteria species</i>		+	+	+	+	
<b>(b) Fungi</b>						
<b>0- and 6-hours</b>						
<b>post-feeding</b>						
<i>Aspergillusniger</i>		+	+	+	+	
<i>Fusarium species</i>		+	+	+	+	
<i>Mucormucedeo</i>		+	+	+	+	
<b>(c) Protozoa</b>						
<b>0 and 6 hours</b>						
<b>post-feeding</b>						
<i>Holotrich specie</i>		+	+	+	+	

<sup>ab</sup> means with different superscripts on the same row are significantly different (P>0.05), SEM= standard error of mean; P= probability level; \* = significant; ns= not significant; TBC= total bacteria count; TFC= total fungi count; TPC= total protozoa count; (x10<sup>5</sup> cfu/ml) = number of viable bacteria/fungi X serial dilution ratio/volume of inoculum; + represents presence of rumen microbe, while - is absence.

**The effects of Cassava leaf meal inclusion on total volatile fatty acid and ammonia-nitrogen concentration of West African Dwarf Does.**

Table 6 shows the effects of Cassava leaf meal inclusion on total volatile fatty acid and ammonia-nitrogen concentration of West African Dwarf Does at zero-hour and six-hours post-feeding. At zero-hour post-feeding, there was insignificant ( $P>0.05$ ) difference in the total volume of volatile fatty acids (VFAs) across the treatment groups. At the sixth hour post-feeding, the diet containing 20% cassava leaf meal had the highest total VFAs, followed by 10%, while 0% and 30% were observed to be the least. The variation in total VFAs between zero and six-hours post-feeding was not significantly ( $P>0.05$ ) different across the different treatments. There was significant difference in the temperature across the different treatments at zero-hour post-feeding. The diets containing 20% and 30% cassava leaf meal had the highest rumen fluid temperature, while 10% and 0% were similar. There was great variation in the temperature between zero-hour and six-hour post-feeding. The treatments with 30% and 20% cassava leaf meal had the highest variation while 10% and 0% were statistically the same. This variation in temperature was expected because, feed consumption is accompanied by heat production (heat of digestion). Also, the sixth hour post-feeding was done later in the day, so the temperature would have risen due to higher solar intensity at those hours. The pH at zero-hour and six-hours post-feeding showed no significant ( $P>0.05$ ) difference across the different treatments. The ammonia nitrogen concentration significantly ( $P<0.05$ ) differed across the treatment groups. The treatment

with 0% cassava leaf meal inclusion had the highest ammonia-nitrogen concentration, while 10%, 20% and 30% were significantly similar. The ammonia concentration at the sixth hours remained significantly ( $P>0.05$ ) similar among treatments.

The total volatile fatty acids (TVFAs) obtained in this study at 0-hour and 6-hour post-feeding were similar to the values of 52.70 mM/100ml to 86.23 mM/100ml reported by (44) and 53.08 to 58.77mM/100ml reported by (45). The same breed of goat that was employed in the *in vivo* experiment may be the cause of this resemblance. The results of (46) were in line with the negligible change in the concentration of total volatile fatty acids between the times of rumen fluid collection. It has been demonstrated that the rumen produces a large amount of volatile fatty acids in conjunction with gastric fermentation. Over 70% of the ruminants' energy comes from these volatile fatty acids. Additionally, they function as building blocks for the synthesis of milk; propionate is utilized to produce glucose, which is required for the synthesis of lactose, the sugar found in milk, and acetate is essential for the development of milk fat (47). The temperature varied significantly between the collecting times in this investigation. Given that heat is produced during digestion, this variance was not surprising. The increased ambient temperature during collecting and the heat generated during digestion could be the cause of the higher temperatures seen six hours after feeding. According to reports, the most crucial nutrient for rumen fermentation is the quantity of rumen ammonia nitrogen ( $\text{NH}_3\text{-N}$ ). The study's rumen ammonia concentration estimates exceeded those of (44) who reported values between 5.99 and

7.29 mg/100 ml. However, these levels fell between 5 to 20 mg/100 ml, which is a range that is appropriate for ruminal microbial activities.

**Table 6: Effects of Cassava leaf meal inclusion levels on Total Volatile Fatty Acids and Ammonia-Nitrogen Concentration of West African Dwarf Does.**

Parameter	Level of inclusion of cassava leaf meal				SEM	P
	0%	10%	20%	30%		
<b>TVFA (mM/100ml)</b>						
0 hr post-feeding	140.00	240.00	160.00	160.00	64.03 <sup>ns</sup>	0.30
6 hr post-feeding	160.00 <sup>c</sup>	180.00 <sup>b</sup>	200.00 <sup>a</sup>	160.00 <sup>c</sup>	10.00*	0.00
Variation	-20.00	60.00	-40.00	0.00	73.48 <sup>ns</sup>	0.43
<b>Temperature (°C)</b>						
0 hr post-feeding	25.78 <sup>b</sup>	24.53 <sup>b</sup>	28.43 <sup>a</sup>	27.400 <sup>a</sup>	0.83*	0.00
6 hr post-feeding	31.05	30.83	30.65	30.48	0.33 <sup>ns</sup>	0.25
Variation	-5.27 <sup>b</sup>	-6.30 <sup>b</sup>	-2.12 <sup>a</sup>	-3.08 <sup>a</sup>	1.08 <sup>ns</sup>	0.01
<b>pH</b>						
0 hr post-feeding	6.46	6.51	6.86	6.22	0.55 <sup>ns</sup>	0.58
6 hr post-feeding	5.13	5.07	5.51	5.58	0.43 <sup>ns</sup>	0.70
Variation	1.33	1.44	1.35	0.64	0.92 <sup>ns</sup>	0.70
<b>NH<sub>3</sub>-N (mg/100ml)</b>						
0 hr post-feeding	47.63 <sup>a</sup>	15.31 <sup>b</sup>	15.31 <sup>b</sup>	22.11 <sup>b</sup>	3.53 <sup>ns</sup>	0.02
6 hr post-feeding	25.52	25.52	20.41	22.11	9.12 <sup>ns</sup>	0.87
Variation	-22.11	-10.21	-5.11	0.00	14.38 <sup>ns</sup>	0.09

abcMean with different superscripts along the same row are significantly different (P<0.05); SEM = standard error of mean; P= probability level; \* = Significant; ns= not significant; TVFA= total volatile fatty acids; NH<sub>3</sub>-N = ammonia nitrogen concentration

### Conclusion and Applications

Sequel to the findings of this study, the following conclusions were drawn:

1. *In vitro* study is time and cost-effective method of evaluating feeds.
2. Up to 30% cassava leaf meal can be included in concentrate diets for improved *in vitro* volatile fatty acid production.
3. Up to 30% cassava leaf meal can be included in concentrate diets for improved protozoa counts.
4. Up to 20% cassava leaf meal can be included in concentrate diets for improved total volatile fatty acid production in West African Dwarf Does.
5. Bacteria species such as *Bacillus species*, *Lactobacillus species*, *Escherichia specie*, *Pseudomonas species*, *Enterobacteria species*, while fungi such as *Aspergillus niger*,

*Fusarium species*, *Mucor mucedeo*, and protozoa such as the *Holotrich species* are rumen microorganisms identified when West African Dwarf does are fed diets containing graded level of cassava leaf meal.

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