Chemical composition and rumen fermentation profile of mangrove leaves (Avicennia marina) from West Sumatra, Indonesia

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Abstract. Jamarun N, Pazla R, Arief, Jayanegara A, Yanti G. 2020. Chemical composition and rumen fermentation profile of mangrove leaves (Avicennia marina) from West Sumatra, Indonesia. Biodiversitas 21: 5230-5236. This study aimed to determine the potential of mangrove leaves of Avicennia marina for ruminant animal feed. Laboratory tests were carried out on A. marina with three replicates. Parameters measured were proximate and fiber contents, rumen fluid profile (pH, NH3, and VFA), digestibility of nutrients (DM, Ash, CP, CF, NDF, ADF, cellulose, and hemicellulose), macro and micro mineral contents, and phytochemical compounds. The results showed the nutritional content of A. marina were CP 13.37%; Ash 7.17%; lignin 7.34%; TDN 79%. Rumen fluid profile is in reasonable condition, digestibility of food substances is more than 50%, rich in macro and micro minerals and contains phytochemical compounds such as phenols, steroids, triterpenoids, and tannins. Macro and micro mineral contents of Ca 0.38%, Na 0.20%, Mg 0.20%, K 0.48%, P 0.51%, S 0.01%, Cl 1.03%, Fe 388 ppm, Zn 164 ppm, Mn 211 ppm, and Cu 128 ppm. This research concludes that A. marina is very potential to be used as a ruminant animal feed.

Keywords: Avicennia marina, mangroves, minerals, phytochemicals, proximate, rumen fluid

INTRODUCTION

Indonesia is a country with the most extensive mangrove forests globally (Richards and Fries 2016; Bunting et al. 2018). Indonesia’s reliable mangrove forests are currently 3,361,216.61 ha (Rahardian et al. 2019). Mangrove forests help to reduce the impact of hurricanes, large waves, and winds from tropical cyclones. Mangrove trees reduce wave energy as they pass through mangrove forests and become barriers between streams and land (United Nations Environment Program 2014). When the sea is high tide, mangrove forests are flooded with water, and at low tide, thick mud covers the surface of the soil, which stores wealthy organic material (FAO 2007).

Avicennia marina (Forsk.) Viehr.) is a mangrove tree species almost always found in major mangrove ecosystems (Tomlinson 1986). Local people use this plant’s stems and twigs for firewood, furniture, building materials, boat balancing joints, and fishing net dyes (Armitage 2002). These products are harvested on a small and large scale, contributing to local livelihoods and national exports.

Avicennia marina leaves have a pointed shape at the tip and are green at the front and grayish at the bottom with about 5-11 cm. The flowers are small round with a diameter of about 0.4-0.5 cm and yellow to orange, while the fruit is round with a pointed tip and smooth-haired surface, green with a length of 1.5-2.5 cm and a width of 1.5-2.0 cm (Kitamura et al. 1997). In the coastal areas of Indonesia, people use their leaves to feed goats. These leaves fall off, and the amount is quite adequate as a forage source for animal feed. Nevertheless, to date, there is little research that explores the potential of A. marina leaves as ruminant feed. This study aimed to evaluate A. marina leaves’ possibility as ruminant feed in terms of nutritional content, phytochemicals, digestibility, and rumen fluid profile in vitro. This research held on Sebelas Tarusan Sub-district, Pesisir Selatan District, Province of West Sumatra, Indonesia. The mangrove area mostly consists of A. marina, but there are also Avicennia alba plant clusters in some locations.

MATERIALS AND METHODS

Sample collection and nutrient analysis

The materials used in this experiment consist of Avicennia marina leaves and fruit, Tithonia diversifolia leaves, Gliciricida sepium leaves, Leucaena leucocephala fruit, and leaves. A. marina leaves were taken from the South Coast mangrove forest, Sebelas Tarusan Sub-district, Pesisir Selatan District, West Sumatra, Indonesia. T. diversifolia, G. sepium, and L. leucocephala leaves were collected from the experimental gardens of the Faculty of Agriculture, Andalas University, Padang, Indonesia.
Phytochemical analysis

Phytochemical analysis was carried out by the extraction method based on Franswort (1996). Before phytochemical analysis, A. marina fruit and leaves, T. diversifolia leaves, G. sepium leaves, L. leucocephala fruit, and leaves were ground into flour, put into a bottle, added with 90% methanol solvent in a ratio of 1:3 (w/v), macerated with solvent methanol 3x24 hours and every 24 hours the methanol solvent was replaced. The maceration results were then filtered using Whatman filter paper no. 42 so that the resulting filtrate. The filtrate was subjected to several phytochemical screening tests, i.e., alkaloid, flavonoid, phenolic, saponin, steroid, and triterpenoid tests. For the alkaloid test, the chloroform layer was added ten drops of H2SO4 and shaken slowly, allowed to form an acidic layer. A layer of acid (the part under the clear ring formed from the addition of H2SO4) was taken, and one drop of Meyer reagent was added. A white mist characterized positive reactions. The flavonoid Test layer of water as much as 2 ml from the preparation stage was taken and put into a test tube. Then 1-2 grains of Magnesium was added, and three drops of HCl were added. Positive samples contain flavonoids. If they form orange to deep yellow, they contain flavonoids. If they form blue to purple, they contain saponins if they are formed permanently, which does not disappear within 15 minutes. Steroid and triterpenoid test was performed by taking the chloroform layer from the preparation stage and put into a Pasteur pipette, which contains charcoal. The filtrate that comes out of Pasteur's pipette was inserted into three holes on the drip plate, adding one drop of anhydrous acetic acid and one drop of H2SO4. Positive samples containing steroid compounds were shown in blue to purple, while positive samples contain triterpenoid compounds if produced in red.

Determination of mineral contents

Avicennia marina leaves and fruits, T. diversifolia leaves, G. sepium leaves, L. leucocephala fruits and leaves were dried in an oven at 60°C for 24 hours. Then the sample was ground and filtered using a 20 mesh filter to obtain a powdered sample. One gram of powdered sample was added with 2 ml of distilled water, then dried in the furnace at 150 °C for 15 minutes. Then the sample was cooled at room temperature. Dilute using aqua dest to a volume of 25 ml, and then the sample was filtered using 45 mesh filter paper. The destruction results were analyzed in the mineral content of Fe, Zn, Mn, Cu, and Co using the Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) tool.

Figure 1. Location of Avicennia marina leaf sampling in Sebelas Tarusan Sub-district, Pesisir Selatan District, West Sumatra, Indonesia.
RESULT AND DISCUSSION

Chemical composition of Avicennia marina leaves

Avicennia marina leaves contain 13.37% crude protein (CP) with 79% Total Digestible Nutrient (TDN) (Table 1). This value makes A. marina leaves included in the category of high-quality forage. (Jamarun and Zain 2013) classify forage quality in three categories based on CP and TDN content, namely low quality forage (CP <4%, TDN> 40%), medium quality forage (CP 5-10%, TDN 40-50%) and high-quality forage (CP> 10%, TDN> 50%). High forage CP and TDN are needed by livestock to optimize their growth and production. Some CP in the rumen will be overhauled into NH₃ by proteolytic enzymes produced by rumen microbes. NH₃ concentration is an important source of N for ruminant microbes and is used for microbial protein synthesis. NH₃ production is influenced by the amount of protein in feed ingredients (Pazla et al. 2018a). High TDN illustrates that these leaves have a high digestibility, so only a few nutrients come out as feces.

The high CP content in A. marina leaves is caused by soil organic matter (OM). (FAO 2007) In mangrove areas, there is high organic matter in thick mud that lines the surface of the land at low tide. Land influences nutrition, plant growth, and development. Plants will grow and develop optimally if the soil conditions in which they live fit the nutritional and nutrient requirements. According to Kennish (2000), mangrove roots can accumulate sediment and play a role in forming soil formations. Mangroves are suppliers of organic material to provide food for organisms that live in the surrounding waters. Sedimentation that occurs in mangrove areas is different from other regions. Sources of sedimentation come from the land, sea, and mangrove areas in the form of deposited leaf deposits, twigs, and dead organisms that are collected so that this region is rich in organic and mineral materials such as N, P, K, Fe, and Mg (Nugroho et al. 2013). A. marina leaves’ crude protein value in this study was higher than reported by (Handayani 2013), 11.04%, and lower than (Ghosh et al. 2015), 15.14%. This variation in crude protein values can be caused by plant age, soil fertility, and the source (Jama et al. 2000).

The high protein content of a feed ingredient will also increase the value of organic matter. This is due to crude protein is part of organic material. Table 1 shows the organic matter content of the leaves is also relatively high, at 92.83%. High organic matter will automatically reduce the value of ash content. The higher the ash content, the worse the quality of feed ingredients (Suparjo 2010). (SNI 2017) suggests that cattle’s low ash content is 12% maximum, while poultry livestock is 8%. The low-crude fat (CF) content in these leaves (3.18%) is advantageous in ruminant animals. The high-fat content in feed ingredients has been reported to be a cause of digestive and metabolic disorders in cattle (Atteh 2002). Preston and Leng (1987) supported this, and Palmquist and Jenkins (1980) stated that ruminant animal feed ingredients’ standard fat content is below 5%.

Crude fiber is needed for ruminants to maintain the development of rumen microbes. Crude fiber that is too low will interfere with the digestive system of ruminants. The A. marina crude fiber content (12.18%) is almost equal to the minimum requirement of crude fiber content in feed ingredients, which is 13% for cattle, according to Sudarmono and Sugeng (2008).

The NDF content is closely related to feeding consumption because all its components meet the rumen space and are slow to digest. The lower the NDF content, the more food can be consumed. ADF’s content (cellulose, lignin, silica) is an indicator of forage digestibility because lignin’s content is part of an indigestible fraction (Pazla et al. 2020). NDF is always higher than ADF because ADF does not contain hemicellulose. NRC (2001) suggests a minimum of NDF in feed 21% with ADF 19%. The percentage of ADF and NDF content given to livestock should be 25-45% ADF and 30-60% NDF from forage dry matter (Anas 2010). The average value of lignin that livestock can tolerate is 7% (Goering and Van Soest 1970). The NDF, ADF, and lignin values of these leaves are still within the tolerance range for ruminant animal feed.

Table 1. Chemical composition of Avicennia marina leaves

| Chemical composition          | %    |
|-------------------------------|------|
| Dry matter                    | 89.19±0.07 |
| Ash                           | 7.17±0.90 |
| Organic matter                | 92.83±0.11 |
| Crude protein                 | 13.37±0.23 |
| Crude fiber                   | 12.18±0.27 |
| Crude fat                     | 3.18±0.39 |
| NDF                           | 45.99±0.41 |
| ADF                           | 35.95±0.43 |
| Cellulose                     | 23.10±0.42 |
| Hemicellulose                 | 10.03±0.67 |
| Lignin                        | 7.34±0.72 |
| TDN                           | 79.00±0.98 |

Table 2. Rumen fluid profile and nutrition in vitro digestibility from Avicennia marina leaves

| Parameters                  | Value  |
|-----------------------------|--------|
| Rumen fluid profile         | 6.79±0.02 |
| pH                          | 117.5±0.04 |
| VFA (mM)                    | 16.88±0.51 |
| NH₃ (mM)                    | 56.68±0.54 |
| Nutrition digestibility (%) | 63.74±0.67 |
| DM                          | 69.96±0.62 |
| OM                          | 61.37±1.58 |
| CP                          | 57.44±0.96 |
| CF                          | 51.44±0.92 |
| NDF                         | 60.24±0.73 |
| ADF                         | 62.03±1.04 |
The high protein content of A. marina leaves in this study was within the normal range for the growth and development of rumen microbes, mostly bacteria (Table 2). The ideal pH for fiber digestion is 6.4-6.8 (France and Siddon 1993). The pH below 6.2 will reduce the microbial population drastically so that the energy generated from the rumen fermentation process is low (Van Soest 1982).

Rumen fluid profile and nutrition digestibility

The pH value of the rumen fluid from A. marina leaves in this study was within the normal range for the growht and development of rumen microbes. The VFA value produced by A. marina leaves sufficient for rumen microbes to grow and develop optimally. Mc Donald et al. (2010) stated that the optimum VFA condition is 80-160 mM. The high value of the resulting VFA indicates that A. marina leaves are a feed material with a high level of fermentability, which is suitable as a source for forage for ruminants. The low lignin content will make it easier for enzymes from rumen microbes to penetrate cellulose and hemicellulose, which are the main components of forming VFA. The high protein content of A. marina leaves also contributed to the high VFA value. There is a positive correlation between high crude protein values and VFA values (Jamarun et al. 2017b; Jamarun et al. 2018).

The concentration of NH₃ in A. marina leaves in this study was included in the amount of NH₃ that supports rumen microbial growth, namely 6 mM-21 mM (Mc Donald 2010). Paengkoum et al. (2006) stated that the maximum NH₃ concentration required for rumen microbes to digest feed was 3.57-14.28 mM. Rumen microbes use NH₃ as a N for microbial protein synthesis source, and its value is also influenced by crude protein levels (Pazla et al. 2018). The pH, VFA, and NH₃ values of A. marina leaves in this study were almost the same as other forages such as T. diversifolia (6.78, 125.88mM, 22.48mM) and Elephant grass (6.79, 87.53 mM 20, 41mM) (Jamarun et al. 2019).

Feed digestibility is a large amount of feed that livestock can utilize to meet basic needs and production. Based on Table 2 above, it can be seen that rumen microbes can digest more than 50% of the nutrients from...
these leaves; this is due to the low lignin content. Lignin in feed ingredients can reduce digestibility, as reported by Jamarun et al. (2017a). Rumen microbes can digest food substances in feed ingredients when the lignin content is low. Imsya et al. (2013) stated that lignin in plant cell walls limits the feed material’s digestibility. Crude protein content in feed ingredients will also affect the digestibility level of a feed ingredient. The high protein content of A. marina leaves will provide more nitrogen for the growth of rumen microbes. Profitable microbial growth will lead to better feed digestibility (Febrina et al. 2016).

Macro and micro mineral contents

The amount of macro minerals (Ca, Na, Mg, K, S, P, and Cl) A. marina leaves is higher than that of A. marina fruit, T. diversifolia, G. sepium leaves, and L. leucocephala leaves (Table 3). The high mineral content is because the soil in the mangrove forest is rich in minerals and organic matter. Nugroho et al. (2013) explained that the sedimentation in the mangrove area is different from other depositional environments. Sources of sediment in mangrove areas come from land and sea (allochthonous) and from the mangrove area itself (autochthonous) in the form of heaps of fallen leaves, twigs, and dead organisms deposited in the mangrove area and contain a lot of organic and mineral matter (N, P, K, Fe, and Mg). The allochthonous sediment is deposited in mangroves through sediment transport, where suspended particles are carried by tidal currents stored in the mangrove area. Because mangroves have a unique root system, they can reduce tidal currents in the mangrove area.

Macrominerals are needed by livestock to build body structures such as bones and teeth (Jamarun and Zain 2013). P mineral is an important mineral to support the growth of rumen microbes digesting fiber (Suyitman et al. 2020). Sulfur minerals are needed by rumen microbes to form amino acids that contain sulfur (Bal and Ozturk 2006). Mineral P and S can stimulate rumen microbial performance to improve feed digestibility (Pazla et al. 2018b). Mineral P, S, and Mg were able to increase rumen VFA concentrations (Febrina et al. 2016). Minerals Ca, P, and Mg at normal levels in the rumen can increase rumen microbial activity in digesting cellulose and VFA (Adriani and Mushawwir 2009). Na functions to increase appetite and maintain osmotic pressure (Jamarun and Zain 2013). A. marina leaves' mineral content is still in the normal range to help supply the mineral needs. According to Mc Dowell et al. (1983) the range of normal values for mineral content in animal feed for Ca is 0.17-1.53 %, Mg 0.05-0.25%, P 0.17–0.59%, K 0.50-0.70%, Na 0.01-0.06%, S 0.08-0.15%.

Fe's mineral content in A. marina leaves relatively high compared to A. marina fruit, T. diversifolia, G. sepium, and L. leucocephala fruit, but L. leucocephala leaves have slightly higher Fe (Table 4). Nugroho (2008) states that Fe content in grass is usually 100-200 ppm while in legume 200-300 ppm. According to Darmono (2007), mineral Fe is used in the enzymatic metabolism of hemoglobin in the livestock body.

The minerals Zn, Mn, and Cu in A. marina leaves show the highest value than other forages in Table 3. Nugroho (2008) states that Mn functions as carbohydrate synthesis, mucopolysaccharide, and enzyme systems, such as pyruvate carboxylase and arginine synthetase. In addition to enzymatic reactions, Mn also functions for growth and reproduction of livestock, Owuka et al. (2001) which states that Mn's mineral content in goats ranges from 2.98-13.9 mg/dl. Based on these data, it can be concluded that the livestock reared with A. marina leaf-based feed does not experience Mn mineral deficiency because the Mn content in the forage is sufficient. Nugroho's (2008) opinion states that Mn mineral deficiency rarely occurs because Mn levels in the feed are enough for livestock needs.

Zinc (Zn) is the micro-mineral often deficient for rumen microbial growth (Leng 1991). To maximize feed degradation in the rumen, the adequacy of Zn minerals is critical, given the specific role of Zn in increasing rumen microbial growth and as an activator of many enzymes (Elihasridas et al. 2012). Mineral Zn can stimulate rumen microbial growth and improve the appearance of livestock. The Zn content in Indonesia's ruminant animal feed ranges from 20-38 mg/kg of dry ration material (Little 1986). This value is far below the need for rumin microbe, namely 130-220 mg/kg of dry ration material (Hungate 1966). Zn deficiency can interfere with rumen microbial metabolism and decrease enzyme activity. Therefore to achieve high feed degradation and microbial growth in the rumen, Zn must be available in sufficient and balanced amounts. The amount of Zn in A. marina leaves still in the range to meet the needs of ruminen microbes.

According to Darmono and Bahri (1989), the low Cu in animal feed sources will adversely affect Fe intake, even though the Fe content in the feed is adequate. It was reported that low Cu content in forage is one of the causes of anemia in livestock. According to Little (1986), several types of grass or forage are used as sources of feed for ruminants in Indonesia, especially on Sumatra, whose Cunya content is below average (low) limits. As reported by Prabowo et al. (1997) and Mathijs (1988) from the results of field examinations that are commonly used as the main feed for goats generally have Cu content below the standard (critical) limit. The Cu content will be even lower during the dry season. This results in animals that consume them, thus experiencing mineral deficiencies. Mc Dowell et al. (1992) states that Cu requirements are influenced by the levels of other mineral rations, which increases the need for ruminants in the presence of high molybdenum (Mo) levels. NRC (1989) recommends a Cu requirement figure of 10 mg/kg for ruminants. The mineral value of Cu in the leaves of the A. marina is sufficient for livestock needs. The definition of Cu will cause bone disorders (paralysis), joint swelling, bone fragility. Pigment deficiency in Cu-deficient animals and humans. However, giving enough copper salt, especially to sheep, will cause accumulation in the liver. Sheep are sensitive to 20-30 mg Cu/kg of Cu ration (Tillman et al. 1998).

Phytochemical contents

The phytochemical contents of the samples were varied (Table 5). Ruminant animals are more resistant to feed
ingredients that contain phytochemicals than poultry. This is due to some phytochemicals that can be used to simplify the process of feed metabolism. Tannins are phytochemicals that function as by-pass protein agents. This means that the protein from feed ingredients eaten by livestock will be protected from rumen bacteria’s degradation to enter the small intestine. This tannin can only release its bonds with feed ingredients by enzymes in the small intestine and low pH levels, while in the rumen, tannins are problematic in the rumen bacterial break and normal rumen pH (Jamarun and Zain 2013). Tannin addition increased neutral detergent insoluble crude protein (NDICP) and acid detergent insoluble CP (ADICP) (Jayaneagara et al. 2019). However, the levels should not be excessive because if excessive phytochemicals can hurt livestock productivity. Phytochemicals in feed ingredients such as T. diversifolia, G. sepium, and L. leucocephala have been tested in livestock, apparently still in normal conditions for consumption by ruminant animals and do not show a negative effect on livestock metabolic activities (Arief et al. 2020; Pazla 2018; Ningrat et al. 2018). Jamarun et al. (2019) tested T. diversifolia by using up to a 100% level, still having a positive effect on the digestibility of dry matter and organic matter and the fermentability of rumen fluids such as pH, NH3, and VFA, even better when compared to elephant grass. Fausyi et al. (2010) identified many phytochemicals found in T. diversifolia such as phytates, alkaloids, saponins, and far more than A. marina leaves. This study confirms that A. marina leaves are entirely safe for livestock consumption. In conclusion, the research showed that A. marina leaves could be used as alternative feed ingredients for ruminant animals with CP content of 13.37%, lignin 7.34%, rich in macro and micro minerals, and containing phytochemical compounds such as tannins, steroids, and triterpenoids.

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