https://doi.org/10.17221/130/2024-CJAS

The potential of tannin from *Sonneratia alba* fruit in defaunation of rumen protozoa and reducing methane gas production: An approach to increase livestock digestive efficiency

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Citation: Pazla R., Antonius A., Ikhlas Z., Jayanegara A., Ardani L.R., Hadiatry M.C., Jumjunidang J., Budiyanti T., Firsoni F., Ardinal A., Iswari K., Febrina D., Ratni E. (2025): The potential of tannin from *Sonneratia alba* fruit in defaunation of rumen protozoa and reducing methane gas production: An approach to increase livestock digestive efficiency. Czech J. Anim. Sci., 70: 223–233.

Abstract: This research explores the addition of mangrove (*Sonneratia alba*) fruit to reduce the production of methane and the total population of protozoa. The dosage for adding mangrove fruit is 0% (without addition), 1.5%, 3%, and 4.5% in sugarcane tops-based feed. Results include ruminal product fermentation, gas and methane emissions, total protozoa, microbial protein production, microbial biomass, and nutrient digestibility. The research findings showed that an additional 1.5% to 4.5% dose can reduce methane gas emissions and the total number of protozoa. The total number of protozoa at 4.5% (T3) reached 9.89×10^4 cells/ml and methane gas was 56.1 ml/g DM (dry matter); 8.41 ml/g OM (organic matter). This effect is attributed to the tannin content in mangrove fruit, which exhibits antimicrobial properties. However, increasing doses also reduced nutrient digestibility. The findings suggest that incorporating 1.5–4.5% mangrove (*Sonnetaria alba*) fruit as a source of tannins causes a positive impact which reduces protozoa populations and methane production without changing the ruminal fermentation product. However, the addition of mangrove fruit in this study caused also reduced nutrient digestibility.

Keywords: anti-nutrition; digestibility; greenhouse gas; ruminal fermentation; sugarcane tops

https://doi.org/10.17221/130/2024-CJAS

Mangrove ecosystems, which cover tropical and subtropical coastal areas throughout the world, are home to various species of plants and livestock and provide a variety of valuable natural resources for humans. *Sonneratia alba*, one of the mangrove species, has emerged as a focus in the field of natural resource utilisation because of its ability to produce different bioactive compounds (Elihasridas et al. 2024). In Indonesia, currently, *S. alba* is not widely commercially available, but its availability is abundant and diverse, especially in coastal areas, in West Sumatra, Indonesia. Mangrove plants have a special ability to adapt to conditions of extreme environments, such as flooded soil conditions, high salt levels and unstable soil conditions (Pazla et al. 2024a).

Studies have specifically concentrated on *S. alba* fruit because it contains significant amounts of tannin which is capable of causing the defaunation of protozoa and reducing methane gas production in the livestock digestive system (Pazla et al. 2024a). Tannins are polyphenolic compounds occurring in plants including mangroves and are being considered for their diverse biological activities in ecological and agricultural research.

Recent investigations have indicated that tannins can influence rumen protozoa activity, which represents one of the major sources of enteric methane emissions by ruminants (Llonch et al. 2017). Methane-producing methanogens are known to be important members of these ecosystems, since they digest both cellulose and complex carbohydrates found in plants through fermentation processes, generating energy for themselves and other organisms in the process (Patra 2016; Ardani et al. 2024).

Methane gas emissions by livestock can harm them because a part of the power captured from their feed consumption is wasted as methane (Ardani et al. 2024). By capturing energy, the surface temperature is raised. It slows down the rate at which this energy escapes to space thereby trapping more energy in the atmosphere resulting in global warming. It can be estimated that it contributes 17–30% of CH_4 pollution in the atmosphere (Kroliczewska et al. 2023).

Various other plants such as mangroves have been found to possess tannins capable of suppressing rumen protozoa growth and activity, thus reducing methane production significantly (Antonius et al. 2024). Several investigations showed beneficial effects of tannin supplementation on livestock performance like an increase in protein retention and feed efficiency.

Previous study by Cardoso-Gutierrez et al. (2021) reported that the use of various types of tropical plants (*Leucaena leucocephala*, *Acacia pennatula*, *Enterolobium cyclocarpum*, *Gliricidia sepium*) rich in tannins is able to mitigate methane emissions most significantly. Due to the high tannin content of *S. alba* fruit, it is considered a possible candidate for future studies aimed at mitigating methane emissions from ruminants.

Sugarcane shoots are a by-product of the sugarcane logging stage. The potential for sugarcane shoots from sugarcane planting areas in Indonesia in 2019 was 453 238 ha with sugarcane production of 2 450 000 tonnes/year. The West Sumatra Province, Indonesia, had the plantation land covering an area of 7 305 ha in 2019, with a yield of sugarcane shoots of 3 247 tonnes/year. In the dry season, obtaining fresh grass forage can be challenging, so breeders around sugarcane plantations will generally use their sugarcane shoots as animal feed (Rohman and Sulistyo 2021).

Sugarcane shoot flour has a nutritional content of dry matter (DM) 89.35%; organic matter (OM) 91.57%; crude fibre (CF) 5.68%; nitrogen-free extract (NFE) 40.06%; acid detergent fibre (ADF) 45.71%; cellulose 28.21%; lignin 15.05%; neutral detergent fibre (NDF) 57.13%; and hemicellulose 11.41% (Pazla et al. 2021). Sugarcane shoots containing low NFE should be added with tannin compounds to kill protozoa to increase feed efficiency.

In this context, this research aims to explore the potential of *S. alba* mangrove fruit as a source of tannins to eliminate the protozoa population, protect protein so that bypass protein occurs, and reduce methane gas in the livestock digestive system. Through this approach, we hope to make a significant contribution to the understanding of sustainable natural resource utilisation and reduce greenhouse gas emissions in the livestock industry.

MATERIAL AND METHODS

Ethical approval

This research did not need an ethical review as no animal subjects were used.

https://doi.org/10.17221/130/2024-CJAS

Experimental design

This research used the addition of mangrove fruit to defaunate the livestock and reduce methane gas in the digestive system of livestock. The rumen fluid was obtained from goats slaughtered at the slaughterhouse. Goats received feed in the form of forage and concentrate at a ratio of 60% to 40%. The nutrient content of goats was met by getting 14% protein and 65% TDN (total digestible nutrient). The nutrient composition of sugarcane tops and mangrove fruit is shown in Table 1. Meanwhile, the nutrient content of sugarcane tops and mangrove fruit based on treatments is shown in Table 2. A randomised block design was implemented in this experiment, incorporating four treatments and four replicative groups as follows:

- T0 = 100% sugarcane tops without the addition of mangrove fruit;
- T1 = 98.5% sugarcane tops + 1.5% mangrove fruit;
- T2 = 97.0% sugarcane tops + 3% mangrove fruit;
- T3 = 95.5% sugarcane tops + 4.5% mangrove fruit.

In vitro fermentation

The *in vitro* experiment follows the Tilley and Terry (1963) method. A total of 2.5 g of the sample was measured and placed into a 250 ml Erlenmeyer flask. To each flask, 200 ml of phosphate-bicarbonate buffer and 50 ml of rumen fluid were added. Next, CO_2 was immediately supplied for 30–60 s so that conditions become anaerobic. The Erlenmeyer tube was sealed with a ventilated rubber cap to allow for gas release. It was then placed in a shaker incubator (Series 126; New Brunswick Scientific, Shanghai, P.R. China), set to a temperature of 39 °C, and incubated for 48 hours**.** Following the incubation period, the flask was cooled in an ice bath to halt the microbial activity. At the end of each incubation period, the pH of the rumen fluid was determined with a pH meter (Shanghai, P.R. China). The resulting mixture of liquid and food particles was subsequently centrifuged using a model S700T Benchtop centrifuge (Kubota, Tokyo, Japan) at 3 000 rpm for 5 min to separate the residue from the supernatant**.** The residue was then used to assess the digestibility of dry matter**,** crude protein, and organic matter**.**

The supernatant was used to measure several parameters including microbial biomass (Elihasridas et al. 2024), ammonia (NH_3) and total volatile fatty acids (VFA) (Conway and O'Malley 1942). VFA production was determined by the steam distillation method and its calculation was performed via the following formula:

$$\text{VFA (mM)} = (\text{ml HCl of blank titration} - \text{ml sample titration}] \times \times \text{N HCl} \times 1\,000/5 \quad (1)$$

Table 1. Nutrient content (% DM)

Nutrient content (% DM)	Materials	
	sugarcane tops	mangrove fruit
Dry matter	89.4	55.8
Organic matter	91.6	94.6
Crude protein	5.68	3.56
Ash	2.80	1.00
Crude fibre	41.9	16.8
Nitrogen free extract	41.2	73.2
TDN	60.7	81.0
NDF	57.1	62.2
ADF	45.7	54.0
Cellulose	28.2	22.6
Hemicellulose	11.4	8.18
Lignin	15.1	23.6
Tannin	0	28.0

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

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where:
VFA – volatile fatty acids;
HCl – hydrochloric acid;
N – normality.

Microbial protein synthesis was determined according to the Lowry method (Lowry et al. 1951) with the following equation:

$$Y = 0.002\ 5X + 0.014\ 6 \quad (2)$$

where:
Y – production absorbance;
X – protein concentration (μ/ml).

The total protozoa population was determined according to Ogimoto and Imai (1981) with the counting chamber used in this study that had a thickness of 0.100 mm, with each box having an area of 0.062 mm; a total 16 boxes were present, and 4 boxes were read.

The population of protozoa was estimated, and the samples were further examined under a microscope fitted with a 40X objective and 10X ocular lens. The protozoa population was then determined using the following formula:

$$\text{Protozoa population} = 1 \times 1\ 000 \times C \times \times Fp/0.1 \times 0.062\ 5 \times 16 \times 5 \quad (3)$$

where:
C – number of calculated colonies;
Fp – correction factor.

Total gas and methane gas production

After completing the incubation period of the syringe with phosphate buffer and noting the final gas volume, the lower end of the syringe was attached to the other lower end of another syringe containing 4.0 ml of 10 M sodium hydroxide (NaOH). NaOH was then transferred from this second syringe into the incubated mixture to prevent gas release. Mixing the contents with NaOH allows the absorption of CO_2, and the remaining gas volume in the syringe is regarded as CH_4. Then, the total gas and methane gas can be calculated.

Statistical analysis

Research data was processed statistically using the analysis of variance (ANOVA). The real difference in mean treatment values was assessed using Duncan's Multiple Range Test (DMRT). Data analysis was performed with the Statistical Package for the Social Sciences (SPSS) v21.0 (IBM Corp., NY, USA).

Table 2. Treatment nutrient content (% DM)

Nutrient content (% DM)	Treatments			
	T0	T1	T2	T3
Dry matter	89.4	89.7	88.3	87.8
Organic matter	91.6	90.2	91.7	91.7
Crude protein	5.68	5.65	5.62	5.58
Ash	2.83	2.80	2.78	2.75
Crude fibre	41.9	41.5	41.1	40.8
Nitrogen free extract	41.2	41.6	42.1	43.6
TDN	60.7	61.0	61.4	62.6
NDF	57.1	57.2	57.3	57.4
ADF	45.7	45.8	46.0	46.1
Cellulose	28.2	28.1	28.0	28.0
Hemicellulose	11.4	11.4	11.3	11.4
Lignin	15.1	15.1	15.3	15.4
Tannin	0.000	0.420	0.840	1.26

ADF = acid detergent fibre; DM = dry matter; NDF = neutral detergent fibre; T0 = 100% sugarcane tops without the addition of mangrove fruit; T1 = 98.5% sugarcane tops + 1.5% mangrove fruit; T2 = 97.0% sugarcane tops + 3% mangrove fruit; T3 = 95.5% sugarcane tops + 4.5% mangrove fruit; TDN = total digestible nutrients

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RESULTS

Rumen fermentation

The effect of adding mangrove (*S. alba*) fruit to feed ingredients on ruminal fermentation can be seen in Table 3.

The analysis of variance revealed that the treatment had no statistically significant effect on NH_3 production and rumen pH ($P > 0.05$). Meanwhile, the treatments had significantly different effects on total VFA concentration ($P < 0.05$). T3 showed that adding mangrove fruit up to 4.5% produces the highest total VFA which is 68.0 mM compared to the treatment without adding mangrove fruit (T0) which is 63.3 mM.

Total protozoa, microbial protein synthesis, and microbial biomass

The addition of mangrove fruit (*S. alba*) to feed ingredients that impacts total protozoa, microbial biomass, and microbial protein synthesis is shown in Table 4. The treatments had significantly different effects on microbial protein synthesis and total protozoa ($P < 0.05$). The addition of 4.5% mangrove fruit in T3 can reduce the total protozoa population to 9.89×10^4 cells/ml and microbial protein synthesis to 18.2 mg/100 ml lower than in the other treatments. However, the treatments have no significantly different effects on microbial biomass ($P > 0.05$).

Total gas and methane gas production

The impact of adding mangrove fruit (*S. alba*) to feed ingredients on the overall production of gas and methane is illustrated in Table 5.

Results of the analysis of variance revealed significant differences in total gas and methane gas production ($P < 0.05$).

According to Table 5, the T3 treatment, which included mangrove fruit at a 4.5% concentration, resulted in the lowest levels of both total gas and methane gas production. Conversely, the T0 treatment, which did not include any mangrove fruit, showed the highest levels of total gas and methane gas production.

Table 3. Effect of mangrove fruit on parameters of *in vitro* rumen fermentation

Treatment	NH_3 (mg 100 ml)	Total VFA (mM)	pH
T0	7.50 ± 0.13	63.3^b ± 2.08	6.83 ± 0.15
T1	7.42 ± 0.05	64.7^b ± 0.58	6.86 ± 0.26
T2	7.31 ± 0.08	65.0^b ± 0.03	6.96 ± 0.19
T3	7.28 ± 0.18	68.0^a ± 2.00	7.00 ± 0.11
SE	0.060	0.740	0.090

^{a,b}Indicate significantly different effects within the column ($P < 0.05$)

NH_3 = ammonia; T0 = 100% sugarcane tops without the addition of mangrove fruit; T1 = 98.5% sugarcane tops + 1.5% mangrove fruit; T2 = 97.0% sugarcane tops + 3% mangrove fruit; T3 = 95.5% sugarcane tops + 4.5% mangrove fruit; VFA = volatile fatty acids

Table 4. Effect of mangrove fruit on total protozoa, microbial protein synthesis, and microbial biomass

Treatment	Total protozoa (cell/ml)	Microbial protein synthesis (mg/100 ml)	Microbial biomass (mg/ml)
T0	16.8×10^{4a} ± 0.50	25.0^a ± 1.78	2.31 ± 0.20
T1	15.1×10^{4b} ± 0.90	20.5^{ab} ± 3.63	1.96 ± 0.28
T2	12.1×10^{4c} ± 0.61	22.3^b ± 0.63	1.96 ± 0.10
T3	9.89×10^{4d} ± 0.47	18.2^b ± 2.10	1.73 ± 0.18
SE	0.330	1.15	0.100

$^{a–c}$Indicate different effects within the column ($P < 0.05$)

T0 = 100% sugarcane tops without the addition of mangrove fruit; T1 = 98.5% sugarcane tops + 1.5% mangrove fruit; T2 = 97.0% sugarcane tops + 3% mangrove fruit; T3 = 95.5% sugarcane tops + 4.5% mangrove fruit

https://doi.org/10.17221/130/2024-CJAS

Table 5. Effect of mangrove fruit on total gas and methane gas production

Treatment	Total gas of digested DM (ml/g)	Methane gas of digested OM (ml/g)	Total gas of digested DM (ml/g)	Methane gas of digested OM (ml/g)
T0	85.2^{a} ± 1.98	12.8^{a} ± 0.73	84.3^{a} ± 1.23	12.6^{a} ± 0.68
T1	73.1^{b} ± 1.56	11.2^{b} ± 0.69	71.8^{b} ± 1.33	10.7^{b} ± 0.79
T2	68.1^{c} ± 1.76	10.3^{c} ± 0.80	66.9^{c} ± 1.02	10.0^{b} ± 0.66
T3	56.4^{d} ± 1.88	9.38^{d} ± 0.83	56.1^{d} ± 1.83	8.41^{c} ± 0.74
SE	1.21	0.620	1.16	0.660

$^{a–c}$Indicate different effects within the column ($P < 0.05$)

DM = dry matter; OM = organic matter; T0 = 100% sugarcane tops without the addition of mangrove fruit; T1 = 98.5% sugarcane tops + 1.5% mangrove fruit; T2 = 97.0% sugarcane tops + 3% mangrove fruit; T3 = 95.5% sugarcane tops + 4.5% mangrove fruit

Table 6. Effect of mangrove fruit on *in vitro* nutrient digestibility

Treatment	IVDMD (%)	IVOMD (%)	IVCPD (%)
T0	47.8^{a} ± 0.59	48.4 ± 0.67	48.0^{a} ± 2.28
T1	46.3^{b} ± 0.76	47.3 ± 0.41	47.8^{ab} ± 0.94
T2	45.9^{b} ± 0.47	46.7 ± 1.11	46.4^{b} ± 0.67
T3	45.7^{b} ± 1.12	46.0 ± 0.73	46.1^{b} ± 0.58
SE	0.390	0.430	0.480

^{a,b}Indicate significantly different effects within the column ($P < 0.05$)

IVCPD = *in vitro* crude protein digestibility; IVDMD = *in vitro* dry matter digestibility; IVOMD = *in vitro* organic matter digestibility; T0 = 100% sugarcane tops without the addition of mangrove fruit; T1 = 98.5% sugarcane tops + 1.5% mangrove fruit; T2 = 97.0% sugarcane tops + 3% mangrove fruit; T3 = 95.5% sugarcane tops + 4.5% mangrove fruit

Nutrient digestibility

The *in vitro* digestibility of nutrients when supplemented with mangrove (*S. alba*) fruit is shown in Table 6. The treatments had significantly different effects on *in vitro* crude protein digestibility (IVCPD) and *in vitro* dry matter digestibility (IVDMD) ($P < 0.05$). Treatment T0 without additional mangrove fruit showed higher digestibility of dry matter and organic matter compared to treatments T1, T2, and T3. Meanwhile, the treatments had no significantly different impact on *in vitro* organic matter digestibility (IVOMD) ($P > 0.05$).

DISCUSSION

Ruminal fermentation

The addition of mangrove (*S. alba*) fruit up to 4.5% did not cause any significant differences in rumen pH and NH_3 production (Table 3). The rumen pH remained within the normal range and showed no significant differences between treatments, suggesting that the addition of *S. alba* at a dose of 4.5% did not directly affect the acid-base balance in the rumen. This may be due to compensation by natural buffer systems in the rumen to maintain pH in the optimal range for rumen microbial activity. Although tannins may influence the activity of certain microbes, the effect may not be great enough to alter the pH balance in the rumen (Jamarun et al. 2025). Neutral rumen pH will help balance the rumen microbiome so that the fermentation process takes place optimally (Antonius et al. 2024). The presence of NH_3 was relatively stable, indicating a limited effect of tannins on the deamination process in the rumen. Even though the levels of NH_3 were stable, digestibility declined for dry matter, organic matter, and crude protein as such in treatment T3. In previous studies, this may be due to the tannin ability to inhibit digestive enzymes as well as ruminal microorganisms involved in crude fibre and crude protein digestion (Elihasridas et al. 2024; Jamarun et al. 2025). Microbes present in the rumen utilise NH_3 for the synthesis of proteins. Adding high amounts of tannins could lead to a decrease in the uptake of NH_3 by microbes thus leading

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to low microbial protein synthesis. Alternatively, there may be other nitrogen sources used by microbes apart from NH_3 (Mahmoudi-Abyane et al. 2020). High doses of tannins have been shown to affect the microbial composition within the rumen and retard the proliferation of microbes efficiently synthesising nutrients (Pazla et al. 2024b).

Meanwhile, the treatments have significant effects on total VFA concentration (Table 3). Treatment T3 had the highest VFA concentration of 68.00 mM, while T0 had the lowest concentration of 63.33 mM. The addition of a 4.5% dose of mangrove fruit to the rations (T3) increased the total VFA content. Tannins at high doses can change the microbial composition in the rumen thereby increasing VFA production even though the digestibility of the main nutrients decreases (Ardani et al. 2024). Further analysis of VFA composition, digestive efficiency, and physiological responses of livestock is needed to assess feed quality. Despite decreased total gas and methane production the high VFA production at T3 suggests that some microbes may be more resistant to tannins or can utilise them as an energy source. Pazla et al. (2024b) suggested that the rise in total VFA may result from the enhanced nutrient use by the remaining rumen microbes. In another report by Zhang et al. (2014), some microbes can use tannins as an energy source, increasing VFA production even though nutrient digestibility decreases.

Total protozoa, microbial protein synthesis, and microbial biomass

The impact of adding mangrove (*S. alba*) fruit up to 4.5% to the rations causes significant differences in the total protozoa population and microbial protein synthesis. Meanwhile, the treatments show no significant differences in microbial biomass (Table 4). The highest counts of total protozoa were found in treatment T0 (16.8×10^4 cells/ml) and the lowest counts were in treatment T3 (9.89×10^4 cells/ml). The T3 treatment resulted in a reduction in the protozoa population in the rumen. Rumen protozoa are among the main producers of methane gas in the digestive system of ruminant livestock. Therefore, reducing the number of protozoa can significantly contribute to reducing methane gas production in livestock receiving feed with added tannin (Pazla et al. 2024a). Antonius et al. (2024) reported that some types of tannins can be toxic to protozoa or interfere with their activity, which can lead to a decrease in protozoa populations within the rumen. Tannins can inhibit the activity of methanogenic microbes, which are responsible for producing methane gas during the rumen fermentation process.

The addition of mangrove fruit as a source of tannins shows a significantly different effect on microbial protein synthesis. The highest synthesis was observed in treatment T0 (25.02 mg/100 ml) and the lowest in treatment T3 (18.18 mg/100 ml) (Table 4). The microbial protein synthesis decreased in T3 compared to the other treatments, this occurred because the tannin content in the T3 treatment was greater than in the others. Huang et al. (2018) stated that tannins at high doses negatively affect some microbial activities that are involved in microbial protein production. Therefore, even though the nutritive value is sufficient for the anticipated dietary needs of the subjects, microbial protein synthesis is reduced due to the impact of tannins (Elihasridas et al. 2024). Some studies have revealed that when tannins are included at high levels, the microbial protein synthesis may be reduced. From these findings, it is clear that high levels of tannins influence the number and intensities of microbes in the rumen resulting in reduced values of microbial protein yield despite not much influence on the concentration of NH_3 and rumen pH. Therefore, it is important to understand that supplementation of tannins can sort proteins in the diet to form non-degradable proteins thereby reducing the possibility of microbial protein production based on NH_3 (Llonch et al. 2017).

However, the treatments do not differ significantly in microbial biomass as depicted in Table 4. Microbial protein is defined as the key structural component of microbial biomass; if the microbial protein synthesis rate was reduced, this might affect the amount of microbial biomass (Matassa et al. 2016). High doses of tannins are known to suppress enzymes needed by the rumen microbes for protein synthesis including nitrogenase which is used in nitrogen fixation by the microbes (Mora-Ortiz and Smith 2018). Tannins can prevent the microbial growth and affect the extent of protein digestion and microbial reproduction process in the rumen (Antonius et al. 2024). According to the prior research by Elihasridas et al. (2024), it was identified that the incorporation of man-

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grove fruit did not have any significantly different impact on the microbial biomass which was found to be 2.07–2.88 mg/ml. Microbial biomass in the above-mentioned study was higher than that obtained in this study.

Total gas and methane gas production

Analysing the data presented in Table 5 on the total gas and methane gas production it is possible to note that this treatment produces a rather different effect. Both increasing and decreasing quantities of mangrove fruit in the diet at various levels will decrease the total amount of gas produced compared to the control. Thus, raising the supply of mangrove fruit for tannin sources corresponds directly with a decline in total gas production. This is in agreement with the earlier study by Patra (2016) identifying that tannins decrease the total and methane gas amount in the rumen of ruminants. This decrease in total and methane gas is a good sign in terms of greenhouse gas emissions that are associated with livestock production (Llonch et al. 2017). The reduction in methane gas production in the T3 treatment is another reason because the tannin element of the feed affects the functionality of enzymes and rumen microbes. The presence of tannins affects the use of some enzymes involved in the production of methane gas, hence a decline in the general methane gas production (Aboagye and Beauchemin 2019).

This finding is supported by several earlier studies that established that tannins can prevent the methane gas formation during the process of ruminant fermentation (Ardani et al. 2024; Pazla et al. 2024a). Valente et al. (2016) noted that at higher levels of supplementary tannins, there is an ability to alter the population of microbes in the rumen which results in either the reduction of the densities of methanogenic microbes or the increase in the densities of non-methanogenic microbes. Consequently, the total production of methane gas can drop. Microbial shifts that affect the function and composition can affect total gas production as well as methane gas production. A decrease in the number or activity of certain microbes responsible for the degradation of nutrient substrates in the rumen may also contribute to a decrease in total gas production (Valente et al. 2016). However, the total gas production is influenced by feed digestibility in ruminant livestock, the higher the feed digestibility, the more gas is produced, and *vice versa* (Hariyani and Chuzaemi 2019; Marlida et al. 2023).

Nutrient digestibility

This research shows that the addition of mangrove fruit at a dose of 4.5% as a source of tannins has a significantly different effect on IVDMD and IVCPD. Meanwhile, the treatments have no significantly different effect on IVOMD (Table 6). The addition of mangrove fruit tends to decrease the digestibility of nutrients including organic matter, dry matter, and crude protein. Therefore, this may also be due to changes in the rumen microbial community, especially in substrate-degrading microbes. This study has consistent results with previous research which also reported the negative effects of tannins on the digestibility of crude fibre and organic matter in ruminant livestock (Patra 2016; Okunade et al. 2022). According to Naumann et al. (2013), tannins are considered to have the ability to precipitate protein and fibre into insoluble precipitates resisting degradation by a ruminal enzyme which is unable to break down into free amino acid. Tannin substances can change the biochemistry and functional end products of rumen microbes (digestibility) due to the poor utilisation of nutrients. Pazla et al. (2024b) reported that tannins inhibit the growth and functioning of fibre-fermenting microbes in rumen.

Other research proves that tannins can chelate with proteins to give products that are tougher to break down and assimilate in the body of the stock (Besharati et al. 2022). The complex formed by tannins and protein can decrease the activity of enzymes responsible for the protein breakdown in the rumen including trypsin and pepsin (Majewska et al. 2023). Furthermore, tannins affect such factors as the microbial population within the rumen since this determines specific fermentation and degradation of organic matter. Rira et al. (2015) stated that because other specific rumen microbes are involved in the breakdown of organic matter, decreased population or metabolic activity of such microbes usually leads to a decrease in the digestibility of organic matter. Tannins can bind to proteins or fibre to rarely let digestive enzymes get access to the nutrients present in the organic materials and thus they reduce their rate of diges-

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tion (Taethaisong et al. 2023). Tannins may reduce some of the microbes involved in the breakdown of specific organic compounds but other microbes may have the ability to carry out the breakdown of affected but not completely impaired organic compounds, and this is why VFA production continues to rise despite a decline in the digestibility of organic matter (Rira et al. 2015).

In this regard, tannins can react with the proteins present in the rumen, thus protecting the proteins from the action of rumen microbes, which makes a balance of RDP to RUP. The content of rumen degradable protein and rumen undegradable protein is critical in feed efficiency regarding ruminant animals. The balance of RDP and RUP is very important for the growth and development of livestock; if more RDP is digested in the rumen, it will produce more ammonia, which can cause poisoning in ruminants (Zain et al. 2023). The effect of tannins on nutrient digestibility can also be influenced by other factors in the diet, such as overall nutritional composition, availability of other substrates, and interactions with complex rumen microbes (Min and Solaiman 2018). This information is important to help develop more effective feed management strategies in maximising the efficiency of nutrient digestion and animal performance.

Current research on the potential of tannins from *S. alba* to modify the rumen metabolism is complex and some points are still open for discussion. Tannins reduce deamination, so less ammonia is available for the microbial protein synthesis. *In vitro* protein digestibility decreases, but tannins form non-degradable protein complexes, so tannins reduce the microbial protein production based on NH_3, but on the other hand, they increase bypass protein to the duodenum. Further research on the effect of tannins on animal productivity is needed through *in vivo* experiments.

CONCLUSION

The study concludes that the addition of mangrove (*Sonneratia alba*) fruit at a dosage of 1.5–4.5% to feed serves as an effective tannin source, reducing protozoa populations and methane gas emissions while maintaining ruminal fermentation products such as volatile fatty acids and rumen pH. These effects highlight its potential in promoting sustainable livestock practices and minimising environmental impacts. However, the inclusion of mangrove fruit also decreases nutrient digestibility, likely due to the anti-nutritional properties of tannins, which can inhibit microbial activity and nutrient breakdown. Further research is necessary to explore solutions for balancing its benefits and mitigating its limitations.

Acknowledgement

The authors would like to express their profound gratitude to the team members who contributed significantly to this research. Special thanks go to Dr. Ramaiyulis for his invaluable support and contributions to this work. We also extend our deepest appreciation to Politeknik Negeri Payakumbuh, Provinsi Sumatera Barat, for their encouragement and the resources they provided.

Conflict of interest

The authors declare no conflict of interest.

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Received: September 9, 2024
Accepted: May 20, 2025
Published online: June 27, 2025