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ISOLATION AND IDENTIFICATION OF MICROBIAL BIOMASS FROM RUMEN FLUID OF YANKASA RAMS FED COWPEA HAY AND GROUNDNUT HAULMS AS SUPPLEMENTS

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ABSTRACT

This study was conducted to assess the influence of cowpea hay and groundnut haulms supplementation on the rumen characteristics of Yankasa rams. The study involved the use of Sixteen (16) Yankasa rams of equal weight randomly assigned to four treatment groups: Rice straw (Treatment I), groundnut haulms (Treatment II), cowpea hay (Treatment III) and combination of cowpea and groundnut haulms (Treatment IV). The experiment was laid out in a Completely Randomized Design (CRD) with four (4) replications for 84 days. About 15 ml of rumen liquor was drawn individually from all the experimental animals from which pH and microbial biomass were determined according to standard protocols. Data obtained for rumen pH and microbial loads were analyzed using Analysis of Variance with Least Significant Difference used to separate significant means at 5% level. Significant difference ($P \leq 0.05$) was found in the rumen fluid pH and microbial counts among the treatment groups. High microbial counts were recorded among Treatment II while Eight different bacterial species were identified with Treatment II having the highest number of bacterial species . Similar result was recorded for fungal species. Three different fungal species were isolated from the rumen fluids in the present study. These are: *Piramonas communis, Sphaeromonas communis* and *Pilobolus* spp. However, Treatment I had the highest number of protozoan species (4 different species). The findings from this study implies that, supplementation of basal rice straw with cowpea hay and groundnut haulm led to improvement in the composition of microbial biomass that aided digestibility and rumen fermentation within Yankasa rams making nutrients available for absorption and assimilation. Treatment II having the highest number of bacterial species is recommended.

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INTRODUCTION

Rumen is a self-sustained ecosystem where feeds consumed by ruminants are fermented by microbes to volatile fatty acids and other potential sources of energy and protein for the host animals (Perez *et al.,* 2024). The rumen microbes played a crucial role in maintaining gastro-intestinal homeostasis, and the entire animal's wellbeing (Zhu *et al.,* 2025). Manipulation of rumen microbial ecosystem for enhancing feed digestibility and ruminants' health to improve performance are some of the most important goals of animal nutritionists (Muhammad *et al.,* 2016). Anaerobic rumen fermentation of feeds is beneficial for the host animal, the co-existence of animal and its microbial eco-system has resulted in stable and the most favored natural selection of bacteria, protozoan and fungi. There exists different kind of symbiotic relationship among different

group of microorganisms due to the diverse nature of these microbial species and their adaptability and interactions in the rumen (Castillo-Gonzalez *et al.,* 2014). It has been reported that ruminal fungi for instance have enzymes which can hydrolyse the majority of the structural polysaccharides found in plant cell wall (Dehority and Tirabasso, 2001).

However, despite the relative importance of rumen microbial biomass to the welfare of the animals and the farmer, rumen microbial composition is easily hampered by a high seasonal variability of feeds availability with low protein concentration thereby reducing their maintenance and production requirements (Fasae *et al.,* 2025). Feeding supplementation have over the years been developed as alternative feeding strategies with economic benefits for utilizing limited feed supply

in ruminant production systems (Moreira *et al.,* 2021). Leguminous plant products such as cowpea hay and groundnut haulms are abundant in Sudano-sahelian zone of Nigeria and are valued for their high-quality protein forage and favorable amino acid profile (Ahmed and Abdelati, 2009), offering significant nutritional benefits as a protein-rich forage to fill dietary gaps for ruminants and manipulate microbial rumen composition (Fasae *et al.,* 2025).

Rumen manipulation improves performance, feed intake and nutrient digestibility using supplementation of diets. The digestibility of fibre fractions like Neutral detergent fiber (NDF), Acid detergent fiber (ADF), hemicellulose and cellulose are increased by supplementation (Santra and Karim, 2002). Supplementation with crude protein to ruminants improves fiber digestibility because of the population of cellulolytic bacteria is increased (Currier *et al.,* 2004). Stabilization of rumen environment favoring development of cellulolytic microbes (Hegarty *et al.,* 1991) and stimulatory effect of rumen ciliate protozoa on rumen bacteria for cellulolysis (Onodera *et al.,* 1988). More so, the results of effect of supplementation on the animal performance are mainly related to animal feed composition and nature of the feeding supplements. The aim of this study therefore is to isolate and characterize the different microbes present in the rumen fluid of Yankasa rams fed cowpea hay and groundnut haulms as supplements.

MATERIAL AND METHODS

Study Location

The study was carried out at the Small Ruminant Unit of Teaching and Research Farm, Department of Animal Science, Aliko Dangote University of Science and Technology, Wudil, Kano State (Latitude 11º 5´N and longitude 9º 40´E and on an altitude of 415m above sea level). The area has a minimum and maximum temperature of about of 26ºC to 36ºC respectively. The mean annual rainfall in the area is 773.4 mm per annum (Adamu *et al.,* 2014).

Collection and Processing of Experimental Materials

Cowpea hay and groundnut haulms were procured from an animal hay seller at Wudil market, Kano state. The experimental materials were bagged separately in clean sacks and labelled accordingly. The rice straw was procured from a rice Farm at Wudil town, Kano. The rice straw was chopped using a forage chopper to size of 4 cm length and bagged. Other feed ingredients were purchased from the same market. All the experimental feeds were bagged properly and stored at room temperature (27±2 ºC) until required for use.

Experimental Animals

Sixteen (16) growing Yankasa rams of equal weight (20.19 ±2.2kg) were procured from Wudil Market, Kano State and were used for the experiment. The animals were quarantined for two weeks, dewormed against internal and external parasites using Ivomec® Super at 200µg/kg body weight prior to experiment.

Experimental Treatments and Design

The experimental animals were randomly assigned into four groups laid down in a Completely Randomized Design (CRD) with four (4) replications. Animals on treatments I were fed rice straw with water *ad libitum* as control, treatment II were fed groundnut haulms (300g /day and rice straw *ad libitum*) and treatment III 300g/day cowpea hay and rice straw *ad libitum* respectively. Those on treatment IV were fed a combination of groundnut haulms and cowpea hay (150g/day Groundnut haulms +150g/day Cowpea hay)

and rice straw *ad libitum*. Wheat bran and sorghum chaff (mixed in a ratio 2:1) plus 1% salt and 1% bone meal were given at 2% body weight to all the experimental animals as concentrate diet. Experimental feeds were offered to the animals twice a day (8:00 am and at 4:00 pm). All the animals received rice straw as basal diet and water *ad libitum*. After the adaptation period, the experiment lasted for 12 weeks (84) days.

Rumen Fluid Collection

Fifteen (15) ml of rumen liquor was drawn individually from the animals, using rubber stomach tube in the morning prior to feeding the animals and at 4 hours after feeding, middle and last week of the experiment. The rumen liquor was stored in sterile glass bottles and kept in a flask containing ice blocks and transported to Microbiology Laboratory, Kano University of Science and Technology, Wudil, for microbial analysis. The bacterial and fungal colonies were observed and identified based on their colony morphology as described by Pelczar *et al*. (2006).

Rumen fluid pH determination

Rumen fluid pH was measured immediately using a digital pH meter (Model: JENWAY 550).

Bacterial isolation and identification

The microbial analysis was conducted using serial dilution technique following the procedure of Adams and Moss (2007). Sterilization of all glass wares was done by washing with detergent rinsed with water and sterilized using hot air oven at 160 ºC for 1 hour while all the liquid media were sterilized in an autoclave at 121ºC for 15 minutes (Prescott *et al*., 2005). One (1) millilitre each of rumen fluid samples were dissolved in a test tube containing 9 ml of sterile distilled water (10^{-1}) dilution. This was shaken to obtain a good suspension. The suspension was then serially diluted to 6 tubes (10^{-6}). From the sixth tube, 1 mi of the suspension containing rumen liquor was inoculated on Nutrients Agar and spread using pour plate technique and incubated at 32 ºC for 24 hours. Colonies formed were counted using colony count metre as described by Prescott *et al*. (2005).

A sterile wire loop of the correct size was dipped into the enrichment culture and inoculated into a small area of a fresh nutrient agar and spread by using a sterile wire loop by streaking and incubated for 24 hours. The pure isolates of each of the colony obtained was transferred into a sterile slant bottles containing fresh nutrient agar and refrigerated at 4ºC for further use (Prescott *et al*., 2005).

Gram staining: Gram staining was carried out according to Prescott *et al*. (2005) method. Smear of bacterial isolates were made on clean glass slide using drop of water with sterile wire loop. It was then allowed to air dry and then passed over a flame in order to fix the smear. The smear was covered with gentian violet for 60 seconds and washed. Iodine was then poured to cover the smear, allowed for 60 seconds and then washed. Ethyl alcohol (ethanol) was used to decolourize the smear and washed immediately with the distilled water, then follow by the application of safranin and left for 60 seconds, and later washed with distilled water. Back of the slide was cleaned with cotton and allowed to air dry. The slide was examined under electrical compound microscope using oil immersion x100 resolution objectives of AMSCOPE microscope (Model: N 400M). The various colonies observed in the plates were distinguished on the basis of their cultural characteristics such as colony size, shape and color as described by Fawole and Oso (1995).

Fungal isolation and identification

Fungal isolation was done using serial dilution technique in which one milliliter of each of the samples was added to 9 ml of distilled water to form 10^{-1}. This was advanced to 10^{-4}. The samples from the last test tube were inoculated on Potato Dextrose Agar (PDA) at 28ºC for 7 days. The total fungal counts were carried out on the samples using Pour plate technique. The plates were subsequently incubated at 32ºC for 72 hours. At the end of incubation, developed colonies were counted in colony forming units per unit milliliter. Discrete colonies were sub-cultured to obtained pure culture which were used subsequently for microscopic identification. Chloramphenicol (30 mg/l) was added to prevent bacterial growth. The distinct colonies formed in the pure culture plates were observed using morphological and cultural characteristics as described by Dashwood *et al.* (1992).

Protozoan isolation and identification

The protozoan counts were used to calculate the protozoal generic distribution and generation time of protozoa according to the following formula:

Generation time in hours = total protozoal counts in fermenter/flow of protozoa in effluent per hour (Sylvester *et al*., 2004). The microorganisms were isolated and identified by conventional method with grams staining for bacterial, fungal and protozoan species.

Statistical Analysis

The data collected were subjected to Analysis of Variance (ANOVA) using SAS (2008) version 9.1; where there is significant difference, the means were separated using Least Significant Difference (LSD) at 5% level of probability.

RESULTS

The results for rumen fluid pH by Yankasa rams fed cowpea and groundnut haulms as supplement to basal rice straw is shown in Table 1. The results showed significant ($P < 0.05$) differences between sampling times. The treatment II recorded a higher acidic rumen fluid pH 4 hours post-feeding than the other treatments, while control (I) had the least acidic value. Whereas the lowest fungal count was found in treatment II and IV before feeding and treatment I and IV after feeding.

Table 1. Rumen pH of Yankasa rams fed cowpea and groundnut haulms as supplement

Sampling Time (Hours)	Treatments				SEM
	I	II	III	IV	
0	6.74	6.67	6.67	6.63	0.06
4	6.29^{a}	6.01^{b}	6.17^{ab}	6.08^{ab}	0.12

^{ab}Means with the same superscript within the same row are not significantly ($p < 0.05$) different, SEM = Standard Error of Mean I= Control Rice straw II= Groundnut Haulms III= Cowpea Hay IV= Cowpea Hay + Groundnut Hay

Influence of Cowpea and Groundnut Haulms Supplementation on Microbial Biomass

The results for the bacterial counts in the rumen fluid of Yankasa rams fed cowpea hay and groundnut haulms as supplements to basal rice straw were presented in Table 2. The results revealed significant variation ($P \leq 0.05$) among the treatments in terms of microbial loads. The result showed that, before morning feeding and at 4 hours after feeding, bacterial counts were recorded higher in treatment II (5.83×10^{6} cfu/ml and 5.87×10^{6} cfu/ml respectively). However, the results for fungal count revealed no significant difference in the fungal count at morning hours before feeding but statistically different ($P \leq 0.05$) after 4 hours of feeding. Similarly, treatment II had higher fungal count (4.26×10^{5}cfu/ml) than all other treatments, followed by treatment III.

Table 2. Rumen microbial counts as influenced by Cowpea and Groundnut haulms supplementation

Parameters	Sampling Time (Hours)	TREATMENTS				SEM
		I	II	III	IV	
Bacterial count x10^{6}(Cfu/ml)	0	5.17^{c}	5.83^{a}	5.46^{b}	5.57^{b}	1.32
	4	5.21^{c}	5.87^{a}	5.59^{b}	5.66^{b}	0.09
Fungal count x10^{5} (Cfu/ml)	0	3.65	3.41	3.75	3.36	1.21
	4	3.31^{c}	4.26^{a}	3.98^{b}	3.26^{c}	0.78

^{abc}Means with the same superscript within the same row are not significantly ($P \leq 0.05$) different, SEM = Standard Error of Mean I= Rice straw, II= Groundnut Haulms, III= Cowpea Hay, IV= Cowpea Hay + Groundnut Haulm

The bacterial species identified in rumen fluids of Yankasa rams fed experimental diets are presented in Table 3. The results indicated the presence of 8 different bacterial species represented by seven (7) were recorded as follows: *Butyrivibrio fibrisolvens, Bacteriodes ruminocola*, *Escherichia coli, Entrococcus spp. Pseudmonas aeruginosa, Fibrobacter succinogenes, Ruminococcus albus and Ruminococcus flavefaciens* with Treatment II

having the highest number of bacterial species (five).

Table 3. Bacterial species isolated from the Rumen Fluids of Yankasa Rams Fed different supplementations

S/N	TREATMENTS			
	I	II	III	IV
1	*Entrococcus* spp.	*Ruminococcus albus*	*Ruminococcus flavefaciens*	*Bacteriodes succinogenes*
2	*Pseudmonas aeruginosa*	*Ruminococcus flavefaciens*	*Butyrivibrio fibrisolvens*	*Ruminococcus albus*
3	*Bacteriodes ruminocola*	*Bacteriodes succinogenes*	*Escherichia coli*	*Escherichia coli*
4		*Butyrivibrio fibrisolvens*		
5		*Escherichia coli*		

Three different fungal species (Table 4) were recorded in the rumen of Yankasa rams fed different feed supplements as follows: *Piramonas communis, Sphaeromonas communis* and *Pilobolus* spp., with treatment II having the highest number of fungal species.

Table 4. Fungal species isolated from the Rumen Fluids of Yankasa Rams fed different supplementations

S/N	TREATMENTS			
	I	II	III	IV
1	*Sphaeromonas communis*	*Sphaeromonas communis*	*Piramonas communis*	-
2	-	*Pilobolus* spp.	-	-

Furthermore, Table 5 revealed the presence of protozoan species in the rumen f Yankasa rams fed different supplements. The result indicated that Treatment I had the highest number of prozoan species (3 species).

Table 5. Protozoan species isolated from the Rumen Fluids of Yankasa Rams Fed different supplementations

S/N	TREATMENT			
	I	II	III	IV
1	*Epidinium caudatum*	*Isotricha intestinalis*	*Entodinium dubardi*	*Eremoplastron rostratum*
2	*Eremoplastron rostratum*	*Epidinium caudatum*		*Entodinium nanum*
3	*Eudiplodinium maggi*			

DISCUSSION

Yankasa rams are grazing animals with the ability to utilize forage sources for maintenance, growth, reproduction and production as such they possess the rumen, which serves as a fermentation chamber composed of high diversity of microorganisms with the ability for degrading fiber or starch rich feed and other types of non-fibrous carbohydrates as reported by Macêdo *et al.* (2022). Bacteria, fungi and protozoans are the three large group of microorganisms inhabiting the rumen environment. As the rumen environment is complex and always dynamic, depending on the existing environmental conditions. The dynamism in the nature and amount of these microbes may occur due to the ability of the microorganisms to compete for available resources for their own development. When diets with high proportion of grain are supplied to animals, the rumen equilibrium is compromised, since the pH of the environment may change from alkaline to acid, then affecting the development of ruminal microorganisms and, consequently, the use of feed as stressed by

Chen *et al.* (2011). The present study reported that, the rumen fluid pH in the treatment groups fed cowpea hay and groundnut haulms supplemented diets were more acidic than those only fed rice straw. This means that, Cowpea hay and Groundnut haulms supplementation provides conducive atmosphere for microbes by modifying the rumen fluid pH as reported by Dan Abba *et al.* (2023). The changes in the rumen pH as reported by Mateos *et al.* (2017) may propitiate the development of pathogenic microorganisms such as *Escherichia coli*, which has high growth rates, multiplies rapidly, and suppress the development of other microbial groups. This microorganism has high degree of pathogenicity, as it may sporulate and produce substances that cause animal metabolic disorders, diarrhea, lack of appetite, hemorrhages and other problems; which can even contaminate the milk and meat of exposed animals.

As the rumen fluid pH decreases below 5.0, the populations of rumen fluids microbes such as bacteria, fungi and protozoa increases and vice versa which might probably be detrimental to the animal. Hence, the shift in the rumen pH reported by this study is more or less to the benefit of the animals. Similar finding was reported by Saricicek and Ozel (2010). The rumen pH was reported by Russell and Rychilk (2001) to be between 5.5-7.0 to enable microbes-aid digestion in ruminant animals as stressed by Saricicek and Ozel (2010).

The bacterial species associated with feeding supplementation as reported by this study highlight the significance of such species in rumen fermentation and feed degradation to enhance performance and well-being of the animals as stressed by Oyaeleke and Okusanmi (2008). Additionally, their presence in the rumen indicated the relative importance of cowpea and groundnuts supplements in providing nourishment. Furthermore, a study by Weimer *et al*. (2008) reported *Ruminococcus albus* and *R. flavefaciens* among the most important bacterial flora in the rumen for their role in cellulose digestion and have been reported in higher roughage diets. Similarly, Yusuf *et al*. (2016) reported the presence of eight different bacterial species in the rumen of sheep. The finding also agrees with that of McAllister (2000) who reported increase in bacterial load with increase in the levels of legumes supplementation.

The presence of three species of fungi in the rumen of the experimental animals is in line with the work of Akin and Borneman (1989) who reported that fungal species play significant role in ruminant nutrition by their ability to colonize extensively the lignin-containing plant cell walls of forages. This may probably be due to the fungal efficient enzymes system that can degrade structural elements of the plant cell wall. Similar report on the protozoan species identified by the present study showed that, the three genera: *Entodinium*, *Diplodinium* and *Isotricha* are the most common protozoan species contributing to the cellulose and starch digestion as reported by Saricicek and Ozel (2010). Previous studies by Finlay *et al*. (1994) and Newbold *et al*. (1995) individually reported that, protozoan species harbor methanogens on their cell surfaces thereby facilitate methane emission. The protozoan species reported by the present study conform to the previous findings of Takenaka *et al*. (2004) and Kamra (2005) who individually reported high protozoan species in bovine rumen with increased in fibre contents. In addition, Hernandez-Sanabria *et al.* (2012) posited that, the entire microbial diversity in rumen as reported by the present study is dynamically modified, since microbial groups present in the rumen are in constant competition for substrates. The development of a particular microbial group, which usually occurs at the expense of another group of microorganisms, depends on the conditions of the environment and the available substrates. The multiplication of these microorganisms occurs as function of degradation of food particles found in the environment, which are further fractionated into small sizes until they become monomers that can be used in the fermentative pathway of ruminal microorganisms. Previous study by Chen (2012) reported that, animals fed on diet consisting of high-grain showed subclinical acidosis, ruminal pH below 5.0, as well as reduction in microbial groups fermenting of structural carbohydrates, decrease of microbial diversity. On other hand, animals fed on the diet consisting of high proportion of the bulky,

showed a ruminal pH close to neutrality, from 5.89 to 6.40, and high development of cellulolytic and hemicellulolytic microorganisms.

CONCLUSION

Eight different bacterial and protozoan species were isolated in the cowpea and groundnut haulms supplemented treatments compared to the control with subsequent improvement in the composition of microbial biomass that aided digestibility and rumen fermentation in the supplemented groups. Three fungal species were also isolated and identified. Cowpea hay and groundnut haulms could be supplemented at 300g/day/head for improving fermentation that could enhance digestion and assimilation with subsequent improvement in growth performance of Yankasa rams.

CONFLICT OF INTEREST

The author declares no conflict of interests.

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