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Impact of Mangrove Leaves (*Rhizophora apiculata*) in Limewater on Nutrients Digestibility, Rumen Ecosystem and *in vitro* Methane Production

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Abstract: The Mangrove is one of the plants that live in coastal areas, because a lot of their availability, mangrove leaves can be used as animal feed. The problem with this mangrove is contained a fairly high tannin which can be harmful to livestock. This study aims to determine the impact of boiled mangrove leaves (*Rhizophora apiculata*) with lime water on tannins, digestibility, rumen characteristics, and *in vitro* methane production. The design used in this study was a randomized block design using 4 treatments and 5 replications. The treatments were boiled with the respective concentrations of quicklime water as follows, P1 (without boiling), P2 (5% lime water), P3 (10% lime water), and P4 (15% lime water) for 10 min. The results showed that boiling mangrove leaves with 5% lime water showed highly significant ($p<0.01$) results on total tannins content, nutrient digestibility, rumen fluid characteristics, and reduced methane gas content. Nutrient digestibility includes among others: Dry matter digestibility (65.18%), organic matter digestibility (66.31%), crude protein digestibility (67.90%), and increased digestibility of fiber fractions including NDF digestibility (59.01%), ADF digestibility (55.75%), cellulose digestibility (64.02%), hemicellulose digestibility (69.61%) and increased rumen fluid characteristics such as NH_3 contents (4.32 Mg/100 mL) and VFA content (130.2 mm) and Methane gas (231.39 mL/gDM). The results of this study concluded that boiling mangrove leaves in lime water at a concentration of 5% for 10 min contributed to the most prominent total tannins (4.54%) and digestibility of nutrients.

Keywords: Boiled, Cellulose, Forages, Fiber Fraction, Tannins

Introduction

Utilization of Forage mangrove leaves as feed ingredients are one of the efforts to find alternative feed sources that are easy to obtain and do not compete with human needs (Jamarun *et al*., 2020). Mangrove plants (*Rhizophora apiculata*) belong to the *Rhizophoraceae* family. Based on the report by Mile *et al.* (2021) the nutritional content of mangrove leaves as follows: Water content 52.38%, ash content 0.22%, crude protein 6.85%, carbohydrates 30.30%, and crude fat 2.3%, it can be used as ruminant animal feed.

The utilization of mangrove leaves as forage has problems in its utilization because it contains quite high tannins. According to Jamarun *et al*. (2021) mangrove leaves contain 11% tannin. Tannins that are too high will harm digestibility by reducing the digestibility of food substances. Stated that increased tannin contents in the ration could contribute to microbial degradation of carbohydrates and proteins and thus reduce enzymatic activity, as a result, the degradation or digestibility of rumen dry matter *in vitro* also decreases so the availability of carbohydrates and protein for microbes is reduced. Therefore, efforts need to be made to reduce tannin levels, so that mangrove leaves can be consumed in large quantities by livestock (Yanti *et al*., 2021a).

Tannin compounds can be removed by alkaline treatment, for example by adding NH_4OH, NaOH, K_2CO_3, or quicklime (CAO) (Jiménez-Martínez *et al*., 2003. Suhirman *et al*. (2015) showed that soaking the treatment of immersing nutmeg juice in 2% lime solution can reduce the tannin content to 4 721.22 mg per 100 g of material. According to Jamarun *et al*. (2021) boiling mangrove leaves (*Avicennia marina*) with husk ash for 10 min, with a concentration of 15% can reduce tannins by 10.27% and increase the digestibility of dry matter by 72.06%, digestibility of organic matter by 73.36%, VFA by 117 mm and NH_3 by 4.57 mg/100 mL.

The use of CAO to reduce tannin levels is based on the binding of tannin compounds by Ca^{2+} ions to form tannic salts. Ca^{2+} ions can also increase the activity of trypsin and chymotrypsin enzymes that function in protein digestion, besides that these ions also provide CA minerals in the ration. The objective of this study was to find the best dose of lime to reduce the tannin in mangrove (*Rhizophora apiculate*) leaves.

Materials and Methods

Experimental Site

This research was conducted at the ruminant nutrition laboratory and the biotechnology laboratory of the faculty of animal science andalas university, Padang, West Sumatra, Indonesia. Mangrove leaves were collected from the Mandeh Island area, Koto Xl Tarusan sub-district, pesisir selatan district of West Sumatra.

Research Material

Materials that are used were Mangrove leaves (*Rhizophora apiculata)*, quicklime, equates, rumen fluid from goat, and Mc. Dougall's solution, sulfuric acid (H_2SO_4), sodium hydroxide (NaOH), Neutral Detergent Solution (NDS), Acid Detergent Solution (ADS), acetone, HCl, Na_2CO_3, alcohol.

The equipment used is a set of tools for the analysis of proximate (AOAC, 2005) and fiber fractions (Van Soest *et al*., 1991), Which includes Whatman filter paper No. 41, Erlenmeyer flask, beaker glass, burette, filter funnel crucible. Porcelain cup, volumetric flask, measuring pipette, analytical balance, spectrophotometer type UV-1280 (Shimadzu), Incubator Shaker Innovs 43, DT-PH1100 Benchtop pH Meter, Centrifuge TDL-80-2B and vacuum pomp rocker 300.

Parameters Measured

The parameters evaluated in this study were tannin contents, Digestibility of Dry Matter (DDM), Digestibility of Organic Matter (DOM), digestibility of crude protein, ruminal pH, Volatile Fatty Acids (VFA), NH_3, methane gas production, and a fraction of fiber such as NDF Digestibility (NDFD) ADF Digestibility (ADFD), Cellulose Digestibility (CelD), Hemicellulose Digestibility (HemiD).

Formula:

$$DDM(\%) = \frac{SW \times DMS - (RW \times DMR - blank)}{SW \times DMS} \times 100\%$$

$$DOM(\%) = \frac{SW \times OMS - (RW \times OMR - blank)}{SW \times OMS} \times 100\%$$

$$NDFD(\%) = \frac{SW \times DM \times NDFD - (RW \times DM - \%NDFR)}{SW \times DM \times NDFS} \times 100\%$$

$$ADFD(\%) = \frac{SW \times DM \times ADFD - (RW \times DM \times \%ADFR)}{SW \times DM \times \%CelS} \times 100\%$$

$$CelD(\%) = \frac{SW \times DM \times CelS - (RW \times DM \times \%CelR)}{SW \times DM \times \%CelS} \times 100\%$$

$$HemiD(\%) = \frac{SW \times DM \times HemiS - (RW \times DM \times \%HeimR)}{SW \times DM \times \%HemiS} \times 100\%$$

Information: Sample Weight (SW), Dry Matter Sample (DMS), Residual Weight (RW), Dry Matter Residual (DMR), Organic Matter Sample (OMS), Organic Matter Residual (OMR), NDF Sample (NDFS), NDF Residual (NDFR), ADF Sample (ADFS), ADF Residual (ADFR), Cellulose Sample (CelS) Cellulose Residual (CelR), Hemicellulose Sample (HemiS), Hemicellulose Residual (HemiR).

Research Procedure

Samples of mangrove leaves were taken as much as 10 kg. Mangrove leaves were collected from the top 4-5 leaves. Mangrove leaves are boiled using lime water with concentrations of 5, 10, and 15% for 10 min. After the leaves were boiled, the leaves were dried at 60°C to dry. The leaves were ground until smooth, and analyzed for proximate analysis (AOAC, 2005), fiber fraction (Van Soest *et al*., 1991), and *in vitro* digestibility (Tilley and Terry, 1963).

In vitro

Prepare McDougall liquid as a buffer in *in vitro* operations. The prepared McDougall solution was stored in a shaker water bath at 39°C before use. Each sample was pondered as much as 2.5 g and put in a 250 mL Erlenmeyer flask. The rumen fluid was mixed with McDougall's solution in a ratio of 1: 4. The Erlenmeyer tubes. Which had been filled with the sample were added to the mixture of 250 mL of rumen fluid with McDougall's solution. Erlenmeyer flasks were incubated inside a shaker water bath at 39°C for 48 h. The fermentation process was stopped to soak the Erlenmeyer flask in ice cubes to stop microbial activity. Tubes were centrifuged for 30 min/1200 rpm. The supernatant was used to test the rumen characteristics and the residue was oven-baked for proximate testing appropriate with the method (AOAC, 2005) and fiber fraction method (Van Soest *et al*., 1991). Where later the digestibility is sought by calculation.

Total Tannin Analysis (Hide-Powder Method)

The Mangrove leaves were ground and 0.5 grams of sample were added with 150 mL of distilled water it was then heated for 30 min at 70°C. The cooled extract was filtered with whattman 1 filter paper. The extraction then being measured with UV-visible (Shimadzu) spectrophotometer at 278.5 nm and pure tannins were used as standard.

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Experimental Design

This research used a Randomized Block Design (RBD) with 4 treatments and 5 replications. Mangrove leaves were boiled with quicklime water for the same time of 10 min for each treatment, where the treatments are as follows:

A = Without boiled
B = Boiled mangrove leaves with 5% lime water
C = Boiled mangrove leaves with10% lime water
D = Boiled mangrove leaves with 15% lime water

All data was observed using a General Linear Model (GLM) and next with Duncan's Multiple Range Tests (DMRT) using IBM SPSS Statistics 26.

Results and Discussion

Tannins Contents

Tannins are polyphenolic compounds with high weight and have the ability to bind proteins. Tannins at high levels negatively impact digestibility. Table 1 explains that in this study, the lowest tannin content was found in the 5% lime water treatment and the highest in the control without boiling lime water. In the 5% treatment, the lime water experienced the highest decline in tannin, this was attributed to the loss of tannin compounds by boiling with alkaline compounds. The use of CAO to reduce tannin levels is based on the presence of a tannin compound binder by Ca^{2+} ions to form tannic salts. Explained that tannins compounds can be removed by alkaline treatment, for example by adding NH_4OH, NaOH, K_2CO_3, or quicklime (CAO).

Table 1 shows that in the 15% treatment tannin compounds increased by 6.31 for too much quicklime water used so the phenolic compounds in the form of tannins were bound back with the help of ongoing antioxidant activity so that heating caused enzyme invasion contributing to degrading tannin in the water. ingredients. Muhammad *et al*. (2015) explained that high temperatures and quicklime water cause catechol oxidase enzyme invasion and a slight enzymatic reaction so that the tannin content increases.

Forage containing a certain amount of tannins can improve dry matter and organic matter digestibility. Jayanegara *et al*. (2009) reported that supplementation of *R. thypina* and *S. alba* containing tannin in low-quality forage (hay and straw) was able to increase digestibility and organic matter. Our results revealed that the tannin at 2% proved to be able to increase digestibility and organic matter.

Digestibility of Dry Matter, Digestibility of Organic Matter, and Crude Protein

Dry matter is a component consisting of organic and inorganic materials. The quality of ration can be seen from the value of digestibility of dry matter, digestibility of organic matter, and digestibility of crude protein. The effect of poaching time of Mangrove (*R. apiculate)* leaves with lime water on the digestibility of dry matter, digestibility of organic matter, and digestibility of crude protein is viewable in Table 2. *R. apiculata* quicklime water 5% increased dry matter digestibility by a rate of 65.18%, Organic matter digestibility by a rate of 66.18%, and crude protein digestibility by a rate of 67.90%. This increase was related to the boiling of mangrove leaves in quicklime water which was capable reduce tannins from 8.41 to 4.54.

This indicates that boiling mangrove leaves with quicklime water can increase the digestibility of dry matter, organic matter, and crude protein. Oliveira *et al*. (2010); Jamarun *et al*. (2018) appointed that the digestibility of dry matter was influenced by the nutritional value of feed, processing of feed, and deficiency of nutrients. The average worth of digestibility of organic matter is straight away proportional to the average digestibility of dry matter value. According to Pazla and Adrizal (2021), organic matter digestibility is tightly linked to each other to dry matter, in that some dry matter consists of organic matter. This result was in agreement who reported that organic matter digestibility and dry matter digestibility are interconnected, where dry matter is composed of two chemicals, namely organic matter and inorganic material.

In treatments C and D (10 and 15% lime concentrations), there was decreased digestibility of dry matter, digestibility of organic matter, and digestibility of crude protein. This decrease was attributed to too high a lime dose and high temperature, causing retrogradation of starch which can change the structure of the fiber making it difficult to digest (Sajilata *et al*., 2006). High alkalinity will also Conditions that are too alkaline will also damage the plant structure, thereby reducing digestibility.

Table 2 showed that crude protein digestibility increased in the 5% lime concentration, with a rate of 67.90%. This increase occurs because of boiling in lime water contributed to the inactivation of active phenolic tannins groups that were binding with proteins. Tannins strongly bind with proteins due to their large number of phenolic groups that were bonded with peptide carboxyl groups. The process of boiling can alter the bonds of tannins and proteins and turn them into a form that can be easily digested. The heating process can improve the digestibility of protein because it opens the structures of a protein that are hard to digest. Mueller-Harvey (2006) stated that several phenolic hydroxyl groups in tannin can make a strong complex with proteins and it can also make weak bonds with metal ions, amino acids, and polysaccharides. The optimal dose of lime used will reduce the tannins in the plant, but too high a dose will damage the plant structure and reduce the digestibility value.

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Table 1: The tannin content of each treatment (%)

Parameter	Treatment A	B	C	D
Tannins	$8.41^{c}±0.42$	$4.54^{a}±0.35$	$5.54^{ab}±0.44$	$6.31^{b}±0.28$

A = without boiled, B = Boiled mangrove leaves with 5% lime water, C = Boiled mangrove leaves with 10% lime water and D = Boiled mangrove leaves with 15% lime water

a-c Mean values in the same row with different superscripts show a significantly different at ($p<0.05$)

Table 2: Dry matter, organic matter, and crude protein digestibility to each treatment (%)

Digestibility	Treatment A	B	C	D
Dry matter	$59.99^{c}±0.78$	$65.18^{a}±0.81$	$63.08^{b}±0.85$	$57.59^{d}±0.86$
Organic matter	$60.35^{b}±0.66$	$66.31^{a}±0.65$	$64.03^{c}±0.85$	$58.50^{a}±0.30$
Crude protein	$62.05^{c}±0.67$	$67.90^{a}±1.02$	$63.09^{b}±1.17$	$60.51^{c}±0.53$

A = without boiled (blank), B = Boiled mangrove leaves with 5% lime water, C = Boiled mangrove leaves with 10% lime water, and D = Boiled mangrove leaves with 15% lime water

a-d count worth to the same line with dissimilar superscripts show a significantly dissimilar at ($p<0.05$)

There was a decrease in crude protein digestibility in 10 and 15% lime treatments due to the high dose of lime used which resulted in the formation of cross-links between protein branch chains causing reduced protein digestibility. Joye (2019) explained that high alkalinity at high temperatures for a long time can form cross-links (disulfide bonds) in proteins and decline the digestibility of protein. In addition, a long heating time can also lessen the protein quality as the effect of denaturation.

Table 2, it can be seen that the increase in digestibility of dry matter, digestibility of organic matter, and digestibility of crude protein had the same pattern. This can happen because organic matter, crude protein, and dry matter are interrelated After all, organic matter is a component of dry matter and crude protein is part of organic matter. The high digestibility of dry matter will lead to an increase in organic matter digestibility (Muhtarudin and Liman, 2006; Pazla *et al*., 2021) stated that an increase in the worth of organic matter digestibility has positively correlated with an intensified in the worth dry matter digestibility worth. Also, the digestibility of crude protein has a positive effect on the digestibility of organic matter. Have a positive correlation with organic matter digestibility.

Fiber Fraction Digestibility

The fiber fractions digestibility (ADF, NDF, Cellulose, and Hemicellulose) of Mangrove leaves treated with lime is provided in Table 3. The top digestibility worth of fiber fraction was in handling B (5% lime water) with an NDF digestibility value of 59.01%, ADF digestibility of 55.75%, cellulose digestibility of 64.02%, and Hemicellulose digestibility of 69.61%. The lowest digestibility was in treatment D (15% quicklime water). This shows that boiling mangrove leaves using quicklime water at a dose of 5% increases the digestibility of the mangrove leaf fiber fraction with a decrease in tannins. (Jamarun *et al*., 2021) said that boiling can reduce tannin levels. According to Yanti *et al*. (2021b) boiling with an alkaline solution can reduce the tannins of a plant.

Boiling mangrove leaves in quicklime water could solve the chain's tannin contents with fiber components. Tannins contain a great amount of phenolic hydroxy groups that enable establish vulnerable cross-links with proteins, carbohydrates, polysaccharides, amino acids, fatty acids, and nucleic acids. Calcium contents of lime prevail in alkaline pH. One of the causes of a decrease in digestibility value is the presence of a fiber component such as starch. In the process when starch is heated, expansion and gelatinization occur, and when heated for 10 min the digestibility increases. After passing through the heating process the granules returned to their initial volume. Heating for 15 min contributes to the caramelization or browning process. This is explained by Agustina *et al*. (2013) who revealed that the starch contents are closely related to the fiber contents so that when subjected to continuous heating, the dietary fiber dissolves or undergoes destruction which causes the starch content (carbohydrates) to decrease.

Tight bonds of tannins and carbohydrates and other substances have an influence on the digestibility of fiber. Crude fiber is built from insoluble polysaccharides such as cellulose, hemicellulose, and lignin. The decrease in the content of active tannins that bind to the constituent components of the fiber components increases the fiber fraction digestibility.

In treatments C and D, the fiber fraction digestibility decreased. This was attributed to the alkaline pH of the boiling and the high temperature so which damages the plant structure and reduces digestibility. Nsa *et al*. (2011) said that boiling for more than 10 min can reduce the protein content and contents of castor beans. The decrease in nutrient contents is attributed to long boiling and causes a decrease in the level of digestibility of food substances. Starch content correlates with fiber contents, where when subjected to continuous. Boiling too long can also cause retrogradation of starch which can change the structure of the fiber making it difficult to digest (Sajilata *et al*. 2006).

Table 3: Digestibility of fiber fraction of mangrove leaves boiled in quicklime water (%)

	Treatment			
Digestibility	A	B	C	D
NDF	53.26^{b}±0.73	59.01^{a}±1.41	54.12^{b}±0.39	51.43^{c}±1.15
ADF	51.14^{c}±0.92	55.75^{a}±0.66	52.42^{b}±1.03	49.39^{d}±0.80
Celulosa	60.09^{b}±0.45	64.02^{a}±1.89	61.49^{a}±0.78	58.14^{b}±1.49
Hemicellulosa	62.86^{b}±0.67	69.61^{a}±0.46	64.58^{b}±1.39	60.20^{d}±1.18

A = without boiled (blank), B = Boiled mangrove leaves with 5% lime water, C = Boiled mangrove leaves with 10% lime water, and D = Boiled mangrove leaves with 15% lime water

$^{a-d}$Mean worth to the same line with dissimilar superscripts show a significantly dissimilar at ($p<0.05$)

Table 4: The value of the rumen ecosystem for each treatment

	Treatment			
Parameters	A	B	C	D
VFA (mM)	116.4^{b}±1.3	125.8^{a}±1.14	117.6^{b}±1.3	111.80^{b}±02.3
NH_3 (Mg/100 mL)	3.79^{bc}±0.05	4.32^{b}±0.11	3.83^{b}±0.09	3.69^{c}±0.11
pH (%)	6.90±0.008	6.92±0.013	6.91±0.013	6.90±0.021

A = without boiled (blank), B = Boiled mangrove leaves with 5% lime water, C = Boiled mangrove leaves with 10% lime water, and D = Boiled mangrove leaves with 15% lime water

$^{a-d}$Mean worth to the same line with dissimilar superscripts show a significantly dissimilar at ($p<0.05$)

Table 5: Methane gas production for each treatment (ml/grDM)

	Treatment			
Parameters	A	B	C	D
Methane gas	130.00^{d}±0.35	231.39^{a}±0.36	221.41^{b}±0.41	201.87^{c}±0.20

A = without boiled (blank), B = Boiled mangrove leaves with 5% lime water, C = Boiled mangrove leaves with 10% lime water, and D = Boiled mangrove leaves with 15% lime water

$^{a-d}$Mean worth to the same line with dissimilar superscripts show a significantly dissimilar at ($p<0.05$)

Rumen Ecosystem

The content of the *in vitro* rumen ecosystem (VFA, NH_3, and pH) of Mangrove leaves boiling in quicklime water is provided in Table 4. The top worth of VFA contents was at treatment B (5% lime water) and the lowest was in treatment D (15% lime water). The highest NH_3 content was found in treatment B (5% lime water) and the lowest in treatment D (15% lime water). Meanwhile, treatment A (blank) got the highest pH and the lowest pH was discovered in treatment D (15% lime water).

Boiling mangrove leaves in lime water could intensify the VFA contents. Volatile Fatty Acid (VFA) is the result of the conversion of organic matter in the form of carbohydrates and fats that was used by ruminant as the main energy sources (Noziere *et al*., 2011). Pazla *et al*. (2021) explained that VFA is adequate energy for a ruminant. The low production of VFA will lead to the low soluble of carbohydrates and proteins and the low digestibility of proteins and carbohydrates caused by the use of lime water in high concentration will lead to a lower nutritional content and reduction of VFA.

The value of NH_3 was improved in 5% lime water because lime water can decompose the bond of tannins and proteins and enhance the solubility of proteins. High soluble protein will lead to a high concentration of NH_3. In the 10 and 15% lime water treatments there was a decrease because the prolonged boiling caused the protein to be difficult to digest. Joye (2019) explained that overheating can make cross-links (disulfide bonds) in proteins that will result in low concentrations of NH_3. Heating for too long can also cause the protein to denature.

Rumen acidity (pH) obtained by each treatment showed no significant difference and the value obtained was good for rumen microorganisms to grow and develop to digest the substances of feed such as carbohydrates (cellulose and hemicellulose) and for microbial protein synthesis in the rumen. Indrayanto (2013) states that the optimum range of rumen pH to digestive activity is between 6.5 -7.0.

Methane Gas Content

The methane gas content of Mangrove leaves treated with boiling lime water is provided in Table 5. The top worth was is with treatment B (5% lime water) and the lowest was in treatment A (blank).

The highest production of methane gas in treatment B (addition of 5% lime water) was up to 231.39 (mL/g DM) and it was attributed to the lower tannin contents in treatment B. The alleviation of methane gas production was caused by the tannin contents, the higher the tannin value, the lower the methane gas production.

(Jayanegara and Sofyan, 2008) Stated that total phenol and tannins affected the production of methane gas.

Treatment A (Blank) showed the lowest methane gas production of up to 135.00 (mL/g DM) for the higher the tannin contents, the lower the methane gas production. Tannins can also alleviate methane gas production by the mechanism of lowering H_2 production or preventing methanogens and protozoa growth. As the digestion of fiber decreases, H_2 production will also be exsiccated so it lowers the methanogenic bacteria due to the lack of hydrogen gas (H_2) supply for their growth. This is in line with (Jayanegara *et al*., 2009) who revealed that the reduction of condensed tannins was an effect of an indirect mechanism that prevents the digestion of fiber and diminishes H2 production while the easily hydrolyzed tannins play a lineal role in the mechanism of inhibiting accretion and methanogenic activity.

Conclusion

Decreased tannin content, increased digestibility of dry matter, digestibility of organic matter, and digestibility of crude protein, as well as the best digestibility of crude fiber, were found in boiling mangrove leaves to 5% lime water so that they can be used as ruminant animal feed.

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Author’s Contributions

Zaitul Ikhlas: Participated in all experiments and contributed to the written of the manuscript and data analysis.

Novirman Jamarun and Mardiati Zein: Designed the research plan and organized and supervised the research.

Roni Pazla and Gusri Yanti: Participated in all experiments and contributed to the written of the manuscript.

Ethics

This article is original and has never been published before. The author has also confirmed to all authors involved in this study to read and agree to the contents of this article and that there are no ethical issues involved.

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