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RESEARCH PAPER

Evaluation of lime treatment in reducing tannin content and enhancing nutrient utilization of mangrove fruits (*Sonneratia alba*) as an animal feed ingredient

Novirman Jamarun[1], Roni Pazla[1], Anuraga Jayanegara[2], Elihasridas[1], Gusri Yanti[3], Antonius[4], Zaitul Ikhlas[5], Laily Rinda Ardani[5]

1 *Department of Animal Nutrition, Faculty of Animal Science, Universitas Andalas, Campus Limau Manis, Padang, 25166 West Sumatera, Indonesia*
2 *Department of Nutrition and Feed Technology, Faculty of Animal Science, IPB University, Jl. Agatis Kampus IPB Dramaga Bogor, 16680, Indonesia*
3 *Department of Agriculture Extension, Faculty of Social, Science and Education, Prima Nusantara Bukittinggi University, Padang, West Sumatra, Indonesia*
4 *Research Center for Animal Husbandry, Research Organization for Agriculture and Food, National Research and Innovation Agency (BRIN), Jl. Raya Jakarta Bogor Cibinong 16915, Indonesia*
5 *Doctoral Program, Department of Agriculture Extension, Faculty of Animal Science, Universitas Andalas, Campus Limau Manis, Padang, 25166 West Sumatera, Indonesia*

Corresponding author: Roni Pazla (ronipazla@ansci.unand.ac.id)

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Abstract

The use of lime water is thought to be able to increase the nutritional value of feed by reducing the tannin content. This study examined the effects of soaking with lime water (calcium hydroxide) on the nutritional quality of mangrove fruits (*Sonneratia alba*) as a ruminant feed ingredient. A factorial experimental design was employed, with mangrove fruits treated with various concentrations of lime water solution (1%, 3%, 5%, and 7%) and soaked for different durations (10, 20, and 30 minutes). The study measured several parameters, including proximate composition, fiber fraction, tannin content, nutrient digestibility, and rumen fermentation characteristics. The results showed a significant reduction ($P < 0.05$) in tannin content with lime water treatment (5%), which demonstrated the most striking effect in enhancing feed quality metrics. Lime water soaking did not alter the pH of the rumen fluid. There was a significantly higher digestibility of dry matter, organic matter, and crude protein in feeds treated with 5% lime water ($P < 0.05$), which was consistent with the higher VFA and NH3 levels ($P < 0.05$), showing enhanced microbial fermentation. Also, the feed treated with 5% lime water had significantly higher ($P < 0.05$) digestible NDF, ADF, cellulose, and hemicellulose characteristics compared to the others. Treatment with 20 minutes of soaking time showed that the digestibility of feed ingredients and rumen fermentation was more optimal than 30 minutes of soaking time. However, the 30 minutes of soaking time treatment reduced methane gas production the most. Thus, the best treatment is 5% lime water and 20 minutes of soaking time. This study proves that carbonation as a cheap technological solution can convert mangrove fruit into a high-value material for livestock in an affordable and environmentally friendly.

Keywords

Lime water soaking, methane, nutrient digestibility, rumen characteristics, ruminant feed

Introduction

International attention is mainly aimed at ensuring sufficient high-quality animal feed materials and some research is being done on improving natural resources that are usually unused. From all these components, the fruits of the *Sonneratia alba* species are the very ones that are found to be promising to be used as ruminant concentrate feed due to their advantageous nutritional profile (Oeturlinsyah et al. 2019; Wintah et al. 2021). However, there is a capacity to exploit fruits but on the other hand, there are high tannin contents which are up to 21.21% the main obstacle. Being rich in tannin, a phenolic compound, can cause harmful effects on the digestion of protein, and therefore may adversely affect the health of the cattle (Elihasridas et al. 2023). Tannins *S. alba* contained that hamper proteins and minerals in ruminant digestion are seen as per the study by Besharati et al. (2022). This will call for improvements in the currently underway processes to minimize the tannin levels in the *S. alba* fruit to ensure that those that will be fed to animals are suitable.

The Indonesian island region, the largest source of mangrove forests in the world, sustains subsistent populations of *S. alba* and offers a great source for economic and ecological activities (Wintah et al. 2021). Recent researches indicate that since mangrove ecosystem management is mostly altruistic, such resources will ensure the maintenance of the mangrove habitat while providing alternative feeds to the livestock animals (Sari et al. 2021; Jamarun et al. 2023). Recently, lime water soaking using calcium hydroxide exhibited an effect of breaking down tannins in various feed ingredients, such as mangrove fruits, without losing their nutritional quality (Sari et al. 2022). In a study by DeLima et al. (2022), tannins, a component in *Bruguiera gymnorrhiza* fruits, can be greatly reduced by soaking with water, as exemplified by a maximum reduction of 27.07%. Likewise, Sari et al. (2022) investigated the changes in mangrove leaf nutritional quality of *Rhizophora apiculata* by using lime water and their best results came after soaking the leaves in a lime-water solution that has a 5% ratio. Soenardjo and Supriyantini (2017) furthered their research, and the connection between lime dosage, soaking time, and the likelihood that the tannin removal was successful was defined. The use of tannin in feed requires certain limits so that it does not affect the ruminant's metabolism. According to Besharati et al. (2022), the use of tannin within a minimum limit of 5 g/kg DM and a maximum of 50 g/kg DM shows optimal results in terms of digestibility of feed ingredients, rumen fermentation and ruminal microbiota. The report added that the use of >50 g/kg DM or a concentration of more than 5% in concentrate can reduce feed consumption and digestibility.

The goal of this research is to find out what influences lime water concentrations and soaking times on tannin content in *S. alba* fruit so that it can be on a path to tannin removal. This study comes up with some valuable information on the preferred situation for soaking which improves tannin content removal and is at the same time nutrition-friendly (deLima et al. 2022). Remarkably, the outcomes are predicted to enable the growth of profitable sustainable, and eco-friendly concentrated feeds for ruminants to guarantee food security for future generations and simultaneously conserve mangrove habitats.

Materials and methods

Ethical approval

This study did not use livestock so it did not require ethical permission from the institution. This study used rumen fluid as part of the experimental material.

Experimental design

The experiment was designed as a randomized complete design with a 4 × 3 factorial arrangement with 3 replications for all the treatments. The two factors in focus were lime water concentration (factor A), which included four variations: 1% (A1), 3% (A2), 5% (A3), and 7% (A4); designating soaking time (factor B) using three intervals of 10 minutes (B1), 20 minutes (B2), and 30 minutes (B3). These elements were mixed up and formed 12 different components; each one of them was tested with 3 replications. The specific data per compound is presented below:

- A1B1: 1% lime water for 10 minutes
- A1B2: 1% lime water for 20 minutes
- A1B3: 1% lime water for 30 minutes
- A2B1: 3% lime water for 10 minutes
- A2B2: 3% lime water for 20 minutes
- A2B3: 3% lime water for 30 minutes
- A3B1: 5% lime water for 10 minutes
- A3B2: 5% lime water for 20 minutes
- A3B3: 5% lime water for 30 minutes
- A4B1: 7% lime water for 10 minutes
- A4B2: 7% lime water for 20 minutes
- A4B3: 7% lime water for 30 minutes

Mangrove natural products from the *S. alba* species were accumulated from the Nagari Sasak region in West Sumatera, Indonesia. They were at that point cut into little pieces. Lime was broken up in refined water agreeing to each treatment, to be specific 1%, 3%, 5%, and 7% lime water. The natural products were cut into pieces and doused in lime water concurring to treatment for 10, 20, and 30 min. After drenching, they were evacuated and washed with running water until clean. At last, the natural product was dried in a broiler at 60 °C for 24 h. After drying, they were squashed with a blender and prepared for examination.

Determination of chemical composition

The amount of nutrients was found using methods from AOAC International (2016). The fiber content was analyzed following the process explained by Van Soest et al. (1991). Table 1 shows the nutritional makeup of the mangrove fruit diet before it was soaked in water.

Table 1. Nutrient content of mangrove fruit food substances before soaking.

Nutrient Content	Mangrove Fruit (%)
Dry Matter	50.80
Organic Matter	94.03
Crude Protein	4.66
Crude Fat	1.03
Crude Fiber	15.73
NFE	72.61
NDF	60.52
ADF	52.01
Cellulose	20.83
Hemicellulose	8.51
Lignin	23.35
Silica	7.83
Tannin	28.02
TDN	80.67

NFE: Nitrogen Free Extract; ADF: Acid Detergent Fiber; NDF: Neutral Detergent Fiber; TDN: Total Digestible Nutrient.

The nutrients of mangrove fruit soaked in water are illustrated in Table 2 and are presented in Table 3 as fiber fraction content. Tannin concentration was measured by a UV-visible spectrophotometer (Shimadzu). This set of samples was mixed with 150 ml of distilled water and the mixture was incubated in a water bath at 70 °C for half an hour. Next, cooling, filtering, and using the extract for analysis followed. The extraction results were measured using a UV-visible spectrophotometer (Shimadzu), at a wavelength of 278.5 nm, and pure tannin (RnD Centre Inc.) was used as a standard.

Table 2. Nutrient content of mangrove fruit food substances after soaking.

Treatments	Nutrient content (%)						
	Dry Matter	Organic Matter	Crude Protein	Crude Fiber	Crude Fat	NFE	TDN
A1B1	48.30	96.55	8.10	15.50	1.11	70.79	80.44
A1B2	48.03	94.57	7.84	15.40	1.10	70.16	79.70
A1B3	49.03	95.06	7.73	15.32	1.33	70.68	80.46
A2B1	47.04	96.05	7.74	15.30	1.42	71.88	81.71
A2B2	50.84	95.42	7.65	15.22	1.44	71.22	81.06
A2B3	49.78	95.71	7.60	15.02	1.09	72.09	81.30
A3B1	49.04	95.95	7.69	15.04	1.32	71.90	81.50
A3B2	49.22	94.99	7.55	14.96	1.22	71.26	80.68
A3B3	50.01	94.94	7.50	14.90	1.04	71.27	80.39
A4B1	48.98	94.92	7.54	14.99	1.07	71.35	80.55
A4B2	49.84	94.78	7.43	14.92	1.09	71.42	80.58
A4B3	48.89	94.39	7.40	14.94	1.06	70.97	80.11

A1B1 (1% lime water soaked for 10 minutes); A1B2 (1% lime water soaked for 15 minutes); A1B3 (1% lime water soaked for 20 minutes); A2B1 (3% lime water soaked for 10 minutes); A2B2 (3% lime water soaked for 15 minutes); A2B3 (3% lime water soaked for 20 minutes); A3B1 (5% lime water soaked for 10 minutes); A3B2 (5% lime water soaked for 15 minutes); A3B3 (5% lime water soaked for 20 minutes); A4B1 (7% lime water soaked for 10 minutes); A4B2 (7% lime water soaked for 15 minutes); A4B3 (7% lime water soaked for 20 minutes); NFE: nitrogen-free extract; TDN: total digestible nutrient.

Table 3. The fiber fraction content of mangrove fruit after soaking.

Treatments	Van soest fiber fraction (%DM)					
	NDF	ADF	Hemicellulose	Cellulose	Lignin	Silica
A1B1	60.80	51.80	9.00	19.80	22.08	9.92
A1B2	60.68	51.28	9.40	19.78	22.30	9.20
A1B3	60.63	52.03	8.60	19.66	22.10	10.27
A2B1	59.80	51.03	8.77	19.04	21.90	10.09
A2B2	59.37	51.33	8.04	19.09	21.83	10.41
A2B3	59.82	51.29	8.53	19.12	21.88	10.29
A3B1	59.78	50.80	8.98	18.98	21.22	10.60
A3B2	59.60	50.78	8.82	18.90	21.13	10.75
A3B3	59.20	50.44	8.76	18.82	21.01	10.61
A4B1	59.68	51.03	8.65	19.03	21.80	10.20
A4B2	60.02	51.10	8.92	19.20	21.83	10.07
A4B3	60.31	51.21	9.10	19.24	21.78	10.19

A1B1 (1% lime water soaked for 10 minutes); A1B2 (1% lime water soaked for 15 minutes); A1B3 (1% lime water soaked for 20 minutes); A2B1 (3% lime water soaked for 10 minutes); A2B2 (3% lime water soaked for 15 minutes); A2B3 (3% lime water soaked for 20 minutes); A3B1 (5% lime water soaked for 10 minutes); A3B2 (5% lime water soaked for 15 minutes); A3B3 (5% lime water soaked for 20 minutes); A4B1 (7% lime water soaked for 10 minutes); A4B2 (7% lime water soaked for 15 minutes); A4B3 (7% lime water soaked for 20 minutes); ADF: acid detergent fiber; NDF: neutral detergent fiber.

In vitro procedures

McDougall buffer solution was prepared to implement the in vitro experiment and consisted of $NaHCO_3$, $Na_2HPO_4.7H_2O$, KCl, $MgSO_4.7H_2O$, NaCl, and $CaCl.2H_2O$. All ingredients were mixed and dissolved using distilled water depending on the number of samples. The solution was prepared one day before in vitro analysis and then placed in a shaker water bath at a temperature of 39 °C which was supplied with CO_2 gas to maintain anaerobic conditions. After measurement, the pH of this solution was close to neutral. Rumen fluid was collected at a slaughterhouse in the morning and brought to the laboratory for in vitro tests. Rumen fluid was collected from three goats, squeezed using gauze, and placed in a thermos in a water bath at a temperature of 39 °C which was supplied with CO_2 gas during the rumen mixing process.

In vitro feed evaluation was conducted using Tilley and Terry's method (1963). A sample of 2.5 g was placed into a 250 ml Erlenmeyer flask. A total of 200 ml of McDougall buffer solution and 50 ml of rumen liquid were added to each Erlenmeyer flask while CO_2 gas was flowing. The flasks were sealed with lids that had an opening for a balloon and a pipe for gas release. The flasks were then placed in a water bath at a temperature of 39 °C and incubated for 48 hours. Methane gas production was measured using the method described by Fievez et al. (2005). After incubation, the flasks were removed and either placed in a refrigerator or basin with ice cubes to stop microbial activity, and the pH was measured immediately.

The next step involved centrifuging for 30 min at 1.200 rpm to separate the supernatant from the residue, using filter paper to retain the residue. The residue on the filter paper was then placed in an oven at 60 °C to dry, while the supernatant was stored in a refrigerator. Once the residue was dry, the filter paper with the residue was weighed, and the residue was mashed using a porcelain mortar and pestle for chemical composition analysis. The parameters measured after in vitro incubation included the digestibility of dry matter, organic matter, and crude protein; the digestibility of fiber fractions (ADF, NDF, cellulose, hemicellulose); rumen fluid characteristics (pH, NH_3, and VFA); and total gas and methane gas production.

Data analysis

The data were analyzed using analysis of variance with the Statistical Package for the Social Sciences software (IBM SPSS Statistics, USA) version 21.0. A post-hoc test namely Duncan multiple range test was employed to statistically distinguish among various treatments (Steel and Torrie 1980).

Result and discussion

Tannin content

The tannin content for each treatment is presented in Table 4. This study revealed that the combination of lime water concentration and soaking time significantly ($P < 0.05$) reduced the tannin content of *S. alba*. Among the 12 treatment combinations tested, A3B2 (5% lime water soaked for 20 min) resulted in the greatest reduction in tannin content, with a decrease of 37.76%. These results demonstrate the significant potential of the lime water soaking method in optimizing mangrove fruit as a source of ruminant feed by reducing its anti-nutrient content.

Table 4. The tannin content of treatments (%).

Factor A (concentrations)	Factor B (minutes)			Average
	B_1	B_2	B_3	
A_1	$24.97^{e} \pm 0.42$	$24.90^{e} \pm 0.31$	$22.80^{d} \pm 0.89$	24.22^{A}
A_2	$23.67^{de} \pm 0.77$	$22.22^{bcd} \pm 2.12$	$21.44^{abc} \pm 0.16$	22.44^{B}
A_3	$22.49^{cd} \pm 0.37$	$20.34^{a} \pm 0.16$	$20.89^{ab} \pm 0.79$	21.24^{C}
A_4	$21.62^{abcd} \pm 0.22$	$20.67^{a} \pm 0.14$	$20.87^{ab} \pm 0.62$	21.05^{C}
Average	23.19^{A}	22.03^{B}	21.50^{B}	SE : 0.46

A_1(1% Lime water); A_2(3% Lime water); A_3(5% limewater); A_4(7% limewater); B_1(10 minutes soaking); B_2(20 minutes soaking); B_3 (30 minutes soaking); SE: standard error.

The significant decrease in tannin content in the A3B2 treatment can be explained by the chemical interaction between tannins and calcium ions in lime water. Calcium ions react with tannins to form complex compounds that are less soluble and can be more easily removed during the soaking process (Slabbert 1992). According to the study by Ojo (2022), Calcium hydroxide proved to be successful in tannin reduction regardless of the type of ruminant feed used. It worked by forming complexes that would make them insoluble. Contrastingly, we did not have a significant ($P > 0.5$) decrease in tannins at the 1% lime water concentration, particularly in 10 and 20 minutes of soaking (A1B1 and A1B2). This implies that following reducing tannin levels, the lower concentration of lime water is less reactive in supplementing the number of calcium ions doubtlessly since there are no Ca ions left in the solution. Furthermore, seems to show that there must be a minimum amount of lime water with which the success of the tannin reduction can be guaranteed. Sari et al. (2022) concluded that ≥5% lime water concentration should procure efficient tannin reduction during plant-based feed ingredients processing.

The interaction between lime water concentration and soaking time shows that longer soaking times do not always result in greater tannin reduction, especially at higher lime water concentrations. This phenomenon can be interpreted as follows: above a certain optimal point, extending the soaking time no longer has a significant impact on tannin reduction. This is in line with the findings of Vijayakumari et al. (1998), which show that there is a saturation point in the effectiveness of soaking to reduce tannins. This study succeeded in showing that a 5% lime water solution applied for 20 minutes effectively reduced the tannin content in *S. alba* mangrove fruit. Tannins act as anti-nutritional components in some feeds, binding to protein and fiber, thereby hindering digestibility and reducing nutrient accessibility for rumen microbes (Min et al. 2003; Patra and Saxena 2011; Ardani et al. 2024). Applying lime water (calcium hydroxide) at optimal doses, as observed in treatment A3, can reduce the inhibitory effects of tannins by either neutralizing or precipitating them. This, in turn, enhances microbial access to fiber and protein, facilitating more efficient fermentation processes, as reflected by the increased VFA production (VFA table).

Nutrient digestibility

This study revealed a significant effect of lime water dosage on the digestibility of both dry matter (Table 5) and organic matter (Table 6) within *S. alba* mangrove fruits. Interestingly, no statistically significant interaction ($P > 0.05$) was observed between the lime water dosage and soaking time. These findings emphasize the crucial role of optimizing lime water dosage in enhancing feedstuff digestibility. Notably, 5% lime water solution (A3) yielded the highest digestibility values, with 55.64% for dry matter and 57.45% for organic matter. In contrast, the highest dose tested (7% concentrations or A4) resulted in the lowest digestibility of 53.42% for dry matter and 55.44% for organic matter, which interestingly was not significantly different from other doses such as A2 (3% concentrations) and A1 (1% concentrations).

This finding aligns with research conducted by Fernandez et al. (2019), who identified an optimal concentration of a tannin-reducing agent that maximizes digestibility without compromising the feedstuff's integrity or other

Table 5. Dry matter digestibility of the treatments (%).

Factor A (concentrations)	Factor B (minutes)			Average
	B_1	B_2	B_3	
A_1	53.48 ± 0.33	54.15 ± 0.19	53.32 ± 0.17	53.65^A
A_2	54.23 ± 1.70	54.40 ± 0.93	54.37 ± 2.72	54.33^A
A_3	54.49 ± 1.72	56.29 ± 0.21	56.14 ± 0.61	55.64^B
A_4	53.63 ± 0.50	54.46 ± 2.01	52.17 ± 0.73	53.42^A
Average	53.95	54.82	54.00	SE: 0.37

A_1(1% Lime water); A_2(3% Lime water); A_3(5% limewater); A_4(7% limewater); B_1(10 minutes soaking); B_2(20 minutes soaking); B_3 (30 minutes soaking); SE: standard error.

Table 6. Organic matter digestibility of the treatments (%).

Factor A (concentrations)	Factor B (minutes)			Average
	B_1	B_2	B_3	
A_1	56.33 ± 0.04	55.69 ± 0.67	56.06 ± 1.19	56.03^A
A_2	55.97 ± 0.97	56.14 ± 0.25	56.63 ± 2.46	56.25^A
A_3	56.13 ± 1.37	57.52 ± 0.14	58.69 ± 0.30	57.45^B
A_4	55.71 ± 0.02	56.87 ± 1.51	53.74 ± 1.08	55.44^A
Average	56.04	56.55	53.74	SE: 0.32

A_1(1% Lime water); A_2(3% Lime water); A_3(5% limewater); A_4(7% limewater); B_1(10 minutes soaking); B_2(20 minutes soaking); B_3 (30 minutes soaking); SE: standard error.

nutritional qualities. Lime water appears to work by reducing tannin contents, thus increasing nutrient availability for microbial digestion in vitro (Min et al. 2003). Nevertheless, more than the optimum concentration like 7% and above may not promote digestibility; instead, it could be dangerous due to over-alkalization that might harm digestive microorganisms or destroy essential nutrients. This finding stresses the importance of choosing correct dose over soaking time when trying to maximize the digestibility of dry matter and organic matter. Such a finding is consistent with earlier research which pointed out that dosage is more important than duration during tannin detoxification process (Carreño et al. 2015). It has been found that using lime water concentration of 5% improves the digestibility of dry matter and organic matter without significant changes at higher or lower concentrations. This implies that one can't expect much change beyond this point if one decides to use different levels of addition. For this reason among others, it would be wise for those involved with the animal feed processing industry especially where chemical interventions are necessary, should take into account these facts because they will help them come up with strategies that can improve digestibility but at the same time keep or even increase the nutritional value of feedstuff as well.

Table 7 shows that lime water concentration significantly affected the crude protein digestibility of *S. alba* mangrove fruits; while immersion period does not have any effect. This effect was prominent at a 5% lime water dose, which resulted in the highest crude protein digestibility at 55.36%. This dose optimally improved digestibility, likely due to limestone's ability to reduce tannin content while maintaining or even increasing the availability of nutrients such as protein for microbial digestion. On the other hand, the lowest crude protein digestibility was observed at the 7% dose. However, this result was not significantly different ($P > 0.05$) from the lower doses of 1% and 3%, indicating that increasing the dose of limestone water above the optimal threshold (5%) did not significantly increase protein digestibility.

Table 7. Crude protein digestibility of the treatments (%).

Factor A (concentrations)	Factor B (minutes)			Average
	B_1	B_2	B_3	
A_1	50.68 ± 0.59	52.10 ± 1.36	52.69 ± 0.87	51.82^A
A_2	52.04 ± 0.39	56.14 ± 0.25	53.98 ± 0.63	52.95^A
A_3	53.66 ± 1.05	57.52 ± 0.14	55.59 ± 0.28	55.36^B
A_4	53.40 ± 0.74	51.52 ± 1.13	50.15 ± 0.56	51.69^A
Average	52.44	53.32	50.15	SE: 0.64

A_1(1% Lime water); A_2(3% Lime water); A_3(5% limewater); A_4(7% limewater); B_1(10 minutes soaking); B_2(20 minutes soaking); B_3 (30 minutes soaking); SE: standard error.

These findings align with research by Chang et al. (1998), which indicated that there is an optimal limit for the use of chemical agents like lime water to improve nutrient digestibility, beyond which its positive effects stabilize or even decrease. This phenomenon could be due to the interaction mechanism between lime water and feed components, where higher concentrations might disrupt protein structures or alter the pH to a level that inhibits digestive microbial activity (Zhang et al. 2014). Tannins in mangrove fruits can form complexes with proteins, making them unavailable for degradation by rumen microbes, which reduces crude protein digestibility (Bhat et al. 2004; Ardani et al. 2024). The process of soaking mangrove fruits in lime water (calcium hydroxide) can neutralize tannins, thereby diminishing their anti-nutritional properties. By breaking the bond between tannin and protein, this method increases the crude protein digestibility by making these proteins easier to be fermented by microorganisms. Lime water dosage is important for increasing crude protein digestibility but soaking time does not matter much since it only indicates that one should use right amount instead of longer hours. Therefore processing methods are recommended to adjust lime water concentration to achieve desired nutrient bioavailability improvement. This finding supports what was discovered by Ikhlas et al. (2023) who found out that among other factors affecting feed quality, lime water optimization remains key.

In their study, Oliveira et al. (2023) have shown that feeding tannin-rich feed after processing can increase the digestion of crude protein by counteracting its negative effect on proteins. This method enabled better microbial fermentation which was indicated by higher levels of NH_3 and this implies that there could be more nitrogen available for rumen bacteria. When crude protein digestibility is improved through soaking mangrove fruits it leads to increased efficiency in using feeds as well as reducing the harm of tannin-rich diets on the environment. A significant benefit of managing tannins effectively in ruminant diets is lower nitrogen excretion into the environment due to enhanced digestibility with nitrogen retention(Jayanegara and Palupi 2010; Ebert et al. 2017; Junior et al. 2022).

Digestibility of fiber fractions

The examination of the digestibility of neutral detergent fiber (NDF) showed that the concentration of lime water largely impacted ($P < 0.05$) NDF disintegration. In other words, chemical modification using lime water treatment directly affects the decomposition process of crude fibers, which can lead to better availability for microorganisms in the rumen. It was observed from Table 8 that treatment A3 having 5% concentration and treatment A2 with a 3% concentration recorded higher rates of NDF digestion compared to those at lower or higher levels.

Table 8. NDF digestibility (%).

Factor A (concentrations)	Factor B (minutes)			Average
	B_1	B_2	B_3	
A_1	51.41 ± 0.10	51.58 ± 0.35	51.40 ± 1.29	51.46^A
A_2	52.00 ± 0.89	52.75 ± 1.71	53.52 ± 1.11	52.75^B
A_3	53.34 ± 0.25	53.90 ± 1.86	53.11 ± 1.49	53.45^B
A_4	51.08 ± 0.75	51.65 ± 0.54	50.95 ± 1.72	51.23^A
Average	51.96	52.47	50.95	SE: 0.37

A_1(1% Lime water); A_2(3% Lime water); A_3(5% limewater); A_4(7% limewater); B_1(10 minutes soaking); B_2(20 minutes soaking); B_3 (30 minutes soaking); SE: standard error; NDF : neutral detergent fiber.

This suggests that an optimal dose of lime water facilitates NDF degradation by rumen microbes, possibly by reducing tannins or other anti-nutrient components that inhibit fiber digestion. In ruminants, the function of lime water dosage in improving Neutral Detergent Fiber (NDF) digestibility shows that you can change feed chemistry to increase the use of fibers. This will improve energy intake through better NDF digestion which supports rumen health since crude fiber is necessary for stimulating fermentation within the rumen where volatile fatty acids (VFAs) are produced as major energy sources for ruminants (Pazla et al. 2023; Jamarun et al. 2023).

The digestibility of Acid Detergent Fiber (ADF) was influenced significantly at different levels by dosage with lime water ($P < 0.05$), similar to Neutral Detergent Fiber (NDF). It means that among all treatments used in this study, treatment A3 which represented a 5% solution had the highest percentage of ADF digestibility thereby indicating its potentiality in breaking lignin and other indigestible components within fibers. Therefore, reducing such types of feeds can improve their quality because when not digested they serve no purpose except acting as a barrier for nutrient absorption by animals leading to inefficient utilization, especially among ruminants. Background The effect of lime water on ADF digestion also proves that feed modification should be done carefully so as not only to break down more fiber but also enhance its utilization without disturbing the ecology of ruminal microorganisms involved in the cellulose breakdown process (Agustin et al. 2024).

Table 9. ADF digestibility (%).

Factor A (concentrations)	Factor B (minutes)			Average
	B_1	B_2	B_3	
A_1	52.98 ± 0.29	53.14 ± 0.07	53.01 ± 0.38	53.04^{AB}
A_2	53.70 ± 1.10	52.88 ± 0.61	54.10 ± 1.47	53.56^{BC}
A_3	54.14 ± 0.61	54.53 ± 0.40	54.30 ± 2.00	54.32^C
A_4	52.69 ± 0.76	51.98 ± 1.38	52.35 ± 1.10	52.34^A
Average	53.38	53.13	52.35	SE: 0.29

A_1(1% Lime water); A_2(3% Lime water); A_3(5% limewater); A_4(7% limewater); B_1(10 minutes soaking); B_2(20 minutes soaking); B_3 (30 minutes soaking); SE: standard error, ADF : acid detergent fiber.

An analysis of the effect of lime water dosage (factor A) on cellulose digestibility showed that factor A had a significant ($P < 0.05$) impact, while soaking time (factor B) had no significant effect (Table 10). Notably, the treatment with 5% lime water (A3) achieved the highest cellulose digestibility at 56.12%, indicating that this dosage was more effective in enhancing cellulose degradation compared to other doses. Treatments A1 (1% lime water), A2 (3% lime water), and A4 (7% lime water) showed no significant ($P > 0.05$) difference in cellulose digestibility, with A4 recording the lowest value at 54.42%.

Table 10. Cellulose digestibility (%).

Factor A (concentrations)	Factor B (minutes)			Average
	B_1	B_2	B_3	
A_1	55.47 ± 0.74	54.72 ± 0.02	54.71 ± 0.47	54.97^A
A_2	54.27 ± 0.32	54.96 ± 1.20	54.91 ± 0.52	54.71^A
A_3	55.34 ± 0.75	56.17 ± 0.93	56.84 ± 1.00	56.12^B
A_4	54.68 ± 1.015	54.44 ± 0.69	53.83 ± 1.09	54.32^A
Average	54.94	55.07	53.83	SE: 0.22

A_1(1% Lime water); A_2(3% Lime water); A_3(5% limewater); A_4(7% limewater); B_1(10 minutes soaking); B_2(20 minutes soaking); B_3(30 minutes soaking); SE: standard error.

This could be because a 5% lime water dose has a positive effect on cellulose digestibility by reducing the levels of tannin and other feed-related anti-nutritional substances. Tannins can bond with cellulose polymers thereby decreasing its breakdown by rumen microbial enzymes (Besharati et al. 2022; Pazla et al. 2024a). Therefore, the treatment of lime water can make it easier for microbial enzymes to access cellulose leading to increased digestion of this compound. The fact that the digestibility of celluloses increases as lime water doses are raised to 5% shows how efficient this technique can be in terms of improving feed conversion rates particularly when dealing with diets rich in fibers. Cellulose accounts for most crude fibers found in ruminant feeds which are essential in supplying energy for microbial fermentation into short-chain fatty acids within the rumen (Pazla et al. 2024b). Improved cellulose digestibility can lead to increased energy intake for ruminants and greater production potential. However, the absence of significant differences among other lime water doses and the lowest value at A4 (7% lime water) suggests there might be an upper limit to the effectiveness of lime water treatment in improving cellulose digestibility. Excessive dosages may not only be ineffective but could also disrupt the rumen microbial balance, potentially impacting cellulose digestibility (Agustin et al. 2024b).

Hemicellulose digestibility showed no significant interaction between factors, but Factor A had a significant ($P < 0.05$) effect, while Factor B had no significant ($P > 0.05$) impact. The treatment with a 5% lime water dose (A3) achieved the highest hemicellulose digestibility at 57.73%, significantly different from treatments A1, A2, and A4. Meanwhile, treatments A1, A2, and A4 were not significantly different from each other, although A4 had the lowest value at 55.30% (Table 11).

Table 11. Hemicellulose digestibility (%).

Factor A (concentrations)	Factor B (minutes)			Average
	B_1	B_2	B_3	
A_1	56.07 ± 0.75	56.49 ± 1.12	56.39 ± 0.61	56.32^A
A_2	55.81 ± 1.22	55.82 ± 0.78	55.11 ± 0.47	55.58^A
A_3	57.05 ± 1.00	57.85 ± 1.61	58.28 ± 0.97	57.73^B
A_4	55.58 ± 1.88	55.00 ± 0.82	55.33 ± 0.05	55.30^A
Average	56.13	56.29	55.33	SE: 0.35

A_1(1% Lime water); A_2(3% Lime water); A_3(5% limewater); A_4(7% limewater); B_1(10 minutes soaking); B_2(20 minutes soaking); B_3(30 minutes soaking); SE: standard error.

The higher digestibility of hemicellulose at a 5% lime water dose compared to other doses suggests that an optimal level of lime water enhances hemicellulose degradation by rumen microbes. Hemicellulose, a key component of plant cell walls, requires specific conditions for its degradation, including appropriate pH and the availability of microbial enzymes. The interaction of mangrove fruits with lime water can change their physicochemical conditions, reduce the inhibiting power of tannins, and increase hemicellulose digestibility by rumen microorganisms (Jayanegara et al. 2012). Proteins may bind with tannin leading to the disruption of plant cell walls and reducing the digestibility of hemicellulose. According to Min et al. (2003), microbial breakdowns for hemicellulosic materials are increased when tannin concentration is lowered through soaking in lime solution hence making it easier to digest such compounds. This means that if we decrease tannin levels by treating them with lime water then this will improve their ability to be digested by microorganisms which supports the idea that hemicelluloses could be more easily broken down once these substances have undergone treatment with lime irrigation systems. In ruminants, where efficient utilization of nutrients is necessary for health maintenance, besides providing energy itself; as an energy source hemicellulose also promotes growth among microorganisms living within the rumen thus improving its utilization represents one way to maximize feed efficiency (Herrick et al. 2012; Jamarun et al. 2017b).

Rumen fermentation characteristics

As shown by this study, the amount of ammonia (NH_3) in the rumen fluid is significantly affected by the combination of lime water dosage and soaking duration. Figuring among them was that treatment A3B2 (5% lime water soaked for 20 minutes) which had produced the highest level ever recorded at 4.55 mg/100 ml while treatment A1B1 (1% lime water soaked for 10 minutes) showed the lowest levels ever observed which were only 3.36 mg/100 ml as seen in Table 12. From this interaction, we can tell that some particular combinations of lime water dosages and soaking times create the best conditions in the rumen for microbial activity where NH_3 serves as a good indicator of protein fermentation.

Table 12. NH_3 concentration (mg/100 ml).

Factor A (concentrations)	Factor B (minutes)			Average
	B_1	B_2	B_3	
A1	3.36^{ab} ± 0.54	3.83^{bcd} ± 0.36	4.21^{cd} ± 1.18	3.80^C
A2	4.29^d ± 0.06	4.25^d ± 0.02	3.57^{ab} ± 0.24	4.04^{BC}
A3	3.53^{ab} ± 0.06	4.55^d ± 0.42	4.51^d ± 0.36	4.19^B
A4	3.61^{ab} ± 0.30	3.66^{abc} ± 0.12	3.27^a ± 0.06	4.89^A
Average	3.70^{AB}	4.07^A	3.27^B	SE: 0.16

A_1(1% Lime water); A_2(3% Lime water); A_3(5% limewater); A_4(7% limewater); B_1(10 minutes soaking); B_2(20 minutes soaking); B_3(30 minutes soaking); SE: standard error.

The availability of nitrogen to rumen microorganisms greatly affects fermentation efficiency and synthesis of microbial proteins hence Ammonia (NH_3) concentration in the ruminal fluid is considered a critical marker for its measurement (Putri et al. 2021; Elihasridas et al. 2023; Marlida et al. 2023; Zain et al. 2023). In other words, new microbial proteins needed during the fermentation process are produced from NH3 by these bacteria as they utilize them being waste products from breakdowns of dietary matter containing various kinds of organic substances including proteins. Thus, proper levels should be maintained because such events may help farmers improve livestock health as well as increase productivity among animals reared under their care (Ardani et al. 2023). According to Getachew et al. (2008), it has been observed that protein degradation in the rumen can be improved by reducing tannin content through treatment with lime water at 5% concentration for 20 minutes where in general tannins bind to proteins thus hindering their breakdown by rumen bugs. This implies that when we lower the levels of tannin there will be more proteins available for microbial fermentation which results in high production of NH3 (ammonia) (Chuzaemi et al. 2022). Such a procedure may increase nutrient availability and enhance protein utilization efficiency in feeds. Nevertheless, the NH_3 level does not always rise with increasing amounts or duration of soaking in limewater because some treatments recorded decreased concentrations of NH3 even though they were done at various dosages. Hence, one should strike a balance between these two points; reducing tannicity and maintaining good conditions for bacteria action within the rumen so that there can be effective synthesis of proteins by them (Pazla et al. 2024b). The research findings also show that ammonia production during feed processing greatly affects the dynamics of ruminal fermentation through control oversoaking times as well as lime water dosages used.

An analysis of rumen pH values showed no significant interaction between the lime water dosage (factor A) and the length of soaking time (factor B). Furthermore, neither factor A nor factor B had a significant ($P > 0.05$) effect on rumen pH, which ranged from 6.88 to 7.40 (Table 13).

Table 13. Rumen pH.

Factor A (concentrations)	Factor B (minutes)			Average
	B_1	B_2	B_3	
A_1	6.89 ± 0.01	6.90 ± 0.02	6.90 ± 0.02	6.90
A_2	6.90 ± 0.01	6.90 ± 0.01	6.88 ± 0.01	6.89
A_3	6.89 ± 0.01	6.88 ± 0.01	6.90 ± 0.02	6.89
A_4	6.90 ± 0.01	6.90 ± 0.02	7.40 ± 0.01	7.07
Average	6.89	6.89	6.90	SE: 0.0025

A_1(1% Lime water); A_2(3% Lime water); A_3(5% limewater); A_4(7% limewater); B_1(10 minutes soaking); B_2(20 minutes soaking); B_3(30 minutes soaking); SE: standard error.

These pH values are within the optimal range for rumen microbial activity and fermentation processes (Pazla et al. 2023; Zain et al. 2023). The highest pH value recorded was 7.40 in treatment A4B3, which used 7% lime water and had a soaking time of 30 minutes. Furthermore, the greatest pH value was exhibited, and yet the pH value variation through the study was still regarded as optimal for better rumen health. Although rumen pH did not change with lime water use, showing the need to balance acid and base in the rumen to ensure optimum growth of microbes and to support an efficient normal fermentation (Jamarun et al. 2017a) A suitable pH level acts as an important factor that ensures the enzymatic activities of microorganisms take place without any obstacle, as this allows the digestion of microbial fiber, the production of microbial proteins, and the generation of volatile fatty acids (VFAs), which is the main energy source for ruminants (Li et al. 2021; Ardani et al. 2023). The pH remains the same, whether it is lime water or the duration of soaking because the lime water treatment process does not interrupt the rumen's acid-base equilibrium. We can say that it is the result of rumen protective mechanisms, which enable microorganisms to decrease the effect of new feed types or chemical treatments on their activity. They do that by maintaining favorable conditions for microbial activity (Calsamiglia et al. 2008; Golder et al. 2012). This agreement results with the research carried out by Fernando et al. (2010), which states that the rumen's ability to adapt to any kind of feed or chemical modification leads to achieved rumen function. This shows that the lime water treatment, in the range of dosage and soaking period that has been tested, is not governed by rumen pH adversely. The stability of pH is one crucial aspect that governs the sustainability of rumen health and fermentation efficiency (Ardani et al. 2023). Therefore, while modifying the feed, the researchers should not forget that the ideal ruminal condition has to be preserved.

Analysis of the impacts of lime water on volatile fatty acid (VFA) concentrations in rumen fluid has resulted in critical insights into feed management and the health of the rumen. The findings gave no significant interaction between the lime water dosage and the soaking time on VFA concentration. Nonetheless, the lime water dosage was found to have a significant ($P < 0.05$) impact on VFA concentrations (Table 14). The treatment with a 5% lime water dosage (A3) gave the highest VFA concentration of 126.67 mM, which indicated that this dosage seemed to promote rumen fermentation. Yet, there was not a significant difference compared to the 3% lime water treatment (A2) with the result of 122.67 mM. The lowest dose (1% lime water, A1) was the one that had the lowest VFA concentration equal to 116.50 mM, while the A4 had 118.33 mM. The differences between A1 and A4 were significant.

Table 14. VFA (mM).

Factor A (concentrations)	Factor B (minutes)			Average
	B_1	B_2	B_3	
A_1	117.50 ± 3.53	117.00 ± 0.31	115.00 ± 0.31	116.50^C
A_2	120.00 ± 4.24	123.50 ± 3.24	124.50 ± 2.12	122.67^A
A_3	123.50 ± 4.95	127.50 ± 3.35	129.00 ± 1.41	126.67^A
A_4	119.00 ± 1.14	119.50 ± 0.71	116.50 ± 4.95	118.33^B
Average	120.00	121.88	116.50	SE: 0.99

A_1(1% Lime water); A_2(3% Lime water); A_3(5% limewater); A_4(7% limewater); B_1(10 minutes soaking); B_2(20 minutes soaking); B_3(30 minutes soaking); SE: standard error.

The significant impact of lime water dosage on VFA concentration is established through the understanding that tannins have an inhibition on the action of rumen microbes. Astringent tannins can combine with proteins and hinder microbial activity, affecting rumen fermentation (He et al. 2020). The tannin reduction through lime water treatment, especially at the lime water concentration of 5% and 3% might result in higher rumen microbial activity, as indicated by increased VFA concentration. VFAs (Volatile Fatty Acids) are the main product of the anaerobic fermentation process in the rumen, being an abundant source of energy for the ruminants and playing a fundamental role in rumen metabolism and health (Vargas et al. 2020; Pazla et al. 2021). On the other hand, the 7% increase in lime water didn't cause a rise in VFA levels but might have a detrimental effect on the microbiota population or disrupt fermentation by undergoing chemical or physical changes in the feed. This result is supported by a study by Pazla et al. (2024a), showing that the chemical alteration of feeds has to be balanced for optimal rumen fermentation. Adding to that, the consistency in soaking duration suggests that the length of the feed that was being exposed to lime water is not the one determining the VFA production rather it is the lime water dosage (Elihasridas et al. 2023). VFAs, which are the main products of microbial fermentation in the rumen, both supply energy to the ruminants and perform pH control in the rumen (Montesqrit et al. 2024). The observed rise in VFA concentration with 5% of limewater treatment (A3) implies complete fibrous fermentation that synchronizes well with better digestibility of NDF, ADF, cellulose, and hemicellulose. VFA readings in higher concentrations suggest improved fermentation efficiency and more nutrients available for ruminants.

Total gas and methane production

A statistical analysis of treatment with lime water defined factor A level combined with factor B intensity level shows that both factors A and B have a significant ($P < 0.05$) impact on total gas and methane yields (Tables 15, 16). The treatment featuring a lime concentration of 7% in a solution and soaking for 30 minutes (A4B3) showed the maximum gas and methane yields (Tables 15, 16, respectively). There was a shift in the activities of microbes as they were able to ferment the organic matter due to the extensive degradation of the same (Elihasridas et al. 2024). Consequently, this particular set of fermentation conditions will typically favour this lime water addition level with an increase in soaking time, and this thereby supports the theory that a combination of a higher lime water concentration and longer soaking time can maximize gas production (Shah and Tabassum 2018).

Table 15. Total gas production (ml/g DOM).

Factor A (concentrations)	Factor B (minutes)			Average
	B_1	B_2	B_3	
A_1	$67.92^a \pm 0.65$	$72.61^c \pm 0.65$	$71.35^c \pm 0.49$	70.63^A
A_2	$69.91^b \pm 0.53$	$72.80^d \pm 0.54$	$71.40^c \pm 0.55$	71.37^A
A_3	$76.69^e \pm 0.74$	$77.10^f \pm 0.75$	$79.27^g \pm 0.46$	77.68^B
A_4	$81.17^h \pm 0.53$	$81.80^i \pm 0.63$	$89.00^j \pm 0.55$	83.99^C
Average	73.92^A	76.08^B	77.75^C	SE: 1.46

A_1(1% Lime water); A_2(3% Lime water); A_3(5% limewater); A_4(7% limewater); B_1(10 minutes soaking); B_2(20 minutes soaking); B_3(30 minutes soaking); SE: standard error.

The studies conducted on anaerobic fermentation, particularly with different concentrations of lime water and soaking times, showed a lot of positive effects. Digestibility and production of volatile fatty acids increased substantially with a 5% lime water concentration. This probably means that the higher the lime-treated water concentration the better substrate availability which again helps in enhanced degradation by microbes and eventually leads to more production of VFAs. A high VFA production shows the proper fermentation of the substrate, as this vital precursor compound of methane synthesis (Antonius et al. 2024; Pazla et al. 2024b).

Furthermore, the tannins in legumes may have an effect that inhibits methane production. Treatment with a maximum level of tannin (A1B1) generated a minimal amount of methane which signifies that tannins have the power to cut back the methane-generating microbes. The role of tannins is hypothesized to be manifested through the tannins ability to block enzymes and viruses that normally produce and release methane (Jayanegara et al. 2015; Hatew et al. 2016).

Table 16. Methane Gas Production (ml/g DOM).

Factor A (concentrations)	Factor B (minutes)			Average
	B_1	B_2	B_3	
A_1	$12.35^a \pm 0.24$	$12.49^b \pm 0.24$	$13.96^c \pm 0.49$	12.93^A
A_2	$13.21^c \pm 0.23$	$16.26^d \pm 0.32$	$17.66^e \pm 0.42$	15.71^B
A_3	$19.36^f \pm 0.25$	$19.65^f \pm 0.31$	$20.74^g \pm 0.51$	19.92^C
A_4	$24.19^h \pm 0.24$	$25.99^i \pm 0.41$	$29.13^j \pm 0.42$	26.44^D
Average	17.28^A	18.60^B	20.37^C	SE: 1.27

A_1(1% Lime water); A_2(3% Lime water); A_3(5% limewater); A_4(7% limewater); B_1(10 minutes soaking); B_2(20 minutes soaking); B_3(30 minutes soaking); SE: standard error.

Conclusion

Soaking mangrove fruit in lime juice can drastically reduce the tannin content. This is important because it greatly increases the digestibility of nutrients and the efficiency of protein utilization in the rumen. The best treatment turned out to be 5% lime water and 20 minutes of soaking time which resulted in an effective balance between tannin reduction and increased feed digestibility. Therefore, this research directly supports ruminant nutrition strategies to improve feed quality. Results like this indicate the need to select the right dose to achieve the desired effect.

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