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Research Paper

The Effect of Boiling Mangrove (*Avicennia marina*) Leaves On*in-Vitro* Nutrients Digestibility and Tannin Content as Animal Feed

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ABSTRACT: *Mangrove (Avicennia marina) is a highly nutritional plant that can be used as an alternative feed for ruminants. However, it has adverse effects when consumed in numerous quantities, due to the presence of anti-nutritional substances. The purpose of this research is to analyze the effect of boiling mangrove leaves (Avicennia marina) in husk ash water (15% w / v) to determine the in-vitro nutrients digestibility as well as the tannin and phenol contents, thereby making it usable for animal feed. The in-vitronutrients digestibility values observed were In-Vitro Crude Fiber Digestibility (IV-CFD), In-Vitro Crude Fat Digestibility (IV-CFtD), and In-Vitro Nitrogen Free extract Digestibility (IV-NFED) as well as tannins and phenols contents. This is an experimental research with a randomized block design (RBD), comprising of 4 treatments and 5 replications. The four treatments are P0: without boiling, P1: boiling for 5 minutes, P2: boiling for 10 minutes, and P3: boiling for 15 minutes. The results of the experiment showed that the boiling time gave a significant effect (P <0.01) on in-vitronutrients digestibility of crude fiber, crude fat, and NFE, as well as tannin and phenol contents of mangrove leaves. Therefore, based on this research, it can be concluded that the optimum boiling time for mangrove leaves was 10 minutes as shown from the high In-Vitro nutrient digestibility that is IV-CFD 77.48%, IV-CFtD 31.80%, IV-NFED 64.57%, and tannin content10.27%, and phenol 99.29 mgGAE.*

KEYWORDS: *Avicennia marina, husk ash, boiling, nutrients digestibility, tannins*

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I. INTRODUCTION

The feed is one of the main factors used to determine a successful livestock business because 60 - 80% of the total production costs for ruminants are associated with this food product [1]. The natural feed for ruminants such as field grass, agricultural waste, and some superior grass are collectively called forages.

However, the increasing limited availability of forages is one of the obstacles recently faced by breeders. [2] stated that the limited availability of forages occurs due to changes in land functions from food and industrial crops to residential areas. Therefore, due to this situation, it is important to determine alternative feed sources by utilizing local raw materials that are available in large quantities, and easy to obtain with high nutrient content.

One of the plants that can be used as an alternative feed for ruminants is mangrove leaves (*Avicennia marina*), a tropical plant from the genus *Avicennia*. According to [3], Indonesia is a country with the largest mangrove forest in the world, which is approximately 22.6% or 3.1 million ha. The most important contribution of mangrove forests to coastal water ecosystems is through the decay of their fallen leaves into the water, which is about 7-8 tonnes/ha/year [4].

Mangrove forests are protected areas that require selective efforts in their utilization. According to forestry law no.41 of 1991 article 8 paragraph (2), forest areas are designated with special purposes as intended for public interests such as research and development, education and research, religion, and culture.

Avicennia marina contains protein, fat, crude fiber, BETN, and ash in percentage values of 14.75%, 4.80%, 13.29%, 42.74%, and 16.02%. However, mangrove leaves also contain anti-nutritional compounds in the form of tannins (18%), therefore, it tends to have a negative effect on livestock when consumed in large quantities [5]. According to [6], the tannin content in mangrove leaves is 13.44%.

Therefore, one of the efforts used to reduce tannin levels in mangrove leaves is by boiling it using husk ash water. This process is defined as the act of mixing water with husk ash through heating and at a temperature of 100^0C. Therefore, the husk ash water has the potential to be an absorbent material for cell fluids, inhibits the oxidation rate of toxic substances, and weakens the alkaline solutions capable of deactivating tannin properties.

Based on the description above, this study was carried out to determine the best time for boiling mangrove leaves (*Avicennia marina*) using husk ash water. Furthermore, this research was carried out to increase digestibility and reduce the tannins content, therefore it can be used as a ruminant feed to determine the in-vitro process on the digestibility of crude fiber, crude fat, BETN, tannins, and phenols.

II. MATERIALS AND METHODS

Research Materials: The materials used were goat rumen fluid, mangrove leaves (*Avicennia marina*) obtained from Nagari Terusan, Pesisir Selatan Regency, husk ash, aquades, Mc. Daugll s solutions as buffers and chemicals for *in-vitro* analysis. This research was carried out from October 2019 to January 2020.

Research Tools: The research tools used consists of laboratory equipment such as scales, centrifuge, thermometer, measuring glasses, test tube, Erlenmeyer, *in-vitro* tube, water shaker bath, oven, etc.

Method: The method used in this study is an experimental method, using a Randomized Block Design (RBD) with 4 treatments and 5 groups, where each treatment uses 15% (w/v) of husk ash water. The treatment consis of P0: without boiling, P1: 5 minutes of boiling, P2: 10 minutes of boiling and P3: 15 minutes of boiling.Boiling is carried out in a solution of husk ash water with a concentration of 15% (w / v), (Nirwani. S and Endang, 2017).

Observed Variables: The variables measured were In-Vitro Crude Fiber Digestibility (IV-CFD), In-Vitro Crude Fat Digestibility (IV-CFtD), and In-Vitro Nitrogen Free Extract digestibility (IV-NFED) using the Tilley and Terry method [7]. Tannin content and total phenol calculations were conducted using the AOAC (1999[8], [9], and [10] methods.

III. RESULTS AND DISCUSSION

The chemical composition of research materialsbased on treatments of mangrove leaves (*Avicennia marina*) is presented in Table 1. In general, the nutritional content of the ingredients fluctuates in each treatment. Dry material is the material left behind after evaporation of the air as a whole contained in the feed ingredients. The average amount of dry matter contained in *Avicennia marina* leaves can be seen in Table 1.The nutritional value of mangrove leaves (*Avicennia marina*) shows that boiling treatment with husk ash water on mangrove leaves has a very significant effect (P <0.01) on dry matter. where there is a significant decrease between P0 (94.89%),P1 (91.34%), P2 (89.05%), and P3 (88.83%).

Table 1. Chemical composition of mangrove leaves for each treatment.

Nutritional Data (%)	Mangrove Leaves (*Avicennia marina*)			
	P0	P1	P2	P3
Dry Matter (%)[a]	91,34	94,89	95,04	95,60
Organic Matter(%)[a]	88,58	89,21	89,41	89,35
Crude Protein (%)[a]	11,40	14,20	13,50	13,04
Crude Fat (%)[a]	2,77	2,75	2,57	3,58
Crude Fiber (%)[a]	8,42	7,28	6,42	9,23
BETN (%)[a]	65,99	64,98	66,92	63,50
Ash (%)[a]	2,76	5,68	5,63	6,25
NDF (%)[b]	30,28	30,22	27,10	32,29
ADF (%)[b]	19,90	19,63	18,14	20,95
Cellulose (%)[b]	8,72	8,37	8,49	8,50
Hemicellulose (%)[b]	10,37	10,16	9,00	10,67
Lignin (%)[b]	10,99	11,00	9,33	12,18
Silica (%)[b]	0,19	0,26	0,28	0,27
Tannin (%)[c]	11,10	14,91	10,27	10,89

Note: P0 (0 minutes), P1 (5 minutes), P2 (10 minutes), P3 (15 minutes).

a) Analysis from Feed Industry Technology Laboratory 2020, [b]) Analysis from Animal Logistics Indonesia Netherlands (ALIN) Laboratory, Bogor 2020 and [c]) Analysis from Agricultural Technology Laboratory 2020.

The protein content of *Avicennia marina* leaves decreased during the boiling process in P2 treatment (10 minutes), while boiling for 5 minutes and 15 minutes increased protein levels. It is assumed that after 10 minutes of boiling, there was a reduction in the mass of *Avicennia marina* leaves and no further decrease in protein and starch levels, so the percentage of these substances increased. Research by [9] states that boiling for a longer time can increase the absorption of water, crude protein and carbohydrate content. The protein content will be increasingly denatured so that it breaks down into more easily digested amino acids. According to [10] that heating treatment can increase the level of protein digestibility by opening protein structures due to denaturation. However, [11] stated that heating treatment can cause a decrease in protein quality due to the denaturation process and the Maillard reaction at high temperatures. The results of the research by [12] stated that boiling the jatropha seeds for more than 10 minutes can reduce the protein and ricin content of the seeds.

In-Vitro Crude Fiber digestibility (IV-KFD), In-Vitro Crude Fat digestibility (IV-KFtD) and In-Vitro NFE digestibility (IV-NFED)

The different treatment effects on the *In-vitro*digestibility of crude fiber, crude fat, and NFE on the boiling process of mangrove leaves (*Avicennia marina*) using husk ash water are shown in Table 2. The variance analysis showed that the use of husk ash water on mangroves leaves had a significant effect (P <0.01) on the *In-Vitro*digestibility of crude fiber, crude fat, and NFE.

Table 2. Average *In-vitro*digestibility of crude fiber, crude fat, and BETN

Treatment	IV-CFtD	IV-CFD	IV-NFED
P0	18,80^{c}	76,95^{b}	56,82^{d}
P1	25,03^{b}	72,31^{c}	60,00^{c}
P2	31,80^{a}	77,48^{a}	64,57^{a}
P3	14,30^{d}	64,26^{d}	60,79^{b}
SE	0,29	0,32	0,39

Note: Different superscripts in the same column are significantly different (P <0.05).
IV = In-vitro, CFD = Crude fiber digestibility, CFtD = Crude fat digestibility, NFED = Nitrogen Free Extract Digestibility. SE: Standard error

Table 2 shows the highest average digestibility of crude fiber, crude fat, and NFE in P2, P1, P0, and P3 treatments, respectively. Therefore, boiling mangrove leaves using husk ash water at a concentration of 15% (w / v) can improve the *In-vitro*crude fiber, crude fat, and NFE digestibility in the rumen during the digestion process.

The average digestibility value showed an increase in each treatment, with the highest value found in P2, which comprises of IV-CFD: 77.48%, IV-CFtD : 31.80%, and IV-NFED 64.57%. [13] stated that digestibility was carried out to determine the nutrients that can be absorbed for maintenance, growth, and production. Therefore, the digestibility was affected by the feeding level, animal species, feed deficient, feed processing, combination of feed ingredients, and gastrointestinal disorders.

The highest value of IV-CFD in this study was 77.48%. This is because at 10 minutes the fiber components was not damaged, the leaves were not softened and the lignin content was decreased at thattreatment. [14] stated when the leave was soaked for 24 hours and boiled for 90 minutes it reduced the composition of crude fiber. According to[15] the crude fiber digestibility was influenced by several factors, the composition of crude fiber and the rumen microorganism's activity.

The average result of the highest crude fat digestibility using husk ash water was 31.80%. This is because the absorbed fat in the treatment material contained triglycerides (simple fat). [16] reported that the high digestibility of crude fat is due to the chemical structure with the average value influenced by several factors. [17] stated that the factors that influence the digestibility value are the amount and content of nutrients in feed. Furthermore, [18] reported that the hydrolysis with husk ash water is more beneficial than other types of alkaline. It also has the ability to increase the nutritional value of feed by adding minerals contained in the ash and by increasing the consumption, digestibility, and palatability values.

The highest average yield of In-vitro Nitrogen Free Extract Digestibility (IV-NFED) was 64.57% due to the high starch content in the treatment. [19] stated that the high NFE content describes the easily digestible carbohydrate fraction such as starch and sugar. According to [20], NFE contains starch such as monosaccharides, trisaccharides, disaccharides, and polysaccharides that are acidic and alkaline with high digestibility. Naturally, BETN is digested more easily by microbes, with an increase in the number of bacteria, thereby, making it possible to maximally degrade complex compounds into simpler compounds.

At 15 minutes of boiling time (P3), there was a decrease in the In-vitro Crude Fiber Digestibility (IV-CFD) and IV-NFED due to the damage of feed substances caused by prolonged boiling time using husk ash water. This result showed that more nutrients are hydrolyzed and anti-nutrients are reattached to reduce digestibility. This is supported by research conducted by [21] which stated that prolonged heating damages and decreases several nutrient substances, including protein, vitamins, and fat. Sometimes, heating is desired in feed

processing, however, when it is prolonged, it becomes detrimental, therefore it needs to be prevented. Meanwhile, the lignin value in P3 treatment was high at 12.18% at an optimal limit of 7% with the rest capable of affecting digestibility. Lignin is a limiting factor in feedcomponent digestibility that is resistant to chemical and enzymatic degradation. The higher the lignin contents in the ration, the lower the digestibility of a feed components [22].

Total Phenol and Total Tannin

Table 3 shown the significant effect (P <0.05) of treatments on total phenol and tannin of mangrove leaves.

The variance results showed that boiling using husk ash water increases and decrease the total phenol of mangrove leaves. The average total phenol in P2 treatment was 99.29 mgMAE, with a decrease in value due to a prolonged heating time with husk ash water. According to [23]and [24], the decrease in total average phenol content occurs due to the unstable destructive effect on heating, which makes polyphenol compounds to be easily dissolved in boiling water. [25] stated that husk ash acts as a good absorbent, therefore it can absorb and bind polyphenol compounds.

Table 3. Average total phenol and tannin

Treatment	Total Phenol (mgGAE)	Total Tannin(%)
P0	79,11^{c}	11,10^{b}
P1	103,19^{a}	14,91^{a}
P2	99,29^{b}	10,27^{c}
P3	95,34^{b}	10,89^{c}
SE	0,62	0,33

Note: Different superscripts in the same column are significantly different (P <0.05). SE: Standard error, P0 = 0 minutes, P1 = 5 minutes, P2 = 10 minutes and P3 = 15 minutes of boiling.

The tannin content at 5 minutes of boiling (P1) increased by 14.91% due to the combination of other secondary metabolites and other polyphenol compounds in initial heating process. [26] stated that at high temperatures, catechol oxidase enzyme becomes inactive with enzymatic reaction, thereby, leading to an increase in the tannin content.

In P2 treatment (10 minutes of boiling), the tannin content decreased by 10.27%. This is because a prolonged boiling process tends to loosen the bonds of mangrove leaves tissue which facilitates the osmosis process.Therefore, the tannins come out and dissolve in husk ash / alkaline water. After boiling, the mangrove leaves are soaked for a while in plain water, and during this period the diffusion process occurs. According to [27], diffusion occurswhen the solution in the cell has a high concentration, therefore, the tannins in mangrove leaves dissolve in alkaline water.

Based on the average results, the tannins found in mangrove (*Avicennia marina) leaves* have been boiled tends to decrease compared to without boilingwith high yield content. Tannins derived from forages are generally condensed and havestronger complex bonds with proteins compared to when it is hydrolyzed. According to [28]and [29], the strong bond between tannins and protein affectson protein digestibility. [30] stated that the condensed tannins are polymers of flavonoid compounds with carbon-carbon bonds in the form of catechin and gallocatechin.

IV. CONCLUSION

The results of the experiment showed that the best boiling time for mangrove leaves (*Avicennia marina*) was P2 (10 minutes boiling), with an average of IV-CFD (77.48%), IV-CFtD (31.80%), and IV-NFED (64.57%). It also showed a decrease in the value of total phenol and tannin of 99.29 mg GAE, and 10.27%, respectively.

V. RECOMMENDATION

Further research needs to be carried out by making complete rations for goats based on mangrove leaves (*Avicennia marina*) in *in-vivo* methods.

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