Research Article

Effectiveness of *Saccharomyces cerevisiae* and Sulfur as Supplements in Ammoniated Citronella Waste Basal Rations on the Nutrient Digestibility, Rumen Fluid Characteristics, and Methane Production

Putri Okta Shafura[1], Mardiati Zain[2]*, Elihasridas[2], Bima Bagaskara[1], Ummi Amanah[1], Laras Sukma Sucitra[1], Bella Veliana Utami[1], Roni Pazla[2], Erpomen[2], Ezi Masdia Putri[3], Rusmana Wijaya Setia Ningrat[2], Riris Delima Purba[3], Ruslan Abdul Gopar[3], Putut Suryo Negoro[3]

[1]Postgraduate Program, Faculty of Animal Science, Andalas University, Jl. Limau Manis, Padang, 25163, Indonesia; [2]Department of Nutrition and Feed Technology, Faculty of Animal Science, Andalas University, Jl. Limau Manis, Padang, 25163, Indonesia; [3]Research Center for Animal Husbandry, Research Organization for Agriculture and Food, National Research and Innovation Agency (BRIN), Jl. Raya Jakarta Bogor Cibinong, 16915, Indonesia.

Abstract | The objective of the study was to find the most suitable formulation for a waste-based ration using ammoniated citronella to increase digestibility and provide optimal supplementation of *Saccharomyces cerevisiae* and mineral sulfur for rumen fluid characteristics. This study employed a completely randomized design comprising four treatments with four replications, with an incubation time of 48 hours. The ration was prepared based on a 50:50 composition of forage and concentrate, including P0 (Control), P1 (0.5% *S. cerevisiae*), P2 (0.3% sulfur), and P3 (0.5% *S. cerevisiae* + 0.3% sulfur). The Tilley and Terry *in-vitro* methods were utilized to measure rumen characteristics, nutrient digestibility, protozoan population, microbial protein synthesis, and methane gas production. The results revealed a significant impact ($P < 0.05$) of these treatments on nutrient digestibility and rumen fermentation characteristics. The combination of 0.5% *S. cerevisiae* and 0.3% sulfur tended to increase nutrient digestibility, total VFA production, and microbial protein synthesis. Additionally, there was a notable difference ($P > 0.05$) in rumen pH. Thus, the ammoniated citronella ration supplemented with 0.5% *S. cerevisiae* + 0.3% sulfur tended to decrease NH_3 production, protozoa population, and methane gas production. The results lead to the conclusion that ammoniated citronella rations supplemented with 0.5% *S. cerevisiae* + 0.3% sulfur have the potential as ruminant feed because they can increase nutrient digestibility and rumen fermentation characteristics and decrease methane gas production.

Keywords | Digestibility, *Saccharomyces cerevisiae,* Sulfur, Ammoniated citronella waste

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***Correspondence** | Mardiati Zain, Department of Nutrition and Feed Technology, Faculty of Animal Science, Andalas University, Jl. Limau Manis, Padang, 25163, Indonesia; **Email:** mardiati@ansci.unand.ac.id
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INTRODUCTION

Citronella (*Cymbopogon nardus L. Rendle*) is a plant known for its main components, citronella, and geraniol, which are used in essential oil production. In Indonesia, citronella plantations covered an extensive area of 19,370 hectares in 2017, yielding a total of 2,340 tonnes per year (Sulaswatty *et al.*, 2019). In West Sumatra,

citronella plantations are located in Solok City, Pasaman Regency, and Pariaman City. The citronella area in Solok is 4.1 hectares with an annual productivity of 3.2 tonnes per hectare (Indriyani, 2021). Meanwhile, Pasaman Regency, spanning 2,863 hectares, achieves a yearly productivity of 186,220 kilograms (BPS-Statistics of Pasaman Regency, 2018).

Citronella that has gone through the distillation process will produce waste that is usually discarded and burned. This waste could potentially be used as an alternative feed for ruminants. Judging from the nutritional content, an alternative feed for ruminants can be effectively derived from citronella. According to Annisa (2020), fresh citronella waste exhibits nutritional content such as BK 61.86%, PK 7.72%, SK 29.19%, TDN 53.07%, NDF 69.93%, ADF 44.45%, Cellulose 30.39%, and Hemicellulose 25.48%. However, citronella waste has a disadvantage, namely a high lignin content of 10.38% (Annisa, 2020), while ruminants are tolerant of maximum lignin content of 7% (Jamarun *et al*., 2020). This high lignin content causes low digestibility, thus requiring chemical processing, one of which is ammoniation.

Processing citronella with ammoniation only gives a low response to increasing the digestibility of food substances and has not provided optimal results. Zain *et al*. (2008) suggest that processing alone does not provide optimal results for livestock. Thus, efforts are needed to optimize bioprocesses in the rumen by increasing rumen microbial activity. One of the efforts that can be used is the supplementation in the ration that will stimulate microbial growth and activity. Supplements that can be used are the addition of *Saccharomyces cerevisiae* and mineral sulfur. The digestive process in the rumen of ruminants heavily relies on the microbial population. The breakdown of feed is primarily carried out by enzymes produced by rumen microbes. Enhancing the microbial population is anticipated to contribute to elevated enzyme concentrations, ultimately leading to improved feed digestibility. This aligns with the findings of Elihasridas *et al*. (2010), supporting the idea that an augmented microbial population not only enhances feed digestibility but also augments the supply of microbial protein, addressing the protein requirements of ruminants.

Saccharomyces cerevisiae, a type of yeast, actively plays a significant role in ruminant nutrition. It acts as a probiotic, improving ruminal fermentation, pH stabilization, and food digestion (Deliberalli *et al*., 2023; Elghandou *et al*., 2019). Enhancements in feed intake, weight gain, and productivity have been demonstrated through the supplementation of yeast in the ruminant diet (Tavarest *et al*., 2021). It also increases the number of anaerobic cellulolytic bacteria and influences mineral absorption (Mohammed *et al*., 2018). Yeast supplementation can treat rumen microbial dysbiosis, leading to improved nutrient utilization and enhanced animal growth and productivity. Yeasts can produce digestive enzymes, growth stimulators, and antimicrobial compounds, promoting the growth and function of beneficial microbiota and inhibiting potential pathogens. The use of *S. cerevisiae* in dairy cows has shown the best response, with increased ruminal cellulolysis and flow of microbial proteins into the intestine (Habeeb, 2017).

S. cerevisiae has been studied in the context of ruminant nutrition. In one study, the combination of *S. cerevisiae* and an *ionophore* improved the performance of finishing sheep in terms of feed intake and feed conversion (Deliberalli *et al*., 2023). Another study evaluated the effects of nitrogen sources and the use of *S. cerevisiae* in sugarcane-based diets for cattle. It was found that the use of *S. cerevisiae* did not significantly affect feed intake or nutrient digestibility (Elghandou *et al*., 2019). Overall, these studies suggest that *S. cerevisiae* can have positive effects on ruminant performance, but its specific impact may vary depending on the specific diet and animal species being studied.

According to Oh *et al*. (2019) the use of *S. cerevisiae* as a direct-fed microbial (DFM) is reported to increase feed digestibility and also help maintain rumen pH stability. The ability of *S. cerevisiae* to enhance digestibility is linked to its capability to decrease oxygen levels in the rumen. This condition protects rumen microbes from oxygen damage, thereby establishing ideal circumstances for the proliferation of cellulolytic bacteria. Consequently, the population of cellulolytic bacteria rises, resulting in enhanced digestion within the rumen (Astuti *et al*., 2022). The addition of *S. cerevisiae* in *in vitro* research at a 0.5% concentration can enhance the microbial population in the rumen (Zain *et al*., 2011).

Mineral sulfur is essential for fiber-digesting microbes. It is required for the synthesis of microbes that contribute to the production of essential amino acids, particularly amino acids containing sulfur groups such as cystine and methionine. Additionally, the synthesis of several vitamins, such as thiamin and biotin, is crucially facilitated by mineral sulfur. The addition of mineral sulfur in *in vitro* studies at a concentration of 0.3% has been shown to enhance the fermentability and degradability of feed (Zain *et al*., 2010).

Sulfur supplementation has demonstrated a significant impact on the ruminant microbiome. Studies indicate that supplementing with sulfur-containing compounds, such as sodium sulfate or methionine, can increase the variety and abundance of microorganisms in the rumen (Tsegay and Tan, 2022). These supplements have been found to increase the proportional quantities of specific bacterial phyla and genera, such as Firmicutes and Ruminococcus 2, while reducing the proportional quantities of others,

such as Prevotella and Bacteroidetes (Zhao *et al*., 2020). Additionally, sulfur supplementation has been shown to boost populations of total bacteria, methanogens, protozoa, fungi, and fiber-utilizing bacterial species in the rumen (Rosamalia *et al*., 2022). These changes in the microbiome have been linked to improvements in rumen fermentation, fiber digestibility, and nutrient metabolism (Hassan *et al*., 2020). Overall, sulfur supplementation appears to modulate the rumen microbial community and enhance microbial protein synthesis, leading to improved rumen function and nutrient utilization in ruminants.

Based on the above description, it is necessary to conduct research to evaluate the supplementation of *Saccharomyces cerevisiae* and mineral sulfur in ammoniated citronella waste-based rations. The aim is to enhance the fermentability and digestibility of these rations in the rumen.

MATERIALS AND METHODS

Research location

This study was conducted at the laboratory specializing in ruminant nutrition, Faculty of Animal Husbandry, Universitas Andalas, Padang, from August to October 2023.

Research materials

The ingredients of the ration included ammoniated citronella waste (ACW), field grass, and concentrate (comprising rice bran, fine corn, tofu, and a mineral mix), supplemented with *S. cerevisiae* and mineral sulfur. This experiment employed a completely randomized design with four treatments and four replications. The treatment rations are detailed below:

P0: Rations (25% field grass+25% ACW + 50% concentrate)
P1: Ration + 0.5 *Saccharomyces* cerevisiae
P2: Ration + 0.3% Sulfur mineral
P3: Ration + 0.5 *Saccharomyces cerevisiae* + 0.3% Sulfur mineral

Ammoniated citronella waste procedure

The procedure for citronella waste ammoniation was first cut into pieces of about 5-10 cm, then urea was dissolved with water with the ratio of urea and water 1:1. The amount of urea used was 4% of the dry matter of citronella waste. Then, citronella waste was mixed with urea solution, put into plastic, and compacted until full. After the plastic was full, it was vacuumed so that the atmosphere in the plastic that contained citronella waste was anaerobic and tied. Then the plastic was stored in a safe and shady place for 3 weeks. After 3 weeks, the plastic bag was opened and the ammoniated citronella waste was removed and aerated to remove excess ammonia.

Sample treatments were milled with a size of 1mm for nutrient content and *in-vitro* analysis. The research parameters included methane gas production, protozoan population, microbial protein synthesis, rumen fluid characteristics (VFA, NH_3, and pH), and digestibility of various feed components, including dry matter, organic matter, crude protein, and fiber fractions (cellulose, NDF, and ADF).

Table 1: Ration constituents.

Feed ingredients	Ration treatment (%)			
	P0	P1	P2	P3
Field grass	25	25	25	25
Ammoniated citronella waste	25	25	25	25
Refined corn	10	10	10	10
Tofu dregs	35	35	35	35
Rice bran	3	3	3	3
Mineral mix	2	2	2	2
Saccharomyces cerevisiae	-	0.5	-	0.5
Sulfur	-	-	0.3	0.3
Nutrients (%)				
Crude protein	13.85	13.85	13.85	13.85
TDN	67.37	67.37	67.37	67.37

In vitro method

The *in-vitro* digestibility test was carried out by preparing the samples to be weighed. Samples were weighed to 2.5 grams and placed into a 250 ml Erlenmeyer flask. Subsequently, 200 ml of McDougall solution buffer and 50 ml of rumen fluid were added to each Erlenmeyer flask. After the mixture of rumen fluid and buffer was added, CO_2 immediately flowed for 30-60 seconds to create anaerobic conditions. Next, the flask was closed with a perforated rubber lid for gas removal. The Erlenmeyer flask (fermenter bottle) was placed in a shaker water bath at 39°C. The flask was then incubated for 48 hours. Afterward, the Erlenmeyer flask was removed and placed in a container containing ice. This was done to inactivate the activity of rumen microbes. Before centrifuging, the pH of the rumen fluid in the Erlenmeyer flask was measured. After that, it was centrifuged for 15 minutes at 3200 rpm at 4°C to separate the supernatant from the residue. Once the supernatant and residue were separated, the supernatant was placed into tubes that had been prepared for the protozoan population, microbial protein synthesis, total VFA, and NH_3 analysis. The remaining substance underwent filtration using pre-weighed Whatman® No. 41 filter paper.

Statistical analysis

The obtained data were analyzed using Analysis of Variance. If there were significant differences, the analysis was followed by the Duncan Multiple Range Test, as per

the instructions provided (Steel and Torrie, 1993).

RESULTS AND DISCUSSION

Effect of *S. cerevisiae* and mineral sulfur supplementation on nutrient digestibility

The supplementation of *S. cerevisiae* and mineral sulfur significantly influenced ($P < 0.05$) the digestibility of crude protein, dry matter, and organic matter. As indicated in Table 2, the supplementation of *S. cerevisiae* and mineral sulfur had a significant impact on nutrient digestibility compared to the control diet. P1, P2, and P3 exhibited similar effects on nutrient digestibility, with P3 showing a tendency to increase nutrient digestibility. The average digestibility of dry matter (DMD), organic matter (OMD), and crude protein (CPD) tended to increase due to the influence of the blend of *S. cerevisiae* and mineral sulfur. *S. cerevisiae* plays a role in increasing the microbial population in the rumen. According to the opinion of Zain *et al.* (2011), supplementation of *S. cerevisiae* can increase the population of fiber-digesting microbes in the rumen, thereby increasing the rate of digestion and overall digestibility. The synthesis of microbial protein heavily depends on the mineral sulfur, as the efficiency of digesting fibrous feed is closely linked to enzymes produced by rumen microbes, a point emphasized by Hassan *et al.* (2020). Suyitman *et al.* (2021) also stated that the mineral sulfur is needed for microbial synthesis, playing a vital role in the provision of essential amino acids, particularly those containing sulfur groups such as methionine and cystine. Additionally, the synthesis of various vitamins (such as biotin and thiamin) and coenzymes relies significantly on mineral sulfur.

Table 2: Effect of treatment on digestibility of dry matter. organic matter. and crude protein.

Treatment	DMD (%)	OMD (%)	CPD (%)
P0	52.45[b]	55.84[b]	62.23[b]
P1	55.55[a]	59.33[a]	65.78[a]
P2	55.37[a]	59.12[a]	65.43[a]
P3	56.48[a]	60.06[a]	66.34[a]

Superscripts [a, and b] in a column showed significantly different ($P < 0.05$). DMD: dry matter digestibility; OMD: organic matter digestibility; CPD: crude protein digestibility.

The supplementation of *S. cerevisiae* and mineral sulfur resulted in a statistically distinct impact ($P < 0.05$) on the breakdown efficiency of fiber components (ADF, NDF, and cellulose). As indicated in Table 3, the breakdown efficiency of fiber components (ADF, NDF, and cellulose) tended to increase in P3. The average digestibility of fiber fractions increased due to the influence of the blend of *S. cerevisiae* and mineral sulfur. This enhancement is attributed to *S. cerevisiae's* ability to increase microbial activity in the rumen, thereby improving fiber digestion. *S. cerevisiae* plays a positive role in rumen metabolism and fermentability by maintaining anaerobic conditions, promoting well-developed microbes, and stimulating beneficial bacteria (Riswandi *et al.*, 2021). Additionally, according to Utama (2011), *S. cerevisiae* can produce cellulase enzymes that increase the digestibility of cellulose and hemicellulose by breaking them down into simple monosaccharides. In addition to *S. cerevisiae*, sulfur minerals also contribute to improving the breakdown efficiency of fiber components. As noted by Pazla *et al.* (2018, 2021), sulfur minerals play a vital role in the digestion of fiber in the rumen. Ensuring an ample supply of sulfur minerals can enhance the digestibility of cellulose by specifically promoting cellulolytic bacteria. The increased activity of cellulolytic microbes leads to enhanced breakdown efficiency of fiber components, emphasizing the importance of mineral sulfur in increasing microbial populations that support cellulolytic activity and cell wall degradation, particularly cellulose. The increased microbial activity with sulfur mineral supplementation results in an optimal fermentation process in the rumen. Treatment with *S. cerevisiae* supplementation combined with mineral sulfur maintains a relatively stable pH for rumen microbial growth, especially cellulolytic bacteria, promoting better microbial growth and increasing the breakdown efficiency of fiber components. Therefore, the combination of *S. cerevisiae* supplementation and mineral sulfur effectively enhances the breakdown efficiency of fiber components.

Table 3: Effect of treatment on the digestibility of ADF, NDF and cellulose.

Treatment	ADF (%)	NDF (%)	Cellulose (%)
P0	42.86[b]	50.23[b]	52.55[b]
P1	46.62[a]	55.11[a]	56.93[a]
P2	46.14[a]	54.91[a]	55.99[a]
P3	47.10[a]	56.28[a]	57.74[a]

Superscripts [a, and b] in a column showed significantly different ($P < 0.05$).

This current study reported that treated ration has a significant effect on nutrient digestibility compared to untreated ration. The addition of 0.5% *S. cerevisiae* combined with 0.3% sulfur minerals in the ration gave a significant difference compared to the ration without treatment. In contrast, the effect of 0.5% *S. cerevisiae* compared to ration treated with 0.3% sulfur minerals and the combination of 0.5% *S. cerevisiae* combined with 0.3% sulfur minerals did not give a significant difference. We suggest increasing the percentage of *S. cerevisiae* and sulfur minerals to get a better effect of ACW in nutrient digestibility.

Effect of *S. cerevisiae* and mineral sulfur supplementation on rumen fluid characteristics (pH, NH_3, and VFA) and microbial protein synthesis

The addition of *S. cerevisiae* and mineral sulfur showed no significant impact ($P > 0.05$) on pH levels. Throughout this investigation, the recorded pH values varied between 6.74 and 6.89 (Table 4), indicating optimal conditions conducive to supporting the growth and activity of rumen microbes. According to Zain *et al.* (2011), the ideal pH value in the rumen falls within the range of 6.0-7.0, conditions that can support the growth of rumen microbes due to the low concentration of lactic acid in the rumen. In alignment with Ardani *et al.* (2023), *S. cerevisiae* plays a role in stimulating the population of bacteria in the rumen that utilizes lactic acid, thereby preventing lactic acid accumulation. Additionally, Pazla *et al.* (2018) stated that *S. cerevisiae* stimulates the growth of bacteria and provides soluble growth factors that positively impact the rumen microbial population and feed utilization in ruminants.

Table 4: Effect of treatment on rumen fermentation characteristics and microbial protein synthesis.

Treatment	pH	NH_3 (mg/100 ml)	Total VFA (mM)	Microbial protein synthesis (mg/10 ml)
P0	6.74	18.38[a]	106.25[b]	13.85[b]
P1	6.88	12.96[b]	141.25[a]	16.71[a]
P2	6.76	13.18[b]	140.00[a]	16.12[a]
P3	6.89	12.11[b]	143.75[a]	19.33[a]

Superscripts [a, and b] in a column showed significantly different ($P < 0.05$).

The supplementation of *S. cerevisiae* and mineral sulfur significantly influenced ammonia levels (NH_3) at a level of significance of ($P < 0.05$). As indicated in Table 4, the supplementation of *S. cerevisiae* and sulfur tended to decrease NH_3 production in the rumen. This reduction can be attributed to the utilization of ammonia by microbial proteins, as evidenced by the increased microbial protein synthesis with the supplementation of *S. cerevisiae in* combination with sulfur minerals. *S. cerevisiae* also plays a role in reducing ammonia levels, particularly beneficial when considering the use of ammoniated citronella waste in the basal ration, which initially leads to an increase in ammonia levels in the rumen. Elevated ammonia levels can be toxic if not efficiently utilized by microbes. Conversely, low ammonia levels are associated with an increased incorporation of ammonia into microbial proteins. According to Putri *et al.* (2021), ammonia serves as a fundamental compound for the synthesis of microbes in the rumen. *S. cerevisiae* contributes to the reduction of ammonia levels by modulating microbial activity and creating a favorable environment for more efficient nitrogen utilization, thereby minimizing ammonia overproduction. Mineral sulfur also plays a crucial role in reducing ammonia concentrations as it is essential for protein synthesis, a key factor for microbial growth in the rumen. It contributes to the effective management of ammonia concentrations. Low ammonia concentrations, coupled with high VFA concentrations, indicate the efficient use of ammonia by bacteria in the rumen for protein synthesis (Putri *et al.*, 2019). The concentration of NH_3 conducive to rumen microbial activity and growth typically falls within the range of 4-12 mM (Hapsari *et al.*, 2018).

The fermentation of carbohydrates results in the main product, Volatile Fatty Acid (VFA), with the principal components being acetic, propionic, and butyric acids (Lamid, 2010). Total VFA concentration, as indicated in Table 4, exhibited a substantial divergence due to the supplementation of *S. cerevisiae* and mineral sulfur, with statistical significance at ($P < 0.05$). In this study, the total VFA concentration ranged from 106.25 to 143.75 mM, supporting microbial growth in the rumen. Maintaining a total VFA concentration within the range of 70 to 160 mM in rumen fluid, as outlined by Indriani and Sutardi (2013), is crucial for supporting microbial growth. Enhanced microbial activity in the rumen is attributed to the increase in total VFA concentration observed with the supplementation of *S. cerevisiae* (0.5%) combined with mineral sulfur (0.3%). *S. cerevisiae* produces microbial growth factors such as organic acids, B vitamins, and amino acids, as noted by Mohammed *et al.* (2018). This improvement in microbial activity influences volatile fatty acids and mineral absorption, contributing to increased feed intake, weight gain, digestion, and ruminal pH. Ardani *et al.* (2023) highlighted that the total VFA concentration increases with *S. cerevisiae* addition, promoting lactic acid-using bacteria and leading to an increase in VFA. Lactic acid-using bacteria utilize lactic acid as their primary substrate, producing VFA. This aligns with He *et al.* (2022), who stated that lactic acid-using bacteria play a role in reducing lactic acid concentration in the rumen by metabolizing it into propionate. Tang *et al.* (2008) added that *S. cerevisiae* increases the proportion of propionate, decreases lactate concentration, enhances cellulolytic bacteria, and improves feed digestibility during rumen fermentation. Furthermore, mineral sulfur contributes to the increase in total VFA. Sulfur mineral supplementation serves as a precursor for microbial protein synthesis in the rumen and plays a role in elevating propionate concentration, as supported by Supapong *et al.* (2019), indicating that sulfur mineral supplementation can increase propionic acid compared to the absence of sulfur mineral supplementation.

From the data provided in Table 4, it is evident that supplementing with *S. cerevisiae* and mineral sulfur had a highly significant effect ($P < 0.05$) on microbial protein

synthesis. The average microbial protein synthesis in this study ranged from 13.85 to 19.33 mg/10 ml. The supplementation of *S. cerevisiae* and sulfur tended to increase microbial protein synthesis. Mineral sulfur plays a vital role in microbial protein synthesis, as the breakdown of fibrous feed heavily depends on enzymes produced by rumen microorganisms, leading to an enhanced rumen microbial population. This observation aligns with the perspective of Zain *et al.* (2019), who stated that the increase in microbial protein synthesis is attributed to an increase in the rumen microbial population. The elevated rumen microbial population enhances microbial activity, subsequently impacting digestibility. This is corroborated by the observed increase in microbial protein synthesis with the supplementation of *S. cerevisiae* combined with mineral sulfur, aligning with the elevated dry matter digestibility as indicated in Table 2.

Effect of *S. cerevisiae* and mineral sulfur supplementation on methane gas production and protozoa population

Supplementation of *S. cerevisiae* combined with mineral sulfur successfully reduced methane gas production and the protozoa population (Table 5). It demonstrates that the supplementation of *S. cerevisiae* and mineral sulfur had a statistically significant impact ($P < 0.05$) of the supplementation on both methane gas production and the protozoa population. The rumen protozoa population is intricately linked to methane gas production in the rumen, as protozoa serve as hosts for methanogenic bacteria. A decrease in the protozoa population affects methane gas production because the addition of sulfur, as noted by Wu *et al.* (2021), can inhibit methane production in ruminants by increasing the population of sulfate-reducing bacteria, which produce hydrogen sulfide instead of methane. Furthermore, the addition of *S. cerevisiae* reduces hydrogen gas production, likely disrupting the symbiotic mechanism between methanogens and protozoa. The decreased methane gas production with the supplementation of *S. cerevisiae in* combination with sulfur minerals is attributed to *S. cerevisiae*'s role in reducing the emission of methane gas by enhancing the organic matter digestibility (Table 2). Carbohydrates, as important organic components, determine the digestibility of organic matter because they produce energy in the form of VFA. An elevated proportion of propionate has the potential to decrease methane gas emissions. In alignment with the perspective of Antonius *et al.* (2023), fermentation in the rumen leading to propionate production results in low methane gas production, with increased production of volatile fatty acids (VFAs) as the main source of energy for ruminants. This is evident in Table 3, where the highest VFA production occurred with the supplementation of *S. cerevisiae* combined with sulfur minerals. This observation indicates that antibacterial activity has the potential to positively reduce methane gas formation by decreasing the protozoan population. Although treated ration had a significant effect compared to untreated ration, ration treated with *S. cerevisiae,* ration-treated sulfur mineral, and the combination of *S. cerevisiae* and sulfur mineral in ration did not affect rumen fermentation characteristics. The increase of *S. cerevisiae* and sulfur mineral level is suggested to be applied in ration to give a significant effect.

Table 5: Effect of treatment on protozoa population and methane gas production.

Treatment	Total protozoa (cells/ml)	Methane gas production (ml)
P0	$6.76 \times 10^{5\,a}$	45.83^{a}
P1	$5.18 \times 10^{5\,b}$	40.04^{b}
P2	$5.28 \times 10^{5\,b}$	41.83^{b}
P3	$3.55 \times 10^{5\,b}$	39.92^{b}

Superscripts [a and b] in a column showed significant differences ($P < 0.05$).

CONCLUSIONS AND RECOMMENDATIONS

This study concludes that supplementation of *Saccharomyces cerevisiae* combined with mineral sulfur in ammoniated citronella waste basal ration can enhance nutrient digestibility, decrease methane gas production, and reduce protozoa population. The supplementation of *S. cerevisiae* (0.5%) combined with mineral sulfur (0.3%) in ammoniated citronella waste basal ration tended to give the best outcomes in terms of increased digestibility of nutrients and reduced production of methane gas and protozoa population, without disrupting the fermentation process in the rumen. We suggest increasing the level of *S. cerevisiae* and sulfur minerals as supplementation in ammoniated citronella waste-based ration. Further research is necessary to examine the impact of *Saccharomyces cerevisiae* supplementation combined with mineral sulfur in ammoniated citronella waste basal rations on livestock *in-vivo.*

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NOVELTY STATEMENT

This research is important to carry out to utilize citronella waste, which is produced quite abundantly, as a fiber source in livestock nutrition for rumination. Its use as a feed source after processing with ammonia does not provide optimal results. Adding feed supplements in the form of sulfur and *Saccharomyces cerevisiae* as feed additives can increase the useful value of feed. No similar studies have been conducted.

AUTHOR'S CONTRIBUTION

Conceptualization: MZ, El, Er, and RWSN. Data curation: RP, MZ, El, Er, and RWSN. Formal analysis: POS, RP, El, Er, and RWSN. Funding acquisition: MZ, El, Er, and RWSN. Labor analysis: POS, BB, UA, LSS, and BVU. Supervision: MZ and El. Validation: MZ, El, Er, RWSN, and RP. Writing-original draft: RP, POS, BB, UA, LSS, and BVU. Writing-review and editing: EMP, RDP, RAG, and PSN. All authors read and approved the final manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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