Research Article

The Effect of *Nothopanax scutellarium* Merr Extracted with Various Solvents in the Feed on Total Gas Production, Methane Gas, and *In Vitro* Fermentability

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Abstract | This study investigated the effects of *Nothopanax scutellarium* Merr. (NSM), extracted with different solvents, on total gas production, methane, ammonia production, and *in vitro* fermentability. The NSM extracts were obtained by maceration with 96% ethanol or water. The basal diet (BD) consisted of 70% roughage and 30% concentrate (based on dry matter). A complete randomized design experiment with four treatments and four replicates was used: T0 = BD only (n=4); T1 = BD + 0.02 g NSM-Simplicia (n=4); T2 = BD + 0.02 g ethanol extract of NSM (n=4); and T3 = BD + 0.02 g water extract of NSM (n=4) then incubated for 72 hours. The data were analyzed using ANOVA, and if significant effects were found, they were subsequently tested using Duncan's test. The treatments significantly influenced methane and ammonia production as well as the rumen protozoa population (P<0.05). Using ethanol as a solvent for extracting the NSM plant resulted in lower methane and ammonia concentrations and a smaller protozoa population compared to water. Methane production was 8.71, 10.41, 7.60, 10.85 mL/g (*SEM=1.42*); ammonia production was 2.63, 2.88, 1.88, 0.76 mL/g (*SEM=0.32*); and protozoa populations were 2.06, 3.06, 1.46, 2.48 (x10^3 sel/ml) (*SEM =0.61*) for T0, T1, T2, and T3, respectively. The treatments had no significant effect (P>0.05) on total gas production, gas production rate, or individual and total fatty acids, total bacteria, or pH of the rumen. It could be concluded that ethanol extract NSM reduced methane and ammonia production, a lower protozoa population.

Keywords | *Nothopanax scutellarium* Merr, Extraction, Gas production, Microbial, *In vitro*, VFA

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INTRODUCTION

Improving and optimizing ruminant livestock production can be achieved through enhanced feed management practices. Feed additives in diets are one of the best strategies to improve productivity. However, the application of chemically based feed additives, such as antibiotic growth promoters, has been prohibited by the European Union since 2006 due to concerns over antimicrobial resistance and food safety (Castanon, 2007). Therefore, alternative solutions are required, one of which is the utilization of natural feed additives. Long-term use of natural feed additives does not leave harmful residues in meat or milk, thereby ensuring the production of safe and hygienic livestock products. *Nothopanax scutellarium* Merr (NSM) is a medicinal plant with potential as a natural feed additive and is known to possess anthelmintic properties. Given its secondary metabolite compounds, NSM has potential as a natural rumen fermentation modifier and methane-reducing agent, warranting further investigation into its efficacy in the ruminant feeding system.

NSM contains essential nutrients, including fat, Ca, P, Fe, and vitamins A, B1, and C. In addition, it contains various secondary metabolites, including flavonoids, alkaloids, saponins, and polyphenols. These secondary metabolite compounds have been reported to exhibit multiple biological functions. The phenolic, tannin, and saponin contents can modulate rumen function, enhance protein and energy utilization (Kholif, 2023; Darlis *et al*., 2021), reduce ammonia production (Jayanegara *et al*., 2020), and improve meat and milk quality (Adriani *et al*., 2024).

In ruminants, digestion is characterized by microbial fermentation to produce volatile fatty acids (VFAs) as the primary energy source and ammonia for microbial protein synthesis (Darlis *et al*., 2021). Darlis *et al*. (2021) reported that water-extracted *Coleus amboinicus* Lour. (CAL) produced higher concentrations of acetic and butyric acids than its alcohol extract, but resulted in lower ammonia concentration and protozoa population. Similarly, supplementation with *Aloe saponaria* in heat-dried and freeze-dried forms as a feed additive improved rumen fermentation through enhanced feed utilization by rumen microbes (Kim *et al*., 2023). In dairy goats, supplementation with 40 g of NSM leaf simplicia reduced mastitis incidence (Adriani and Yurleni, 2021), while 20 g of tannin and saponin protected NSM simplicia, increasing milk yield and feed intake (Adriani *et al*., 2024). The objective of this research is to determine the extraction method that can provide the best results for rumen fermentation patterns *in vitro*.

MATERIALS AND METHODS

Experimental design

This study used a completely randomised design (CRD) consisting of four treatments and four replicates. The basal diet (BD) used to evaluate the effect of NSM extracted with different solvents consists of 70% forage and 30% concentrate on a dry matter basis. The treatment consists of: T0 = BD only (n=4); T1 = BD + 0.02 g simplicia of NSM (n=4); T2 = BD + 0.02 g ethanol extract of NSM (n=4); and T3 = BD + 0.02 g water extract of NSM (n=4) furthermore incubated 0-72 hours with measurement time points 2, 4, 6, 8, 12, 16, 24, 48, 72 hours (Figure 1). The amount of extract used in the treatment is based on the research results of Darlis *et al*. (2021), which indicate that the water solvent yields better results for microbial activity in ruminant feed and *in vitro* degradation, with of 2%/kg dry matter feed.

Diet

The research feed was formulated according to the needs of lactating female goats, consisting of 70% forage and 30% concentrate on a dry matter basis as a basal diet (NRC, 2003). The fodder used was *Pennisetum purpureum*. The concentrate consisted of bran, soybean meal, palm kernel meal, and topmix. The extract was added according to the treatment. The chemical compositions of basal feed are shown in Table 1.

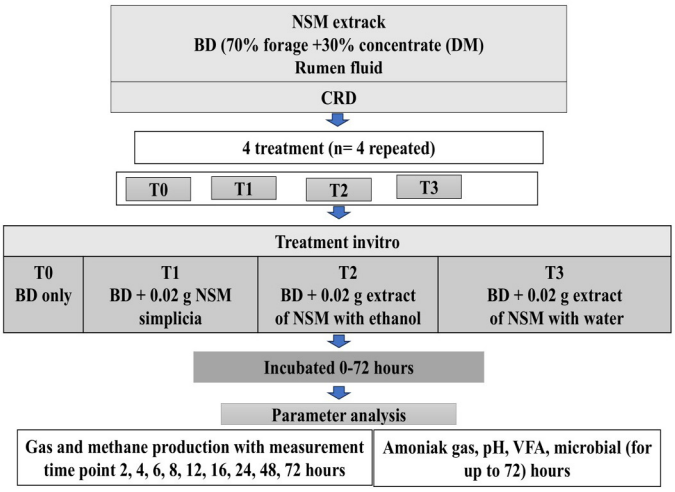

Figure 1: Materials and methods experiment.

Table 1: Nutrient content of research feed.

Nutrition	Forage*	Concentrate*	Feed**
Dry matter (%)	21.10	68.24	35.24
Crude fiber	38.56	6.72	29.01
Ash (%)	10.54	10.14	10.42
Crude protein (%)	10.35	21.23	13.61
Crude fat (%)	2.87	3.56	3.08
Ether ekstract (%)	37.68	58.35	43.89
Ca (%)	2.17	0.98	1.81
P (%)	0.31	0.47	0.36
Energy (kcal/kg)	3756.00	4029.00	3837.90

Note: * Laboratory analysis results, ** Calculation results

Leaf collection and NSM extraction

NSM simplicia was prepared by harvesting fresh leaves, followed by cleaning and draining to remove surface moisture. The cleaned leaves were cut into small pieces and dried naturally under shade (UV plastic greenhouse). The dried material was then ground and sieved through a 1.5 mm sieve to produce simplicia. A portion of the simplicia is macerated with 96% ethanol or water, then filtered and solvent removed under reduced pressure. The resulting ethanol and water extracts were used in the *in vitro* fermentation process.

The extraction process was adapted from the method described by Ahirwar and Tembhre (2016) and Adebayo *et al*. (2014). NSM extraction with ethanol was carried out by soaking NSM simplicia with ethanol (96%) at room temperature for 3 days with a 1:5 (w/v) ratio of NSM simplicia to ethanol. After soaking, the solution was filtered,

and the solvent was removed using a rotary evaporator, then stored at 4°C for further use. The extraction of NSM with water was done by boiling at 90°C. NSM simplicia was mixed with distilled water at a ratio of 1:5 (w/v) and left at room temperature for 48 h, followed by filtration. The aqueous filtrate was then concentrated using a rotary evaporator. All extracts were stored at 4 °C until further use (± 1 day). Each extract of NSM was analysed for total tannin and phenol compounds according to Makkar (2003). Table 2 presents the results of the analysis of secondary metabolite compounds found in NSM leaves using various extraction solvents.

Table 2: Bioactive content of NSM leaves under extraction based on treatments.

Nutrition	Simplicia of NSM	Ethanol extract of NSM	Water extract of NSM
Flavonoid (%)	0.88	1.87	1.69
Saponin (%)	1.32	2.54	2.33
Tannin (%)	1.07	1.25	1.34

Note: NSM (*Nothopanax scutellarium* Merr).

In vitro procedure

Rumen fluid was collected from cattle slaughtered at a slaughterhouse in Jambi City. The cattle came from smallholder farms fed green fodder. The rumen fluid was squeezed, filtered using gauze, placed in a thermos (37°C), and transported to the laboratory for *in vitro* analysis. Filtered rumen fluid was mixed with buffer solution in a ratio of 4:1 for rumen fluid and buffer solution, respectively. Fifty millilitres of this mixture was transferred into a serum bottle (100 ml capacity) containing 1 g of feed sample and added NSM simplicia, NSM extract, which was extracted with water and ethanol (each 0.02 g). The incubation was then carried out for 72 h. Total bacterial and protozoa counts were measured at 72 hours of incubation, gas production and methane gas were measured at 2, 4, 6, 8, 12, 16, 24, 48, and 72 h of incubation (consisting of treatments T0, T1, T2, T3, and an *in vitro* blank). The *in vitro* blank contained rumen fluid without feed, whereas ammonia gas was measured at the end of the *in vitro* process. After 72 h, the tubes were opened, and microbial activity was stopped by adding one drop of $HgCl_2$ solution. The solution was then centrifuged at 2000 rpm for 30 min until the solids settled and separated from the supernatant. The supernatant was used for total and partial VFA analyses using the steam distillation method (General Laboratory Procedure, 1996).

The variables observed were total gas, methane gas, ammonia production, VFA production and microbial population. VFA production using the steam distillation method (General Laboratory Procedure, 1996), Ammonia measured using the Conway Microdiffusion Method (1950), bacterial counts determined by counting live bacterial colonies and rumen fluid protozoa according to the Ogimoto and Imai (1981), and rumen fluid pH measured using a pH meter.

Data analysis

The data obtained were analysed using SAS (SAS Institute, 2004). Data were analyzed using ANOVA, with significant effects further examined using Duncan's multiple range test.

RESULTS AND DISCUSSION

Gas production *in vitro*

Fermentation activity in the rumen is caused by the presence of rumen microbes, especially bacteria and protozoa. This microbial activity can be characterised by the gas it produces. Table 3 presents the average total gas and methane production, ammonia, and rate of gas production (c) from the treatment with NSM extract different.

The addition of NSM extract with a solvent different on the diet had a significant effect ($P<0.05$) on methane rumen and a more significant effect ($P<0.01$) on ammonia production in the rumen. However, no significant effects ($P>0.05$) were observed on total gas production, and the rate of gas production rumen (Table 3). Standard Error *in vitro* cumulative gas and methane production curve of extract NSM with different solvent 0–72-hour measurement period is shown in Figures 2 and 3. Figure 2 shows that gas production is not different at every time point measurement. Whereas, Figure 3, methane production at time point measurement 48 and 72 hours resulted in higher at T3 compared T2 and did not differ values T0 and T1 treatment.

Table 3: Average total gas and methane production, ammonia, and gas production rate based on treatment.

Parameter	T0	T1	T2	T3	SEM	P value	Sig
Total gas (mL/g)	88.44	100.81	83.38	91.63	8.09	0.06	ns
Methane (mL/g)	8.71^{ab}	10.41^{a}	7.60^{b}	10.85^{a}	1.42	0.02	*
Ammonia (mL/g)	2.63^{b}	2.88^{b}	1.88^{c}	3.38^{a}	0.32	0.0002	**
Gas rate production(mL/h)	0.028	0.047	0.018	0.015	0.007	14.73	ns

Note: ns= no significant, a single asterisk (*) indicates a significant difference at $P < 0.05$, whereas a double asterisk (**) indicates a significant difference at $P < 0.01$., T0= Basal Diet (BD) only, T1= BD + 0.02 g, simplicia of NSM, T2 = BD + 0.02 g ethanol extract of NSM with ethanol, T3 = BD + 0.02 g water extract of NSM, SEM (standard error of means).

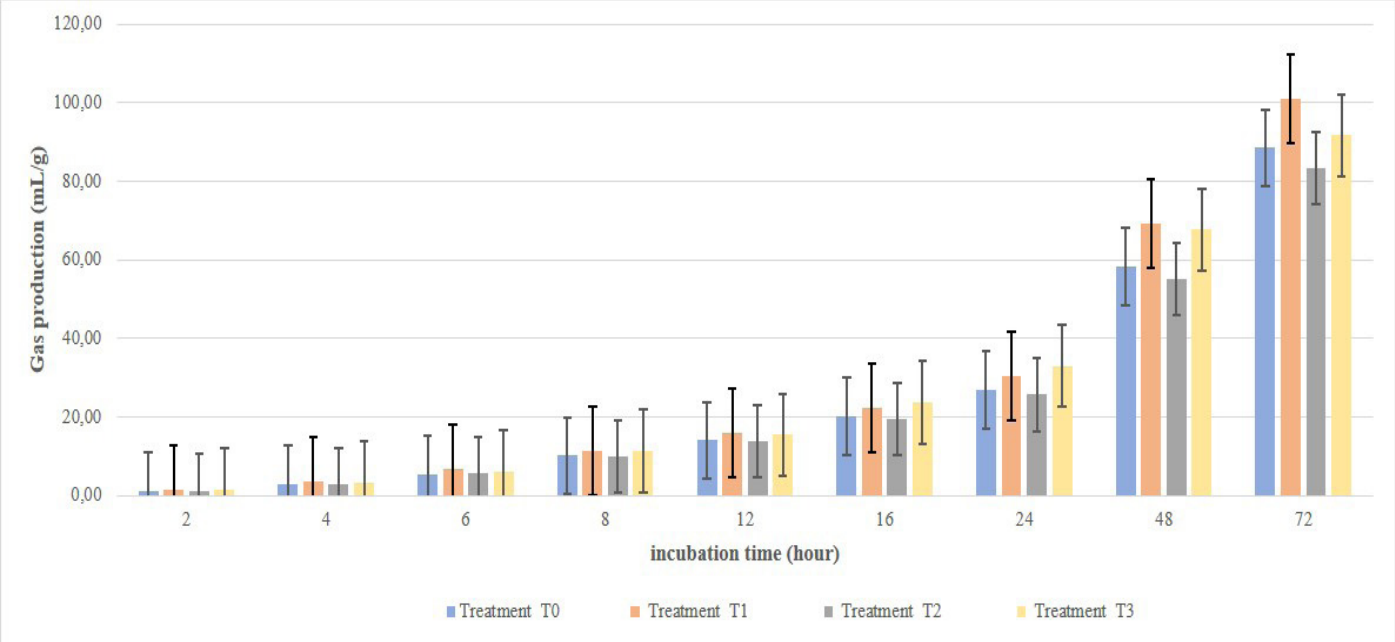

Figure 2: Cumulative gas production *in vitro* with curve standard error using NSM extract and various solvents.

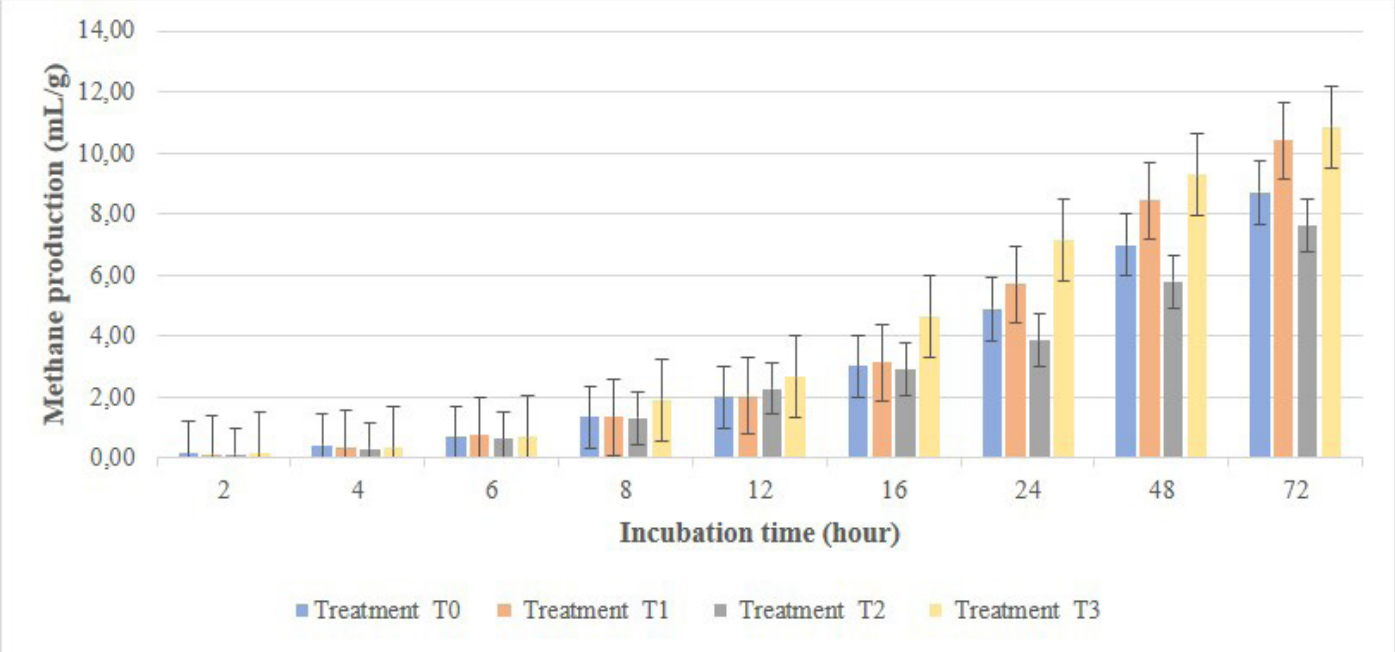

Figure 3: Cumulative gas methane *in vitro* with curve standard error using NSM extract and various solvents.

The results showed that methane and ammonia production ranged from 7.60–10.85 mL/g (*SEM = 1.42*) and 1.88–3.38 mL/g (*SEM = 0.32*), respectively. The amount of energy and protein content in the basal diet in this study was the same. Post-hoc analysis indicated that methane (10.85 mL/g) and ammonia (3.38 mL/g) concentrations in T3 were significantly higher than T2.

The significant difference in methane and ammonia production between NSM extracted with water and ethanol solvents is due to differences in solvent polarity and the composition of the extracted compounds. The water solvent extract produces more water-soluble compounds and fewer phenolic compounds that are antimicrobial and nitrogen-degrading enzymes, so that nitrogen decomposition and ammonia production remain high. In contrast, the ethanol extract produces more phenolic compounds with antimicrobial/urease properties, thus suppressing the activity of protein-degrading microbes and reducing ammonia formation in the rumen *in vitro* (Arya *et al*., 2025; Mehmood *et al*., 2022; Liu *et al*., 2019; Mwangi *et al*., 2024).

In this study, ethanol extraction yielded higher concentrations of secondary metabolites, particularly flavonoids, tannin, and saponins, compared with water

extraction (Table 2). The gas produced during ruminal fermentation is associated with feed utilization efficiency, which can also be influenced by the presence of secondary metabolites by modulating rumen microbes, particularly by inhibiting the growth and activity of proteolytic and deaminative microbes, which are responsible for protein degradation and formation, so that to reduce methane and ammonia gas production. Ammonia is produced when rumen microbes, mainly proteolytic bacteria, break down proteins into peptides and amino acids. Furthermore, the amino groups of amino acids are deaminated to ammonia (Wang *et al*., 2018).

Flavonoids in NSM extract may inhibit enzymatic activity and chelate essential metal ions required by microorganisms for fermentation (Bodas *et al*., 2012), and have also been shown to possess antimicrobial activity capable of inhibiting the growth of anaerobic microorganisms such as Clostridium aminophilum and Peptostreptococcus anaerobius, which play key roles in ammonia formation from amino acids (Liu *et al*., 2020; He *et al*., 2022).

Saponins can alter fermentation pathways by increasing propionic acid production, which competes with methanogenic bacteria for hydrogen utilization (Jayanegara *et al*., 2014), and reducing ruminal protozoa populations that contribute to protein degradation and ammonia release (Kholif, 2023; Patra and Saxena, 2009; Liu *et al*., 2020; He *et al*., 2022). Tannins form stable complexes with dietary proteins, thereby reducing the availability of protein for microbial degradation and lowering ammonia production and directly suppressing the growth of cellulolytic and methanogenic bacteria, thereby contributing to reductions in total gas and methane production (Goel and Makkar, 2012). Several previous studies have demonstrated that the use of ethanol as a solvent for extracting flavonoids and saponins is more effective than using water (Gichuki *et al*., 2023; Lezoul *et al*., 2020). These compounds work through different but complementary mechanisms to inhibit gas-producing microorganisms, particularly methane and ammonia producers. This result differs from the study by Darlis *et al*. (2021) on *Coleus amboinicus* L. extract, where the lowest ammonia production was found in the extract using water as a solvent.

Furthermore, secondary metabolites in NSM leaf extract may enhance the efficiency of nitrogen utilization by rumen microbes by channeling nitrogen toward microbial protein synthesis rather than accumulating as free ammonia. This not only reduces nitrogen losses through urinary excretion but also contributes to improved feed utilization efficiency and livestock growth performance (Newbold and Ramos-Morales, 2020).

Rumen fermentability *in vitro*

The average concentrations of individual and total volatile fatty acids (VFA) treated with the NSM extract are presented in Table 4. VFA are the primary energy source for ruminants. Increased VFA production can be an indicator of more efficient fermentation. The addition of extract NSM using different solvents had no significant effect (P>0.05) on individual or total VFA concentrations. The concentrations of total VFA, acetate, propionate, and the acetate: Propionate ratio ranged from 74.38, 50.50, 19.27, and 2.62 mM (T0), respectively, to 112.83, 85.24, 22.88, and 3.73 mM (T1), respectively. Although there was no significant difference statistical, the treatments with NSM extract (T1, T2, and T3) showed a numerical increase in VFA concentration compared to the basal diet only (T0).

The addition of NSM extract containing active compounds cannot significantly alter rumen fermentation patterns, particularly in the carbohydrate degradation process that produces VFA as the product of fermentation. The same crude fiber content in the feed did not produce significant differences in the final products of rumen fermentation (Suwandyastuti, 2013). This is in step with the similar crude fiber levels among the treatment feeds. The trend towards increasing VFA concentrations may also be attributed to the presence of tannins and saponins in NSM. These compounds can modulate rumen microbial composition

Table 4: Average concentrations of VFA based on treatment.

Parameter	T0	T1	T2	T3	SEM	P value	Sig
Total VFA (mM)	74.38	112.83	89.38	78.48	23.54	0.14	ns
As. asetat (mM)	50.50	85.24	63.02	54.78	20.43	0.13	ns
As. propionat (mM)	19.27	22.88	21.67	19.20	7.20	0.85	ns
Asetat: propionat ratio	2.62	3.73	2.91	2.85	1.37	0.81	ns
Butirat (mM)	3.46	3.55	3.71	3.43	0.81	0.96	ns
Iso butirat (mM)	1.22	1.01	0.91	1.01	0.31	0.57	ns
Valerat (mM)	1.15	1.17	0.99	0.95	0.43	0.93	ns
Iso valerat (mM)	1.38	1.43	1.00	1.31	0.56	0.68	ns

Note: ns= no significant, T0= Basal diet (BD) only, T1= BD + 0.02 g simplicia of NSM, T2 = BD + 0.02 g ethanol extract of NSM with ethanol, T3 = BD + 0.02 g water extract of NSM, VFA (Volatil Fatty acid), SEM (standard error of means).

Table 5: Average pH, bacteria, and protozoa populations based on treatment

Parameter	T0	T1	T2	T3	SEM	P value	Sig
pH	6.91	6.87	6.99	6.92	0.07	0.20	ns
Bacteri 10^9 (sel/mL)	11.25	6.00	7.50	13.75	9.64	0.67	ns
Protozoa 10^3 (sel/mL)	2.06^{cb}	3.06^{a}	1.46^{c}	2.48^{ab}	0.61	0.02	*

Note: ns= no significant, a single asterisk (*) indicates a significant difference at P < 0.05, T0= Basal Diet (BD) only, T1= BD + 0.02 g simplicia of NSM, T2 = BD + 0.02 g ethanol extract of NSM with ethanol, T3 = BD + 0.02 g water extract of NSM, SEM standard error of means.

by suppressing methanogenic archaea, indirectly increasing fermentation efficiency and VFA production (Cardoso-Gutierrez *et al*., 2021). As polyphenolic compounds, tannins have antimicrobial and antifungal properties; However, at optimal concentrations, tannins can support rumen fermentation by binding to proteins, thereby reducing the rate of degradation, allowing more protein to reach the small intestine, balancing rumen fermentation, and improving microbial nitrogen utilization. Tannins can stimulate hydrolase enzyme activity and influence fermentation patterns, although the effects are complex and can vary depending on the type of tannin, concentration, and environmental conditions (Suryani *et al*., 2024). Freitas *et al*. (2024) found that the use of ethanolic (EE) and hydroalcoholic (HE) extracts of Urochloa brizantha in basal diets tended to produce higher VFA, although the results were not significant.

Other research suggests that feed additives are known to increase fermentation efficiency by influencing specific microbial populations, resulting in increased volatile fatty acid production, although this response has not yet been statistically significant. Darlis *et al*. (2021) found that using water as a solvent to extract plant CAL resulted in higher VFA values, particularly acetate and butyrate, compared to using basal diets alone.

The insignificant research results obtained are thought to be caused by several factors, including the suboptimal level of NSM extract use, the adaptability of rumen microbes to bioactive compound (Zhang *et al*., 2024; Yang *et al*., 2022), relatively stable rumen fermentation conditions in all treatments (Bouzazi *et al*., 2025; Formato *et al*., 2022), basal diet composition, and interactions between microorganisms in the rumen (Darlis *et al*., 2021; Zhang *et al*., 2024; Yang *et al*., 2022), the type and characteristics of the substrate or feed (Zhang *et al*., 2024; Dhakal *et al*., 2024; Palmonari *et al*., 2023), This indicates that ekstrac NSM still have the potential to improve rumen fermentation characteristics, especially when applied at more optimal doses or combinations.

Rumen microbial population

Feed fermentation in the rumen is a complex process involving interactions between rumen conditions, such as pH, and microbes, such as bacteria, fungi, and protozoa. The average numbers of bacteria, protozoa, and pH levels treated with the NSM extract are shown in Table 5.

The study results indicate that the addition of NSM has a significant effect (P<0.05) on protozoan populations but no significant effect (P>0.05) on bacterial populations and rumen pH. The average protozoa population in treatment T2 was lower than that observed in treatments T0, T1, and T3. This trend was consistent with the ammonia production results, indicating that ethanol-extracted NSM was effective in suppressing protozoal growth. The reduction in protozoa population was closely associated with decreased ammonia production (Table 3). A reduction in the ruminal protozoa population can serve as an indicator of decreased ammonia production. In the present study, a decline in ammonia gas production was observed, which was related to pH. The range rumen pH in this study was within the normal range (6.87–6.99), indicating that ruminal microbial activity was likely maintained at an optimal level throughout the experimental period. These conditions support good microbial activity in the rumen.

Protozoa constitute a substantial proportion of the rumen microbial community, accounting for more than 50% of total microbial biomass (Williams and Coleman, 2020; Newbold *et al*., 2015), where protozoa prey on bacteria (±24% of bacteria/day) and hydrolyze approximately 50% of bacterial protein into peptides and free amino acids, which are then fermented by ammonia-producing bacteria (AAFB) to ammonia (Park and Yu, 2023). In *in vitro* rumen fermentation, protozoan numbers are positively correlated with ammonia production: Higher protozoan densities result in greater release of peptides/amino acids and substrates for ammonia-producing bacteria, thus increasing ammonia concentrations, and vice versa. Protozoa play an important role in nitrogen recycling and ammonia formation. Many studies manipulate or measure protozoan populations and then observe changes in ammonia concentrations. In their paper, Spangero *et al*. (2022) stated that there is a strong relationship between the number of protozoa and ammonia concentration (R^2 = 0.58).

Rumen pH is an important indicator of normal rumen

function. The rumen pH range in this study was 6.87–6.99, which is considered the optimum pH during *in vitro* experiments. According to Kamra (2005), the optimum pH range for rumen microbial activity is generally between 6.0 and 6.9. Supplementation with NSM extract in this *in vitro* study was able to maintain rumen pH within the optimum range, so that carbohydrate fermentation in the rumen was not disturbed, as indicated by total gas and methane production, which did not differ significantly. However, the decrease in ammonia concentration and protozoan population indicates the presence of bioactive compounds such as tannins, flavonoids, and saponins, which act as modulators of rumen fermentation.

CONCLUSIONS AND RECOMMENDATIONS

It could be concluded that ethanol extract NSM reduced methane and ammonia production, a lower protozoa population. This study recommends the use of NSM extract extracted using ethanol solvent for in vivo trials, appropriate dosage use, and safety examination of its use *in vivo*.

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NOVELTY STATEMENT

This study examined the effect of NSM extract in feed with various solvents on total gas production, total methane, ammonia production, rumen microbial population, and vitro digestibility. These results suggest that using ethanol as a solvent to extract NSM in feed reduces protozoa populations and ammonia gas.

AUTHOR'S CONTRIBUTION

Yurleni: Designed the study, conducted research, and wrote the manuscript. Darlis and Muhammad Afdal: Collected and analyzed the data. Adriani: Edited the manuscript and analyzed the data. All authors have read, reviewed, and approved the final manuscript

Generative AI and AI assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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