Examining the Changes in Rumen Bacterial Populations Associated with Improved Rumen Fermentation and Reduced Methane Production in Yankasa Rams Fed Fermented *Hibiscus sabdariffa* Seed Meal

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Abstract

This study investigated the effects of dietary inclusion of fermented *Hibiscus sabdariffa* L. (roselle) seed meal (FRSM) on rumen fermentation parameters, methane emission, and microbial populations in Yankasa rams. Sixteen rams with an average body weight of 18.66 ± 2.84 kg were randomly allocated to four treatment groups receiving diets containing 0%, 10%, 20%, and 30% FRSM for 84 days in a randomized complete block design (RCBD). Fermented roselle seed meal was prepared by soaking cleaned seeds in airtight water-filled containers for 72 hours, followed by seven days of sun-drying and coarse grinding. The experimental diets were formulated with standard feed ingredients and analyzed for proximate and fibre fractions using AOAC and Van Soest methods. Rumen fluid was collected at 0 before feeding and 3-hours post-feeding for pH determination, microbial counts, and bacterial identification. Methane production was estimated using the model: CH_4 (L/day) = 0.34 × BW + 19.7 × DMI + 12. Results showed that dry matter content increased from 92.31% (control) to 94.63% (30% FRSM), and crude protein rose from 11.30% to 19.67% at 20% inclusion. Rumen pH ranged from 6.36 to 6.89, with no significant difference ($P>0.05$) between treatments or sampling times, maintaining optimal microbial conditions. Estimated methane output ranged from 34.98 to 38.52 L/day, with the 20% FRSM group showing a numerical reduction, though not ($P>0.05$) statistically significant. Rumen microbial counts increased post-feeding, ranging from 4.3×10^6 to 6.7×10^6 CFU/mL, and dominant bacteria identified included *Escherichia coli*, *Ruminococcus albus*, *R. flavefaciens*, and *Salmonella* spp. Inclusion of

FRSM significantly (P<0.05) improved the nutritional composition of the diets without disrupting rumen fermentation or microbial balance. The study concludes that FRSM can be incorporated up to 30% in Yankasa ram diets, with 20% offering a potential methane mitigation effect while enhancing nutritional intake and supporting microbial stability in the rumen.

Keywords: Yankasa rams, *Hibiscus sabdariffa*, methane emission, rumen fermentation, microbial profile.

Introduction

Ruminant livestock production is essential for global food security, economic stability, and farmer livelihoods. However, sustainable feeding strategies remain a significant challenge, particularly in regions with limited or expensive conventional feed resources (Millam *et al.,* 2020). The search for alternative feed ingredients that improve animal performance while minimizing environmental impact has led to increased interest in unconventional feedstuffs. *Hibiscus sabdariffa* L. (roselle) seed meal, an agro-industrial by-product, has garnered attention due to its nutritional potential: 94.34%DM, 38.06%CP,23.80%lipid, 4.40%ash (Yashim*et al.,* 2016).

Fermentation is a well-established method for enhancing the nutritive value of plant-based feed ingredients (Ikyume*et al.,* 2018). This process can increase protein content, reduce anti-nutritional factors, and promote beneficial microbial activity, making fermented roselle seed meal a promising feed supplement for ruminants (Yusuf *et al.,* 2013). Furthermore, concerns about methane emissions from ruminants, a significant contributor to global greenhouse gas emissions (McDonald *et al.,* 2010), have driven research into strategies that improve rumen fermentation efficiency and reduce methane output (Anya *et al.,* 2018).

Yankasa rams, a prominent sheep breed in Nigeria, are vital for meat production. Their adaptability to diverse diets makes them suitable for evaluating alternative feed resources (Herrero, 2020). Investigating the inclusion of fermented roselle seed meal in Yankasa ram diets could provide valuable insights into its effects on rumen fermentation, microbial balance, and methane production (Ari *et al.,* 2012). Therefore, this study aims to evaluate the impact of varying levels of fermented roselle seed meal on rumen fermentation characteristics, methane emissions, and microbial populations in Yankasa rams.

Materials and methods

Experimental area

This experiment was conducted at the livestock section of the Federal University Dutse Teaching and Research Farm, located in Dutse, the capital city of Jigawa State. Dutse lies between 11°N and 13°N latitude and 8°E and 10°35'E longitude, at an altitude of 485 meters above sea level. Jigawa State, with an estimated land area of 23,154 km^2 (Google Earth, 2012), is situated within the Sudan Savannah vegetation zone,

with some areas of Guinea Savannah in its southern region. The area is characterized by a prolonged dry season lasting 7-8 months (Olofin, 2008).

Experimental materials

Roselle seeds were collected from farms in neighboring villages surrounding the Federal University Dutse Teaching and Research Farm. The seeds were cleaned by winnowing and hand-picking to remove stones and debris, and then sun-dried for seven (7) days. For fermentation, the seeds were placed in a muslin cloth, completely submerged in clean tap water, and soaked for three days under airtight conditions. After three days, the water was drained, and the fermented seeds were sun-dried for seven (7) days, as described by Ari *et al.* (2012). The dried, fermented seeds were coarsely ground to produce fermented roselle seed meal (FRSM) and bagged. Other feed ingredients used in the study included wheat offal, soybean meal, cowpea haulms, rice milling residue, sorghum husk, bone meal, and common salt.

Experimental animals, management and design

Sixteen Yankasa rams, with an average live weight of 18.66 ± 2.84 kg, were purchased from a local sheep market for this study. Before the experiment, pens were thoroughly cleaned and disinfected. The animals were dewormed with albendazole (2 ml/10 kg body weight) and treated with tetracycline long-acting (TLA) antibiotic (1 ml/10 kg body weight) to prevent bacterial infections. Ivermectin (1 ml/50 kg body weight) was administered to control ectoparasites, following the manufacturer's instructions. Each ram was housed individually in a 2x2 meter pen with an aluminium roof and raised slatted floor. The rams were randomly assigned to four treatment groups, with four animals per group, for an 84-day experimental period, using a randomized complete block design (RCBD). All animals received *ad libitum* groundnut haulms and a daily concentrate supplement of 250g containing fermented roselle (*Hibiscus sabdariffa* L.) seed meal. The rams were balanced for weight across the four dietary treatments (Table 1).

Data collection

Rumen fluid collection

At the end of the experiment (84 days), rumen fluid was collected from three randomly selected rams per treatment at 0 and 3hours post-feeding. Approximately 10 ml of rumen liquor per ram was collected from the mid-rumen using a suction tube, as described by Babayemi and Bamikole (2006). Rumen samples were filtered through four layers of muslin cloth to obtain rumen liquor. The strained rumen liquor (SRL) was immediately preserved with a few drops of saturated mercury (II) chloride solution and stored in labeled polypropylene bottles at -20°C until analysis.

Rumen fluid pH determination

Rumen liquor pH was measured three (3) times immediately using a digital pH meter (Model: JENWAY 550).

Determination of methane gas production

Methane (CH4) gas production was calculated using the model developed by Yan *et al.* (2006) and Ida *et al.* (2012), which forms the basis for many current estimations of ruminant methane production, as shown below:

Methane (L/d) = 0.34×BW (kg) + 19.7× DMI (kg/d) +12

Where BW = body weight.

DMI = dry matter intake.

L = feeding level.

d = da

Table 1. Ingredient Composition (%) of the experimental diets

	Inclusion level of fermented roselle seed meal			
Ingredients	**0%**	**10%**	**20%**	**30%**
Fermented Roselle Seed Meal	0	10	20	30
Wheat Offal	25	24	24	20
Soya Bean Meal	3	3	3	3
Cowpea Haulms	19	14	11	8
Rice Milling Residue	26	25	25	20
Sorghum Husk	26	23	16	18
Common Salt	0.5	0.5	0.5	0.5
Bone Meal	0.5	0.5	0.5	0.5
Total	**100**	**100**	**100**	**100**
Calculated analysis				
Crude Protein (%)	18	17.89	17.78	17.68
Energy (ME Kcal/kg)	2807.0	2926.2	3068.0	3311.0
Crude Fibre (%)	17.24	17.90	18.24	18.29
Ether Extract (%)	6.63	7.62	8.45	8.58
Cost (₦/kg)	70.62	69.69	69.21	65.56

Rumen microbial count

The total number of viable bacteria was estimated using anaerobic roll tubes, based on the methods described by Holdeman *et al.* (1977) and Joblin (1981). Rumen fluid samples were fixed in methyl green-formalin-saline (MFS) solution. The concentrations of 2,6-diaminopimelic acid (DAP), aminoethylphosphonic acid (AEP), and chitin were determined in the rumen fluid samples as microbial markers for bacteria, following the procedure of Olubobokun*et al.* (1988).

Proximate and fibre fractions analysis

The determination of dry matter, crude protein, crude fiber, ether extract, nitrogen free extract and ash of the browse pods and faecal samples were carried out according to the methods of AOAC (2000). While, Fiber fraction: acid detergent fiber, nitrogen detergent fiber, lignin and hemicellulose were determined according to the procedure put forward by Van soest *et al.* (1991).

Dry matter (DM)

The sample was ground and mixed thoroughly. The initial weight of the petri dish was measured. A sample of 10 g was poured into the petri dish and the petri dish with the sample material was placed into a hot air oven and oven dried at 105 ^{o}C for 24hrs. After that the sample was removed from the oven and placed in a desiccator to allow proper cooling and absorption of excess moisture and then weighed. This procedure was repeated until the difference between two successive weighing is less than 1 g in other words until a constant weight is obtained.

The moisture by weight will be calculated using the equation below:

$$\% \text{ Moisture by weight} = \frac{W1-W2}{W1-W} \times 100$$

Where:

W1= weight in gram of petri dish with sample before drying

W2= weight in gram of petri dish with dried sample

W= weight in gram of empty dish

DM= 100- moisture percentage

Crude protein (CP)

Crude protein was determined using Kjeldhal methods. Basically, crude protein analysis involved three major steps; digestion, distillation and titration.

Digestion:

A sample of two grams was weighed and placed in a digestion tube. Catalyst (Kjeldhal digestion tablet) was added to each of the digestion tube with sample to be analyzed. Later 20 ml of concentrated sulphuric acid was added to each of the digestion tube with the sample. The digestion tube stand was placed with the sample in front of the digesta on the tube shelve. Fluorine rubber ring and collecting pipe was put on the main flame and thereafter connected with tap and pump. The digesta was connected to the power line and turn on. The sample was digested at 420 ^{o}C for 3-4 hrs, the tube together with the sample was then removed from the digesta and allowed to cools. As soon as the sample sufficiently cool, a quantity of 80 ml distilled water was added and then mixed thoroughly.

Distillation:

The tap and the switch were turned on, then 20 ml of the digested sample was placed in the digestion tube and fix to the digestion tube tray on the digestion unit. Fifty milliliters of 4% boric acid are taken into the conical flask and placed on the tray, the alkaline nob was turned on and allowed to rich 20 ml before it is then switch off. The distillation machine was switched on and the sample distillated for at least 5-15 minutes until when the boric acid change color to blue or green. Once it changes color it indicates that

there is presence of protein in the sample.

Titration:

The distillate was titrated with 0.1 N hydrochloric acid (HCl normal) and the amount of acid used was recorded.

The crude protein was calculated with the equation below:

$$\% \text{ Protein} = \frac{(A-B)\times N\times 14.007\times F}{2\ Gram\ of\ sample} \times 100$$

Where:

A = Milliliters of acid for titration

B = Blank (Milliliters of acid for titrating blank)

N = Normality of acid use for titration (0.1)

F = Factor (6.25)

Ether extract (EE)

The crude fat was determined using soxlet method: A sample of 1 g was weighed accurately and then placed in a thimble. The mouth of the thimble was then covered with cotton wool, and placed in the fat extractor thereafter. The weight of flat bottom flask was taken. The fat extractor and the flask were fixed together and 150 ml of diethyl ether was added to it and then fixed to the condenser and the heating mantle was turned on which last for 6hours. After the extraction is completed, the thimbles were removed from the extractor along with the residue. After successful collection the chamber was disconnected and then, allowed excess ether to evaporate. The flask and the fat were placed in a hot air oven for 30 minutes at 105 ˚C. The flask and the fat were removed and then placed in a desiccator for 30 minutes to cool. The flask was removed from the desiccator and weighed.

Thereafter, the crude fat was calculated with the equation below:

$$\% \text{ Fat} = \frac{W1-W}{M} \times 100$$

Where:

W1 = weight of fat and flask

W = weight of empty flask

M = weight of sample before extractor

Crude fiber (CF)

The crude fiber was determined using trichloroacetic acids (TCA) methods as described by AOAC (2000): One gram of the sample was accurately weighed and placed in a 250 ml flat bottom flask and 250 ml TCA reagent was added and boil for 40 minutes timing from the time heating commences. 3 ft long air condenser or jacketed condenser was used to reduce loss of liquid. After digestion, the apparatus was removed and filter paper was weighed while the digested sample was filtered with a known weighed filter paper and washed with hot water seven times and the last time with petroleum spirit. The sample was allowed to dry overnight and the filter paper oven dried with the sample in a weighed crucible. The crucible was placed in a desiccator for 30minuts. The crucible then weighed.

The amount of crude fiber was calculated from the equation below:

$$\% \text{ CF} = \frac{Weight\ of\ oven\ dried\ sample - weight\ of\ empty\ crucible - weight\ of\ filter\ pape}{Initial\ weight\ of\ the\ sample} \times 100$$

Determination of ash

One gram of the sample was weighed into a previously weighed crucible. The crucible with the sample was placed in a furnace at 550˚C for at 5 hours. The sample was then be removed from the furnace and placed in a desiccator for 30 minutes to allow to cool. Sample was remove afterward and then weighed.

The amount of ash was calculated with the equation below:

$$\% \text{ Ash} = \frac{W1 - W2}{W} \times 100$$

Where:

W1 = Weight of crucible with the sample

W2 = Weight of empty crucible

W = Initial weight of the sample.

Statistical analysis

Data were expressed as means with standard errors. Proximate and fiber fraction analyses were subjected to one-way analysis of variance (ANOVA) using the General Linear Model (GLM) procedure of Statistical Analysis System (SAS, 2013). Means found to be statistically different (P <0.05) were separated using the Least Significant Difference (LSD) test within SAS. Prism software (version 8) was used to compare means for pH, estimated methane production, and bacterial counts. All results are presented as mean ± SEM in figures and tables.

Results and discussion

Chemical composition of the experimental diets

Table 2 presents the chemical composition of the experimental diets, revealing significant differences (P < 0.05) among treatments. The dry matter (DM) content of the diets was higher than the 85.00% to 88.50% reported by Yusuf *et al.* (2013) and the 83.23% to 85.60% reported by Ajagbe *et al.* (2020) for cassava peel meal, indicating that the inclusion of fermented roselle seed meal increased the nutrient concentration of the diets. The crude protein (CP) content of all diets exceeded the minimum 7% requirement for small ruminant production recommended by the NRC (2007) and was also higher than the 10.00% to 16.0% reported by Yusuf *et al.* (2013). The DM and CP contents observed in this study were sufficient for the maintenance and growth of Yankasa rams, as per NRC (2007).

The crude fibre (CF) content was lower than the 5.50% to 25.00% reported by Bello and Tsado (2013) for sorghum stover supplemented with poultry droppings. Similarly, the ether extract (EE) content was lower than the 5.00% to 20.00% reported by Bello and Tsado (2013) for Yankasa rams. The ash content values were lower than the 10.50% to 10.89% reported by Bello (2017) for basal diets. The nitrogen-free extract (NFE) values were higher than the 7.29% to 14.56% reported by Ukanwoko and Ibeawuchi (2009) for soybean meal.

These variations in nutrient composition may be attributed to differences in feed ingredients,

processing methods, soil conditions, and climatic factors. The fermented roselle seed meal (*Hibiscus sabdariffa* L.) contains substantial protein, neutral detergent fiber (NDF), and acid detergent fibre (ADF) compared to other legumes. Moderate NDF and ADF values, as observed in this study, enhance energy availability for ruminants (Belewu*et al.,* 2010). Plant byproducts like *Hibiscus sabdariffa* L. seeds are generally nutritionally adequate for production (Anele et al., 2012).

Table 2. Chemical compositions (%) of the experimental and basal diet

	Inclusion Level of fermented Roselle Seed Meal					
Parameters	**0%**	**10%**	**20%**	**30%**	**GNH**	**p-values**
DM	93.93±0.27^{b}	94.55±0.06^{a}	94.24±0.14^{b}	94.63±0.00^{a}	92.31±0.56^{c}	0.0004
CP	11.30±0.25^{c}	17.89±0.31^{b}	19.67±0.06^{a}	17.87±0.03^{b}	13.74±0.07^{b}	0.0001
CF	14.46±1.11^{b}	14.22±0.23^{b}	17.35±0.24^{a}	19.34±0.47^{a}	22.15±0.28^{a}	0.0001
EE	6.50±0.01^{b}	6.74±0.01^{b}	8.13±0.04^{a}	8.14±0.01^{a}	2.12±0.01^{c}	0.0001
ASH	1.66±0.02^{b}	1.91±0.02^{b}	2.33±0.01^{a}	2.16±0.02^{a}	5.27±0.04^{a}	0.0001
NFE	60.01±1.37^{a}	53.48±0.56^{b}	47.07±0.29^{b}	47.12±0.54^{b}	55.11±0.01^{c}	0.0001
NDF	24.78±0.01^{b}	26.71±1.16^{b}	26.60±0.60^{b}	28.81±0.56^{a}	45.20±0.03^{a}	0.0015
ADF	18.54±0.09^{b}	15.90±0.05^{c}	19.56±0.07^{b}	24.86±0.07^{a}	28.34±0.38^{a}	0.0001
ADL	6.19±0.00^{b}	5.45±0.01^{b}	6.59±0.00^{a}	8.33±0.02^{a}	5.27±0.03^{c}	0.0001

^{abc}: Means on the same row having different superscripts are significantly different = significant at P<0.05; and not significant at P>0.05, P – Parameters, DM-Dry matter, CP-Crude protein, CF-Crude fibre, EE-Ether extract NFE-Nitrogen free extract, NDF - neutral detergent fibre, ADF – acid detergent fibre, ADL – acid detergent lignin, T_1 = control diet contains 0% fermented Roselle seed meal, T2 = diet contain 10% fermented Roselle seed meal, T_3 = diet contain 20% fermented Roselle seed meal, T_4 = diet contain 30% fermented Roselle seed meal, GNH = Groundnut Haulms, ±SEM = Standard Error of Mean

Effects of varying levels of fermented Roselle Seed meal in diets of Yankasa rams on rumen pH and methane (estimate of methane produced)

Figures 1 and 2 present the rumen fluid pH and estimated methane emissions of Yankasa rams fed diets containing varying levels of fermented roselle seed meal (FRSM). No significant differences (P > 0.05) were observed in pH or estimated methane emissions between sampling times (0 hours before feeding and 3 hours after feeding). The recorded pH values fell within the optimal range of 6.00 to 7.20, which is conducive to rumen microbial growth and activity (Jallow & Hsia, 2011). This aligns with Kamra's (2005) finding that a pH range of 6.0 to 6.9 supports optimal rumen bacterial growth.

Rumen fluid pH analysis revealed that rams-fed diets containing 20% and 30% FRSM exhibited slightly more acidic pH values compared to the control group. This observation contrasts with Olafadehan*et al.* (2016), who reported

that higher rumen pH is associated with increased rumination and salivary secretion, buffering ruminal pH, potentially due to higher fibre levels and a lower roughage-to-concentrate ratio. The present study's pH findings suggest that FRSM-supplemented diets maintained a suitable environment for rumen microbes. Rumen microbial populations (bacteria, fungi, and protozoa) generally decrease with increasing pH and vice versa (Saricicek& Ozel, 2010). Ruminant digestion relies on these diverse reticulorumen microorganisms, primarily anaerobic bacteria and ciliate protozoa (Saricicek& Ozel, 2010). The measured rumen pH values were consistent with the normal range of 6.0 to 7.0 (Petrovski, 2017).

Methane emission is a critical factor in ruminant nutrition studies. Feed intake is a major determinant of total methane emissions (Ramin &Hubtanem, 2013). Methane production is influenced by feed type and quantity (Shibata &Tarada, 2010). Dry matter intake is a primary driver of daily methane output, although methane output can decrease with increased feeding levels, diet digestibility, and concentrate inclusion (Grainger *et al.,* 2007; Beauchemin, 2009).

In this study, the 20% FRSM inclusion level resulted in numerically lower methane emissions, suggesting a potential influence of feed type on methane production. However, overall methane emissions were not significantly affected by FRSM inclusion. This aligns with Getabalew*et al.* (2019), who identified feed intake, type, and quality as factors influencing ruminant methane production. High-concentrate diets have been reported to reduce methane production (Finlay et al., 1994; Van Soest, 1982), potentially explaining the observed trend.

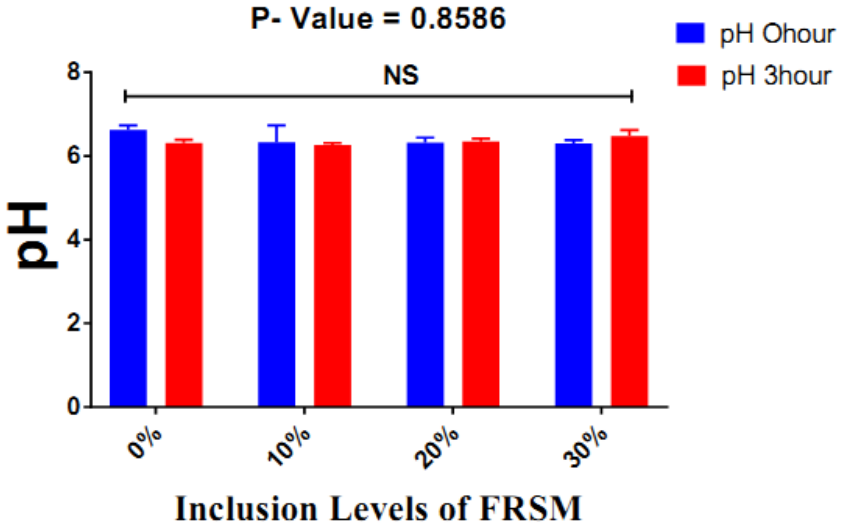

Figure 1. Effects of varying levels of fermented (*Hibiscus sabdariffa L.*) seed meal in diets of Yankasa rams on rumen pH zero hour (0h) before and (3hrs) after feeding

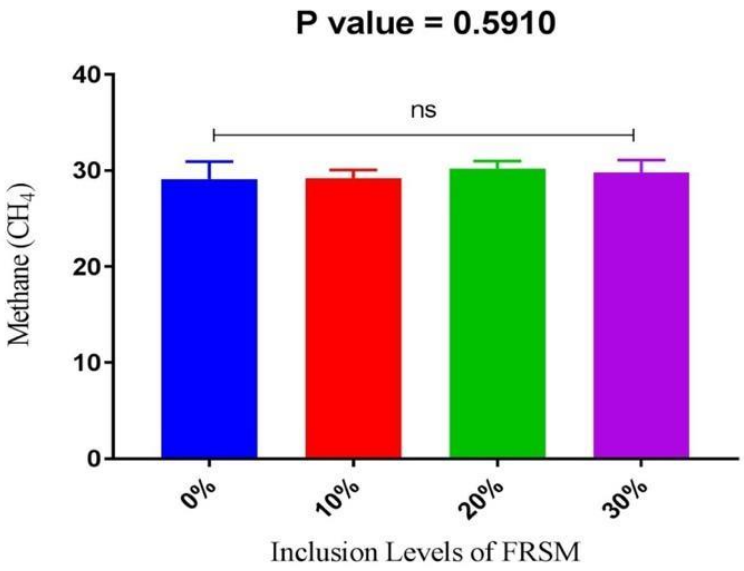

Figure 2. Effects of varying levels of fermented (*Hibiscus sabdariffa L.*) seed meal in diets of Yankasa rams on an estimate of methane produced

Rumen bacterial count (CFU/mL) of Yankasa rams fed varying inclusion levels of fermented (Hibiscus sabdariffa L.) seed meal

Figure 3 presents the bacterial counts in the rumen fluid of Yankasa rams-fed diets containing varying levels of fermented roselle seed meal (FRSM). No significant differences (P > 0.05) were observed in bacterial counts between 0 and 3-hours post-feeding across all treatments (0%, 10%, 20%, and 30% FRSM inclusion). Bacterial counts were generally lower at 0 hours, likely due to pre-feeding starvation. The observed increase in bacterial counts at 3 hours post-feeding is likely attributable to increased bacterial activity in the rumen. This aligns with McAllister (2000), who reported significant changes in rumen microbes following feed consumption, particularly when transitioning from roughage-based to concentrate-based diets. Varal and Dehority (1989) also noted increased bacterial counts with concentrate and basal feed. The present study's results demonstrate a clear increase in bacterial counts 3 hours post-feeding compared to pre-feeding level.

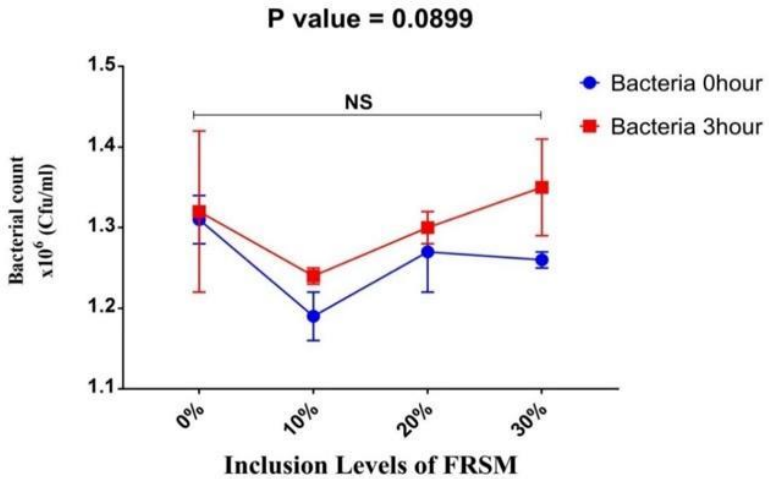

Figure 3. Rume ; of fermented (Hibiscus sabda ee (3hrs) after feeding

Rumen bacterial identification in Yankasa rams fed fermented roselle seed meal

Table 3 presents the bacterial species identified in the rumen fluid of Yankasa rams fed diets containing varying levels of fermented roselle seed meal (FRSM). Five bacterial species were identified: *Escherichia coli*, *Ruminococcus albus*, *Ruminococcusflavefaciens*, *Ruminocola* (likely a typo for *Ruminococcus*), and *Salmonella* spp. The presence of these diverse bacterial species highlights the influence of diets containing FRSM on the rumen microbial community.

Ruminococcus albus and *R. flavefaciens*, identified in this study, are considered key bacterial flora in the rumen, playing a vital role in cellulose digestion, and are often associated with high-protein and roughage diets (Weimer *et al.,* 2008; Church, 1984). The number of bacterial species identified in this study is lower than the eight species reported by Yusuf et al. (2016) in sheep rumen.

Anaerobic bacteria, such as *Escherichia coli* (which constitutes approximately 0.1% of gut microbiota, Eckburg *et al.,* 2005), are essential for rumen degradation processes. The increased bacterial counts observed with increasing FRSM inclusion levels align with McAllister (2000), who reported higher bacterial loads with increased dietary levels. This trend is also consistent with Varel and Dehority (1989), who observed increased bacterial counts with increased test ingredient levels. However, this contradicts Church (1984), who reported a decrease in rumen microorganism counts during starvation.

Table 3. Rumen Bacterial Identification in Yankasa Rams Fed Fermented Roselle Seed Meal

	Inclusion level of fermented roselle seed meal			
Parameter	**0%**	**10%**	**20%**	**30%**
Bacteria	(1) *Escherichia coli*	(1)*Ruminocolaspp,* (2) *Escherichia coli*	(1) *Escherichia coli,* (2)*Ruminococcus albus*	(1*) Salmonella spp,* (2) *Escherichia coli,* (3) *Ruminococcusflavefaciens,*

T_1 = control diet contains 0% fermented Roselle seed meal, T2 = diet contain 10% fermented Roselle seed meal, T_3 = diet contain 20% fermented Roselle seed meal, T_4 = diet contain 30% fermented Roselle seed meal

Conclusion

In conclusion, this study demonstrated that fermented *Hibiscus sabdariffa* (roselle) seed meal can be incorporated into Yankasa ram diets at inclusion levels up to 30% without detrimental effects on rumen fermentation, microbial balance, or overall performance. The diets provided adequate crude protein for growth and maintenance, while variations in crude fibre and ether extract were observed. Rumen pH and microbial populations remained within

optimal ranges for digestion. Notably, the 20% inclusion level showed a numerical reduction in methane emissions, suggesting a potential role for fermented roselle seed meal in methane mitigation strategies. The presence of beneficial rumen bacteria, such as *Ruminococcus albus* and *Ruminococcusflavefaciens*. This study highlights the potential of fermented roselle seed meal as a viable alternative feed resource for enhancing livestock productivity while contributing to sustainable animal agriculture.

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