P-ISSN: 2304-3075; E-ISSN: 2305-4360
International Journal of Veterinary Science
www.ijvets.com; editor@ijvets.com

Research Article https://doi.org/10.47278/journal.ijvs/2024.182

Optimizing Nutrient Digestibility Through Fermentation of Mangrove (*Sonneratia alba)* Fruit with *Aspergillus niger*: Implications for Livestock Feed Quality Improvement

Roni Pazla[1], Elihasridas[1]*, Novirman Jamarun[1], Gusri Yanti[2], Antonius[3], Ezi Masdia Putri[3], Zaitul Ikhlas[4], Shahid Ullah Khan[5], Faheem Ahmed Khan[3], Sharli Asmairicen[3], Maman Surachman[3], I Wayan Angga Darmawan[3], Sindu Akhadiarto[3] and Zul Efendi[3]

[1]Department of Animal Nutrition, Faculty of Animal Science Universitas Andalas, Campus Limau Manis, Padang, 25166 West Sumatera, Indonesia
[2]Department of Agricultural Extension, Faculty of Social, Science and Education, Prima Nusantara Bukittinggi University, Bukittinggi 26122, 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
[4]PhD Student, Faculty of Animal Science Universitas Andalas, West Sumatera, Indonesia
[5]Integrative Science Center of Germplasm Creation in Western China (CHONGQING) Science City and Southwest University, College of Agronomy and Biotechnology, Southwest University, Beibei, Chongqing 400715, P.R. China
***Corresponding author:** Elihasridas@ansci.unand.ac.id

Article History: 24-470	Received: 14-Apr-24	Revised: 12-May-24	Accepted: 13-May-24	Online First: 21-May-24

ABSTRACT

Utilizing mangrove (*Sonneratia alba*) fruit as an alternative feed source presents a viable avenue for enhancing livestock feed efficiency and sustainability. However, the intrinsic high tannin content of *S. alba* poses a significant barrier to its digestibility and utility as a feed component. The application of fermentation processes facilitated by *Aspergillus niger* emerges as a strategic intervention to diminish tannin impediments and elevate the feed material's nutritional profile. This investigation delves into the ramifications of varied fermentation periods with *A. niger* on the digestibility, nutrient quality, and fermentative by-products of *S. alba* fruit. Adopting a completely randomized block design, the study administers treatments spanning fermentation durations of 7, 10, 13, and 16 days, scrutinizing their influence on a spectrum of digestibility indices, ruminal fluid properties, microbial protein synthesis, and gaseous production. The findings articulate that protracted fermentation markedly augments the digestibility of dry matter, organic matter, and crude protein, alongside elevations in volatile fatty acids and ammonia levels, while sparing rumen pH and overall gas output from significant alterations. Notably, a 16-day fermentation tenure culminates in optimal feed digestibility and nutritional amelioration, underscoring the potency of extended *A. niger* fermentation in curtailing tannin contents and fostering the adaptability of *S. alba* fruit as livestock feed, thereby advocating for advanced, sustainable feed formulation methodologies.

Key words: *Aspergillus niger*, *In vitro* digestibility, livestock feed, Mangrove fruit, *Sonneratia alba*, Tannin

INTRODUCTION

The utilization of alternative feed sources, including mangrove plants, has become a focal point in efforts to enhance feed availability for livestock (Sari et al. 2022; Ikhlas et al. 2023; Jamarun et al. 2023; Pazla et al. 2024a). The fruit of *Sonneratia alba*, a type of mangrove, exhibits promising potential as a substitute feed source for costly concentrates. Its carbohydrate content reaches 75.1% (Ardiansyah et al. 2020); however, a significant challenge in feed digestibility and utilization is its high tannin content, which amounts to 41.6% (Bay 2016). Tannins are secondary metabolite compounds in plants (Kondo et al. 2016; Jamarun et al. 2020), capable of significantly impacting livestock digestion, affecting feed efficiency and overall animal health (Popova and Mihaylova 2019; Verma et al. 2021).

Nonetheless, the use of microorganisms, such as *Aspergillus niger*, in fermentation has emerged as a promising approach to reducing the tannin content in

Cite This Article as: Pazla R, Elihasridas, Jamarun N, Yanti G, Antonius, Putri EM, Ikhlas Z, Khan SU, Khan FA, Asmairicen S, Surachman M, Darmawan IWA, Akhadiarto S and Efendi Z, 2024. Optimizing nutrient digestibility through fermentation of mangrove (*Sonneratia alba)* fruit with *Aspergillus niger*: implications for livestock feed quality improvement. International Journal of Veterinary Science 13(6): 862-869. https://doi.org/10.47278/journal.ijvs/2024.182

mangrove fruits (Pakaweerachat and Chysirichote 2022; Elihasridas et al. 2023a). The tannase enzyme produced by *A. niger* has the potential to alter the tannin structure, which in turn can improve feed digestibility and utilization by livestock (Espitia et al. 2022). However, to optimize this fermentation process, a deeper understanding of the influence of fermentation duration and optimal fermentation parameters is required.

Previous studies, such as those conducted by Purnama (2004), Probowati et al. (2012), and Khasnabis et al. (2015), have provided initial insights into the potential of *A. niger* to reduce tannin content in feed materials. However, further comprehensive and detailed research is necessary to expand this knowledge and apply it practically in livestock production.

In this context, the present study aims to investigate the impact of the fermentation duration of *S. alba* with *A. niger* on tannin content, as well as the overall digestibility and nutritional quality of the resulting feed. A profound understanding of this fermentation process is expected to offer valuable insights into developing higher quality and more efficient livestock feeds.

MATERIALS AND METHODS

Ethical approval

This study does not employ experimental animals; therefore, ethical approval is unnecessary.

Research methods

The method employed in this study is a Completely Randomized Design with four treatments and four repetitions. The treatments in the study are as follows:

T1: Fermentation of Mangrove (*S. alba*) fruit for 7 days
T2: Fermentation of Mangrove (*S. alba*) fruit for 10 days
T3: Fermentation of Mangrove (*S. alba*) fruit for 13 days
T4: Fermentation of Mangrove (*S. alba*) fruit for 16 days

Fermentation utilizes *A. niger* mold at a dose of 6% of the dry weight of the sample in each treatment. The fermentation procedure of mangrove fruit refers to Elihasridas et al. (2023a). The mathematical model used in the design is based on the design of Steel and Torrie (2002). The chemical composition of *S. alba* mangrove fruit before and after fermentation can be seen in Table 1.

In vitro preparation

The stages of *in vitro* preparation were conducted through several steps, namely:

1. Collection of rumen fluid

Rumen fluid was collected from a slaughterhouse and placed into a thermos that had been previously filled with warm water; afterward, the warm water was discarded to maintain the temperature at 39°C. This ensured that the microbes in the rumen fluid did not die, and anaerobic conditions were maintained. The rumen fluid was then filtered using four layers of cheesecloth.

2. Preparation of Mc. Doughall's solution

Mc. Doughall's solution was used as a buffer in *in vitro* fermentation. The chemicals used to make Mc. Doughall's solution included NaHCO3 (9.80g/L), Na2HPO4.7H2O (7.00g/L), KCl (0.57g/L), MgSO4.7H2O (0.12g/L), NaCl (0.47g/L) and CaCl2·2H2O (0.05g/L) (Tilley and Terry 1963).

All chemicals were dissolved in distilled water. The buffer solution was prepared before fermentation and placed in a shaker water bath at 39°C while CO_2 gas flowed for 20min to maintain anaerobic conditions.

3. *In vitro* evaluation

A sample of 2.5g was weighed and placed into a 250mL Erlenmeyer flask, to which 200mL of buffer and 50mL of rumen fluid were added in a 4:1 ratio. CO_2 gas was flowed for 60sec to maintain anaerobic conditions in the flask. A blank containing rumen fluid and buffer was also prepared. The flask was sealed with a ventilated rubber stopper to release fermentation gases. Fermentation was carried out in a shaker water bath at 39°C for 48hrs. After fermentation, the flask was placed in a basin containing ice chunks to stop microbial activity and measure pH. The mixture was then centrifuged for 5min at 1500rpm until separation occurred, with residue settling at the bottom and the supernatant at the top. The supernatant was transferred into a bottle and stored in a freezer for VFA and NH_3 analysis. The sediment from the mixture of supernatant and residue was filtered with Whatman No. 41 paper and dried in an oven at 60°C for 24hrs before analysis. Nutrient contents were analyzed using the AOAC Procedure (2016).

Parameter measured

The study measured several parameters, including dry matter digestibility, organic matter, crude protein, rumen fluid characteristics (pH, VFA, and NH_3), microbial protein synthesis, and total gas production.

Data analysis

The data obtained from the study were statistically processed using variance analysis. All collected data were processed and analyzed for variability using ANOVA with SPSS software version 25.0, followed by Duncan's test (Duncan's Multiple Range Test / DMRT).

RESULTS AND DISCUSSION

Dry matter digestibility

The influence of tannins on dry matter digestibility is an essential aspect in the fermentation research of *S. alba* fruit with *A. niger*. Tannins possess anti-nutritional properties that can bind proteins and digestive enzymes, limiting the accessibility and digestibility of nutrients in livestock feed. However, fermentation induced by *A. niger* for 16 days (Treatment T4) proved effective in reducing tannin content, which significantly ($P<0.05$) improved dry matter digestibility (Table 1).

A study by Pakaweerachat and Chysirichote (2022) indicated that the fermentation process using *A. niger* could decrease the tannin content in feed materials, enhancing dry matter digestibility by facilitating the decomposition of fiber and increasing nutrient accessibility. This research aligns with the results found in the fermentation of *S. alba*, where an increase in dry matter digestibility after 16 days of fermentation indicates a reduction in the inhibitory effects of tannins on digestive enzymes (Mueller et al. 2019).

Table 1: Chemical composition of *Sonneratia alba* mangrove fruit before and after fermentation.

Chemical Composition	Content (%)				
	Before Fermentation	Fermentation 7 day	Fermentation 10 day	Fermentation 13 day	Fermentation 16 day
Ash	5.43	6.60	8.03	8.41	8.65
Dry matter	55.82	51.48	42.83	32.50	28.37
Organic matter	94.57	93.40	91.97	91.59	91.35
Crude fiber	16.81	15.02	14.64	14.54	13.56
Crude fat	1.00	0.97	0.88	0.43	0.16
Crude protein	3.56	4.65	4.72	8.24	12.94
Nitrogen free extract	73.20	72.76	71.73	68.38	64.69
TDN	63.03	64.42	63.63	64.28	66.61
Tanin	21.21	19.36	18.30	17.25	16.03

Furthermore, res0earch by Schmitt et al. (2020) underscored that tannins can reduce nutrient digestibility by forming stable complexes with proteins, which are challenging to hydrolyze by digestive enzymes. This affirms the importance of strategies such as fermentation to reduce tannin content in feed, improve dry matter digestibility, and enhance feed efficiency.

Fermentation with *A. niger* not only reduces tannin content but also produces secondary metabolites that can positively affect rumen microflora, further supporting dry matter digestibility. Research by Pazla et al. (2023a) demonstrated that microbial fermentation can enhance the nutritional profile of feed by producing enzymes targeting the degradation of anti-nutritional components like tannins and improving the availability and digestibility of nutrients.

Organic matter digestibility

Fermentation duration is directly related to the reduction of tannin content in *Sonneratia alba*. As shown in Table 1, fermentation for 16 days results in the lowest tannin content and a significant increase in dry matter digestibility (Table 2), reflecting the reduction of tannin's inhibitory effects on digestion. Tannins, with their anti-nutritional properties, can bind proteins and carbohydrates, reducing the digestibility of organic matter. The decrease in tannin content due to fermentation allows rumen microbes to access and degrade organic matter more efficiently, increasing the digestibility of organic matter (Elihasridas et al. 2023a).

Table 2: Nutrient digestibility of the treatments

Parameter	Treatments				
	T1	T2	T3	T4	SEM
Dry matter	23.06^{c}	26.19^{b}	28.79^{b}	36.28^{a}	1.25
Organic matter	22.84^{d}	28.29^{c}	$3\ 1.95^{b}$	42.16^{a}	1.24
Crude Protein	54.46^{b}	73.03^{a}	74.06^{a}	75.70^{a}	1.96

Values (mean±SD) marked with differing letters in the same row indicate a significant difference at P<0.05.

The fermentation process with *A. niger* for 16 days (T4) not only reduces tannins but also affects the overall nutritional composition, including an increase in the availability of crude protein and a reduction in crude fiber (Table 1). The content of crude fiber in feed leads to a low degradation value, as crude fiber consisting of cellulose and hemicellulose often binds with lignin and will be challenging to break down by digestive enzymes (Pazla et al. 2020; Ajayi et al. 2021). The digestibility of organic matter is defined as the amount of nutrients contained in feed materials, including protein, carbohydrates, fats, and vitamins that the body can digest. Organic matter in a complete feed that is easily digestible is organic matter that is soluble, whether derived from protein, carbohydrates, or fats (Agustin et al. 2024). This transformation improves the nutritional profile of feed materials, making them easier for livestock to digest. These nutrient composition changes enhance organic matter's digestibility, an essential indicator of improved feed quality (Pazla et al. 2024b).

A. niger plays a crucial role in the fermentation of *S. alba* by producing enzymes such as cellulase, which can degrade plant cell walls (Zohri et al. 2022). This enzymatic activity breaks down components of organic matter that are difficult to digest into substrates more easily accessed and utilized by rumen microbes. As a result, the digestibility of organic matter is increased, providing a better source of energy and nutrients for livestock. The enzymes produced by *A. niger* play a crucial role in enhancing the digestibility of dry matter and organic matter, demonstrating the potential of fermentation in improving feed efficiency (De Vries and Visser 2001).

Crude protein digestibility

The reduction in tannin content during the 16-day fermentation is critical in improving the digestibility of crude protein (P<0.05) in *S. alba*. Tannins, known for their anti-nutritional solid properties, can form bonds with proteins and inhibit the digestion and absorption of proteins in the digestive tract (Patra and Saxena 2011). This effect negatively impacts the availability of nutrients for rumen microbes, resulting in low crude protein digestibility. A study by Elihasridas et al. (2023a) showed that fermentation with *A. niger* can effectively reduce the tannin content in *S. alba*, leading to a significant improvement in crude protein digestibility in T4, reaching up to 75.70% (Table 2).

A. niger is also known as a producer of protease enzymes (Wahab and Ahmed 2018). The fermentation process with *A. niger* induces the production of proteolytic enzymes that break down tannin-protein bonds, releasing crude protein that was previously unavailable due to interaction with tannins. Furthermore, these enzymes assist in degrading protein into peptides and amino acids more easily digested by rumen microbes, thereby enhancing microbial protein synthesis and nutrient availability for livestock. Research conducted by Li et al. (2021) supports these findings, indicating that fermentation reduces tannin content and increases enzymatic activity that improves crude protein digestibility. Pazla et al. (2023c) state that the crude protein digestibility level varies from each feed material due to plant type and anti-nutritional content.

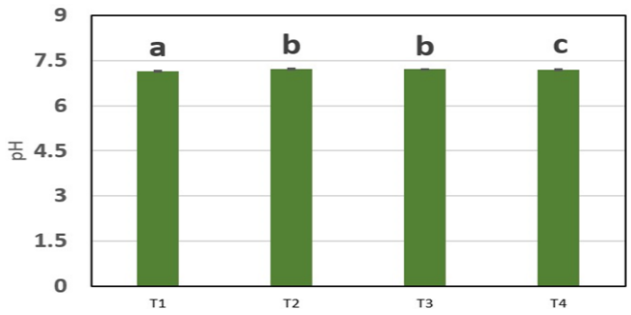

Fig. 1: pH of rumen fluid in the treatments: Treatments do not show a significant variation (P>0.05).

pH of rumen fluid

The pH value of rumen fluid is presented in Fig. 1. The analysis of variance results showed that the duration of fermentation treatments had no significant effect (P>0.05) on the pH of the rumen fluid. The obtained pH values ranged from 7.14 to 7.23, which are still considered normal for the activity of rumen microbes. The ideal pH of the rumen ranges between 6.9 and 7.3, where such pH conditions can support the growth of rumen microbes (Souza et al. 2022). According to Bach et al. (2023), pH varies depending on the type of feed provided. Fermentation of *Tithonia diversifolia* with *Aspergillus ficuum* and *Lactobacillus plantarum* produced a pH of 6.72-6.85 (Pazla et al. 2021a). Fermentation of sugarcane tops using *Pleurotus ostreatus* and *Aspergillus oryzae* resulted in a pH of 6.83-6.98 (Pazla et al. 2021b). Fermentation of *Tithonia diversifolia* using *Lactobacillus bulgaricus* produced a pH of 7.19-7.31 (Pazla et al. 2023b).

The stability of rumen fluid pH despite the fermentation process indicates that the fermentation of *S. alba* with *A. niger* does not produce by-products that can significantly alter the acid-base balance in the rumen. This suggests that the fermented substrate may have been well adapted by the rumen microbes without causing extreme pH fluctuations, which could disrupt the activity and population of rumen microbes. This pH regulation is essential because a pH that is too low or too high can inhibit microbial activity and nutrient digestion, particularly of fiber. Rumen fluid with a pH below 6.0 can inhibit proteolysis and deamination processes, disturb the life of cellulolytic microbes, and decrease fiber digestibility. If the pH exceeds 7.3, the ammonia absorption process is accelerated because the formation of non-ionized ammonia that more easily passes through the rumen wall occurs (Russell et al. 2009; Hackmann and Firkins 2015).

In the fermentation process, *A. niger* can produce enzymes that break down organic matter into components that are more easily digested by rumen microbes without drastically changing the production of VFA and other gases that affect rumen pH. These results indicate that fermentation with *A. niger* may optimize the degradation of organic matter and crude protein without causing an increase in acid production that could lower rumen pH. According to Luo et al. (2017), the pH of the rumen plays a vital role in regulating processes within the rumen, such as supporting rumen microbes in producing VFA and NH_3.

Total VFA concentration

In this study, the research results showed that the concentration of VFA ranged between 78.00mM and 83.25Mm (Fig. 2), indicating a significant variation related to the duration of fermentation. Statistical analysis revealed that the difference in fermentation duration had a very significant effect (P<0.05) on the VFA concentration, with the 16-day fermentation treatment (T4) producing the highest value of 83.25mM. In contrast, the 7-day fermentation (T1) produced the lowest value, 78.00mM. These findings affirm that the duration of fermentation plays a crucial role in increasing the VFA concentration, which is a direct result of the reduction in tannin content during the fermentation process (Table 1).

The decrease in tannins observed in each treatment contributes to the increased amount of soluble carbohydrates and protein escaping degradation, thus increasing the amount of glucose available to be fermented by microbes into VFA. This aligns with the literature stating that fermentation can increase VFA concentration through more efficient feed degradation processes, facilitating the fermentation of carbohydrates in the rumen into a vital energy source for livestock (Jamarun et al. 2017; Jin et al. 2023).

Furthermore, the role of *A. niger* in producing cellulase enzymes during the fermentation of *S. alba* strengthens the degradation process of cellulose into glucose (Probowati et al. 2012). This glucose, in turn, is used as a vital carbon and energy source for livestock, given that glucose is essential for meeting the energy needs of rumen microbes (Bureenok et al. 2024). The optimal VFA concentration, as produced in this study, supports the growth and activity of microbes in the rumen, consistent with the view that a VFA concentration between 70 and 150mM is conducive to microbial growth (Putri et al., 2021). An increase in VFA production indicates the ease of feed degradation by rumen microbes, which can also indicate feed fermentability (Zain et al. 2024).

NH_3 concentration

The data show a variation in NH_3 concentration from 3.53 to 5.03mM (Fig. 3). Statistical analysis indicates that differences in fermentation duration have a significant effect (P<0.05) on NH_3 concentration, with the most pronounced increase recorded in the treatment involving 16 days of fermentation (T4), resulting in the highest NH_3 concentration of 5.03mM. On the other hand, a shorter fermentation duration of 7 days (T1) resulted in the lowest NH_3 concentration, at 3.5mM. These results affirm that fermentation duration plays a crucial role in modulating NH_3 concentration, with an increase in fermentation duration potentially raising NH_3 levels.

The decrease in tannin content in mangrove fruit and fermentation treatment plays a significant role in this phenomenon. Analysis shows that the reduction of tannins from 19.36% (T1) to 16.03% (T4) contributes to increased NH_3 concentration and crude protein content, which grew from 4.65 to 12.94%. This increase in NH_3 can be explained by the reduced tannin-protein interaction that inhibits protein degradation. With lower tannin levels, proteins are more readily degraded into NH_3, providing a nitrogen source for rumen microbes, thus supporting their growth (Getachew et al. 2008; Chuzaemi et al. 2022).

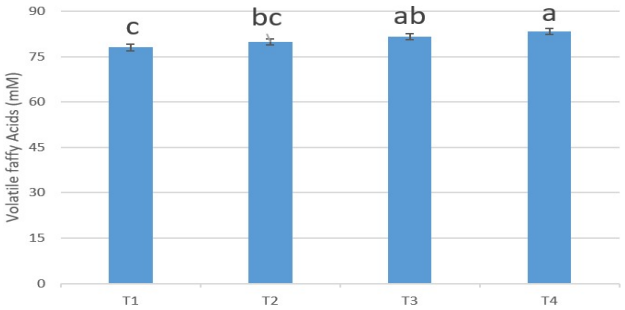

Fig. 2: Volatile fatty acid concentration of the treatments: There is a significant difference among treatments (P<0.05).

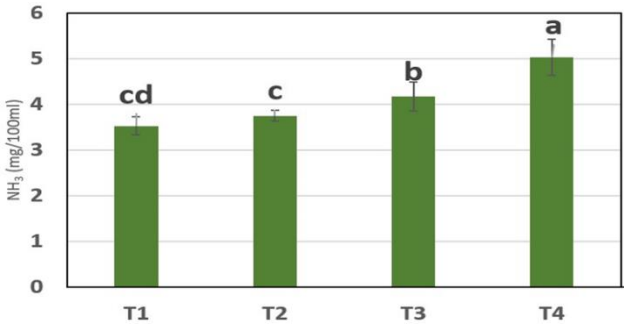

Fig. 3: NH_3 concentration of the treatments: There is a significant difference among treatments (P<0.05).

The use of *A. niger* in fermentation, known to produce tannase enzyme, plays a vital role in reducing tannin content and enhancing the crude protein content of mangrove fruit. The ability of *A. niger* to produce a tannase enzyme, which effectively lowers tannin content, facilitates the increased accessibility of protein for degradation into NH_3. The lower NH_3 concentration in the initial treatment (T1) indicates that with a significant decrease in tannin content, the available crude protein is sufficient to meet the nitrogen needs of rumen microbes. Jayanegara et al. (2020) reported that ammonia concentration decreases when tannin concentration increases due to the formation of tannin-protein bonds, which results in protein precipitation, then forming insoluble complex compounds.

Microbial protein synthesis

The variance analysis results show that the duration of mangrove fruit fermentation significantly differs (P<0.05) in microbial protein synthesis among treatments, with microbial protein production during the 48-hour incubation period ranging from 0.65mg/100mL to 1.38 mg/100mL (Fig. 4) Specifically, the treatment involving 16 days of fermentation (T4) stands out, producing the highest microbial protein synthesis of 1.38mg/100mL. This confirms the importance of fermentation duration in influencing microbial protein synthesis.

This study reveals that fermenting mangrove fruit with *A. niger* induces significant changes in the fruit's composition, particularly a decrease in tannin content. The tannase enzyme activity produced by *A. niger* plays a vital role in this process, degrading tannins into gallic acid and glucose, thereby reducing the tannin content. Khasnabis et al. (2015) support these findings by demonstrating the effectiveness of *A. niger* in reducing tannin content in fermented tea leaves.

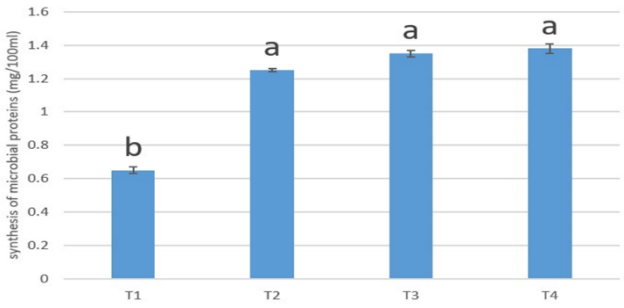

Fig. 4: Synthesis of microbial protein of the treatments: There is a significant difference among treatments (P<0.05).

The treatment with the highest tannin content (T1) produced the lowest microbial protein synthesis, attributable to the negative impact of tannins on rumen bacteria. Farha et al. (2020) explain that tannins can damage bacterial cell wall polypeptides, causing bacterial lysis and reducing microbial biomass in the rumen, ultimately affecting microbial protein production. Research by Harun (2019) also affirms that microbial biomass is an essential factor influencing microbial protein synthesis.

Furthermore, tannin interaction with proteins inhibits protein degradation by rumen microbes, resulting in low NH_3 production. This negatively impacts microbial protein synthesis due to unmet NH_3 requirements. Jayanegara et al. (2020) added that an increase in tannin concentration decreases ammonia concentration, limiting the availability of essential nitrogen for microbial protein synthesis. This study found that NH_3 concentration ranged from 3.53-5.03mM, with only the T4 treatment reaching the optimal ammonia concentration for microbial growth, according to the standard set by Russell and Strobel (1987).

Increased fermentability and decreased tannin levels through mangrove fruit fermentation improve feed material degradation in the rumen, enhancing the availability of energy (VFA) and ammonia (NH_3) for microbial protein synthesis. This is supported by findings that VFA ranged from 78.00 - 83.25mM, reaching optimal values to support microbial activity, according to McDonald et al. (2010). Optimal NH_3 and VFA production in T4 from this study successfully increased microbial protein synthesis, indicating that microbes obtain adequate energy and nitrogen sources for protein synthesis. According to Zain et al. (2024), microbial protein synthesis is highly influenced by the availability of ammonia and energy from carbohydrate degradation fermentation (VFA). Pazla et al. (2018) reported a linear increase in microbial protein synthesis in fermented palm fronds with increased NH_3 and VFA concentrations.

Compared with the study by Ramaiyulis et al. (2019), the microbial protein synthesis results in this study are lower, indicating varying responses depending on the type of feed and substrate degraded. Chuzaemi et al. (2022) and Elihasridas et al. (2023b) state that differences in microbial protein synthesis are influenced by the type of feed consumed by livestock and the substrate degraded. This phenomenon highlights the complexity of the relationship between feed composition, tannin-protein interactions, and their influence on microbial protein synthesis in the rumen.

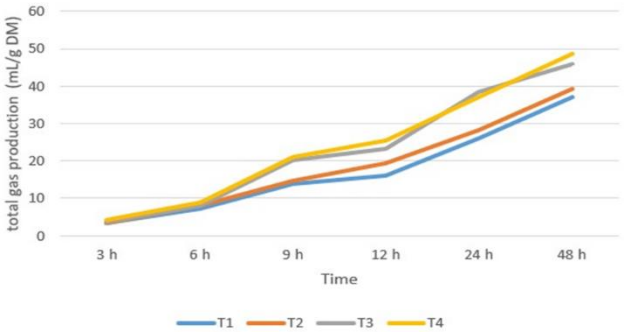

Fig. 5: Total gas production of the treatments: Treatments do not show a significant variation (P>0.05).

Total gas production

The dynamics of total gas production from the fermentation of mangrove fruit (*S. alba*) with *Aspergillus niger* are presented in Fig. 5. Although statistical analysis did not indicate a significant difference (P>0.05) among treatments regarding total gas production, there was a consistent increase in gas production as the incubation time progressed. Over 48 hours of incubation, total gas production ranged from 36.97mL/grDM to 48.62mL/gr DM, with the highest result achieved in the T4 treatment, fermentation for 16 days. This data suggests that fermentation time is directly proportional to gas production.

The fermentation process alters the composition of mangrove fruit, specifically reducing tannin levels through the activity of the tannase enzyme produced by *A. niger*. This reduction facilitates the degradation of organic material by rumen microbes, triggering increased gas production. Khasnabis et al. (2015) support these findings by demonstrating that *A. niger* is effective in reducing tannin content in fermented tea leaves.

The treatment with the shortest fermentation duration, T1 (7 days of fermentation), produced the lowest gas production at 48hrs of incubation (36.97mL/grDM). This condition is due to the higher tannin content that inhibits the degradation of organic material (Pellikaan et al. 2011; Hassanat and Benchaar 2013; Besharati et al. 2022). Anti-nutritional substances like tannins can bind various organic compounds, limiting their digestion by rumen microbes.

The success of the T4 treatment in producing the highest total gas production indicates the positive effect of reduced tannin activity through longer fermentation on the degradation of organic material in the rumen. Although there were no statistically significant differences between treatments, the increase in gas production with longer fermentation time indicates better digestion of organic material. *In vitro* gas production originates from the direct fermentation of substrates (e.g., CO_2 and CH_4) and indirectly through buffering mechanisms, with CO_2 released from bicarbonate during fermentation (Jayanegara et al. 2009). The fermentation of carbohydrates into acetate, propionate, and butyrate contributes to the primary gas production (Hatew et al. 2016). This study affirms that the reduction of tannin levels through fermentation enhances substrate availability for microbial fermentation, which ultimately increases gas production, supporting findings by Pazla et al. (2021c) that fermentation can facilitate the degradation of organic material by rumen microbes and enhance gas production.

Conclusion

The fermentation of *S. alba* with *A. niger* demonstrates significant potential in enhancing digestibility and nutritional quality of feed, reducing tannin content, and supporting microbial protein synthesis. These results offer new insights for the development of more effective and sustainable feeding strategies for ruminant livestock.

Competing Interest: None

Acknowledgments

The authors express their sincere gratitude to the Research and Community Service Institute of Universitas Andalas (Lembaga Penelitian dan Pengabdian Masyarakat Universitas Andalas), Indonesia, for providing financial support for this research (indexed publication research) via contract number T/62/UN.16.17/PT.01.03/Pangan-RPT/2022.

Author contribution

RP, GY, and ZI collecting data and preparing the manuscript. E, Nj conceptualization and supervision. A, EMP data analysis and review of the manuscript. SUK, FAK, SA, MS, IWAD, SA, ZE review of the final manuscript.

REFERENCES

Agustin F, Jamarun N, Ningrat RWS, Pazla R and Suryadi H, 2024. Decreasing cyanide acid content through soaking in betel lime: Effect on chemical composition and nutrient digestibility of Cassava peel. International Journal of Veterinary Science 13(3): 349-356. https://doi.org/10.47278/journal.ijvs/2023.104

Ajayi F, Babayemi O and Taiwo A, 2021. Ruminal degradability of mixtures of Panicum maximum and four forage legume hays in N'dama steer. Nigerian Journal of Animal Production 37(2): 247-257. https://doi.org/10.51791/njap.v37i2.1358

AOAC International, 2016. Appendix F: Guidelines for Standard Method Performance Requirements. AOAC Official Method of Analysis. AOAC International 1-18.

Ardiansyah P, Wonggo D, Dotulong V, Damongilala L, Hari S, Mentang F and Sanger G, 2020. Proksimat pada Tepung Buah Mangrove Sonneratia alba. Media Teknologi Hasil Perikanan 8(3): 82-87. https://doi.org/10.35800/mthp.8.3.2020.27526

Bach A, Baudon M, Elcoso G, Viejo J and Courillon A, 2023. Effects on rumen pH and feed intake of a dietary concentrate challenge in cows fed rations containing pH modulators with different neutralizing capacities. Journal of Dairy Science 106(7): 4580-4598. https://doi.org/10.3168/jds.2022-22734

Bay RH, 2016. Analisis kadar tanin pada buah, daun dan kulit batang mangrove *Sonneratia alba* dengan metode Lowenthal-procter. Thesis. Universitas Negeri Gorontalo, Gorontalo, Indonesia.

Besharati M, Maggiolino A, Palangi V, Kaya A, Jabbar M, Eseceli H, De Palo P and Lorenzo JM, 2022. Tannin in Ruminant Nutrition: Review. Molecules 27(23): 8273. https://doi.org/10.3390/molecules27238273

Bureenok S, Pitiwittayakul N, Saenmahayak B, Saithi S, Yuangklang C, Cai Y and Schonewille JT, 2024. Effects of fibrinolytic enzyme supplementation on feed intake, digestibility, and rumen fermentation characteristics in goats fed with Leucaena silage. Small Ruminant Research 231:107200. https://doi.org/10.1016/j.smallrumres.2024.107200

Chuzaemi S, Mashud M, Ndaru P, Huda A and Siswoyo E, 2022. Effect of Condensed Tannin and Myristic Acid in Corn Straw-Based Complete Feeds on NH_3 Concentration and Microbial Protein Synthesis. Advances in Biological Sciences Research 21: 29-34. http://dx.doi.org/10.2991/absr.k.220401.007

De Vries RP and Visser JA, 2001. *Aspergillus* enzymes are involved in the degradation of plant cell wall polysaccharides. Microbiology and Molecular Biology Reviews 65(4): 497-552. https://doi.org/10.1128/mmbr.65.4.497-522.2001

Elihasridas, Pazla R, Jamarun N, Yanti G, Sari RWW and Ikhlas Z, 2023a. Pretreatments of *Sonneratia alba* fruit as the potential feed for ruminant using *Aspergillus niger* at different fermentation times: tannin concentration, enzyme activity, and total colony. International Journal of Veterinary Science 12(5): 755-761. https://doi.org/10.47278/journal.ijvs/2023.021

Elihasridas, Zain M, Pazla R, Sowmen S and Aini Q, 2023b. *In vitro* digestibility of ammoniated aromatic supplemented lemongrass waste. Advances in Animal and Veterinary Sciences 11(8): 1368-1376. https://doi.org/10.17582/journal.aavs/2023/11.8.1368.1376

Espitia P, Ruelas-Chacón X, Mónica L, Ascacio-Valdés J, Flores-Naveda A and Sepúlveda L, 2022. Solid-state fermentation of sorghum by *Aspergillus oryzae* and *Aspergillus niger*: effects on tannin content, phenolic profile and antioxidant activity. Foods 11(19): 3121. https://doi.org/10.3390/foods11193121

Farha A, Yang Q, Kim G, Li H, Zhu F, Liu H, Gan R and Corke H, 2020. Tannins as an alternative to antibiotics. Food Bioscience 38: 100751. https://doi.org/10.1016/j.fbio.2020.100751

Getachew G, Pittroff W, Putnam D, Dandekar A, Goyal S and Depeters E, 2008. The influence of addition of gallic acid, tannic acid, or quebracho tannins to alfalfa hay on *in vitro* rumen fermentation and microbial protein synthesis. Animal Feed Science and Technology 140: 444-461. https://doi.org/10.1016/J.ANIFEEDSCI.2007.03.011

Hackmann TJ and Firkins JL, 2015. Maximizing efficiency of rumen microbial protein production. Frontiers in Microbiology 6: 465. https://doi.org/10.3389/fmicb.2015.00465

Harun A, 2019. Factors Affecting Rumen Microbial Protein Synthesis: A Review. Veterinary Medicine – Open Journal. https://doi.org/10.17140/vmoj-4-133

Hassanat F and Benchaar C, 2013. Assessment of the effect of condensed (acacia and quebracho) and hydrolysable (chestnut and valonea) tannins on rumen fermentation and methane production *in vitro*. Journal of the Science of Food and Agriculture 93 (2): 332-9. https://doi.org/10.1002/jsfa.5763

Ikhlas Z, Jamarun N, Zain M, Pazla R and Yanti G, 2023. Impact of Mangrove leaves (*Rhizophora apiculata*) in limewater on nutrients digestibility, rumen ecosystem and *in vitro* methane production. American Journal of Animal and Veterinary Sciences 18(1): 40-46. https://doi.org/10.3844/ajavsp.2023.40.46

Jamarun N, Zain M, Arief and Pazla R, 2017. Effects of calcium (Ca), phosphorus (P) and manganese (Mn) supplementation during oil palm frond fermentation by *Phanerochaete chrysosporium* on rumen fluid characteristics and microbial protein synthesis. Pakistan Journal of Nutrition 16(6): 393-399. https://doi.org/10.3923/pjn.2017.393.399

Jamarun N, Pazla R, Arief, Jayanegara A and Yanti G, 2020. Chemical composition and rumen fermentation profile of mangrove leaves (*Avicennia marina*) from West Sumatra, Indonesia. Biodiversitas Journal of Biological Diversity 21(11): 5230-5236. http://dx.doi.org/10.13057/biodiv/d211126

Jamarun N, Pazla R, Arief, Elihasridas, Yanti G, Sari RWW and Ikhlas Z, 2023. The Impact of Mangrove (*Rhizophora apiculata*) Leaves Hay and Fermented *Tithonia diversifolia* on Intake, Nutrient Digestibility, and Body Weight Gain of Goat. Advances in Animal and Veterinary Sciences 11(9): 1441-1450. https://dx.doi.org/10.17582/journal.aavs/2023/11.9.1441.1450

Jayanegara A, Sofyan A, Makkara HPS and Becker K, 2009. Kinetika produksi gas, kecernaan bahan organik dan produksi gas metana *in vitro* pada hay dan jerami disuplementasi hijauan mengandung tanin. Media Peternakan 32(2): 120-129.

Jayanegara A, Yogianto Y, Wina E, Sudarman A, Kondo M, Obitsu T and Kreuzer M, 2020. Combination effects of plant extracts rich in tannins and saponins as feed additives for mitigating *in vitro* ruminal methane and ammonia formation. Animals 10(9): 1531. https://doi.org/10.3390/ani10091531

Jin C, Su X, Wang P, Liang Z, Lei X, Bai H, Liang G, Li J, Cao Y and Yao J, 2023. Effects of rumen degradable starch on growth performance, carcass, rumen fermentation, and ruminal VFA absorption in growing goats. Animal Feed Science and Technology 299: 115618. https://doi.org/10.1016/j.anifeedsci.2023.115618

Khasnabis J, Rai C and Roy A, 2015. Determination of tannin content by titrimetric method from different types of tea. Journal of Chemical and Pharmaceutical Research 7(6): 238-241.

Li YJ, Zhao GY, Guo YQ and Hu JG, 2007. Comparison of two buffers on *in vitro* gas production, pH, and NH_3-N concentration of feedstuffs for ruminants. Journal of Animal Feed Science 16(Suppl. 2): 512-516. https://doi.org/10.22358/jafs/74595/2007

Luo J, Ranadheera CS, King S, Evans C and Baines S, 2017. *In vitro* investigation of the effect of dairy propionibacteria on rumen pH, lactic acid, and volatile fatty acids. Journal of Integrative Agriculture 16(7): 1566-1575. https://doi.org/10.1016/S2095-3119(16)61556-3

Kondo M, Jayanegara A, Uyen Y and Matsui H, 2016. Variation of tannin contents in selected agro-industrial byproducts and their biological activity in precipitating protein. Animal and Veterinary Science 4(2): 66-70. http://dx.doi.org/10.14737/journal.aavs/2016/4.2.66.70

McDonald P, Edwards RA and Greenhalg JFD, 2010. Animal Nutrition. 6th Edition. Prentice Hall, London.

Mueller HI, Bee G, Dohme-Meier F, Hoste H, Karonen M, Kölliker R, Lüscher A, Niderkorn V, Pellikaan W, Salminen J, Skøt L, Smith L, Thamsborg S, Totterdell P, Wilkinson I, Williams A, Azuhnwi B, Baert N, Brinkhaus A, Copani G, Desrues O, Drake C, Engström M, Fryganas C, Girard M, Huyền N, Kempf K, Malisch C, Mora-Ortiz M, Quijada J, Ramsay A, Ropiak H and Waghorn G, 2019. Benefits of Condensed Tannins in Forage Legumes Fed to Ruminants: Importance of Structure, Concentration, and Diet Composition. Crop Science 59(3): 861-885. https://doi.org/10.2135/CROPSCI2017.06.0369

Pakaweerachat P and Chysirichote T, 2022. Valorization of tannin-rich triphala waste for simultaneous tannase and gallic acid production under solid state fermentation by *Aspergillus niger*. Chemical Engineering Communications 210(9): 1420-1433. https://doi.org/10.1080/00986445.2022.2107509

Patra A and Saxena J, 2011. Exploitation of dietary tannins to improve rumen metabolism and ruminant nutrition.. Journal of the Science of Food and Agriculture 91(1): 24-37. https://doi.org/10.1002/jsfa.4152.

Pazla R, Jamarun N, Zain M and Arief, 2018. Microbial protein synthesis and *in vitro* fermentability of fermented oil palm fronds by *Phanerochaete chrysosporium* in combination with tithonia (*Tithonia diversifolia*) and elephant grass

(*Pennisetum purpureum*). Pakistan Journal of Nutrition 17(10): 462-470. https://doi.org/10.3923/pjn.2018.462.470

Pazla R, Jamarun N, Agustin F, Zain M, Arief and Cahyani NO, 2020. Effects of supplementation with phosphorus, calcium, and manganese during oil palm frond fermentation by *Phanerochaete chrysosporium* on ligninase enzyme activity. Biodiversitas 21(5): 1833-1838. https://doi.org/10.13057/biodiv/d210509

Pazla R, Jamarun N, Zain M, Yanti G and Chandra RH, 2021a. Quality evaluation of Tithonia (*Tithonia diversifolia*) with fermentation using *Lactobacillus plantarum* and *Aspergillus ficuum* at different incubation times. Biodiversitas 22(9): 3936-3942. https://doi.org/10.13057/biodiv/d220940

Pazla R, Jamarun N, Warly L, Yanti G and Nasution NA, 2021b. Lignin Content, Ligninase Enzyme Activity and *In vitro* Digestability of Sugarcane Shoots using *Pleurotus ostreatus* and *Aspergillus oryzae* at Different Fermentation Times. American Journal of Animal and Veterinary Sciences 16(3): 192-201. https://doi.org/10.3844/ajavsp.2021.192.201

Pazla R, Jamarun N, Agustin F, Zain M and Cahyani NO, 2021c. *In vitro* nutrient digestibility, volatile fatty acids, and gas production of fermented palm fronds combined with tithonia (*Tithonia diversifolia*) and elephant grass (*Pennisetum purpureum*). IOP Conference Series: Earth and Environmental Science 888(1): 012067. https://doi.org/10.1088/1755-1315/888/1/012067

Pazla R, Putri EM, Jamarun N, Negara W, Khan FA, Zain M, Arief, Yanti G, Antonius, Priyatno TP, Surachman M, Darmawan IWA, Herdis, Marlina L, Asmairicen S and Marta Y, 2023a. Pre-treatments of *Mirasolia diversifolia* using *Lactobacillus bulgaricus* at different dosages and fermentation times: Phytic acid concentration, enzyme activity, and fermentation characteristics. South African Journal of Animal Science 53(3): 429-437. http://dx.doi.org/10.4314/sajas.v53i3.11

Pazla R, Jamarun N, Arief, Elihasridas, Yanti G and Putri EM, 2023b. *In vitro* evaluation of feed quality of fermented *Tithonia diversifolia* with *Lactobacillus bulgaricus* and *Persea americana miller* Leaves as Forages for Goat. Tropical Animal Science Journal 46(1): 43-53. https://doi.org/10.5398/tasj.2023.46.1.43

Pazla R, Zain M, Despal D, Tanuwiria UH, Putri EM, Makmur M, Zahera R, Sari LA, Afnan IM, Rosmalia A, Yulianti YI, Putri SD, Mushawwir A and Apriliana RA, 2023c. Evaluation of rumen degradable protein values from various tropical foliages using *in vitro* and in situ methods. International Journal of Veterinary Science 12(6): 860-868. https://doi.org/10.47278/journal.ijvs/2023.045

Pazla R, Jamarun N, Elihasridas, Yanti G, Ikhlas Z and Juniarti, 2024a. Effect of combination of mangrove (*Rhizophora apiculata*) leaves and *Mirasolia diversifolia* in rations on production performance of goats. International Journal of Veterinary Science 13(4): 435-446. https://doi.org/10.47278/journal.ijvs/2023.118

Pazla R, Jamarun N, Arief, Elihasridas, Antonius, Yanti G, Indah DN and Saputra I, 2024b. *In vitro* digestibility combination of avocado leaves (*Persea americana Miller*) with fermented tithonia leaves (*Tithonia diversifolia*) in the rumen and post-rumen. AIP Conference Proceedings 2957(070042). https://doi.org/10.1063/5.0184240

Pellikaan W, Stringano E, Leenaars J, Bongers D, Schuppen S, Plant J and Mueller-Harvey I, 2011. Evaluating effects of tannins on extent and rate of *in vitro* gas and CH4 production using an automated pressure evaluation system (APES). Animal Feed Science and Technology 166: 377-390. https://doi.org/10.1016/J.ANIFEEDSCI.2011.04.072.

Popova A and Mihaylova D, 2019. Antinutrients in plant-based foods: a review. Open Biotechnol Journal 13(1): 68-76. https://doi.org/10.2174/1874070701913010068

Probowati RC, Sutrisno CI and Sumarsih S, 2012. Kadar VFA dan NH_3 secara *in vitro* pakan sapi potong berbasis limbah pertanian dan hasil samping pertanian difermentasi dengan A. Niger. Animal Agriculture Journal 1(2): 258-265. https://ejournal3.undip.ac.id/index.php/aaj

Purnama IN, 2004. Kajian potensi isolat kapang pemecah ikatan tanin pada kulit buah kakao (*Thebroma cacao L*). Thesis. Institut Pertanian Bogor, Bogor, Indonesia.

Putri EM, Zain M, Warly L and Hermon H, 2021. Effects of rumen-degradable-to-undegradable protein ratio in ruminant diet on *in vitro* digestibility, rumen fermentation, and microbial protein synthesis. Veterinary World 14(3): 640-648. http://www.doi.org/10.14202/vetworld.2021.640-648

Ramaiyulis, Ningrat RWS, Zain M and Warly L, 2019. Optimization of rumen microbial protein synthesis by addition of gambier leaf residue to cattle feed supplement. Pakistan Journal of Nutrition 18(1): 12-19. http://dx.doi.org/10.3923/pjn.2019.12.19

Russell JB, Muck RE and Weimer PJ, 2009. Quantitative analysis of cellulose degradation and growth of cellulolytic bacteria in the rumen. FEMS Microbiology Ecology 67(2): 183–197. https://doi.org/10.1111/j.1574-6941.2008.00633.x

Russell J and Strobel H, 1987. Concentration of ammonia across cell membranes of mixed rumen bacteria. Journal of Dairy Science 70 (5): 970-976. https://doi.org/10.3168/JDS.S0022-0302(87)80101-7

Sari RWW, Jamarun N, Arief, Pazla R, Yanti G and Ikhlas Z, 2022. Nutritional analysis of mangrove leaves (*rhizophora apiculata*) soaking with lime water for ruminants feed. IOP Conference Series: Earth and Environmental Science 1020: 012010. https://iopscience.iop.org/article/10.1088/1755-1315/1020/1/012010

Schmitt M, Ward D and Shrader A, 2020. Salivary tannin-binding proteins: A foraging advantage for goats. Livestock Science 234: 103974. https://doi.org/10.1016/j.livsci.2020.103974

Souza JG, Ribeiro CVD and Harvatine KJ, 2022. Meta-analysis of rumination behavior and its relationship with milk and milk fat production, rumen pH, and total-tract digestibility in lactating dairy cows. Journal of Dairy Science 105(1): 188-200. https://doi.org/10.3168/jds.2021-20535

Steel RGD and Torrie J, 2002. Prinsip dan Prosedur Statistik Suatu Pendekatan Biometrik. Alih Bahasa: Sumantri B. Gramedia, Jakarta.

Verma S, Taube F and Malisch CS, 2021. Examining the variables leading to apparent incongruity between antimethanogenic potential of tannins and their observed effects in ruminants: a Review. Sustainability 13(5): 2743. https://doi.org/10.3390/su13052743

Wahab W and Ahmed S, 2018. Response surface methodology for production, characterization and application of solvent, salt and alkali-tolerant alkaline protease from isolated fungal strain Aspergillus niger WA 2017. International Journal of Biological Macromolecules 115: 447-458. https://doi.org/10.1016/j.ijbiomac.2018.04.041

Zain M, Tanuwiria UH, Syamsu JA, Yunilas Y, Pazla R, Putri EM, Makmur M, Amanah U, Shafura PO and Bagaskara B, 2024. Nutrient digestibility, characteristics of rumen fermentation and microbial protein synthesis from Pesisir cattle diet containing non-fiber carbohydrate to rumen degradable protein ratio and sulfur supplement. Veterinary World 17(3): 672–681. https://doi.org/10.14202/vetworld.2024.672-681

Zohri A, Ali M and Moamen S, 2022. Evaluation of Cellulases Production by Aspergillus niger Using Response Surface Methodology. Egyptian Sugar Journal 19: 18-28. https://doi.org/10.21608/esugj.2022.155979.1017