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Effect of tannin degradation of mangrove (*Sonneratia alba*) fruit on nutrient digestibility, protozoa population and methane gas production

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Abstract: This study examined the fermentation duration in relation to the effectiveness of tannin levels in mangrove (*Sonneratia alba)* fruit during the fermentation process using *Aspergillus niger*. The tested durations were 7, 10, 13, and 16 days in anaerobic conditions. The outcomes measured included crude fat, crude fibre, and nitrogen-free extract digestibility. Microbial biomass, protozoa population, and methane gas production were also recorded as responses to the rumen microbial activity. The results showed that 16 days of fermentation gave the highest average for all responses. The protozoa population reached around 4.07×10^5 cells/ml and methane gas amounted to 33.9 ml/g of dry matter (DM). This is caused by a decrease in the anti-nutrient tannin content in mangrove (*S. alba*) fruit due to fermentation by *A. niger* according to treatment. The conclusion of this research is that the 16-day fermentation treatment (T4) of mangrove fruit is the optimal time to be used as a source of concentrate feed for livestock in terms of the increase in nutritional value and gastrointestinal microbes represented by total protozoa population, crude fibre digestibility, crude fat digestibility, and the highest nitrogen-free extract digestibility. However, microbial biomass and methane gas production were not significantly different in this study.

Keywords: animal feed; *Aspergillus niger*; fermentation; *in vitro*; rumen

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Indonesia has two-thirds of its territory covered by water, making it suitable for mangrove development and growth. It has 3.36 million ha of mangrove forests (Rahadian et al. 2019). According to FAO (2007), Papua, Kalimantan, and Sumatra are the primary homes of Indonesia's mangrove forests. Mangrove plants are the leading vegetation in coastal areas. Mangroves are important to the ecosystem because they can prevent coastal erosion and abrasion. Mangroves consist of fruits, leaves, stems, and roots. These mangrove plants serve various purposes, beginning with the utilization of their wood as a construction material, and some people use it as firewood, meanwhile, in the previous study, the mangrove (*Rhizophora apiculata*) leaves are used as animal feed (Sari et al. 2022). However, local people must understand the benefits of mangrove fruit, because many of these fruits end up as marine waste. This study used mangrove fruit from the species *Sonnetaria alba*.

S. alba is a mangrove plant that grows in coastal areas. Its population surpasses that of the other mangrove species based on seedlings and adult trees from the underwater edge to the land boundary. *S. alba* exhibits remarkable adaptability and boasts an abundance of seeds, facilitating rapid growth in new habitats within a short annual timeframe (Primavera and Esteban 2008). Mangrove fruits mature simultaneously across different parts of Indonesia. The species *S. alba* bears fruit in two fruiting periods: April–June and September–November. In addition to its relatively fast fruiting period, *S. alba* mangrove trees can produce 2 kg of fruit per day. However, the fruit remains underutilised because many still fall each season (Tahir et al. 2023). Additionally, mangrove fruit contains complete nutrition, serving as a source of carbohydrates and calories, thus making it suitable for use as an energy source in concentrate feed. The nutritional content of old *S. alba* mangrove flour is based on dry matter (DM) moisture (9.63%), ash 5.39%, protein 8.34%, fat 1.54%, and carbohydrates 75.1% (Ardiansyah et al. 2020) and the tannin content is 41.6%, which can be categorised as quite high (Bay 2016).

The primary challenge in utilizing mangrove fruit lies in its high tannin content. Tannins, which are polyphenolic compounds found in plants, act as antinutrients (Jamarun et al. 2017; Pazla et al. 2023). When consumed by livestock, high tannin levels can have a negative impact. Macromolecules such as structural carbohydrates, proteins and starch can bind tannins, so that their digestion decreases. Tannins can reduce the production of ammonia in ruminants (Besharati et al. 2022; Ardani et al. 2024). However, providing tannins in low concentrations can support the rumen microbial activity. In contrast, high concentrations of tannins can reduce the ration consumption due to their astringent taste, reduce digestibility, and have a toxic effect on rumen microbes because they can inhibit the enzyme activity (Verma et al. 2021). The reduction of tannin content can be done by boiling, soaking, and fermenting (Elihasridas et al. 2023a).

Fermentation is one way to improve the quality of animal feed ingredients using microorganisms (Pazla et al. 2021, 2024). In the fermentation process, careful consideration must be given to both the dosage and the duration of fermentation. The dosage level is related to the microbial population and determines the rate of microbial development, influencing the enzyme production and ultimately impacting the outcome. Meanwhile, the length of fermentation will affect microbial growth. Fungi are the most commonly used microorganisms for tannase production (Anwar et al. 2013). *A. niger* produces a tannase enzyme, which is used to reduce tannin content (Elihasridas et al. 2023a). The tannase enzyme acts as a catalyst, breaking down ester bonds in hydrolysed tannins, including those between glucose and esters. *A. niger* fungi can be used in the fermentation of *S. alba* mangrove fruit to reduce tannin content so as it will not interfere with the digestive process in the rumen.

The inclusion of tannins in feed rations has been shown to reduce the population of protozoa and methane-producing bacteria in the rumen (Antonius et al. 2024). Consequently, a decrease in protozoan populations can lead to an increase in the rumen populations of amylolytic and cellulolytic bacteria (Jamarun et al. 2017). Rumen bacteria play a crucial role in degrading feed and contribute significantly to the protein supply for the host livestock (Pazla et al. 2021). However, the provision of tannins at high concentrations can disrupt rumen microbial activity, thereby affecting microbial protein synthesis. Tannins bind to feed proteins, making them resistant to degradation, which deprives rumen microbes of nitrogen sources necessary for their growth (Rira et al. 2022).

This study aims to investigate the impact of the duration of *S. alba* mangrove fruit fermentation with

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A. niger mould on reducing tannin content, as well as its effects on digestibility, protozoa population, microbial biomass, and methane gas production when used as concentrate feed ingredients.

MATERIAL AND METHODS

Preparation of *Aspergillus niger* inoculum

A total of 100 g of rice bran was weighed, and distilled water was added until the water content was 60%, then it was homogenised in a heat-resistant plastic container. Next, it was autoclaved at 121 °C for 30 minutes. After cooling to a temperature of 35–37 °C, one test tube was inoculated with a slant medium containing *A. niger*. It was incubated for 7 days; after that, the inoculum was ready for use.

Mangrove fruit fermentation with the fungus *Aspergillus niger*

A total of 100 g of the sample was prepared and put into a plastic container, added distilled water to a level of 60%, and homogenised. Next, it was autoclaved at 121 °C for 30 min and allowed to cool. Then it was inoculated with 6 g of *A. niger* inoculum and homogenised. The samples were covered tightly with tape, small holes were made, and they were incubated for 7, 10, 13, and 16 days. The chemical composition of mangrove fruit can be seen in Table 1. The treatments were as follows: T1 (7 days fermentation); T2 (10 days fermentation); T3 (13 days fermentation); T4 (16 days fermentation).

Tannin analysis

An aliquot of 200 g of the sample was weighed and put in a test tube. Using a vortex, the sample was extracted with 10 ml of 50% methanol for 10 minutes. The mixed sample was centrifuged at a speed of 5 000 rpm for 5–10 minutes (S700T Benchtop Centrifuge, Kubota, Tokyo, Japan). After centrifugation, the liquid was discarded and 1 ml of bovine serum albumin (BSA) standard was added to 1 ml of the centrifuged sample. The resulting precipitate was dissolved with 4 ml of SDS-TEA, transferred into a test tube, and 1 ml of ferric chloride (FeCl) solution in 0.010 hydrogen chloride (HCl) was added. The mixture was homogenised by vortexing and allowed to stand at room temperature. The absorption was measured using a spectrophotometer (Double Beam Spectrophotometer UH5300, Hitachi, Tokyo, Japan) at a wavelength of 510 nm. Standard solutions were prepared by adding 50 mg of tannic acid to absolute methanol (1 mg/ml). Six test tubes each containing 1, 2, 3, 4, and 5 ml of standard solution were prepared and then adjusted to 10 ml each.

Table 1. Chemical composition of mangrove (*Sonneratia alba*) fruit

Chemical composition (% DM)	Before fermentation	Fermentation time (days)			
		7	10	13	16
Ash	5.43	6.60	8.03	8.41	8.65
Dry matter	51.8	51.5	42.8	32.5	28.4
Organic matter	94.6	93.4	91.9	91.6	91.4
Crude fibre	16.8	15.0	14.6	14.5	13.6
Crude fat	1.00	0.977	0.889	0.436	0.168
Crude protein	3.56	4.65	4.72	8.24	12.9
Nitrogen free extract	73.2	72.7	71.7	68.4	64.7
NDF	62.2	61.6	61.6	62.7	60.0
ADF	54.0	54.1	54.5	49.1	46.1
Cellulose	22.6	21.2	20.9	18.2	16.8
Hemicellulose	8.18	7.51	7.07	13.6	13.9
Silica	7.85	10.2	10.7	8.01	6.54
Lignin	23.5	22.7	22.8	22.8	22.7
TDN	63.0	64.4	63.6	64.3	66.6
Tannin	21.2	19.7	18.3	17.3	16.0

ADF = acid detergent fibre; DM = dry matter; NDF = neutral detergent fibre; TDN = total digestible nutrients

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Standard solution concentrations were 0.000, 0.100, 0.200, 0.300, 0.400, and 0.500 mg/ml. An amount of 1 ml of each standard solution was pipetted into a test tube, and 1 ml of BSA solution was added (2 mg/ml). The mixture was incubated at 5 °C for 20 minutes. The resulting colour should be blackish purple. After centrifugation, the liquid was discarded and the precipitate was washed three times with slowly dripping pH 5 acetate buffer through the walls of the tube and standard solution. Finally, the absorption was measured using a spectrophotometer.

The tannin percentage was calculated using the following formula:

$$\%\,\text{tannin} = \frac{\text{dilution factor} \times \text{sample}}{200\,\text{mg}} \times 100\% \quad (1)$$

In vitro fermentation

In vitro fermentation was conducted based on the Tilley and Terry method (Tilley and Terry 1963). The prepared McDougall solution was stored in a shaking water bath at 39 °C until use. Rumen fluid was collected in the morning from the slaughterhouse. A 2.50 g sample was placed into a 250 ml Erlenmeyer flask along with 50 ml of rumen fluid and 200 ml of McDougall buffer. CO_2 gas was then introduced into the Erlenmeyer flask for 30 s to create anaerobic conditions. The flask was sealed with a vented rubber stopper and placed in a shaking water bath set at 39 °C for 48 hours. After 48 h, the flask was cooled in an ice bath to deactivate the microbes. The liquid and food particles resulting from the incubation were then centrifuged at 3 000 rpm for 5 min to separate the residue and supernatant. The residue was used to evaluate the digestibility of crude fibre, crude fat, and nitrogen-free extract.

Total protozoa analysis

Total protozoa analysis was performed following the Ogimoto and Imai method (Ogimoto and Imai 1981). The used counting chamber had a thickness of 0.100 mm, with each box having an area of 0.062 mm; a total of 16 boxes were present, and four boxes were read. Total protozoa were observed using a microscope (Binocular Microscope 107bn) with a 40 × objective lens and a 10 × ocular lens.

Total protozoa were calculated using the formula:

$$\text{Total protozoa (cell/ml)} = \frac{1 \times 1\,000 \times C \times \text{FP}}{0.1 \times 0.062\,5 \times 16 \times 5} \quad (2)$$

where:

C – number of counted colonies;

FP – dilution factor.

Microbial biomass analysis

An amount of 1.50 ml of supernatant was pipetted into an Eppendorf tube and capped. The tube was centrifuged at 12 000 rpm for 15 min at 4 °C to precipitate the microbes. The supernatant was discarded, and the microbial precipitate in the Eppendorf tube was washed with sodium chloride (NaCl) solution using the same centrifugation procedure. Subsequently, it was washed with 50% methanol and centrifuged again. The washed precipitate was then dried in a 60 °C oven and weighed to obtain the dry weight. The microbial biomass (MB) can be calculated using the following formula:

$$\text{MB (mg/ml)} = \frac{\text{DW of percipitate (g)} - \text{EW (g)}}{\text{supernatant (ml)}} \quad (3)$$

where:

DW – dry weight;

EW – Eppendorf weight.

Methane gas production

Measurement of methane gas was conducted using the indirect Fieves method (Fieves et al. 2005). Readings of methane gas production were recorded in 3, 6, 9, 12, 24, and 48 hours. The gas drawn into the syringe during the measurement of total gas production was sprayed into a vacuum bottle with a rubber cap containing 35% NaOH. An empty syringe was attached to the bottle, through which methane gas escaped, while NaOH captured a portion of the CO_2 gas. Methane gas measurement was performed by observing the volume scale on the second syringe.

Statistical analysis

The data obtained from the research were statistically processed using ANOVA. Data analysis used Statistical Package for the Social Sciences (SPSS) v25.0 software (IBM Corp., New York, USA).

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RESULTS

Nutrient digestibility

The average digestibility of nutrients includes crude fibre, crude fat and nitrogen-free extract at different fermentation times of mangrove (*S. alba*) fruit using *A. niger*; it is shown in Table 2. Crude fibre digestibility ranged from 22.23% to 33.32%. Meanwhile, crude fat digestibility ranged from 25.67% to 32.34%. The digestibility of nitrogen-free extract was in the range of 22.67% to 36.10%. Treatment T4 showed higher digestibility, which covers crude fibre, crude fat and nitrogen-free extract, compared to treatments T1, T2, and T3.

Microbial biomass

The average microbial biomass of mangrove (*S. alba*) fruit feed ingredients fermented at different fermentation times in each treatment is shown in Figure 1. The results of the ANOVA indicated no significantly different effect of the treatment on microbial biomass in the rumen fluid ($P > 0.05$). Microbial biomass ranged from 2.07 to 2.28 mg/ml during 48 h of incubation (Figure 1). Treatment T4 resulted in the highest microbial biomass of 2.28 mg/ml.

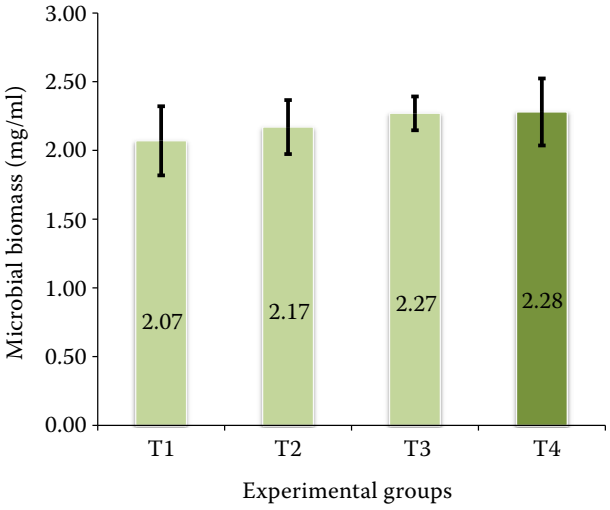

Figure 1. Microbial biomass of the treatments
Treatments showed non-significantly different results ($P > 0.05$)
T1 = 7 days fermentation; T2 = 10 days fermentation; T3 = 13 days fermentation; T4 = 16 days fermentation

Total protozoa

The average total protozoa from mangrove (*S. alba*) feed ingredients fermented at different fermentation times in each treatment is shown in Figure 2. The results of the ANOVA showed a highly significant effect of the treatment ($P < 0.01$) on the population of protozoa in the rumen fluid *in vitro*. Treatment T4 had the highest total protozoa of 4.07×10^5 cells/ml, followed by treatment T3 with 3.55×10^5 cells/ml. Treatments T1 and T2 had the lowest total protozoa of 0.00×10^5 cells/ml (Figure 2).

Methane gas production

Table 3 presents the average methane gas production from mangrove feedstuffs (*S. alba*) fermented at different fermentation times in each treatment during 48 h of incubation. The table shows that the treatment has an influence on methane gas production that is not significantly different ($P > 0.05$). However, methane gas production tended to increase during each incubation period. Treatment

Table 2. Nutrient digestibility in each treatment

Treatment	Crude fibre digestibility (%)	Crude fat digestibility (%)	NFE digestibility (%)
T1	22.2^{c}	25.7^{c}	22.7^{c}
T2	24.3^{b}	28.9^{b}	23.8^{c}
T3	26.3^{b}	29.8^{b}	26.4^{b}
T4	33.3^{a}	32.3^{a}	36.1^{a}
SE	0.455	0.698	0.575

^{a,b,c}Means followed by the letter are significantly different at $P < 0.05$
NFE = nitrogen-free extract; SE = standard error; T1 = 7 days fermentation; T2 = 10 days fermentation; T3 = 13 days fermentation; T4 = 16 days fermentation

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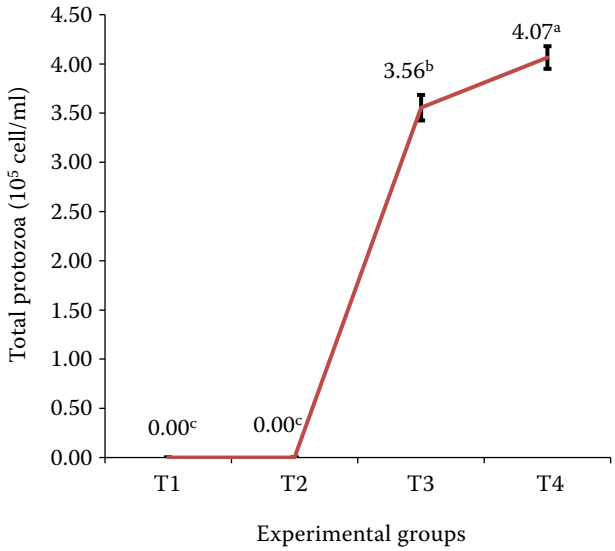

Figure 2. Total protozoa of the treatments

[a,b,c]Means followed by the letter are significantly different at $P < 0.05$

T1 = 7 days fermentation; T2 = 10 days fermentation; T3 = 13 days fermentation; T4 = 16 days fermentation

Table 3. Average methane gas production (ml/g)

Treatments	Incubation time (hour)[ns]					
	3	6	9	12	24	48
T1	2.16	5.56	10.6	12.1	18.8	26.0
T2	2.24	5.90	10.7	13.2	20.6	30.9
T3	2.38	6.13	14.7	15.9	25.7	31.9
T4	3.04	6.20	14.9	17.4	26.4	33.9
SE	0.280	0.370	1.31	1.46	2.01	2.95

SE = standard error; T1 = 7 days fermentation; T2 = 10 days fermentation; T3 = 13 days fermentation; T4 = 16 days fermentation

T4 exhibited the highest methane gas production after 48 h of incubation at 33.96 ml/g, surpassing treatments T3, T2, and T1.

DISCUSSION

Nutrient digestibility

The highest crude fibre digestibility was found in T4 at 33.3%. Meanwhile, T4 showed the highest crude fat and nitrogen-free extract (NFE) digestibility at 32.3% and 36.1%, respectively. This could happen due to a decrease in tannin content along with the increasing fermentation time of mangrove fruit. By reducing the tannin content, there is an adequate supply of nutrients for microbial growth, thereby increasing the digestibility of crude fibre. The increased rumen microbial growth causes increased digestibility of feed, including crude fibre, because rumen microbes produce enzymes that can degrade crude fibre (Jamarun et al. 2017; Pazla et al. 2021). A previous study by Espitia-Hernandez et al. (2022) reported that in sorghum the content of hydrolyzed tannins could decrease by 2–3% and condensed tannins decreased by around 6–23% using *Aspergillus oryzae* via solid-state fermentation (SSF). However, the lowest crude fibre digestibility was found in T1 at 22.2%, primarily due to the high presence of antinutrients, such as tannins, in mangrove fruit. Tannins binding to proteins hinder their digestion by protease enzymes, resulting in the limited amino acid formation and affecting growth (Ardani et al. 2024). This is consistent with Beauchemin et al. (2007), who stated that the high tannin content in feed can reduce the digestibility of fibre in the rumen by binding to cellulose and hemicellulose, thereby inhibiting digestion. The crude fibre digestibility in the study was low due to antinutrients present in mangrove (*S. alba)* fruit, such as tannins, which hinder protein digestion by protease enzymes, resulting in the limited amino acid formation and growth impairment. Additionally, high silica and lignin content, indigestible fibre components, contribute to low digestibility (Pazla et al. 2021; Elihasridas et al. 2023b; Marlida et al. 2023)

The reduction in tannin content accompanies the prolonged fermentation of mangrove fruit, leading to higher rumen microbial biomass. Increased microbial biomass can enhance microbial activity due to adequate nutrient requirements between ammonia (NH_3) and volatile fatty acid (VFA), thereby maximising the microbial formation. Kusumaningrum et al. (2012) reported a decrease in crude fat content in fermented rations due to the presence of glucose, which stimulates fungal biomass growth, consequently enhancing the production of lipase enzymes for crude fat breakdown. Lipase enzymes from *A. niger* effectively break down fat into fatty acids and glycerol, serving as energy sources for growth. The nutrient-rich environment in the T4 treatment supports optimal microbial growth, facilitating efficient fermentation with lipase activity facilitating crude fat degradation. Crude fat digestibility was lowest in T1

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because the fat content in this treatment was higher than in T2, T3, and T4, which resulted in a tendency to decrease crude fat digestibility even though the fat content in each treatment was still within the average threshold (< 5%). This is supported by Shirley (1986), who stated that the standard crude fat content in ruminant feed ingredients is below 5%. The feed that contains a lot of fat is not good for animal health because it oxidizes more easily and causes an unpleasant odour (Montesqrit et al. 2024). If the fat content of the feed is too high, it will harm feed degradation by microbes in the rumen (Enjalbert et al. 2017).

The digestibility of NFE is influenced by other nutrient components, including crude protein, ash, crude fat, and crude fibre (Arief and Pazla 2023; Agustin et al. 2024). NFE content represents the fraction of easily digestible carbohydrates such as starch and sugar. NFE contains monosaccharides, disaccharides, and polysaccharides, mainly starch, and all of them are soluble in acids and bases and have high digestibility (McDonald et al. 2010; Agustin et al. 2024). The observed NFE digestibility in this study was lower compared to that reported by Yunita (2018), where the use of gamal leaves and sweet corn in ruminant rations did not significantly impact the digestibility of nitrogen-free extract, with an average digestibility ranging from 66.6% to 67.9%.

Microbial biomass

Microbial biomass indicates the number of microbes in the rumen fluid that influence feed degradation. Rumen bacteria constitute the largest population in the rumen as they originate from feed ingredients and direct contact with materials containing bacteria (Verma et al. 2021). The fermentation of mangrove fruit at different durations in each treatment had no statistically significant effect on microbial biomass, although there was an increase in biomass in each treatment. This increase is attributed to the fermentation of mangrove fruit with *A. niger,* which reduces tannin levels, thus affecting the growth of rumen microbes and enhancing the degradation of feed ingredients by microbes (Jamarun et al. 2017).

The increase in microbial biomass is related to the production of NH_3 and VFA in the rumen fluid. This rise in microbial biomass production in the rumen fluid is a result of the balanced availability of NH_3 and VFA from the fermentation of organic matter, including crude fibre, crude fat, and nitrogen-free extract (Elihasridas et al. 2023b). Afzalani et al. (2023) also stated that supplementation of an extract from sengon leaf flour (ETDS), containing 1–3% condensed tannins, led to an increase in the protozoa population. However, when the ETDS levels exceeded 3%, there was a decrease in the protozoa population. In this study, the microbial biomass ranged from 2.07 to 2.28 mg/ml. T4 treatment had the highest microbial biomass yield although it was not different from other treatments. The microbial biomass results in this study were higher compared to previous studies reported by Bretschneidera et al. (2007), where supplementation of corn silage in grazed heifers showed no significant effect on microbial biomass, with production ranging from 1.70 to 1.91 mg/ml. Meanwhile, Ramaiyulis et al. (2016) reported that the higher microbial biomass production by adding gambier pulp to supplements with tannin levels of 2.50–5.00% resulted in higher microbial biomass ranging from 2.39 to 2.90 mg/ml.

Total protozoa

Based on the study results, the average total protozoa increased. The highest total protozoa count was recorded in treatment T4 at 4.07×10^5 cells/ml, followed by treatment T3 at 3.55×10^5 cells/ml. Conversely, treatments T1 and T2 exhibited the lowest total protozoa count, both registering 0.000×10^5 cells/ml. In treatments T1 and T2, the lowest total protozoa were caused by higher tannin content than in the other treatments. The highest tannin content in treatment T1 was 19.4%. According to Jamarun et al. (2017), tannin levels above 5% can limit the digestibility and potential inhibitory effects on methanogens and protozoa.

In this study, there was an increase in total protozoa as the fermentation progressed, due to the decreasing levels of tannins in the fermented *S. alba* mangroves. Fermentation with *A. niger* can alter the chemical composition of mangrove fruit. According to Anantasook et al. (2013), the reaction between tannins and protozoan cell walls disrupts the cell wall permeability, leading to protozoa mortality. Therefore, it can be inferred that the decrease in tannin levels in feed ingredients due to the fermentation process increases the protozoa

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population as the fermentation time increases. This assertion was supported by Elihasridas et al. (2023a), who stated that the tannase enzyme produced by *A. niger* can dissolve insoluble tannin compounds into soluble gallic acid and glucose. According to Majewska et al. (2021), the presence of protozoa in the rumen functions as a buffer by swallowing starch granules and slowing down fermentation, thereby preventing acidosis and rapid fluctuations in rumen pH values. In addition, protozoa can detoxify toxins that enter the host's body with feed. The presence of protozoa plays an essential role in fermentation; for example, oligotrich species can ingest feed particles but cannot utilize cellulose (McDonald et al. 2010).

Methane gas production

The lowest methane gas production was measured in treatment T1, which was 26.01 ml/g and the highest production was found in T4, which was 33.9 ml/g at 48 h incubation time. The low results of methane gas production are related to tannin content. The high tannin content will result in low methane gas production since it plays a role in inhibiting growth and methanogenic activity. The increase in methane gas production observed in this study was also directly related to an increase in the total protozoa produced and a decrease in tannin compound content (Antonius et al. 2024). In this study, the total protozoa population ranged from 0.00 to 4.07 × 10^5 cells/ml. An increase in methane gas production tends to accompany an increase in the protozoa population in the rumen because protozoa become hosts for methanogenic bacteria during the process of producing hydrogen gas (H_2), which then utilises the H_2 produced by the protozoa and converts it into methane gas (CH_4).

Tannins can directly and indirectly inhibit methanogen activity, decreasing H_2 availability (Hassan et al. 2020). Furthermore, their ability to modify the ruminal microbiome can reduce protein degradation and diminish methanogenesis (Canul-Solis et al. 2020). Ningrat et al. (2020) reported that tannin supplementation could decrease methane gas production and enhance feed digestibility in Simmental Cattle. The fungus used for fermentation in this study was *A. niger*, which produces a tannase enzyme capable of dissolving tannins. This aligns with the finding of Espitia-Hernandez et al. (2022), who stated that the tannase enzyme produced by *A. niger* can dissolve insoluble tannin compounds into soluble gallic acid and glucose. According to Widiawati and Puastuti (2016), the chemical compound tannin can reduce methane gas resulting from the enteric fermentation of ruminants. The magnitude of the effect of tannin compounds varies in reducing methane depending on the level and source of the compound.

CONCLUSION

Based on the research results, it can be concluded that fermenting mangrove (*S. alba*) fruit with *A. niger* for 16 days of fermentation was able to increase itsnutritional value in terms of the level of crude fibredigestibility, crude fat digestibility, and the highest nitrogen-free extract digestibility. However, microbial biomass and methane gas production were not significantly different in this study.

Conflict of interest

The authors declare no conflict of interest.

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