2019 Journal of Agricultural Production and Technology ISSN: 2360-9364

MICROBIAL POPULATION AND PHYSICO-CHEMICAL CHARACTERISTICS OF RUMEN LIQUOR IN WAD RAMS FED ENSILED MAIZE FORAGE AND *Mucuna pruriens* FOLIAGE

[*1]Alabi, B. O., [2]Ososanya, T. O., [3]Alabi, O. M. and [2]Amuda, A. J.

[1]Department of Animal Science, Osun State University, Osogbo, Osun State, Nigeria.
[2]Department of Animal Science, University of Ibadan, Ibadan, Oyo State, Nigeria.
[3]Department of Animal Science and Fisheries, Bowen University, Iwo, Osun State, Nigeria.

****Corresponding author's e-mail:** banwo.alabi@uniosun.edu.ng

ABSTRACT

The microbial population, Physical and Chemical characteristics of rumen liquor of West African dwarf (WAD) rams fed ensiled Maize Forage (MF) and Mucuna pruriens foliage (MPF) was assessed. MF was ensiled with MPF for 21 days to form four silages and were fed to 20 WAD rams (12.00±0.25 kg) in a completely randomized design for 105 days growth study. Prior to end of the growth study, rumen liquor (10 mL) each was collected from all the WAD rams before the morning feeding using suction tube. Rumen physical and chemical characteristics, microbial Counts in rumen liquor (10 mL) collected from the rams were enumerated using standard procedures. Data obtained were analyzed using descriptive statistics and ANOVA at $\alpha_{0.05}$. *The colour, odour and consistency of all the silages were brownish green, aromatic and slightly viscous, respectively. Acetate was significantly higher in T1 (53.81) and T2 (53.52) compared with T3 (50.24) and T4 (49.02) while propionate was significantly higher in TI (34.74), T2 (34.08) and T3 (33.21), respectively and lower in T4 (31.72). Total Bacterial Count ranged from 7.80±0.42 (T2) to 5.28±0.08 (T4). The rumen ecology of all the West African dwarf rams fed the silage combinations was in normal condition that allowed proliferation of useful microorganisms for effective rumination.* .

Keywords: *Mucuna pruriens,* Maize forage, silage, Rumen liquor, WAD rams
J. Agric. Prod. & Tech.2019; 8:50-57

INTRODUCTION

The rumen is an open ecosystem where all microbial species have the opportunity to grow. The rumen microbial ecosystem is complex and highly dependent on the diets (Mako, 2009). In the rumen the main agents that breakdown carbohydrates are anaerobic bacteria, protozoa and fungi. The anaerobic bacteria are the principal agents for fermenting plant cell-wall carbohydrates but the anaerobic phyco-phycomycetous fungi, may at times be extremely important (Bauchop, 1981).

Bacteria along with protozoa are the predominant microbes in the rumen and by mass account for 40-60% of total microbial matter in the rumen. Protozoa are present in much smaller number than bacteria, but being larger, may equal the latter in total mass (McDonald *et al*., 1988). Fungi are made up of 5-10% of microbes and are always present in the diet high in fibre. They play significant role in the rumen because they hydrolyze some ester linkage between lignin and hemicellulose and cellulose and digesta particulate.

Growth and multiplication of microbes depend greatly on rumen pH and supply of energy and protein. Rumen microbes ferment carbohydrates to make Volatile Fatty Acids (VFAs) and gasses (McDonald *et al.*, 1988). Volatile fatty acids are the major source of energy for ruminant and the amount of each VFA

produced depends on the form and nature of feed consumed. This study was therefore designed to determine the microbial population, physical and chemical of rumen liquor in WAD rams fed silage combination of maize forage and *Mucuna pruriens* foliages.

MATERIALS AND METHODS

Study location: The study was carried out at the sheep unit of Teaching and Research Farm, University of Ibadan and at the Microbiology Laboratory, Department of Animal Science, University of Ibadan, Ibadan, Nigeria.

Experimental Animals: Twenty (20) post weaned WAD rams were used for this experiment. The rams were randomly allotted to four (4) silage combinations (dietary treatments) in a completely randomized design. The silage combinations were:
T1= 100% MF
T2= 75% MF: 25% MPF
T3 = 50% MF: 50% MPF
T4= 25% MF: 75% MPF
MF – Maize Forage
MPF – *Mucuna pruriens* Foliage

Collection of rumen liquor: Seven days prior to the end of 105 days growth experiment, rumen liquor was collected through the stomach suction tube as described by Menke and Steingass (1988) from five animals per dietary treatment.

Data collection: Data were collected on rumen liquor physical characteristics (colour, odour, consistency), chemical characteristics (pH, temperature, ammonia – nitrogen and volatile fatty acids) and microbial population.

pH: The pH of the rumen was measured immediately after withdrawal of rumen liquor using a digital meter.
Temperature: Rumen liquor temperature was immediately measured after the withdrawal of rumen liquor using a digital thermometer.
Amonia Nitrogen, *NH_3-N (mg/dl):* This was determined by steam distillation method (AOAC, 1990).
Volatile fatty acids: The propionic, acetic and butyric acids were determined by using spectrophotometry method as described by Kayouli and Lee (1999).
Microbial counts: Total bacteria and fungi counts were done according to the standard pour-plate method by Harrigan and McCance (1976), while total protozoa count was done using the Neubaurer chamber count as described by Frumholtz *et al*. (1989). Pure culture of bacteria and fungi were determined according to the method of Frumholtz *et al*. (1989).

Experiment Design: The design for this experiment was completely randomized design (CRD)

Statistical Analysis: Data obtained were subjected to analysis of variance (ANOVA) using the procedure of SAS (2010). Statistically significant observed means were compared using LSD of the same package.

RESULTS

Table 1 shows the physical characteristics of rumen liquor in WAD rams fed silage combination of maize forage and *Mucuna pruriens* foliage. The colour, odour and consistency observed were similar across treatments. The rumen liquor collected from all the WAD rams fed the silages was brownish green in colour with aromatic odour and slightly viscous in consistency.

Table 2 shows pH, temperature, Ammonia Nitrogen and volatile fatty acids of rumen liquor of WAD rams fed silage combination of maize forage and *Mucuna pruriens* foliage. All the WAD rams that were fed ensiled maize forage and *Mucuna pruriens* foliage have similar values for rumen pH, temperature and ammonia nitrogen.

Table 1: Physical Characteristics of rumen liquor in WAD rams fed ensiled maize forage and *Mucuna pruriens* foliage.

Parameters	T1	T2	T3	T4
Colour	Brownish green	Brownish green	Brownish green	Brownish green
Odour	Aromatic	Aromatic	Aromatic	Aromatic
Consistency	Slightly viscous	Slightly viscous	Slightly viscous	Slightly viscous

MF = Maize Forage; MPF = *Mucuna pruriens* Foliage; T1 = 100% MF; T2 = 75% MF: 25% MPF; T3 = 50% MF: 50% MPF; T4 = 25% MF: 75%MPF.

Table 2: Chemical Characteristics of rumen liquor in WAD rams fed ensiled maize forage *and Mucuna pruriens* foliage

Parameter	T1	T2	T3	T4	SEM
pH	6.87	6.73	6.70	6.70	0.14
Temperature (°C)	38.70	38.80	38.87	38.72	2.06
NH_3-N (mg/dL)	7.02	7.21	7.32	7.84	0.01
Acetate (mg/L)	53.81[a]	53.52[a]	50.24[b]	49.02[c]	0.03
Propionate (mg/L)	34.74[a]	34.08[a]	33.21[a]	31.72[b]	0.06
Butyrate (mg/L)	7.45[a]	7.15[a]	6.55[b]	6.26[b]	0.29

[a-c]Mean values within the same row with different superscripts are significantly different (P< 0.05). SEM = Standard error of means; MF = Maize Forage; MPF = *Mucuna pruriens* Foliage; T1 = 100% MF; T2 = 75% MF: 25% MPF; T3 = 50% MF: 50%MPF; T4 = 25% MF: 75%MPF.

However, there were significant (*p < 0.05*) differences in volatile fatty acids (VFAs) in the rumen liquor of WAD rams across treatments. Acetate (mg/l) ranged from 49.02 in T4 (25% MF: 75% MPF) to 53.81 in the rumen liquor of WAD rams on T1 (100% MF), propionate (mg/l) ranged from 31.72 in the rumen liquor of WAD rams on T4 (25% MF: 75% MPF) to 34.74 in T1 (100% MF), while the value of butyric (mg/l) in the rumen liquor of WAD rams fed ensiled maize forage and *Mucuna pruriens* foliage followed the same trend.

Table 3 shows the probable bacteria and fungi species in rumen liquor of WAD rams fed silage combination of maize forage and *Mucuna pruriens* foliage. Probable bacteria species found in the rumen liquor of WAD rams on T1 (100% MF) were *Fibrobacter spp, Ruminococcus spp, Streptococcus spp, Eubacterium spp, Ruminicoccus spp*and *Eubacterium spp.* Probable bacteria species found in the rumen liquor of WAD rams on T2 (75% MF: 25% MPF) were *Lactobacillus spp, Eubacterium spp, Ruminicoccus spp, Streptococcus spp* and *Fibrobacter spp*. Probable bacteria species found in rumen liquor of WAD rams on T3 (50% MF: 50% MPF) were *Lachnospira spp, Selenomonasspp,*while the probable bacteria species found in the rumen liquor of WAD rams on T4 (25% MF: 75% MPF) were *Streptococcus spp, Lacthnospira spp, Lactobacillus spp,*

Enterobacter spp. and *Methanobrevibacter spp*. Probable fungi species in the rumen liquor of WAD rams on T1 (100% MF) were *Aspergillus fumigatus, Aspergillus niger, Fusarium spp, penicillium expansium*. Probable fungi species found in the rumen liqour of WAD rams on T2 (75% MF: 25% MPF) were *Aspergillus fumigatus, Aspergillus niger, Aspergillus terreus, Aspergillus flavus*. Probable fungi species found in the rumen liquor of WAD rams on T3 (50% MF: 50% MPF) were *Fusarium spp, Aspergillus niger, Aspergillus terreus, penicillinum expansium,* while probable fungi species in the rumen liquor of WAD rams on T4 (25% MF: 75% MPF) were *Aspergillus flavus, Aspergillus fumigatus, Penicillium expansium.*

Table 4 shows the microbial count in rumen liquor of WAD rams fed silage combination of maize forage and *Mucuna pruriens* foliage. There were significant ($P<0.05$) differences in the rumen protozoa and bacteria counts across the dietary treatments. Protozoa count was significantly highest $1.90\times10^3/cm^3$ in the rumen liquor of WAD rams on T1 (100% MF) and lowest $1.30\times10^3/cm^3$ in the rumen liquor of WAD rams on T4 (25% MF: 75% MPF), bacteria counts was significantly highest 7.80×10^4 cfu/mL in the rumen liquor of WAD rams on T2 (75% MF: 25% MPF) and lowest 5.28×10^4 cfu/mL in the rumen liquor of WAD rams on T4 (25% MF:75% MPF). However no significant ($p>0.05$) difference was obtained for fungi count in the rumen liquor of WAD rams across the dietary treatments.

Table 3: Probable bacteria and fungi species in rumen liquor of WAD rams fed ensiled maize forage and *Mucuna pruriens* foliage

Micro Org.nism	T1	T2	T3	T4
Bacteria	*Streptococcus spp.* *Ruminococcus spp.* *Fibrobacter spp.* *Eubacterium spp.* *Selenomonas spp.*	*Streptococcus spp.* *Ruminococcus spp.* *Fibrobacter spp.* *Eubacterium spp.* *Lachnospira spp.*	*Lactobacillus spp.* *Ruminobacter spp.* *Selenomonas spp.* *Eubacterium spp.*	*Streptococcus spp.* *Enterobacter spp.* *Lachnospira spp.* *Methano-brevibacter spp.* *Lactobacillus spp.*
Fungi	*Aspergillus spp.* *Penicillium spp.* *Fusarium spp.*	*Aspergillus spp.*	*Aspergillus spp.* *Penicillium spp.* *Fusarium spp.*	*Aspergillus spp.* *Penicillium spp.*

MF = Maize Forage; MPF = *Mucuna pruriens* Foliage; T1 = 100% MF; T2 = 75% MF: 25% MPF; T3 = 50% MF: 50% MPF; T4 = 25% MF: 75%MPF.

Table 4: Microbial count in rumen liquor of WAD rams fed ensiled maize forage and *Mucuna pruriens* silage

Parameters	Treatment				
	T1	T2	T3	T4	SEM
Protozoa ($\times10^3/cm^3$)	1.90^a	1.50^b	1.50^b	1.30^c	0.02
Bacteria ($\times10^4$cfu/mL)	7.40^a	7.80^a	7.62^a	5.28^b	0.56
Fungi ($\times10^4$cfu/mL)	1.76	1.74	1.72	1.70	0.03

$^{a-b}$Means within the same row with different superscripts are significantly different ($P < 0.05$). SEM = Standard error of means; MF = Maize Forage; MPF = *Mucuna pruriens* Foliage. T1 = 100% MF; T2 = 75% MF: 25% MPF; T3 = 50% MF: 50% MPF; T4 = 25% MF: 75% MPF.

DISCUSSION

Physical characteristics of rumen liquor

Physical characteristics of rumen liquor that was observed instantly upon collection include colour, odour and consistency. The physical characteristics directly depend on the animal's diet and general digestive stable health (McDonald *et al*., 1995). There are possible causes for various differences in those physical characteristics because diet often times contributes to the colour of the rumen fluid.

McDonald *et al*. (1995) observed a range of colour or rumen liquor of animals fed various diets. Animal fed on corn silage/straw diet will have rumen fluid with yellow-brown colour while those fed on concentrate diet will have rumen fluid with brown/olive colour. Animals fed on pasture diet will have a green rumen fluid while the appearance of milk gray/brown rumen fluid indicates lactic acidosis (McDonald *et al*., 1995). The brownish green colour of rumen liquor observed for all the WAD rams in this study was the same across the dietary treatments and was an indication that the experimental animals were fed either on pasture diet or on corn silage/straw diets.

Rumen fluid odour is classified either as aromatic (for normal animal), acidic/sour (lactic acidiosis) or as rotten (rumen purification/infection) (McDonald *et al*., 1995). The aromatic odour observed in the present study was the same for the rumen fluid of WAD rams across the dietary treatments, which was an indication that the animal's rumen function is normal.

Rumen fluid can be described in term of excess viscosity which is an indicative of high saliva content, watery rumen fluid with few particles which is an indication of anorexia in animal and bubbles in the rumen fluid which indicate bloat (Bowen, 2009). Slightly viscous rumen fluid was observed for all the rams across the dietary treatments in the present study which indicate the living condition of most microorganisms in the rumen.

Chemical characteristics of rumen fluid of experimental WAD rams

pH: The microbes break down feed through the process of fermentation. Under normal condition, the pH of the content of the rumen and reticulum is maintained in the range of 6 to 7. It may become lower in grain-fed cow (Hungate, 1975). The stable pH range is maintained by continual removal, via the rumen wall, of acidic end products of microbial fermentation, and by the addition of bicarbonate from saliva. The pH range of 6.70-6.87 obtained in the present study was in line with the ranged value 6 - 7 for normal rumen pH that can promote efficient microbial growth (Hungate, 1975). Bowen (2009) documented range of pH in the rumen of animal and its direct implications. pH of 8 and above is an indicative of saliva contamination and putrification, pH of 7- 8 will cause reduced feed intake, pH of 6-7 is normal pH of cattle and other ruminants, 5-5.6 pH range is an indicative of high grain diet or pasture fed/early lactic acidosis while rumen pH of 5.5 and below is an indicative of lactic acidiosis.

Rumen Temperature: McDonald *et al*. (1995) reported that the rumen temperature remains stable at around 39^0C (range 38-42^{o}C) which is suitable for the growth of a range of microbes. The temperature range values of 38.70 to 38.87^{o}C obtained in this study was similar to the range of values reported by the same author.

Rumen Ammonia-Nitrogen: Rumen microbes are the major source of protein in ruminants' diet. They break down rumen degradable protein (RDP) to amino acids, then ammonia. Ammonia is a major source of nitrogen for microbial growth. The microbes also convert non-protein nitrogen to ammonia (Moran, 2005). If energy is limited, microbes become less efficient at using ammonia. Instead of being converted

to microbial protein, the ammonia is absorbed across the rumen wall and into the bloodstream and transported to the liver where ammonia is then converted to urea (Moran, 2005). NH_3-N (mg/dL) obtained in the present study ranged between 7.02-7.84 which suggests that the microbes in the rumen are efficient at using ammonia nitrogen for protein synthesis. This is within the range of 5-8 mg/dL reported by Satter and Sylter (1974) for maximum microbial synthesis.

Volatile fatty acids: The volatile fatty acids (VFAs) are the most important end products of carbohydrate break down in the rumen. These acids are important because they are the major source of energy for the ruminants (McDonald *et al*., 1995). The levels in which they are produced determined the fat and protein contents of milk. The three major volatile fatty acids produced are acetate (or acetic acid), propionate (or propionic acid) and butyrate (or butyric acid) (Moran, 2005). The ratio of the various volatile fatty acids produced depends on the type of feed being digested. The value of acetate (mg/L) in the present study ranged from 49.02 in T4 (25% MF: 75% MPF) to 53.81 in T1 (100% MF) while the ranged of 31.72 to 34.74 and 6.26 to 7.45 were obtained for propionate and butyrate, respectively. The acetate followed the same trend. Significant ($p<0.05$) differences observed for all the VFAs across the dietary treatments shows that *Mucuna pruriens* foliage has low energy content but have high fibre. Moran (2005) noted that acetate is an end product from fermentation of fibre, as highly fibrous, low energy such as pasture hay lead to microbial populations which produce high ratio of acetate to propionate. Acetate is necessary for production of milk fat. Propionate is an end- product of fermentation of starch and sugars. Feeds high in rapidly fermentable carbohydrates such as cereal grains lead to populations of bacteria which produce relatively more propionate and butyrate than acetate (Moran, 2005). Butyrate is metabolized in the liver into ketone bodies which are used as a source of energy for fatty acid synthesis, skeletal muscles and other body tissues. High proportion of acetate to propionate and butyrate in the present study might be due to composition of dietary treatments which are mainly fibrous maize forage and *Mucuna pruriens* foliage silage.

Rumen microorganisms in experimental WAD rams

The identified microbes in the rumen of WAD rams fed ensiled maize forage and *Mucuna pruriens* foliage include bacteria, protozoa and fungi. These microbes believed to have feed on the silage ingested by the WAD rams, and by fermentation, produced end products that are utilized by the rams as well as by the microbes themselves for their own reproduction and cell growth. Most of the bacterial species identified in the present study are cellulolytic, while some are fibrolytic and amylolytic. This was in line with Moran (2005) that different species of bacteria perform different functions, some digest starch and sugar with other digest cellulose. There is close relationship between fungi and other microbes since the fungi appear to be the first organism to invade plant cell walls, thereby hydrolyze some esters of lignin and hemicelluloses or cellulose, which allow bacterial fermentation to be initiated (Bauchup, 1981; Ørskov, 1994). Most of the identified fungi species in the present study are fibre degrading anaerobic fungi.

Rumen microbial population in experimental WAD rams

Protozoa count ($\times 10^3/cm^3$) ranged from 1.30 in the rumen of animals on T4 (25% MF: 75% MPF) to 1.90 in those on T1 (100% MF). There were significant ($p<0.05$) differences in the protozoa count across the dietary treatments. Protozoa

population reduced with increased level of *Mucuna pruriens* foliage in the silage.These reductions might be due to the presence of some secondary metabolites in the silage that might have contributed to the reduction in the microbial population especially the presence of tannin both in maize forage and *Mucuna pruriens* foliage that may act as defanuating agent (Matenga *et al*., 2003; Ivan *et al*., 2013). Bacteria count ($\times 10^4$ cfu/mL) was highest (7.40) in T1 (100%, MF) and lowest (5.28) in T4 (25% MF: 75% MPF). There was reduction in bacteria count with increased level of *Mucuna pruriens* foliage in the silage. The result suggests that increased level of *Mucuna pruriens* foliage caused a sharp decrease in the amount of soluble carbohydrates which is the principal agent of fermentation to encourage the proliferation of anaerobic bacteria (McDonald *et al*., 1988). There were no significant ($p>0.05$) differences in the fungi count across the dietary treatments. That shows that the silage compositions do not have any influence on the population of fungi as fungi population was similar across the dietary treatments.

CONCLUSIONS

- Combination of different proportions of maize forage and *Mucuna pruriens* foliage in silage treatments can be fed ruminants without any negative effects on rumen ecology and rumen microbial population.
- However, silage that contained 75% Maize forage and 25%*Mucuna pruriens* foliage gave better results in term volatile fatty acids production and rumen microbial population growth when compared with other dietary treatments.

REFERENCES

A.O.A.C. 1990. The official methods of Analysis. Association of Official Analytical Chemist, 16th Edition ,Washinton D.C.pp.69-88.

Bauchup, T. 1981. The anaerobic fungi in rumen fibre digestion, *Agriculture and Environment*, 6: 339 – 348.

Bowen, R. 2009. Rumen physiology and Rumination retrieved from Colorado state university: http://www.vivo.colosate.edu/hbooks/pathphys/digestion/herbivores/rumination.html.

Frumholtz, P.P., Newbold, C.J. and Wallace, R.J. 1989. Influence of *Aspergillus oryzae* fermentation extract on the fermentation of a basal ration in the rumen simulation technique (Rusitec). *J. Agric. Sci.*, Camb. 113: 169 -172

Harrigan, W.F. and McCance, M.E. 1976. Laboratory Methods in Microbiology, Academic press, London and New York.

Huhtanen, P., Nousiainen, J. And Rinne, M. 2006. Recent developments in forage evaluation with special reference to practical applications. *Agricultural Food Science*. 15: 293– 323.

Hungate, R.E. 1975. The rumen microbial ecosystem. Annual Review of Ecology Systematic. 6:39 – 66.

Ivan, M., Petit, H.V., Chiquette, J. and Wright, A.D. 2013. Rumen fermentation and Microbial population in lactating dairy cows receiving diets containing oilseed rich in C – 18 fatty acids. *British Journal of Nutrition.* 109 (7): 1211 - 1218

Kayouli, C. and Lee, S. 1999. Silage from by-products for smallholders. Proc.FAO e-Conf.on Tropical Silage. FAO Plant Production and Protection Paper 161. Rome. 85-96.

Mako, A.A. 2009. Water Hyacinth (*Eichornia crassipess* Mart Soms-lau bach) as a potential feed for the West African Dwarf Goats. Ph.D. Thesis, University of Ibadan, Ibadan pp. 1–249.

Matenga, V.R., Ngongoni, N.T., Titterton, B. and Maasdorp, B.V. 2003. *Mucuna* seed as feed ingredient for small ruminants and effect of ensiling on its nutritive value. *Tropical and Subtropical Agroecosystems*. 1 (2-3): 97-105

McDonald, P., Edward, R.A. Greenhalgh, J.F.D. and Morgan, C.A. 1995. Animal

Nutrition. 5th Ed. Longman Scientific and Technical, England.

McDonald, P., Edwards R.A. and Greenhalgh, J.F.D. 1988. Animal Nutrition, 4th Ed., Essex, U.K., Longman Publishers, 479p.

Menke, K.H. and Steingass, H. 1988. Estimation of the energetic feed value from chemical analysis and *in vitro* gas production using rumen fluid. *Anim. Res. Dev*. 28: 7 – 55.

Moran, J. 2005. Tropical Dairy Farming: Feeding management for smallholder dairy farmers in the humid tropics. *Land link press.* Pp. 321.

Ørskov, E. 1994. Recent advances in understanding of microbial transformation in ruminants. *Livestock Production Science*. 39: 53 – 60.

SAS. 2010. Statistical Analysis Systems, User'Guide,Version 8 ed., SAS Institute Inc.SAS Campus Drive Cary, North Carolina, USA.

Satter, L.D. and Slyter, L.L. 1974. Effect of ammonia concentration on rumen microbial protein production *in vitro. British Journal of Nutrition* 32: 194 – 208.