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# Rumen Microbiology of West African Dwarf Goats Fed Cassava Peels-Based Diets with Varying Levels of *Leucaena leucocephala*

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## ABSTRACT

**Background and Objective:** In Nigeria, *Leucaena leucocephala* is a significant source of goat feed. Four iso-nitrogenous and iso-caloric diets were formulated for *L. leucocephala* to determine the effect of a diet containing *L. leucocephala* with varying levels (0, 5, 10 and 15%) on the rumen microbial count of West African Dwarf (WAD) goats in southwestern Nigeria. **Materials and Methods:** The 70 day trial consisted of four inclusion levels with four replicates making a total of 16 treatments with four WAD goats weighing 6.50±0.06 kg per treatment. Animals in each treatment were served wilted Panicum maximum cv Jacq. On the final day of the experiment, rumen fluid samples were taken for bacterial, protozoal and fungal counts investigation. **Results:** The goats fed a diet containing 5% of *Leucaena* had the highest ($p \leq 0.05$) value for total anaerobic bacteria count ($0.93 \times 10^6$ CFU $mL^{-1}$), whereas goats fed a diet containing 15% of *Leucaena* had the lowest ($p \leq 0.05$) value ($0.67 \times 10^6$ CFU $mL^{-1}$). Goats fed a feed containing 15 % *Leucaena* showed the highest levels of total fungus and protozoa, at 0.07 and 0.47 CFU $mL^{-1}$, respectively. The least ($p \leq 0.05$) value for the total protozoa count was recorded in the rumen of goats fed the control diet, which had a value of 0.27 CFU $mL^{-1}$. **Conclusion:** According to data obtained, adding *L. leucocephala* to goat meals at a rate of 5% raised bacterial counts while lowering fungus counts. Consequently, it is advised that *L. leucocephala* be fed to WAD goats at a level of inclusion of 5%.

## KEYWORDS

*Leucaena leucocephala*, rumen protozoa count, *Panicum maximum*, WAD goats, rumen fungi count, rumen bacteria count



## INTRODUCTION

Protozoa, fungi and bacteria make up the ruminal microbial population, which is essential for ruminant digestion[1]. It is thought that most cellulose hydrolysis occurs in this bacterial population[2]. The ruminal bacterial community is of particular interest in neo-tropics, where tree-based browse usage can decrease reliance on grasses.

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Leucaena, a locally available tree legume, is being used to feed ruminants due to its good feeding values especially high protein and sulfur content. This improves the health of livestock while also positively affecting microbial populations[3]. Furthermore, Leucaena's high digestibility of protein and dry matter (DM) content is up to 70% when adequately supplemented with low-quality feed. The use of high proteinaceous legumes as animal feed is important for improving the nutritional value of poor-quality feeds. Leguminous plants such as *L. leucocephala* grow abundantly in tropical regions and were considered to be good-quality forage because of their high protein content, which also provided a good balance of amino acids.

In mitigating the effect of the high cost of feeding in livestock production, several crop residues and agro-industrial by-products have been in use for decades, including cassava peel. Reports of Babayemi and Bamikole[4] showed that peels from several varieties of cassava have been used for decades to feed livestock, particularly ruminants and pigs. Aside from the lower protein in cassava peel[5] when compared with maize, its utilization in poultry has been limited due to a large amount of cyanogenic glycoside, high phytate content and quick spoilage if left unprocessed[6,7], however, played a major role in ruminant nutrition[5]. Thus, an effort to combine such crop residue with locally available feed resources in a least-cost feed combination will greatly improve feed security in ruminant diets and hence food security.

The West African Dwarf goat plays an important economic factor in the livelihood of the people in Southwestern Nigeria, hence feeding efforts to increase their production is a panacea to increasing its importance. There have been a series of feeding trials in improving the productivity of WAD and hence this present study aimed to evaluate the effect of *L. leucocephala* inclusion in cassava peel-based concentrate-supplemented diets on the rumen microbial populations in goats.

## MATERIALS AND METHODS

**Experimental site:** The experiment was carried out between April and June, 2018 at the Small Ruminant Research Station of the College of Animal Science and Livestock Production (COLANIM) farm and the laboratories of the Department of Animal Nutrition, Federal University of Agriculture, Abeokuta (FUNAAB), Ogun State. FUNAAB is located in the tropical rainforest zone in Nigeria within 7°10'N and 3°2'E. The area has a bimodal rainfall pattern with peaks in June and October, an average rainfall of 1100 mm, a mean ambient temperature of about 34°C and an average relative humidity of 82%[8].

**Experimental animals and management:** Sixty-four West African Dwarf (WAD) goats of both sexes aged six to seven months with an average live weight of 6.50±0.06 kg sourced from Ibarapa Village, Iseyin Local Government, Oyo State were used for this study. The experiment comprised 16 treatments, including diets and replicates laid in a Completely Randomized Design (CRD). Goats representing each dietary treatment were housed together in a 6×6 feet standard goat pen with a feeder and water provided. The pens were well cleaned and fumigated three weeks before the commencement of the trial, while the experimental goats were acclimatized and dewormed with Albendazole using the recommended dosage. The experimental diets consisted of an iso-nitrogenous diet containing four levels of inclusion of *Leucaena leucocephala* at 0, 5, 10 and 15%. Animals within each group were fed the experimental diet at 3% body weight in dual split forms in the morning (07:00 hrs) and in the evening (16:00 hrs) daily while a 3-5 cm copped and wilted *Panicum maximum* was offered at 900 g per goat/day. Clean water was provided *ad libitum* daily. Every required routine management for ruminants was observed with adequate records taken through the 70 days of the experiment.

**Experimental diets:** The experimental diets (Table 1) consisted of concentrate with varying levels of dried *Leucaena leucocephala* and a basal diet of *Panicum maximum* (Guinea grass). *Panicum maximum* was harvested in the natural pasture around the premises of the Small Ruminant Research Station, College of

Animal Science and Livestock Production (COLANIM-FUNAAB) and was wilted overnight before being fed to the animals. On the other hand, the *Leucaena leucocephala* leaves (before flowering) were sourced from the same location and air-dried until a constant weight is reached and preserved. The experimental diets were formulated as shown below.

**Collection of data:** About 100 mL of rumen fluid was collected before the commencement and at the end of the experiment. The rumen fluid was collected from each of the experimental goats using a suction tube as described by Francis *et al*.[9] before they were offered morning feed and six hours after feeding. The collected samples were immediately checked for temperature and pH using a Kedida CT-6020 pen pH meter manufactured by Shenzhen Kedida Electronics Co. Ltd., Baoan District City, Fuyong, Shenzhen Street Peace Community, China. thereafter sieved through four layers of cheesecloth and then transferred into universal bottles for laboratory analysis of microbial population. The estimation of total anaerobic bacteria, total fungi count and protozoa count in rumen fluid was done according to Franzolin and Dehority[10]. This method was used in measuring microbial population by Total Direct Count (TDC) of bacteria, protozoa and fungal zoospores.

**Microbial count**

**Total bacteria count:** The test procedure for the estimation of total anaerobic bacteria count in rumen samples was done according to the method of Franzolin and Dehority[10]. One milliliter of rumen sample was thoroughly homogenized with 9 mL of sterile distilled water. Eight sterile test tubes were arranged and 9 mL of sterile water was placed. Thereafter, 1 mL from the initial dilution was serially diluted in the eight test tubes and 1 mL was discarded from the last tube 8. One milliliter of the diluted sample from tube 8 was spread on egg yolk lactose agar and allowed to be absorbed. The plates were incubated at 37°C for 18 to 24 hrs in a gas pack containing an anaerobic generating kit (GasPak™) to remove oxygen. The bacteria colonies seen were counted and estimated accordingly.

**Total fungi count:** The test procedure for the isolation and identification of fungi isolates in rumen fluid was done by measuring 1 mL of rumen sample which was thoroughly homogenized with 9 mL of sterile distilled water. Eight sterile test tubes were arranged and 5 mL of sterile water was placed. One milliliter from initial dilution was serially diluted in the 8 test tubes and 1 mL was discarded from the last tube 8. Thereafter, 1 mL of diluted sample from tube 8 was spread on potato dextrose agar supplemented with ciprofloxacin (20 mg $mL^{-1}$) and allowed to be absorbed. The plates were incubated at room temperature for three days and the fungi colonies seen were counted, identified and estimated accordingly.

**Total protozoa count:** The test procedure for the isolation and identification of protozoa isolate from rumen fluid was done by measuring 4 mL of rumen fluid which was diluted in 26 mL of saturated saline solution. About 1 mL will be put into the Macmaster slide and allowed to settle for a few minutes. Thereafter, the identified protozoan eggs were counted and estimated accordingly.

**Statistical analysis:** Data generated from this experiment were subjected to One-way Analysis of Variance (ANOVA) for a Completely Randomized Design (CRD) using the procedure of Makkar *et al*.[11]. The significant difference between individual means was determined using Duncan's Multiple Range Tests (DMRT). The significant difference was tested at a 5% palatability level.

**Ethical consideration:** The rights and welfare of humans and animals involved in this study were upheld in accordance with the Nigerian Constitution and the National Health Research Ethics Code. Ethical considerations were given to ensure the protection of their dignity, privacy and well-being, adhering to the principles of justice, beneficence and non-maleficence.

**Ethical approval:** This study received ethical approval from the Research Ethics Committee of the Federal University of Agriculture, Abeokuta, following the guidelines provided by the Nigerian National Code for Health Research Ethics. Hence, all necessary measures were taken to protect the rights and well-being of the participants and animals involved in the study.

## RESULTS

**Experimental diets:** The chemical composition of the experimental diets is presented in Table 1. The crude protein, metabolizable energy, ash, total digestible nutrient, crude fiber and ether extract have the obtained ranges crude protein (14.08-14.36%), metabolizable energy (2,787.33-2,813.60 Kcal $kg^{-1}$ DM), total ash (5.03-7.33%), total digestible nutrient (27.92-39.85%), crude fiber (10.52-13.33%) and ether extract (5.21-6.04).

**Effect of varying levels of *Leucaena leucocephala* inclusion in a cassava peel-based concentrate supplement on the rumen microbial count of WAD goats:** The highest ($1.25\times10^6$ CFU $mL^{-1}$) value for total anaerobic bacteria count was recorded from the rumen of the goat-fed diet containing a 5% inclusion level of *L. leucocephala* at 0 hr post-feeding period, the least ($0.40\times10^6$ CFU $mL^{-1}$) value for the corresponding parameters was recorded from the rumen of the goat-fed diet containing a 0% inclusion level of *L. leucocephala*.

Results recorded for the total protozoa count showed that the highest ($p>0.05$) value of $0.25\times10^6$ CFU $mL^{-1}$ was recorded from the rumen of the goat-fed diet containing a 15% inclusion level of *L. leucocephala*. While, the lowest $0.05\times10^6$ CFU $mL^{-1}$ ($p>0.05$) value for the corresponding parameters was recorded from the rumen of the goat-fed diet containing a 15% inclusion level of *L. leucocephala* (Table 2).

Table 1: Composition of experimental concentrate diet (%)

| Ingredients | 0 | 5 | 10 | 15 | |
|---|---|---|---|---|---|
| Cassava peel | 38 | 40 | 40 | 40 | |
| *Leucaena* | 0 | 5 | 10 | 15 | |
| *BDG | 34 | 31 | 28 | 25 | |
| **PKC | 22 | 18 | 16 | 14 | |
| Oyster shell | 4 | 4 | 4 | 4 | |
| Salt | 2 | 2 | 2 | 2 | |
| Total | 100 | 100 | 100 | 100 | |
| Calculated analysis | | | | | SD |
| Crude protein (%) | 14.18 | 14.08 | 14.22 | 14.36 | ±0.11 |
| Metabolizable energy (kcal $kg^{-1}$ DM) | 2787.33 | 2794.49 | 2811.21 | 2813.60 | ±12.79 |
| Ash (%) | 7.33 | 7.15 | 5.03 | 6.69 | ±1.05 |
| Total digestible nutrient (%) | 39.85 | 35.75 | 32.36 | 27.92 | ±5.06 |
| Crude fibre (%) | 13.33 | 12.26 | 11.39 | 10.52 | ±1.20 |
| Ether extract (%) | 6.04 | 5.96 | 5.58 | 5.21 | ±0.38 |

*BDG: Brewery Dried Grain, **PKC: Palm Kernel Cake and SD: Standard Deviation

Table 2: Effect of varying levels of *L. leucocephala* inclusion in a cassava peel-based concentrate supplement on the rumen microbiology parameters of WAD goats

| | | Levels of inclusion of *L. leucocephala* (%) | | | | | |
|---|---|---|---|---|---|---|---|
| Parameters | Interval | 0 | 5 | 10 | 15 | SEM | p-value |
| Bacteria ($\times10^6$ CFU $mL^{-1}$) | 0 hr post-feeding | $0.40^{c}$ | $1.25^{a}$ | $0.75^{b}$ | $0.60^{bc}$ | 0.099 | 0.00 |
| | 6 hrs post-feeding | 0.90 | 0.93 | 0.73 | 0.67 | 0.128 | 0.90 |
| | Difference | 0.50 | -0.32 | -0.02 | 0.07 | 0.170 | 0.45 |
| Total fungi count ($\times10^6$ CFU $mL^{-1}$) | 0 hr post-feeding | 0.15 | 0.00 | 0.00 | 0.00 | 0.027 | 0.10 |
| | 6 hrs post-feeding | 0.00 | 0.03 | 0.03 | 0.07 | 0.019 | 0.73 |
| | Difference | $-0.15^{b}$ | $0.03^{ab}$ | $0.03^{ab}$ | $0.07^{a}$ | 0.036 | 0.11 |
| Total protozoa count ($\times10^6$ CFU $mL^{-1}$) | 0-hrs post-feeding | $0.05^{b}$ | $0.00^{b}$ | $0.20^{a}$ | $0.25^{a}$ | 0.035 | 0.00 |
| | 6 hrs post-feeding | 0.27 | 0.33 | 0.40 | 0.47 | 0.040 | 0.35 |
| | Difference | $0.22^{b}$ | $0.47^{a}$ | $0.13^{b}$ | $0.15^{b}$ | 0.051 | 0.04 |

**$^{a,b,c}$Means with different superscripts in the same row are significantly different ($p<0.05$) and SEM: Standard error mean

Table 3: Total bacteria count in the rumen of West African Dwarf goat fed *L. leucocephala*-based diet

| | | Levels of inclusion of *L. leucocephala* (%) | | | |
|---|---|---|---|---|---|
| Parameters | Intervals | 0 | 5 | 10 | 15 |
| *Clostridium sporogenous* | 0 hr post-feeding | + - | + - | + + | - - |
| | 6 hrs post-feeding | + + - | + - + | + - - | + + - |
| *Clostridium bifermentum* | 0 hr post-feeding | - + | + + | - + | - + |
| | 6 hrs post-feeding | + + + | - + - | + + + | - + + |
| *Streptococcus bovis* | 0 hr post-feeding | + - | - + | + + | + + |
| | 6 hrs post-feeding | - - + | + + - | - + - | + - + |
| *Streptococcus specie* | 0 hr post-feeding | + - | + - | + - | + - |
| | 6 hrs post-feeding | + + - | + - + | + - + | + + - |
| *Escherichia coli* | 0 hr post-feeding | - + | - - | - + | - + |
| | 6 hrs post-feeding | + - + | - + - | - + + | - + + |
| *Pseudomonas fluoresce* | 0 hrs post-feeding | + - | + - | + - | + - |
| | 6 hrs post-feeding | + + - | - + + | - - - | + - - |
| *Peptococcus specie* | 0 hr post-feeding | - + | - + | - + | - + |
| | 6 hrs post-feeding | - + + | + - - | + - + | - + + |

+: Present and -: Absent

Table 4: Total fungi count in the rumen of West African Dwarf goat-fed *L. leucocephala*-based diet

| | | Levels of inclusion of *L. leucocephala* (%) | | | |
|---|---|---|---|---|---|
| Parameters | Intervals | 0 | 5 | 10 | 15 |
| *Penicillium notatum* | 0 hr post-feeding | + - | - - | - - | - - |
| | 6 hrs post-feeding | - - - | - - - | - - - | - - - |
| *Fusarium oxysporum* | 0 hr post-feeding | - - | - - | - - | - - |
| | 6 hrs post-feeding | - - - | - - - | - - - | - - - |
| *Aspergillus flavus* | 0 hr post-feeding | + - | - - | - - | - - |
| | 6 hrs post-feeding | - - - | - - + | - - + | - + - |
| *Aspergillus fumigatus* | 0 hr post-feeding | + - | - - | - - | - - |
| | 6 hrs post-feeding | - - - | - - - | - - - | - - - |

+: Present and -: Absent

**Effect of anaerobic bacteria organisms in the rumen of WAD goats fed varying levels of *L. leucocephala*-based diet**

**Zero hour post-feeding phase:** Except for *Clostridium sporogen* which was recorded to be absent in the rumen of the animals fed a diet containing 15% inclusion levels of *L. leucocephala*, all other anaerobic bacteria organisms were observed in the rumen of the goats fed the experimental diets containing varying levels of inclusion of the test ingredient (Table 3).

**Six hours post-feeding phase:** The outcome of the current study trial showed that except for *Escherichia coli* and *Pseudomonas fluoresce* were observed to be absent from the rumen of the animal-fed diets containing 5 and 10% levels of inclusion of *L. leucocephala* every other anaerobic bacteria organism observed in this trial were recorded to be a presence in all the dietary treatments (Table 3).

**Effect of fungi organisms in the rumen of West African Dwarf goats fed varying levels of *L. leucocephala*-based diet**

**Zero hour post-feeding phase:** As presented in Table 4, it was observed that *Penicillium notatum*, *Aspergillus flavus* and *Aspergillus fumigatus* were present only in the rumen of the animal-fed diet containing 0% level of *L. leucocephala* while these organisms were absent in other dietary treatments. *Fusarium oxysporum* was observed to be in the rumen of animals fed all the dietary treatments (Table 4).

**Six hours post-feeding phase:** All the species of the fungi organism observed in this trial were observed to be absent in the rumen of the animals fed the dietary treatment, except *Aspergillus flavus* which was recorded to be present in the rumen of the animal fed diets containing 5, 10 and 15% levels of inclusion of *L. leucocephala* (Table 4).

Table 5: Total protozoa count in the rumen of West African Dwarf goat fed *L. leucocephala*-based diet

| | | Levels of inclusion of *L. leucocephala* (%) | | | |
|---|---|---|---|---|---|
| Parameters | Intervals | 0% | 5% | 10% | 15% |
| *Nematodius* spp. | 0 hr post-feeding | - - | - - | + + | - - |
| | 6 hrs post-feeding | - - - | + - + | + - - | - - + |
| *Holotrich* spp. | 0 hr post-feeding | + - | - - | - + | + + |
| | 6 hrs post-feeding | - + + | + + - | - - + | + + - |
| *Trichuris* spp. | 0 hr post-feeding | - - | - - | - - | - + |
| | 6 hrs post-feeding | + - + | + + - | + + - | - + + |

+: Present and -: Absent

**Effect of protozoa organisms in the rumen of West African Dwarf goats fed varying levels of *L. leucocephala*-based diet**

**Zero hour post-feeding phase:** It was observed from the results of this study that no definite pattern was observed in the presence of the protozoa organisms in the rumen of goats fed experimental diets. For instance, *Nematodius* spp., was only present in the rumen of the goats fed a diet containing *L. leucocephala* at a 10% level of inclusion, *Holotrich* spp., was present at 0, 10 and 15% while *Trichuris* spp., only recorded from the rumen of the animals fed a diet containing *L. leucocephala* at a 15% level of inclusion (Table 5).

**Six hours post-feeding phase:** Except for *Nematodius* spp., which was observed to be absent in the rumen of the animal-fed diet containing *L. leucocephala* at a 5% level of inclusion, results showed that both *Nematodius* spp. and *Trichuris* spp., were present in the rumen of the goat fed all the experimental diets (Table 5).

## DISCUSSION

The chemical composition of experimental concentrate diets shows that the diets were both iso-nitrogenous and iso-caloric with a crude protein (CP) of 14% and metabolizable energy of about 2800 (kcal $kg^{-1}$ DM). This level of the CP recorded was within the adequate range (12-16%) for maintenance and moderate growth in goats[12] and higher than the minimum recommended CP values of 7.0-8.0% for the efficient functioning of rumen micro-organism[13], hence, the diets will create an enabling environment for the rumen microbes, which in turn support optimal growth and performance of goat by facilitating the utilization of the diets. The outcome of this study showed that at the 0 hr post-feeding phase, an increase was observed at all treatment levels of bacteria and protozoa except for total fungi count which was not influenced by the effect supplementation of *L. leucocephala* leaves in the diets of the WAD goats. The optimum population for both bacteria and protozoa was recorded from the rumen of goats fed diets containing 5, 10 and 15%, respectively levels of inclusion of *L. leucocephala* leaves.

The reduced bacterial population in the rumen of the goat might be due to the presence of certain substances that inhibit the population of these organisms at higher quantities. Such might be tannin, which was earlier reported by Vasta *et al.*[14], wherein a lamb fed a diet rich in tannins a replica of Leucaena showed a rise in the protozoa population in the rumen. However, prior research suggested that tannin's mode of action on protozoa involved altering the permeability of the protozoa cell membrane, which led to the membrane's eventual disintegration[9]. While, a higher ($p<0.05$) protozoa population was recorded with an increase in the level of *L. leucocephala* leaves in the diets, this might have resulted from increased protein contents that favors the rumen environment and hence, the increase in the protozoa population.

In addition to the benefits of *Leaucaena* in ruminant feeding is the influence of the presence of secondary compounds (such as tannins) on the rumen ecology[15]. When consumed in an appropriate amount, these compounds typically have beneficial effects and do not reduce voluntary feed intake. In an aqueous solution, the phenolic hydroxyl groups of tannins bind to dietary protein, causing the formation of a complex with proteins, primarily and to a lesser extent, with metal ions, amino acids and polysaccharides, preventing their degradation in the rumen, increasing the amount of bypass protein to the lower parts of the gastrointestinal tract (abomasum) and increasing the amount of essential amino acid supply, leading to higher animal production and several other effects on animal nutrition reported by researchers[16-18]. In the tropics, *Leucaena leucocephala* is a legume species used in animal feed due to its high content of protein. Thus, making the use of *Leucaena leucocephala* for ruminant nutrition widely implemented. Leucaena has been potential for increasing productivity and sustainability of farming systems using ruminants in the tropics either as a high-quality forage or possibly contributing to the reduction of greenhouse gas emissions[15].

However, an inverse relationship was noticed in the population of both bacterial and protozoa concerning the level of inclusion of *L. leucocephala* leaves in the diet fed WAD goat, as the highest population of bacterial was recorded from the diet where the lowest population of protozoa was recorded. The decrease in protozoa population and observed increase in the bacterial population has been reported by Vasta *et al.*[14] as high levels of protozoa in the rumen can engulf ruminal bacteria. Hence, rumen micro-organisms react differently to similar feed substances.

The changes in the rumen protozoa population found in this study could be influenced by other factors such as diet composition and feed level. This further supports the report by Barros-Rodríguez *et al.*[3], who noted that *L. leucocephala* contains a high level of tannin, which is a factor encouraging microbial growth. The outcome of this study will be useful for goat farmers in improving the nutrition of their flock by the utilization of some browse materials like *L. leucocephala* in the diet of their animals. However, efforts can be further made in the inclusion of other browse plants either as a sole material or mixture at varying levels and this is therefore recommended.

## CONCLUSION

Based on this study, it could be concluded that the inclusion of *L. leucocephala* at the level of 5% increases the bacteria population and decreases the protozoa population but has no effect on fungal zoospores. The increase in the population of rumen bacteria will help to break down the feed eaten by the animal thereby turning the feed into energy and protein for the goat. This is very important for feed utilization and the productivity of the animal. Therefore, the use of locally available feed resources like *L. leucocephala* can be very helpful in achieving this frontier in production at a reduced cost.

## SIGNIFICANCE STATEMENT

This study further strengthens the use of *L. leucocephala* as a feed material for goats in southwestern Nigeria. The outcome of this study will not only help the farmer in the utilization of *L. leucocephala* to improve microbial activities for increased feed utilization and hence the productivity of the WAD goat but will also help in reducing the cost of feed thereby increasing the profitability. In addition, *L. leucocephala* will largely contribute to the feed basket of WAD goats in the region, which can be used as alternative feed resources and used to improve other fibrous feed materials.

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