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Research Paper

Rumen Fermentation Parameters and Microbial Population of West African Dwarf Rams Fed Diets Containing *Tetrapleura Tetraptera* Fruit Meal.

[1]Jinadu, K. B., [2]Oluwatosin, B.O., [3]Akingbade, A. O.,[3]Adekanbi, A.O.,[3]Saka, A.A.,[3]Olaniyi, T.A. and [3]Abdulsalam, S.

[1]*Centre of Excellence in Agricultural Development and Sustainable Enviroment, FUNAAB.*
[2]*Institute of Food Security, Environmental Resources and Agricultural Research, FUNAAB.*
[3]*Federal College of Animal Health and Production Technology, Ibadan Nigeria.*

ABSTRACT
This study investigated the potential of Tetrapleura tetraptera as feed additive alternative to non- nutritive chemical and antibiotics to enhance rumen fermentation parameters and subsequently influence feed utilization efficiency. Thirty five (35) WAD rams with an average live weight of 13.20 ± 0.2kg were used in a completely randomized design for 140 days. Five concentrate diets containing varying levels (0, 0.5, 1.0, 1.5 and 2.0%) of TTFM were formulated while Panicum maximum was fed as a basal diet. Rumen fluid was collected at the onset and at the end of feeding trial to determine rumen fermentation parameters and microbial loads. Data collected were subjected to one way Analysis of Variance (ANOVA). Results showed that the acetate and propionate with total volatile fatty acids increased between 1.5 and 2.0% inclusion levels of TTFM in the diet of rams. The total volatile fatty acids (VFAs) increased significantly ($p<0.05$) as the inclusion levels of TTFM increased and highest level was obtained on diet 2.0% TTFM. Addition of TTFM significantly ($p<0.05$) influenced the total coliforms, fungi and protozoan counts in the rumen. It can be concluded that Tetrapleura tetraptera fruit meal can be incorporated into the diets of rams between 1.5 and 2.0% to improve the total volatile fatty acids which can subsequently increase the energy status and growth rate of West African Dwarf rams without any adverse effects on the health status of experimental rams.

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I. Introduction

The major constraint for sheep production most especially during the dry season is scarcity and poor quality of forages (Baba *et al*., 2015), coupled with highly diverse methanogenic microbes present in the rumen which has been implicated in global warming and attempts to manipulate rumen microbial fermentation through application of feed additives to the diets remain the high priority (Patra and Sexena, 2010). Rumen modifiers are defined as feed additives which alter ruminal fermentation, microbial growth and positive impact on feed utilization efficiency (Calsamighia *et al.*, 2007). Feed additives are typically non-nutritive compounds or additive added to diets to improve dietary nutrient utilization, enhance performance, minimize the risk of metabolic diseases and curtail adverse impacts of diets on the environment (Acamovic and Brooker, 2011).

A lot of research and production strategies have been employed, including the use of antibiotics to achieve the aim of better net returns of quality meat (Zawadzki *et al.*, 2011). Several antibiotic compounds such as Monensin, Hainanmycin and Virgininamycin have been used to improve ruminal fermentation and the efficiency of nutrient utilization (Candanosa *et al*., 2012; Wang *et al.*, 2015). Although antibiotics achieved good performance, their potential side effects became a real public health concern globally (Rusell and Houlihan, 2003). However, the increased use of these antibiotics has raised concerns about the product safety and environmental health. The use of antibiotics as additives in the animal feed has been banned by the European Union due to its potential hazards to animal and human health (FAO/OIE/WHO, 2004; Chesson, 2006).

II. Materials and methods

The study was conducted at the small ruminant unit of the Federal College of Animal Health and Production Technology, Moor Plantation, Ibadan. The unit is located in the south western part of Nigeria. The area lies within the rain forest ecological zone and fall within longitude and latitude 7^0-27^0N and 3^0-25^0E respectively and altitude of 220-300m above sea level with the average rainfall of about 1250mm. The temperature and relative humidity ranges from 30-35^0C and 76-84% respectively. Thirty five (35) West African dwarf rams randomly allotted to five dietary treatments in a completely randomized design with 5 replicates chosen from each treatment between 6 and 8months of age and weighing between 12.80 and 13.00kg were used for the experiment. The fresh *Tetrapleura tetraptera* fruits were purchased from a reputable market in Ibadan, Oyo State Nigeria. This was identified and authenticated at the Herbarium unit of the Forest Research Institute of Nigeria (FRIN) Ibadan, Oyo state, Nigeria. The authenticated fruits were rinsed in sterile water and air-dried for two (2) consecutive weeks at room temperature and later milled into powdery form before compounding with other feedstuffs as fruit meal at 0%, 0.5%, 1.0%, 1.5% and 2.0% inclusion levels for treatments 1, 2, 3, 4 and 5 respectively. Each animal was served with *Panicum maximum* grass *ad-libitum* and concentrate diets at 3% body weight twice daily.

Table 1. Gross compositions of concentrate diets containing varying levels of *Tetrapleura tetraptera* fruit meal for West African dwarf rams

	Inclusion levels of TTFM (%)				
	0	0.5	1.0	1.5	2.0
Ingredients					
Corn bran	30.00	30.00	30.00	30.00	30.00
Palm kernel cake	25.00	25.00	25.00	25.00	25.00
Rice bran	20.00	20.00	20.00	20.00	20.00
Wheat offal	15.00	15.00	15.00	15.00	15.00
Groundnut cake	5.00	5.00	5.00	5.00	5.00
TTFM	-	+	++	+++	++++
Dicalcium phosphate	3.00	3.00	3.00	3.00	3.00
*Premix	1.00	1.00	1.00	1.00	1.00
Salt	1.00	1.00	1.00	1.00	1.00
Total	100.00	100.00	100.00	100.00	100.00

TTFM: *Tetrapleura tetraptera* fruit meal
+ (0.5kg TTFM), ++ (1.00kg TTFM), +++ (1.50kg TTFM), ++++ (2.00kg)

*contains Vitamin A (I.U.) 10,000.000; Vitamin D_2 (I.U.) 2,000,000; Vitamin E (I.U) 20,000; Vitamin K (mg) 2,250; Riboflavin (mg) 5000; Pyridoxine (mg) 275; Biotin (mg) 50; Pantothenic acid (mg) 7500; Vitamin B_1 (mg) 175; Vitamin B_{12} (mg) 15.0; Niacin (mg) 27,500; Folic acid (mg) 7500. Choline Chloride (mg) 400; Antioxidant (mg) 125; Fe (g) 20.0; Zn (g) 50.0; Mn (g) 80.0; Cu (g) 5.0g; I (g) 12.0; Co (mg) 200; Se (mg) 200

Table 2: Chemical compositions of experimental diet containing varying levels of *Tetrapleura tetraptera* fruit meal

	Inclusion levels of TTFM (%)				
Parameters	0	0.5	1.0	1.5	2.0
Dry matter	81.50	81.40	81.10	80.90	81.55
Crude protein	15.20	15.28	15.34	15.38	15.43
Ether extract	8.40	8.70	8.95	8.96	9.02
Ash	11.00	10.95	10.75	11.02	10.93
Crude fibre	15.89	15.91	16.05	16.10	16.23
Nitrogen free extract	49.51	49.16	48.71	48.54	48.39
Neutral detergent fibre	48.64	52.62	54.69	58.19	60.38
Acid detergent fibre	34.64	36.84	38.93	43.64	47.19
Acid detergent lignin	9.87	11.64	14.62	16.32	17.11
Hemicelluloses	14.00	15.78	15.76	14.55	13.19
Cellulose	24.77	25.20	24.31	27.32	30.08
Tannin	0.32	0.38	0.45	0.56	0.74
Saponin	0.71	0.73	0.78	0.84	0.95
Flavonoid	2.32	2.44	2.67	2.82	3.54

Alkaloid	1.87	1.86	1.90	2.01	2.23
Hydrogen cyanide	0.12	0.15	0.22	0.25	0.26
Sterol	0.76	0.96	1.11	1.36	1.45
Macrominerals (g/kg)					
Calcium	0.84	0.92	1.24	1.68	2.31
Phosphorus	1.12	1.32	1.65	1.97	2.22
Magnesium	2.47	2.54	2.95	3.54	4.01
Potassium	0.74	0.56	0.98	0.79	0.98
Sodium	0.24	0.28	0.28	0.31	0.34
Microminerals(mg/kg)					
Manganese	234.12	242.23	251.23	264.33	267.67
Iron	184.60	177.80	173.30	195.45	205.54
Copper	11.34	8.79	10.33	11.65	10.98
Zinc	55.32	44.76	45.65	51.21	48.87

Rumen fermentation parameters and microbial population determination

Twenty- five (25) West African Dwarf rams were used for this experiment. Rumen fluid was collected using suction tube as described by Preston *et al*. (1995). Rumen fluid pH, Rumen fluid temperature was determined on the farm using digital thermometer and p^H scale.

Microbial population in terms of Bacteria, fungi, Protozoa and microbial populations were determined according to Galyen, 1989. Ammonia Nitrogen was also determined using steam distillation (AOAC, 2000).

III. Result and discussion

Table 3 shows the rumen fermentation parameters of West African dwarf rams fed diets containing varying levels of *Tetrapleura tetraptera* fruit meal. The pH values ranged from 5.79- 6.14 at the onset of the experiment which were not significantly different (P>0.05). The final value observed for rumen fermentation fluid pH ranged from 5.84-6.08. The rumen fluid temperature, propionic and butyric acids values observed in this study ranged from 36.12-37.60^{o}C, 18.90- 20.53 and 17.67- 19.30 nmol/100mol for initial values respectively which were not significantly affected (p>0.05). Significant differences (P>0.05) were not observed at the onset of this study for acetic acids with the lowest values obtained with 2.0% TTFM, inclusion level while the highest acetate was recorded with animals offered 1.5% TTFM at the initial stage. The final acetic acids recorded in this present study was significantly differed (p<0.05) with marked differences in animals fed diets containing 2.0% TTFM having 44.62nmol/100mol while the least value was obtained with animal offered diets inclusion of 2.0% TTFM. The total volatile acid recorded in this study varied from 116.60 to 129.86nmole/100mol. The highest total volatile acids (129.86nmole/100mol) was observed with rams fed diets containing 2.0% TTFM.The range of lactic acid obtained in this study 48.20-56.36 which was not significantly affected (P>0.05) by the inclusion levels of TTFM

Table 3: Rumen fermentation parameters of West African dwarf rams fed diets containing varying levels of *Tetrapleura tetraptera* fruit meal

Parameters	Inclusion levels of TTFM (%)						
	0	0.5	1.0	1.5	2.0	SEM	P- value
Ph							
Initial	5.79	6.14	5.81	5.93	6.08	0.12	0.65
Final	5.93	5.98	5.90	5.84	6.08	0.27	0.51
Variation	0.14	0.16	0.09	-0.09	0.00	0.32	0.42
Temperature (^{0}C)							
Initial	37.02	36.44	37.60	36.54	36.12	0.23	0.78
Final	35.82	36.06	36.44	34.10	35.28	0.06	0.56
Variation	-1.20	-0.38	-1.16	-2.44	-0.84	0.28	0.43
Acetic acid (nmole/100ml)							
Initial	19.20	17.67	19.30	17.80	18.96	0.26	0.54
Final	41.38^{ab}	39.60^{b}	43.04^{ab}	43.38^{ab}	44.62^{a}	0.16	0.06
Variation	22.18	21.93	23.74	25.58	25.66	0.10	0.34
Propionic acid (nmole/100ml)							
Initial	18.90	20.38	20.53	20.18	19.58	0.41	0.80
Final	41.28	42.26	41.48	42.04	46.20	0.12	0.61
Variation	22.38	21.88	20.95	21.86	26.62	0.29	0.74
Butyric acid (nmole/100mL)							

Initial	17.46	17.26	17.20	16.94	15.90	0.41	0.93
Final	34.52	35.62	35.52	37.78	39.04	0.30	0.32
Variation	17.06	18.36	18.32	20.84	23.14	0.11	0.17
Total volatile fatty acids(nmole/100mL)							
Initial	55.56	55.38	56.95	54.92	53.95	1.13	0.38
Final	117.18^{b}	116.60^{b}	120.92^{ab}	123.20^{ab}	129.86^{a}	1.88	0.02
Variation	61.62	61.22	63.97	68.28	75.91	1.02	0.67
A:P							
Initial	1.02	0.95	0.86	0.97	0.88	0.14	0.38
Final	1.00^{a}	1.02	0.95^{ab}	0.97^{ab}	1.03^{b}	0.06	0.01
Variation	-0.02	0.07^{ab}	0.13^{ab}	0.00^{ab}	0.15	0.07	0.13
Valeric acid (nmole/100mL)							
Initial	2.48^{a}	2.29^{ab}	2.15^{ab}	1.98^{b}	2.04^{b}	0.06	0.01
Final	2.69	2.76	2.73	2.40	2.56	0.28	0.33
Variation	0.21	0.47	0.58	0.42	0.52	0.22	0.24
Ammonia Nitrogen- NH_3N (mg/dL)							
Initial	8.30	7.51	8.89	8.00	8.26	0.20	0.27
Final	8.73	9.27	9.35	9.48	9.58	0.46	0.36
Variation	0.43	1.76	0.46	1.52	1.32	0.26	0.49
Lactic acids(nmole/mol)							
Initial	55.32	48.76	56.36	48.74	48.20	0.25	0.96
Final	56.32	45.60	53.74	53.36	50.58	0.12	0.57
Variation	1.00	-3.16	-2.62	4.62	2.38	0.43	0.46

Rumen microbial counts of West African Dwarf rams fed *Tetrapleura tetraptera* fruit meal

Table 4 reveals the rumen microbial counts of West African Dwarf rams fed diets containing varying levels of *Tetrapleura tetraptera* fruit meal. The total bacteria counts at the initial and final were not significantly affected (P>0.05) by the dietary inclusions of TTFM. The values observed in this study for total bacteria counts ranged from 3.34- 3.69x10^{7}cfu/mL. The highest total coliforms count 6.45 was observed with animals offered 1.5% TTFM which was significantly different from other treatments. The negative values in the variation showed a reduction in the total coliform at the end of the experiment. Range of 2.12-2.85 was found for total fungi counts which were significantly influenced ($p<0.05$) by the inclusion of TTFM to the diets of rams. The total protozoa in the rumen fluid at the end of the experiment recorded in this study was 3.09-4.41 with the highest value recorded with 0% TTFM which was significantly different ($p<0.05$) from other treatments.

Table 4: Rumen microbial counts of West African dwarf ram fed diets containing *Tetrapleura tetraptera* fruit meal

Parameters	Inclusion levels of TTFM (%)						
	0	0.5	1.0	1.5	2.0	SEM	P-value
TBC (×10^{7}cfu/mL)							
Initial	3.63	3.59	3.34	3.69	3.59	0.07	0.18
Final	3.73	3.74	3.71	3.76	3.52	0.25	0.71
Variation	0.10	0.15	0.37	0.07	-0.07	0.19	0.45
TCC (×10^{2}cfu/mL)							
Initial	6.09	6.03	5.92	5.84	5.88	0.29	0.18
Final	6.00^{b}	6.16^{ab}	6.45^{a}	5.83^{b}	6.16^{ab}	0.13	0.04
Variation	-0.09^{b}	0.17^{ab}	0.53^{a}	-0.01^{ab}	0.28^{ab}	0.07	0.01
TFC (×10^{3}cfu/mL)							
Initial	1.91	1.94	1.95	1.75	1.76	0.29	0.39
Final	2.12^{b}	2.24^{ab}	2.55^{a}	2.39^{ab}	2.12^{b}	0.10	0.03
Variation	0.21	-0.31	0.59	0.64	0.36	0.05	0.63
TPC (×10^{5}cfu/mL)							
Initial	3.91	4.12	3.69	3.88	3.87	0.15	0.54
Final	4.41^{a}	3.47^{b}	3.64^{ab}	3.65^{ab}	3.09^{b}	0.05	0.04
Variation	0.50^{a}	-0.66^{b}	0.46^{ab}	-0.23^{ab}	-0.78^{b}	0.19	0.02

^{a,b,c}Means with different superscripts along the same row are significantly different ($p>0.05$)

TTFM: *Tetrapleuratetraptera*fruit meal, TBC- Total bacteria count, TCC- Total coliform count, TFC- Total fungi count, TPC- Total protozoan count.

The result revealed that the ruminal pH was significantly influenced by the inclusion levels of TTFM, the trend of the result obtained could be attributed to the fact that fibre digesting bacteria growth is favoured with the pH from 6.00- 6.80. The pH of 5.70 to 6.72 observed in this study is slightly higher than 5.5 to 6.00 reported by Cardoso (2002) who stated that the type of diet could shift values when high forages or grasses rations were fed because forages stimulates higher rate of saliva secretion and it contains bicarbonate which buffer the rumen and therefore increases acetate production. pH decreased as the release of sugar increased (Beever and Drackley, 2013). Van soest*et al.*1994 recommended a range of 6.5- 6.7 for a normal physiological activity in the rumen. However, the rumen pH value obtained in this present study agreed with the report of Dembe, 2001 and Maria *et al*. (2020) with the ranges of 6.5- 6.72 and 6.3-6.50 respectively for maximum microbial growth. Depeters and Bath, 1996 also reported that the pH value between 6.20 and 6.80 reflects the normal microbial ecosystem and cellulolytic bacteria activity. Ruminal pH, ammonia nitrogen and volatile fatty acids are important indicators of ruminal fermentation and the stability of the rumen ecosystem (Li *et al.* (2017). Different responses of ruminal fermentation resulting from feeding animals different substances can be attributed to to different responses of rumen microbes (Golder *et al.*, 2012). Several authors reported increase in the rumen pH (Ben Salem *et al*. 2000), a decrease in ruminal fluid pH (Bhatta *et al.* 2007) and on change in pH (Jolazadeh *et al.*2015) of ruminal pH related to the effects of dietary tannins on the rumen ecology. The ruminal fluid temperature observed in this experiment ranged from 38.09- 38.55^{o}C which was slightly reduced to the range reported by Jinadu *et al.*(2018c) on West African dwarf sheep fed diets containing varying levels of sugarcane waste silage. The temperature difference might due to higher rumen modulation effects of TTFM as a result of difference in heat eructation from microbial fermentation as reported by Hall, 2001.

The volatile fatty acids are produced from the breakdown of amino acids skeletons by rumen microbes (Hassanat and Benchaar, 2013). The improvement in the total fatty acids produced depends on the manipulation of gut function and rumen microbiota with the feed additives which are recognised as an important strategy for improving growth performance and feed efficiency in sheep (Swanson, 2016). Rumen volatile fatty acids are the main energy source for ruminants (Vansoest *et al*., 1994). Consequently, a reduction in their production would be nutritionally unfavourable for the animals. Higher concentration of total volatile fatty acids and propionate in the rams fed diets containing 2.0% inclusion level of TTFM could be an indication of better ruminal fermentation attributes that might have led to improved C:N balance for rumen microbial efficiency. This in line with the finding reported by Animut *et al.* (2008) that total volatile acids and the acetate to propionate ratio increased significantly with increased levels of kobele-spedeza which contained 151g/kg DM of condensed tannins. The increase in acetic: propionic (C_2:C_3) ratio reflects an increase in acetic acid and slight decrease in propionic acid concentration (Ehsan*et al.*, 2013). The acetate: propionate range observed in this study is in line with the range of 3.88 and 4.29 reported by Luana *et al*. (2018) for cattle fed tropical forage supplemented with protein in the rumen, abomasums or both. The better feeding efficiency exhibited by animals on treatment 5 was promoted by the increase in rumen propionate concentration. The total volatile acid results in this study were in agreement with the studies of Devant *et al.*(2000) and Spanhero *et al.* (2017) *invivo* and *invitro* that the total volatile fatty acids concentration and molar concentration of VFA were not affected by the protein degradability. In contrary to these observations in this present study, Chen *et al*. (2015) observed no significant changes in total volatile fatty acids and pH when coriander (6g/head/day) was added to the diets of sheep. No significant difference observed in TVFA due to application of volatile oil to the diets of sheep (Newbold *et al*., 2004). No significant difference observed by Busquet *et al.* (2006) in a laboratory experiment with some medicinal plants. Mohammed *et al*. (2018) observed also no significant difference on TVFA when 3g/L extract of coriander seed powder added to the diets of Awassi ewes. The difference might be attributed to a reduction in TVFA production as a result of hydrolysis of dietary protein to amino acids which are deaminated before conversion to volatile fatty acids (France *et al.*, 2005). A decrease in protein degradation could lead to a reduction of volatile fatty acid originating from deamination of amino acids. Patra and Saxena, 2011; Vasta *et al*.(2010) explained that tannin supplementation can influence lipid bio-hydrogenation in sheep rumen by increasing the accumulation of volatile fatty acids and decreasing the acetate: propionate ratio. Patra and Sexena (2009) also emphasized on the effects on tannins which reduces C2:C3 ratio which in turns reduces the amount of available hydrogen for methanogenesis.

The decay of rumen protein indicated by the production of ammonia nitrogen (NH_3-N), higher NH_3N concentration may be due to higher protein solubility (Neumann *et al.*, 2013) which promoted crude protein digestibility. The NH_3N range of 7.48 – 11.32mg/dL obtained in this present study was below the range of 15 and 30mg/dL reported by Ghorbani *et al.* (2010) for normal physiological rumen activity. The reduction in ruminal ammonia nitrogen might be attributed to a reduction in proteolysis and deamination and/or higher incorporation of NH_3N to microbial protein. The ammonia nitrogen reduction could be related to the ability of polyphenols to affect the growth of rumen proteolytic bacteria, either directly by reducing the activities of protease enzymes or indirectly by their ability to bind proteins as demonstrated though invivo and invitro studies reported by Abarghuei *et al.* (2010) and Ishlak *et al.* (2015). Similar reduction in ammonia nitrogen was in

accordance with previous work on ruminants by Sliwinski *et al*. (2002) who investigated the influence of dietary tannins or polyphenols on the reduction of rumen ammonia nitrogen production. Carulla *et al*. (2005) reported lower concentration of ammonia nitrogen in the rumen fluid of sheep fed diets containing Acaiamearnsii with abundant tannins. Also, a lower concentration of NH_3-N was reported by Puchala *et al*. (2018) in a yearling meat goats consuming a diet with high level of Laspedea compared with alfalfa hay based diet. The values of NH3-N observed in this study were similar to the finding of Kholif *et al*. (2015) who reported a range of 0.45 to 1.24mg/dL in young cattle fed untreated rice straw and increased to 9mg/dL when the straw was treated with urea. The most suitable rumen ammonia nitrogen level for micro-organism activities were 5-20mg/dL in small ruminants fed low quality roughages and 13.6- 34.3/100ml for microbial protein synthesis and digestibility most especially in buffaloes (Wanapat *et al*., 2011).Ammonia nitrogen NH_3N was significantly affected progressively across the treatment. The result of rumen ammonia concentration of rams agreed with that of Hidayah, 2004 who reported that levels of ammonia in the rumen of sheep fed with hay increased to peak levels three hours after feeding. Similarly, Goel *et al*. (2008) stated that for microbial growth, ammonial is required in the level of 0.35 to 29mg/100ml of the third. The values obtain in this study is higher than the range reported by Ososanya *et al*. (2014) that a good profile as a minimum rumen third to maximize rumen microbial protein synthesis. the value observed in this study were similar to the finding of (Kisseda *et al*.,2010) who reported a range of 0.45 to 1.24mg/dl in young cattle fed untreated rice straw ammonia nitrogen (NH_3-N) was less than 2mg/dl and increased to 9mg/dl when the straw was treated with urea. The most suitable rumen ammonia nitrogen level for micro-organism activities were 5 to 20mg/100ml in ruminant fed low quality roughages. (Agle *et al*., 2010) found that optimum range of (NH_3-N) was 13.6 to 34.3mg/100ml for microbial protein synthesis and digestibility in buffaloes. Acetic acid concentration ranged from34.67 in (T3) to 39.43Mmol/100ml ($3.55x10^6$cfu/ml) in (T5) exhibited the best total volatile fatty acid with marked significant difference of butyric acid produced in T4 with (61.04Mmol/100ml). A micro-organism yield in the rumen is an index or a function of the amount of microbial protein made available to the ruminant daily (Jyotti *et al*., 2002). Marked significant difference was observed in rumen protozoan for ram in diet 1 with $3.55x10^6$cfu/ml.

The rumen is colonized by several facultative anaerobic bacteria communities (Vohra *et al*. (2016) which play important roles in fermenting and digesting nutrients to provide energy and protein resources (Vymazal and Kropfelova, 2013), maintaining major biological activities and promoting growth and performance (Morgavi et al. (2018). Moreover, these bacteria communities are living in a dynamic environment that can be affected by many factors such as diurnal variation, diet structure and feed additives (Guan *et al*. (2008); Menezes *et al*. (2011). Previous studies have demonstrated that greater population of ruminal micro-organisms can improve resistance to external stress, stabilize the ruminal micro-ecosystem and enhance production performance (McCann, 2001; Cani and Deizenna, 2009). The significant increase in total bacteria count in the rumen of rams fed with the 2.0% TTFM could be attributed to the effects of bioactive compounds present in the TTFM. The flavonoids directly or through new derivatives produced upon transformation or degradation affect the rumen microbial activity (Hang *et al*., 2018). Flavonoid generally acts against microorganism through inhibition of cytoplasmic membrane fixation, inhibition of cell wall synthesis or inhibition of nuclei acids synthesis (Cushnie and Lamb, 2011). Presence of polyphenols in the TTFM included in the diets of rams can influence the bio hydrogenation process by reducing the activity and growth of rumen micro organisms (Cabiddu *et al*. (2009). Different responses of ruminal fermentation resulting from feeding animals with different substances could be attributed to different responses of rumen micro organisms (Golder *et al*., 2003). The increase in the total bacteria count across the treatment could be due to a number of factors including interactions between the fibrolytic and proteolytic populations and changes in digesta composition and the rate of outflow from the rumen. (Silanikove*et al*.,2001). Significant decrease in the number of protozoa in this present study is in agreement with previous experiment with Lespedeza condensed tannins (Animut *et al*.(2008) and Puchala *et al*.(2012). Presence of phenolic compounds, flavonoids and other anti oxidants can inhibit protozoans growth (Freitas *et al*., 2015), this is also in line with the findings of Mazza *et al*.(2020) with the decrease in the levels of protozoa when acerola (*Malpighiae marginata*) fruit pulp added to the diet of lambs. Despite the maintenance of rumen fluid pH in this study, the protozoa population was affected by the presence of these bioactive compounds which is in agreement with the findings reported by Deyani *et al*. (2007) who observed significant decrease in the protozoa population despite maintenance in pH when feeding ewes with diets containing medicinal plants. Protozoa constitute up to 60% of the microbial biomass, although they rarely exceed 20% of the microbial protein flow in the small intestine (Newbold *et al*., 2014)

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