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Effect of boiling on *in-vitro* nutrients digestibility, rumen fluid characteristics, and tannin content of mangrove (*Avicennia marina*) leaves as animal feed

N Jamarun¹, R Pazla¹, G Yanti²

¹ Staff of Animal Science Faculty, Andalas University

² PhD Student at Graduate School Faculty of Animal Science, Andalas University

E-mail: novirman55@gmail.com

Abstract. The feed is one of the main costs of ruminant animal production. The main is the limited availability of forages. Therefore, it was necessary to found alternative forages with large quantities, easy to get, and low cost. One of the alternatives is Mangrove (*Avicennia marina*) leaves. Although it is available in large quantities, the presence of tannins and polyphenol causes low nutrients digestibility. Boiling mangrove leaves in water can reduce tannin concentrations and increase nutrients digestibility. This study aims to find the best boiling period effect on *Avicennia marina* leaves on *in-vitro* nutrients digestibility, rumen fluid characteristics, and tannin content. The research used Randomised Block Design (RBD) with four treatments and five replications. The treatments were boiling time of *Avicennia marina* leaves in husk ash water, 15% (w/v) for 0 minutes (P0), 5 minutes (P1), 10 minutes (P2) and 15 minutes (P3). The results showed that the hydrolysis time in husk ash water contributed significantly on *in-vitro* digestibility of dry matter, organic matter, VFA, and NH₃ production, and tannin content. The best boiling time for *Avicennia marina* leaves was 10 minutes, producing the *in-vitro* digestibility of dry matter (72.06%), *in-vitro* digestibility of organic matter (73.36%), VFA (117 mM), NH₃ (4.57 mg/100 ml), pH (6.73) and tannin content (10.27%). The result was suitable for the growth and production of rumen microbes.

1. Introduction

Forages, commonly used as feed for Ruminant animals, can be obtained from field grass, agricultural by-products, agriculture waste products and larger grasses. The issues were the limited forages availability at any season. The main inhibiting factor of the supply of forages was the change of the land function to the crop's plantation, real estates, and industrial plant. This situation can be overcome by using alternative forages for animal feeds [1].

Mangrove (*Avicennia marina*) is one of the plants that can be used as alternative forages for ruminants feed. *Avicennia marina* plants are a type of tropical mangrove plant from the genus of *Avicennia*. The plants have not been used as ruminants feed in Indonesia yet. Therefore, it is necessary to research this mangrove for animal feed without risking the animal and the mangrove ecosystem.

Farmers live around the Red Sea, India and Australia have used mangrove leaves for their animals feed such as camel, goat, and sheep. This shows that mangrove leaves have the potential to be used as



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animal feed. The research conducted by [2] reported that the protein content of mangrove leaves was 17.31%. Mangrove also grows better in extreme environments. The problem for mangroves as animal feed is their secondary metabolites such as tannin and other polyphenols. Tannin is used by plants to protect themselves from pest and insecticides and to grow better. Mangrove leaves contain 13.44% tannins [3], which would be harmful to the animal.

Tannins are polyphenol compounds which by its chemical structure can be classified into two types, hydrolysed tannins (*hydrolysable tannins*) and condensed tannins. Green plants generally produce tannins at different concentrations. Tannins from forage (legumes) generally in the form of condensed tannins and have complex bonds with proteins that are stronger than hydrolysed tannins [4]. According to [5], an increasing percentage of tannins binds carbohydrates and proteins so that these proteins are difficult to be degraded by rumen microbes and also decreasing enzyme activity. The degradation or digestion of nutrients in the rumen will also decrease, and the availability of carbohydrates and proteins if rumen microorganisms were decreased.

Mangrove boiling by using an active organic mineral can reduce the tannin levels in mangrove leaves. [6] reported that boiling at temperatures of 98-101°C can break down tannin into glucose and gallic acid. Boiling can also reduce levels of tannins and other anti-nutrients contents because phenolic acids can dissolve in hot water [7]. Husk ash is an alkaline compound that can activate active phenolic tannin groups [8]. The use of husk ash with different doses had a significant effect on the decrease of tannin content with the addition of 15% husk ash [9]. This is the rationale that the husk ash could decrease the amount of tannin that can be found in mangrove leaves, which can improve the digestibility of nutrients such as dry matter and organic matter of mangrove leaves.

2. Material and Methods

This research used mangrove (*Avicennia marina*) leaves, husk ash, a set of test kits and proximate analysis materials, a set of test kits and *in-vitro* materials and a test tool for rumen fluid characteristics. The method used is a Randomised Block Design (RBD) with four treatments and five groups, where each treatment uses 15% (w/v) of husk ash water. The treatment consists of P0: without boiling, P1: 5 minutes of boiling, P2: 10 minutes of boiling, and P3: 15 minutes of boiling.

In-vitro method: The *In vitro* analysis was carried out to determine the digestibility of mangrove leaves (*Avicennia marina*) for each feed treatment. The rumen process was stopped by immersing the Erlenmeyer tube in ice water to stop the microbial activity, then measuring the pH using a pH meter. The next step was to separate the supernatant from the residue. The mixture obtained from *in vitro* analysis was centrifuged for 30 minutes at a speed of 3000 rpm and a temperature of 4°C until there was a separation between the supernatant and the residue. The residue was filtered using Whatman No.41 filter paper and then dried in an oven at 60°C, prior to nutrient digestibility analysis.

The tannin content was measured by the Colorimetric method with a spectrophotometer. The *in-vitro* dry matter and organic matter digestibility was analysed referring [10], and the rumen fluids were characterised. Data obtained from this research were statistically analysed using SPSS software version 21.0.

3. Results and Discussion

3.1. *In-vitro* dry matter and organic matter digestibility and tannin content

Table 1 shows the average dry matter digestibility ranging from 58.97% to 72.06%. The highest digestibility of dry matter was obtained at 10 minutes of boiling (P2) (72.06%), and the lowest was at a boiling time of 15 minutes (P3) (58.97%). The digestibility of dry matter in this study was higher compared to that of Gibran's [11] who measured the effect of the ratio of rice straw and mangrove (*Avicennia marina*) leaves resulting in the digestibility of dry matter between 44.39% to 56.08%. This indicates that boiling mangrove leaves with husk ash water can increase the digestibility of dry matter. Dry matter digestibility increased in each treatment until P2 treatment. This increase occurs because the boiling of mangrove leaves using husk ash water (alkaline) is able to break the complex bonds between

tannins and other polyphenols with protein, carbohydrates, vitamins, and minerals. The temperatures of 98-101^o C can break down the tannin's component into glucose and gallic acid [12]. According to [13], husk ash is a basic compound containing KOH that is able to absorb cell fluids, binding poison groups, or anti-nutrient substances. In the treatment of P3 (boiling time of 15 minutes) indicated the decreased dry matter digestibility. According to [14], this decline was due to the excessive boiling time, which caused starch retrogradation which could alter the structure of the fibre that difficult to digest by rumen microbes.

Table 1. *In-vitro* dry matter and organic matter digestibility and tannin content.

Digestibility (%)	Treatment			
	P0	P1	P2	P3
Dry matter	66.63 ^b	66.69 ^b	72.06 ^a	58.97 ^c
Organic matter	67.58 ^b	67.80 ^b	73.36 ^a	60.50 ^c
Tannin content	11.10 ^b	14.91 ^a	10.27 ^c	10.89 ^c

^{a,b,c} superscript means significantly different in a row (p<0.05)

Table 1 shows that the average digestibility of the organic matter of mangrove leaves boiled using husk ash water ranges from 60.50% to 73.36. The highest digestibility of organic matter was obtained at 10 minutes of boiling (P2: 73.36%), and the lowest digestibility of organic matter was at 15 minutes of boiling (P3: 60.50%). The average digestibility of organic matter is directly proportional to the average digestibility of dry matter. The dry matter consists of organic matter [15]. Table 1 shows the best digestibility of organic matter, and the dry matter is P2 treatment. The highest increase in digestibility of organic matter is on P2 (10 minutes of boiling). Boiling the leaves with husk ash water can break the bonds of tannins or other polyphenols with protein, carbohydrates, vitamins, and minerals so that their digestibility will increase. Husk ash can activate phenolic hydroxyl groups in tannins due to its alkaline properties [13, 8].

In P3 treatment (boiling of 15 minutes), there was a decrease in the digestibility of organic matter that was in line with the digestion of dry matter. This decrease was due to changes in the structure of food substances such as protein and carbohydrates by an excessive boiling time. Continuous heating will damage food fibres so that the concentrations of carbohydrates will also be decreased [16].

The effect of boiling using husk ash water on tannin content was shown in Table 1. Tannins could be hydrolysed by acids, bases, and enzymes. Tannins will break down into glucose and gallic acid, if heated at temperatures of 98.89°C - 101.67°C [17]. Tannins dissolve easily in water and their solubility increases when dissolved in hot water [18]. The husk ash also causes an increase in tannin dissolve during the boiling process.

The P1 treatment (boiling of 5 minutes) indicated increases the tannin level up to 14.91%. This occurs due to at the first boiling several polyphenol substances will make a complex substance each other and will be counted as a total tannin substance in analysed. [18] stated that at high temperatures, enzymes catechol oxidase is inactivated and caused an increase in the total tannin.

At treatment P2 (10 minutes of boiling) tannin content showed decreased (10.27%) due to a long time of boiling, which could loosen ties between mangrove's leaves fibre. This condition will make osmosis processes happen in the leaves and push tannin out and soluble in alkaline water by diffusion activity, thus reduce the tannin content [18].

3.2 Characteristics of rumen fluids

The average pH of rumen fluid from Mangrove (*Avicenna marina*) leaves are shown in Table 2. Boiling of mangrove leaves does not affect the pH of rumen fluid. This means that the rumen conditions are in an ideal atmosphere for rumen microbes. The pH of the rumen liquid obtained was ranging from 6.64 to 6.73, which is relatively normal conditions for the growth and development of rumen microbes. The normal value of acidity in the rumen is between 6.0 - 7.0 [19].

Table 2. Characteristics of rumen fluids.

Parameter	Treatments			
	P0	P1	P2	P3
pH	6.71 ^a	6.64 ^b	6.73 ^a	6.68 ^b
VFA (mM)	89 ^c	101 ^b	117 ^a	108 ^b
NH ₃ (mg/100ml)	3.51 ^c	4.25 ^b	4.57 ^a	3.72 ^c

^{a,b,c} superscript means significantly different in a row (p<0.05)

Volatile Fatty Acid (VFA) is a fermentation product from carbohydrates and fats that are used as an energy source and as a carbon framework for rumen microbes. The effect of boiling of Mangrove (*Avicenna marina*) leaves in husk ash water with different times can be seen in Table 2. The average value of VFA production in this study ranged from 89 - 117 mM. The production of VFA in this study is able to support microbial growth and activities. The optimal VFA production to support microbial growth was 80-160 mM [19]. High VFA production provides an energy source for the growth of rumen microbes. This energy will support more microbial cells to produce enzymes. It also increases the degradation of feed in the rumen while increasing its digestibility.

The average value of VFA production also indicates the length of boiling time. The longer the boiling time, the more tannin bonds are degraded so that the soluble protein and carbohydrates were increased [20]. An increase in the amount of carbohydrate (cellulose, hemicellulose, and pectin) tends to increase its digestibility. This increased digestibility affects the production of VFA, because cellulose, hemicellulose, and pectin were degraded by rumen microbes and then converted into simple sugars. Simple sugar is converted to pyruvic acid and then converted to VFA [21].

The VFA production in P3 was due to the excessive boiling time (15 minutes). This boiling damaged nutritional content (crude fibre, crude protein, and crude fat) of mangrove. The decrease in nutrient content, especially carbohydrates, will effects on the production of VFA because the carbohydrates are converted into volatile fatty acid. The boiling time over 10 minutes in castor beans decreases the nutrient content of that beans [22].

Ammonia is a product of protein degradation by rumen microbes. NH₃ in the rumen could be used by microbes as a source of nitrogen for protein synthesis. An increase in NH₃ can improve the growth and development of microbes so that the feed's digestibility in rumen increased. The effect of the boiling time of Mangrove (*Avicenna marina*) leaves in husk ash water on NH₃ rumen fluid concentration can be seen in Table 3. The concentrations of NH₃ in rumen fluid ranges from 3.51 - 4.57 mg / 100 ml. The average concentration of NH₃ was lower compared to that of normal values. NH₃ concentration of 5 mg / 100 ml or 4.58 mM of rumen fluid is the minimum concentration of NH₃ to support rumen growth [23]. The factors influencing the production of NH₃ in rumen fluid are the time of feed in the rumen, carbohydrates content in the ration, solubility, and amount of feed and rumen pH [23].

The increasing average NH₃ concentration of rumen fluid in each treatment is as shown in Table 2. The highest NH₃ production is shown in P2 treatment while the lowest was in the P0 treatment, showing that the length of boiling mangrove leaves in husk ash water have significantly effect on NH₃ concentration. According to [4], tannins have a very strong bond to proteins because they contain a large number of phenolic groups that are bonding points with the groups of carboxyl peptide. The husk ash is capable of breaking the bonds between tannins and proteins, increasing the soluble protein content. The higher the soluble protein caused, the higher the production of NH₃.

4. Conclusions

The best boiling time of Mangrove (*Avicenna marina*) leaves was in 10 minutes which producing the highest *in-vitro* digestibility of dry matter (72.06%), organic matter digestibility (73.36%), VFA (117 mM), NH₃ (4.57 mg/100 ml), pH (6.73) and lower the tannin content (10.27 %). These properties make the mangrove leaves suitable for the growth and production of rumen microbes.

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