

Evaluation of the Potential Combination of Mangrove Fruit and Leaves as Alternative Ruminant Feed: In-Vitro Approach

G. Yanti, R. Elmiati, O. R. Anggraini, and H. D. Triani

Agricultural Extension Program, Faculty of Social Sciences and Education, Prima Nusantara University, Bukittinggi, Indonesia

Corresponding author: gusriyanti@upnb.ac.id

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ABSTRACT

This study aimed to determine the optimal combination of mangrove leaves (*Rhizophora apiculata*) and pidada fruit (*Sonneratia alba*) to improve nutrient digestibility as ruminant feed through in-vitro methods. The experiment used a Completely Randomized Design (CRD) with four treatments: P0: Mangrove leaves (100%); P1: Pidada fruit (100%); P2: Mangrove leaves (50%) + Pidada fruit (50%); P3: Mangrove leaves (75%) + Pidada fruit (25%), with each treatment replicated four times. Nutrient digestibility was assessed using the Tilley and Terry method. The results showed that treatment P3 (the combination of 75% mangrove leaves and 25% pidada fruit) achieved dry matter digestibility of 59.38% and organic matter digestibility of 62.56%, with a total volatile fatty acid (VFA) concentration of 125.54 mM and ammonia (NH₃) concentration of 16.76 mg/100 ml. This combination exhibited high digestibility, indicating its potential as an alternative ruminant feed.

Keywords: mangrove, forage, ruminants, *in-vitro*,

INTRODUCTION

The mangrove forests in West Sumatra, covering areas such as Pesisir Selatan, Mentawai, Padang, Padang Pariaman, Pariaman, Agam, and Pasaman Barat, span an area of 39,832 hectares. Dominant mangrove species include *Rhizophora apiculata* (mangrove), *Sonneratia alba* (pidada), and *Avicennia marina* (api-api). In certain areas like Pesisir Selatan and Padang Pariaman, mangrove plants have been utilized to meet food needs, fish feed, and, more recently, livestock feed for ruminants (Yanti, 2022).

The leaves of *Rhizophora apiculata*, in particular, hold nutritional potential for developing ruminant feed diversification. Several studies have reported that these leaves contain sufficient nutrients and minerals for ruminant feed (Jamarun et al., 2021; Ahoungan et al., 2021 and 2022; Yanti, 2023). The fruit of *Sonneratia alba* also has nutritional content that can be used as a feed source and concentrate (Elihasridas, 2023). Combining these two ingredients could produce a new ruminant feed source, especially for coastal communities.

Rhizophora apiculata leaves contain 15.3% protein and 12.32% crude fibre, rich in macro and micro minerals (Sari et al., 2021). Similarly, *Sonneratia alba* fruit contains 8.73% protein, 1.44% fat, 75.1% carbohydrates, and 5.18% ash content (Ardiansyah et al., 2020).

However, these materials also contain anti-nutritional compounds that could hinder absorption efficiency as feed. Therefore, a feasibility test is needed to ensure the combination is safe for consumption and improves livestock growth.

Ruminant livestock in West Sumatra, such as cows, goats, and buffalo, have become vital food commodities. In the coastal areas, particularly in the south, the Pesisir Cow is a notable livestock commodity. According to data from the Livestock Office of West Sumatra Province, Pesisir Cows make up 20% of the total cattle population in the region. However, despite being a commodity, farmers still rely on conventional feed, such as grass, which is costly and must be sourced from other areas.

In addition to Pesisir Cows, many farmers in the region raise goats that graze freely due to a lack of pasture land. Ruminant livestock in the coastal region is relatively tolerant of feed with low nutritional content (Adrial, 2010; Wahyuni, 2018). The issue of feed scarcity for ruminants in coastal areas is expected to be addressed by utilising mangrove plants as feed. Moreover, mangroves are locally available, and their nutritional value could provide additional benefits for ruminant feed in the area (Yanti, 2022). This study aimed to determine the best combination of mangrove leaves and pidada fruit to improve nutrient digestibility *in vitro*.



MATERIALS AND METHODS

This research on combining mangrove leaves and pidada fruit as ruminant feed in vitro was conducted at the Ruminant and Livestock Biotechnology Laboratory, Faculty of Animal Husbandry, Andalas University. The study took place from June to September 2024. The experimental design used was a Completely Randomized Design (CRD) with four treatments: P0 (100% Mangrove leaves); P1: 100% Pidada fruit; P2 (50% Mangrove leaves + 50% Pidada fruit); P3 (75% Mangrove leaves + 25% Pidada fruit). Each treatment was replicated four times.

The mangrove leaves and pidada fruit used in the research were collected from Nagari Sasak, Ranah Pasisie Subdistrict, Pasaman Barat Regency. After collection, the leaves and fruit were dried in an oven at 50°C until completely dry. The dried leaves and fruit were then finely ground and mixed according to the predetermined treatment combinations.

Tannin Content Analysis

50 mg of sample was extracted using 70% ethanol for 30 minutes to measure tannin content. One millilitre of the extract was mixed with 1 ml of Folin-Ciocalteu reagent, shaken, and left to stand for 5 minutes. Afterwards, 15% Na₂CO₃ was added, and the mixture was allowed to stand for 15 minutes. The absorbance was measured at a wavelength of 725 nm. The total tannin content was calculated as gallic acid equivalents (GAE).

Polyphenol Content Analysis

For polyphenol content analysis, 1 ml of sample extract was mixed with 0.2 ml of Folin-Ciocalteu reagent in a test tube and vortexed for 1 minute. Afterwards, 0.2 ml of Na₂CO₃ (7.5%) solution was added. The mixture was then kept in a dark room for 30 minutes. The absorbance was

read using a spectrophotometer at a wavelength of 656 nm. The result was expressed in gallic acid equivalents (GAE).

Mineral Content Analysis

The mineral content of calcium (Ca), phosphorus (P), magnesium (Mg), and sulfur (S) in the mangrove leaves and pidada fruit combination was determined using the dry destruction method. The samples were subjected to dry destruction and then analyzed using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) to measure the mineral content of Ca, P, Mg, and S.

Dry Matter and Organic Matter

Digestibility (Tilley & Terry, 1963)

For the digestibility test, 0.5 grams of feed sample was placed in a fermentation tube, and 10 ml of McDougall buffer solution and 40 ml of rumen fluid were added. The mixture was stirred and flushed with CO₂ gas for 30 seconds before the tube was sealed tightly. The fermentation tube was then incubated at 39°C for 48 hours. After incubation, 2-3 drops of saturated HgCl₂ were added to the tube to stop microbial activity. The mixture was centrifuged at 3000 rpm for 15 minutes, and the supernatant was collected. Next, 50 ml of 0.2% pepsin HCl solution was added to the tube. The enzymatic digestion process was carried out aerobically for another 48 hours.

The digestion residue was filtered using Whatman No. 41 filter paper. The filtered residue was then placed in porcelain crucibles and dried in an oven at 105°C for 24 hours to obtain the dry matter (DM residue). The residue was then incinerated in a furnace at 600°C for 5-6 hours to determine the organic matter (OM residue). The percentage of DM and OM digestibility was calculated by:

$$\% \text{ DM Digestibility: } \text{DM incubated} - \text{DM residue} / \text{DM incubated} \times 100\%$$

$$\% \text{ OM Digestibility: } \text{OM incubated} - \text{OM residue} / \text{OM incubated} \times 100\%$$

Rumen Fluid pH Measurement

The pH of the rumen fluid obtained from the in-vitro process was measured using a pH meter. The pH meter electrode was immersed in the rumen fluid and recorded the pH reading.

Ammonia (NH₃) Concentration Measurement

The NH₃ concentration in the rumen fluid was measured using the Conway

microdiffusion method. The supernatant from the in-vitro process was centrifuged at 3500 rpm for 15 minutes. Then, 1 ml of supernatant was placed on one side of the Conway dish, while 1 ml of saturated Na₂CO₃ Yuk solution was added to the other. A boric acid indicator was placed in the centre of the Conway dish. The dish was sealed tightly, and the Na₂CO₃ reacted with the supernatant to release ammonia gas. The mixture was left to stand for 24 hours. Afterwards, the

solution was titrated with 0.005 M H₂SO₄ until the colour changed from blue to pink.

Volatile Fatty Acid (VFA) Concentration Measurement

To measure the total VFA, 5 ml of the sample was placed in a distillation flask, and 1 ml of 15% H₂SO₄ was added. The VFA produced was captured using 5 ml of 0.5 N NaOH in an Erlenmeyer flask containing two drops of phenolphthalein indicator. The distillate was collected until it reached 250 ml, then titrated with 0.5 N HCl until the endpoint of the titration was reached.

RESULTS AND DISCUSSION

Tannin and Polyphenol Content

The tannin and polyphenol content from the combination of mangrove leaves and pidada fruit are presented in Table 1. Different combinations had a significant effect on tannin and polyphenol content. Treatment P1 (100% pidada fruit) had the highest levels of tannin (23.24%) and polyphenol (36.68 mg GAE/g), while treatment P3 (75% mangrove leaves + 25%

pidada fruit) had the lowest levels of tannin (14.21%) and polyphenol (23.45 mg GAE/g).

Tannins and polyphenols are bioactive compounds commonly found in plants and taste bitter and astringent. These compounds play an important role in protecting plants. Tannin and polyphenols have some benefits, such as protein protection and reducing methane gas production through defaunation. However, excessive use can cause anti-nutritional effects. Therefore, an appropriate usage strategy is needed to increase feed efficiency and support livestock health.

Tannins interact with proteins derived from feed, thereby reducing the availability of these proteins to rumen microorganisms (Besharati et al., 2022). This condition can positively increase the digestibility of forage within certain limits because tannins protect proteins from degradation by rumen microorganisms. These metabolites are mainly found in the post-rumen digestive tract. The tannin-protein binding complex can be released at low pH in the abomasum, allowing protein degradation by the enzyme pepsin. Thus, the amino acids contained in the protein become available to livestock.

Table 1. Tannin and Polyphenol content of the combination of pidada fruit and mangrove leaves

Parameters (%)	Treatment			
	P0	P1	P2	P3
Tannin (%)	15.76 ^c ±0.012	23.24 ^a ±0.102	22.23 ^b ±0.014	14.21 ^c ±0.024
Polyphenol (mg GAE/g)	24.51 ^c ±0.007	36.68 ^a ±0.021	29.68 ^b ±0.026	23.45 ^c ±0.015

Note: P0: Mangrove’s leaves (100%); P1: Pidada’s fruit (100%); P2: Mangrove’s leaves (50%) + Pidada’s fruit (50%); P3: Mangrove’s leaves (50%) + Pidada’s fruit (25%).

Mineral Content

The mineral content of calcium (Ca), phosphorus (P), magnesium (Mg), and sulfur (S) in the combination of mangrove leaves and pidada fruit is presented in Table 2. Mangrove plants are known to be rich in macro and micro minerals due to their coastal habitat, which is high in mineral content. Minerals such as calcium, magnesium, and phosphorus are absorbed by the plant roots from the environment and distributed to various plant parts, including leaves and fruit. These minerals play an essential role in supporting metabolic functions and the health of livestock.

In general, the calcium content of the mangrove leaf and pidada fruit combination ranged from 0.258% to 0.372%, sufficient to meet the calcium requirements for ruminants. Calcium and phosphorus are closely related in metabolism, particularly in bone formation and

enzymatic processes. Maintaining Ca and P intake balance is critical for livestock health and reproduction. According to Chaudhary and Singh (2004), phosphorus deficiency can cause reproductive disorders, such as abortion, while excessive phosphorus intake can increase susceptibility to uterine infections. Thus, balancing Ca and P in feed is vital for the health and reproduction of ruminants.

The magnesium content in the combination of mangrove leaves and pidada fruit ranged from 0.6% to 0.83%. According to McDowell (1985), the regular mineral content in forage should be: calcium (Ca) 0.17-1.53%, phosphorus (P) 0.17-0.59%, magnesium (Mg) 0.05-0.25%, and sulfur (S) 0.01-0.1%. Magnesium is a general enzyme cofactor important in metabolising carbohydrates, fats, and proteins (Taith & Cashman, 2015).

Table 2. Mineral Content of the Combination of Mangrove Leaves and Pidada Fruit

Parameters (%)	Treatments			
	P0	P1	P2	P3
Calcium	0.372±0.015 ^a	0.334±0.032 ^a	0.342±0.017 ^a	0.258±0.022 ^b
Phosphor	0.143±0.021 ^b	0.162±0.022 ^a	0.163±0.022 ^a	0.103±0.100 ^c
Magnesium	0.831±0.018 ^a	0.802±0.016 ^a	0.812±0.032 ^a	0.604±0.021 ^b
Sulfur	0.007±0.032 ^b	0.011±0.031 ^a	0.008±0.018 ^b	0.006±0.018 ^b

Note: P0: Mangrove's leaves (100%); P1: Pidada's fruit (100%); P2: Mangrove's leaves (50%) + Pidada's fruit (50%); P3: Mangrove's leaves (50%) + Pidada's fruit (25%).

The mineral content of this combination showed significant variation and could meet the mineral needs of ruminants. Studies by Kaswarjono et al. (2016) indicate that macro and micro minerals in forage are closely related to reproductive capability in ruminants. A deficiency in minerals can cause nerve dysfunction, decreased appetite, reduced sugar and mineral absorption, and, ultimately, weight loss. Maxwell and Lai (2012) concluded that mangrove leaves could be a candidate for nutritional mineral supplementation to improve dairy cow milk production, highlighting the potential of forage rich in minerals like mangrove leaves for improving livestock productivity.

Dry Matter Digestibility, Organic Matter Digestibility, and Rumen Fluid Characteristics

The average dry matter digestibility of the combination of mangrove leaves and pidada fruit is shown in Table 3. The combination of these plant materials significantly affected ($P<0.05$) both dry and organic matter digestibility. Higher digestibility values indicate a higher nutritional quality of the feed. Dry matter digestibility is a crucial indicator in assessing feed quality and the ability of livestock to utilize it.

Table 3. Dry Matter Digestibility, Organic Matter Digestibility, and Rumen Fluid Characteristics

Parameters	Treatments			
	P0	P1	P2	P3
DM digestibility (%)	58.60±0.31 ^a	49.63±0.12 ^c	50.24±0.13 ^b	59.38±0.14 ^a
OM digestibility (%)	61.74±0.11 ^a	51.21±0.08 ^b	50.86±0.19 ^b	62.56±0.22 ^a
pH	6.77±0.12	6.98±0.08	6.94±0.09	6.79±0.015
VFA (mM)	121.54±0.13 ^b	114.43±0.13 ^c	116.32±0.21 ^c	125.54±0.11 ^a
NH ₃ (mg/100ml)	16.68±0.10 ^a	11.67±0.01 ^b	12.02±0.26 ^b	16.76±0.15 ^a

Note: P0: Mangrove's leaves (100%); P1: Pidada's fruit (100%); P2: Mangrove's leaves (50%) + Pidada's fruit (50%); P3: Mangrove's leaves (50%) + Pidada's fruit (25%).

Dry and organic matter digestibility in treatments P1 and P2 decreased, while they increased in treatments P0 and P3. This indicates that the best combination for enhancing dry matter and organic matter digestibility is P3 (75% mangrove leaves + 25% pidada fruit).

The increased digestibility in treatment P3 is related to the lower tannin content than other treatments. Tannins are secondary compounds commonly found in forage plants. They can have both positive and negative effects on digestibility. In moderate concentrations, tannins can protect proteins from excessive degradation in the rumen, enhancing nitrogen utilization and organic matter digestibility (Min et al., 2003). However, in excessive concentrations, tannins can bind to proteins and fibres, reducing feed digestibility (Makkar,

2003). Studies by Jayanegara et al. (2012) have shown that supplementation with *Rhizophora thypina* and *Sonneratia alba*, which contain tannins, can increase organic matter digestibility, supporting the idea that tannins in moderate amounts can improve feed digestibility. In this study, P3 increased dry matter digestibility to 59.38% and organic matter digestibility to 62.56%. Thus, this combination effectively reduces tannin content to an optimal level, improving feed digestibility as an alternative ruminant feed.

The rumen fluid pH values of the combination ranged from 6.77 to 6.94, which is within the normal range for optimal rumen microbial activity. Variations in rumen pH across treatments reflect the effect of the feed composition on rumen conditions.

Lower pH values in P0 and P3 indicate increased microbial activity in utilizing feed nutrients in the rumen, producing volatile fatty acids (VFA). The pH levels observed in this study support the idea that rumen microbial activity is optimized within the range of 5.5 to 7.5 (Le et al., 2022; Jamarun et al., 2020).

The ammonia (NH₃) concentration in rumen fluid across treatments ranged from 11.67 to 16.76 mg/100 ml. The NH₃ concentration was closely linked to organic matter digestibility. As explained by Sa'dan et al. (2021), protein in ruminant feed is broken down by microbes into ammonia, which is necessary for microbial protein synthesis and nitrogen availability. According to Phesatcha et al. (2022), sufficient ammonia concentrations are required for efficient microbial protein synthesis.

The total volatile fatty acids (VFA) produced ranged from 114.43 mM to 125.54 mM, indicating a good level of fermentation. The optimal range for VFA production is between 70-150 mM, which indicates effective feed fermentability. Increased VFA levels correlate with microbial activity involved in the fermentation of fibre and non-fibre carbohydrates (Mamuad et al., 2019). This demonstrates that the microbial population in the rumen is functioning efficiently with optimal substrate utilization.

CONCLUSION

Based on the research results, the combination of 75% mangrove leaves and 25% pidada fruit (P3) showed a dry matter digestibility of 59.38%, organic matter digestibility of 62.56%, total volatile fatty acids (VFA) of 125.54 mM, and ammonia (NH₃) concentration of 16.76 mg/100 ml. This combination exhibited good digestibility, demonstrating significant potential as an alternative ruminant feed. The benefits of tannins in this study helped to reduce protein degradation in the rumen and control ammonia production, as shown by the optimal NH₃ concentration. In addition, pidada fruit as a source of fermentation energy helped to balance the effects of tannins so that rumen fermentation continued to run well without adverse antinutritional effects.

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