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Research Paper

In-vitro Nutrient Digestibility of combination Mangrove Leaves (*Avicennia marina*) And Rice Straw as Ruminant Feeds

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ABSTRACT
This study aims to determine the in-vitro nutrient digestibility of combination mangrove leves (Avicennia marina) and rice straw as ruminants feed. The nutrient measured are digestibility of dry maters, organic matters, crude protein, crude fiber, crude fat and rumen fluid characteristics (ph, NH_3 and VFA content). The experiment using randomized block design (RBD) with 4 treatments and 4 replications for each treatment. This treatments are P0 : 25% mangrove leaves and 75% rice straw., P1: 50% Mangrove leaves and 50% rice straw)., P2 : 75% mangrove leaves and 25% rice straw., and P3: 100% mangrove leaves only. The parameters measured were digestibility of dry matters, organic maters, crude protein, crude fiber, crude fat and rumen fluid characteristics. To determine the differences between treatment mean was using Duncan's multiple range test (DMRT). Based on the analysis of variant the results of experiment show that the combination of mangrove leaves and rice straw has a very significant effect (P <0.01) on the dry matter digestibility and rumen characteristics. The best treatment from this study was the combination of 75 % mangrove + 25 rice straw as animal feeds.

***KEYWORD**: Mangrove, rice straw, nutrients digestibility, and rumen fluid characteristic*

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I. INTRODUCTION

Feed is a major factor in ruminant production. Ruminant feed consists of forages, concentrates, vitamins and minerals sources. Forages that are commonly used as fodder in rural areas are field grass and agricultural by-products, as well as some introduced grasses. The availability of forages has been decreasing time by time. This is caused by increasing the price of feeds for animal and the narrowing of land for the planting of grasses, due to the conversion of land for food and settlement purposes. The solution to deal with such conditions, it is necessary to look for another potential new resources to be used as alternative feeds. The effort that can be done is to utilize agricultural waste as feed for livestock (Syamsu *et al*, 2006). One of the ingredients that can be used as feed for livestock that is available in large quantities and is easily to obtained are rice straw and mangrove.

Rice straw is a by-product of rice plants which are widely used as a source of feed for ruminants, especially by traditional farmers in developing countries, including Indonesia. According to Antonius (2009) the dry matter content of rice straw was 44.88%, crude protein 4.55%, crude fiber 30.31%, acid detergent fiber (ADF) 46.72% and total digestible nutrient (TDN) 51.47%. Sutardi (1982) stated that the crude fat content in rice straw was 1.55%, and digestible energy (DE) 1.9% kcal/kg. The cell wall contained NDF 45-71%, cellulose 25-33%, lignin 5-12% and silica 16-22% (Roxas, 1984).

In Indonesia, rice straw is an agricultural waste as alternative feed and also for plant fertilizer. With so much availability, farmers use rice straw as animal feed, especially during the dry season where some farmers also find it difficult to obtain high-quality forages (Roxas, 1984). With this rice straw can be said as a source of feed for livestock when there is a lack of available good quality forages.

In addition to rice straw which can be used as animal feed, one of the potential forages as feed but not yet widely used as animal feed is mangrove leaves (*Avicennia marina*). In terms of nutritional content, mangrove leaves can be used as source of feed protein. So far, mangroves function only to prevent sea abrasion, maintain water quality and become a habitat for a variety of living things. Judging from the number of mangrove plants that grow in coastal areas, this might be best opportunity to the farmers to utilize them as

animal feeds. Until now there have not been many studies on the potential of mangrove leaves as feed for ruminants.

Avicenia marina leaves analysis showed contain vitamin B 2.64 mg /100 g, vitamin C 15.32 mg/100 g, crude fiber 8.7% and carbohydrates 13% and also high in minerals. *Avicenia marina* also contains dry matter 32.42%, ash 15.3%, crude protein 15.14%, crude fat 2.17%, Ca 0.625% and P 0.28% (Ghost et al., 2015). This is very much different compared to rice straw where the protein content of rice straw is only 3-5%. Rice straw contains high crude fiber, and also of lignin and silica which can interfere the digestion process in the rumen so that the digestibility of rice straw is very low. In ruminants the largest process of digestion of food for forage material occurs in the rumen with the help of rumen microbes. The characteristics of rumen are influenced by the type of feed consume, the nutrients content especially

According to Tillman et al (1991) feed digestibility is very important to know because it can be used to determine the quality of the feed. There are several methods used to determine the digestibility level of a feed ingredient, including using *in-vitro, in-sacco* or *in-vivo* methods. The in-vitro method is a method of estimating indirect digestion carried out in the laboratory by mixing the proces that occur in the rumen of ruminants.

Mangrove leaves have not been widely used as fodder forage, because there is no knowledge of the utilization of mangrove leaves by farmers on the coast, therefore it is necessary to conduct research to determine the digestibility of mangrove leaves, so that after doing research it can be compared with rice straw, whether the digestibility of mangrove leaves is better than rice straw or vice versa. The results of the research are expected to determine the best value of combination of rice straw and mangrove leaves as ruminant feeds.

II. MATERIALS AND METHODS

The materials used in this study were rice straw, mangrove (*Avicennia marina)* leaves, aquades, rumen liquid, McDougall 'solution as a buffer and chemicals for the analysis of *in-vitro* nutrient digestibility and rumen characteristics.

The equipment used is a set of tools for measuring in-vitro such as shaker waterbath, gauze, fermentor, filter paper, vacuum pumps, water flasks, cups, rubber covers, ovens, desiccators, scale, aluminum foil, ph meter, thermometers, and test tubes. The tools used to make Mc Dougalls' solution are beaker glass, measuring flask, magnetic stirrer, erlenmeyer, pH meter, pipette and a set laboratory equipments.

The method used in this study is an experimental method, using a randomized block design (RBD) with 4 treatments and 4 replications. The treatments was P0: 75% rice straw + 25% mangrove leaves., P1: 50% rice straw + 50% mangrove leaves., P2: 25% rice straw + 75% mangrove leaves and P3: 100% mangrove leaves.

Implementation

Mangrove leaves are taken at coastal areas of Tarusan village and rice straw is taken in paddy fields in Padang. There are dried by drying in the sun, then grind to be flour. Rumen fluid is taken at the Padang abattoir. In-vitro digestibility are based on principles of Tilley and Terri (1963). The prepared ration sample was weighed as much as 2.5 grams and put into a fermentor, added with artificial saliva solution (McDougall solution) as much as 200 ml at 39^{o}C and pH ± 6.9 and rumen liquid as much as 50 ml as inoculum.

Anaerobic incubation be done for 48 hours in a shaker waterbath at 39^{o}C. The fermentation liquid is centrifuged at a speed of 3000 rpm for 5 minutes, separating the supernatant and the precipitate, filter with whatman paper No. 41. Supernatant is used to analyzed the characteristics of rumen fluid while the sediment is dried in an 60^{0}C oven for 48 hours, then ready to be analysed.

Dry matter are measured by drying method Thermogravimetri. Organic matter measured using direct method, crude fat using Soxhlet method, crude protein using micro Kjeldahl, and crude fiber using Gravimetri method. For measuring the characteristics of rumen fluid, pH is measured using a pH meter, NH3 is carried out by Conway microdifusion, and VFA levels by Steam destillation techniques.

III. RESULTS AND DISCUSSION

Digestibility of Dry matter, Organic matters, Crude Protein, Crude Fiber and Crude Fat

Average nutrients digestibility of dry matter, organic matter, crude protein, Crude fiber and crude fat on combination treatments of rice straw and mangrove leaves (*Avicennia marina*) can be seen in Table 1. The results of the analysis showed very significantly different (P <0, 01) to the digestibility of dry matter, organic matter, crude protein, crude fiber and crude fat among the treatment means.

Table 1. *In-vitro* Nutrient digestibility of mangrove of leaves (*Avicennia marina*) and its combination with rice straw.

Digestibility	Treatments				SE
	P0	P1	P2	P3	
Dry matter	44,39[a]	46,68[ab]	50,14[b]	56,08[c]	1,25
Organic metter	46,40[a]	51,10[ab]	57,51[bc]	63,74[c]	2,58
Crude protein	41,83[a]	44,06[a]	46,58[bc]	51,14[c]	0,97
Crude fiber	61,53[a]	64,07[ab]	67,43bc	69,96[c]	1,70
Crude Fat	66,87[a]	67,80[a]	71,40[ab]	74,02[b]	1,67

Note: Different superscripts in the same row very significant different (P <0.01)
SE: Standard Error

From the research it was found that the higher percentage of rice straw in each treatment, the lower dry matter digestibility, this was caused by the lower nutrient composition of rice straw especially crude protein and higher the fiber content such as lignin, cellulose, and silica, that caused difficult to digest by rumen microbes. Arora (1995) states that feed will be easily digested if the crude fiber content especially lignin and silica are low. Lignin is a component that when digested does not provide the final results of the digestive process and lignin is also will inhibit the digestion process in animals. According to Tillman et al (1989) stated that lignin content of the plants highh as plants age increase and highest level when the plants mature.

The result of the research as indicated by Table 1, the highest digestibility of nutrients is found in treatment P3 (100% Mangrove Leaves). This is due to there is no combination with rice straw. This experiment shows that the combination with rice straw as indicated by treatments P0, P1, and P2 were greatly affects the digestibility of dry matter, organic matter, crude protein, crude fiber and crude fat. Rice straw contains high enough crude fiber and many other limiting factors making it difficult for animals to digest. According to Sutardi (1982) the limiting factors in the utilization of rice straw are lignin content which form complex compounds with cellulose so that the structure of cellulose are difficult to digest by rumen microbes. In rice also cell walls are covered by silica crystals that also so difficult to be hydrolyzed by enzymes in the rumen animal. Rice straw has a very low protein content amount of 3-5%.

From the Table. 1 shows that the digestibility of organic matter is directly proportional to the digestibility of dry matter. Acccording to Ismail (2011) digestibility of organic matter is closely related to digestibility of dry matter, because in dry matter composition include some organic materials. Tillman et al (1989) explain the digestibility of organic matter is higher than the digestibility of dry matter due to increased digestibility of organic matter will also increase the digestion of dry matter. This is consistent with the experiment obtained where the digestibility of organic matter is higher than the digestibility of dry matter. The same results are also found in the average digestibility of crude protein and crude fiber. The highest nutrients digestibility was found in the P3 treatment and the lowest was in the P0 treatment. This shown that the combination of rice straw with mangrove leaves has not increased the digestibility of crude protein and crude fiber of the combination feeds.

From Table.1 there is an indication that increase the digestibility of crude fiber of the combination feed along with the increase the percentage of mangrove leaves, because Mangrove Leaves has hight in crude protein content. Budiman et al (2006) stated that supply of ready-to-digest energy and protein in feed are capable to initiate the growth of fiber-digesting bacteria.

The higher protein content in feed can help provide food for rumen microbes to carry out metabolic processes, so that the fermentation process in the rumen can take place properly and also increase the digestibility of crude protein. Mcdonald et al (1988) explained that the digestibility of crude protein is closely related to the protein content of the feed where the higher the protein the higher the protein digestibility in the rumen. The crude protein content of mangrove leaves is quite high at 13.37% that can be very utilized as animal feed ingredients. The protein content of this research slightly different compared to Reddy and Yang (2006) that is protein content of 11.53%. This difference can occur because it is influenced by many factors : habitat, soil, age, climate and metabolic rate.

The nutrients composition of mangrove leaves is quite different compared to rice straw that is mangrove leaves have a high protein content, on the other hand the rice straw high fiber content. Lopez (2005) states that the nutrients of the rice straw consists of 40% cellulose, 30% hemicellulose, 15% lignin and 15% silica, that causes the digestibility of rice straw will decrease. This is consistent with the results of this research that the digestibility of the combination feed with rice straw result in low digestibility. The lowest digestibility were found in the P0 treatment with highest level of rice straw, 75% combination with 25% mangrove leaves.

The crude fat digestibility as shown in Table 1indicate the highest fat digestibility in P3 treatment 74.62%. Based on the analysis of variance that the treatment P3 (100% mangrove leaves) gave the highest nutrients digestibility and very significantly effect (P <0.01) on the digestibility of crude fat. Arora (1995) states that the digestibility of a feed depent on the quality of nutrients contained in the feed, so that it affects on the growth of rumen microorganisms. Chemical composition of feed affected on nutrients digestibility due to the digestibility itself depends on the harmony of food substances contained.

Characteristics of rumen

Average values of acidity (pH), NH_3 and VFA of rumen fluid from combination of feed rice straw and mangrove leaves can be seen in Table 2. The results of the analysis showed very significant different (P<0,01) to the value of NH_3 and VFA.

Table 2. Rumen Characteristic of mangrove leaves (*Avicennia marina*) and its combination with rice straw.

Characteristics	Treatment				SE
	P0	P1	P2	P3	
pH	6,86	6,85	6,88	6,79	0,02
NH_3 (mg/100ml)	8,50c	10,62b	11,79b	16,68a	0,57
VFA (mM)	92,50c	105,00b	116,25a	117.50a	1,76

Note: Different superscripts in the same row very significant different (P <0.01)
SE: Standard Error

Rumen pH obtained in this study ranged from 6.79 to 6.88 that is still in normal conditions for the growth of rumen microbes. Jamarun and Mardiati (2013) explain that the normal degree of acidity (pH) in the rumen is between 6.0 to 7.0. According to Ismail (2011) and Arora (1995) there was no significant difference between treatment on the pH of rumen due to the presence of balancing between VFA and NH_3. The pH value of the rumen fluid is determined by the amount of bicarbonate (HCO_3) and phosphate (HPO_4^2) originating from the flow of saliva that enters the rumen.

The highest NH_3 level was obtained in the treatment of P3 (100% mangrove leaves) which was 16.68 mg/100 ml, this showed that the combination of rice straw and mangrove leaves was able to increase rumen microbial activity. High levels of NH_3 in treatments P1, P2 and P3 are due to contribution of crude protein present in mangrove leaves. The lowest NH_3 levels were obtained in the P0 treatment (75% rice straw+25% mangrove leaves) which was 8.50 mg/100ml. Nolan (1993) stated that the concentration of ammonia in the rumen depends on the protein content in the feed. In this study the levels of ammonia obtained had exceeded the minimum requirement of NH_3 in the amount of 5 mg/100ml. While the optimum amount of NH_3 levels in the rumen ranges from 85-300 mg/l or 8.5-30 mg/100ml (McDonald et al., 2002). Ammonia concentrations area very important measure to determine the rate of microbes growth and activities in the rumen. Moante et al. (2004) explained that the concentration of ammonia was determined by the level of feed protein consumed, rate of passage, and the level of acidity (pH) of the rumen.

The average VFA production in this study sequentially from the P0 to P3 treatments was 92.50 mM; 105 mM; 116.25 mM; 117.50 mM. According to Mcdonald et al (2002) the normal VFA range contained in the rumen is 80-160 mM. High VFA production is influenced by the digestibility of organic matter. Rahmawati (2001) explained that the digestibility of organic material can also indicate the production of ammonia and VFA in the rumen, the higher the digestibility value of organic material indicates that the production of ammonia and VFA in the rumen is also high.

IV. CONCLUSION

From this study it can be concluded that the highest nutrients digestibility of the feed combination mangrove leaves and rice straw it can be show in P3 treatment (mangrove leaves without rice straw) based on digestibility of dry matter, Organic matter, crude protein, crude fiber, crude fat and the rumen characteristics. It can be stated that rice straw can not improved the digestibility feed combination due to very low nutrients quality.

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