Research Article

In vitro Evaluation of Mangrove Leaves (*Rhizophora apiculata*) and Native Grass Based on Phytochemical, Nutritional and Fiber Degradability, Rumen Liquid Characteristics and Gas Production

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Abstract | This study aimed to evaluate the combination of mangrove leaves (*Rhizophora apiculata*) and native grass based on phytochemical contents, nutrient and cells fiber digestibility, rumen fluid characteristic, and gas production using an in vitro methodology. Samples of Hay Mangrove Leaves (HML) (*Rhizophora apiculaca*) and Native Grass (NG) were arranged into dietary tretmeants (Dry Matter (DM) Basic). The experimental design used is a randomized block design consisting of 4 treatments with 5 replications. The treatment was P1=60% Hay Mangrove Leaves (HML)+40% Native Grass (NG), P2=50% HML+50% NG, P3=40% HML+60% NG, and P4=30% HML+ 70% NG. Data were analyzed using General Linear Model and Duncan's Multiple Range Test to reveal the significant differences between different treatments applied. The result showed that different ratios between HML and NG contributed significantly differences ($p<0.05$) in phytochemical contents, in vitro nutrient and fiber fraction digestibility, Volatile Fatty Acid (VFA), Ammonia (NH_3), and gas production. No significantly differences ($p>0.05$) were observed in the pH among the treatments. The nutritional degradation, fiber degradation, VFA and NH_3 in the P3 higher than other treatments ($p<0.05$). P3 had digestibility of dry matter, organic matter, crude protein, acid detergent fiber (ADF), neutral detergent fiber (NDF), cellulose, and hemicellulose of 62.82%;65.69%;66.17%;50.67%; 57.22%; 55.84%; 63.27%. VFA and NH_3 is 156.20%;9.84%. The methane gas production in P3 was lowest 20.57 ml/gr DM. This research concludes that P3 (40% hay mangrove leaves + 60% native grass) had the best resulted effect on nutrient and fiber in vitro digestibility, rumen fluid characteristic, and gas production.

Keywords | Hay mangrove leaves, *Rhizophora apiculata,* Native grass, *In vitro*, Digestibility pathway.

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INTRODUCTION

Rhizophora apiculata are dominant plants that can grow in mangrove ecosystems (Dahuri, 2003). *Rhizophora apiculata* has characteristics of modified roots as a support for standing, with root color dark brown, brownish yellow speckled; the color of the main stem is grayish white; brownish old branch color (Rizki et al., 2016). Mangrove's potential can be feed for ruminants, especially the leaves part. The nutritional composition of mangrove leaves contain 11.40% crude protein and 63.50% NFE (Sari et al., 2020). Mangroves contain high crude protein and play a role in rumen microbial growth. There is a breakdown of protein into ammonia which will be used as the main nitrogen resource for microbial synthesis in the livestock's rumen (Russell et al., 2009). The mangrove plant lived in the littoral area are considered to have the potential become forage source (Yanti et al., 2021a; Sari et al., 2021).

Anti-nutritional substances liked polyphenol compounds and tannin are contain in mangrove's leaves (Sari et al. 2022). Hay mangrove leaves processing aims to reduce water content due to harvesting carried out in large quantities, so it is hoped that the nutritional content is maintained. In addition, the tannin content will decrease so that it is not harmful to livestock. Hay processing function also increasing durability for stored and does not rot easily (Suherman, 2019).

Fiber feeds source was native grass which is quite likely by ruminants, especially sheep and goats. Field grass is abundant and easy to obtain, but the nutrient content of native grass is relative low. Rusdin et al. (2009) stated that the content of native grass produces crude protein 3.10-5.89%, crude fiber 34.89-40.68%, and nitrogen free extract 40.35-46.35%. This causes native grass to be combined with other feed ingredients such as mangrove leaves to fulfilled livestock nutritional needs, especially in the coastal area.

The nutrients supply mainly protein of native grass was limited compared to other plants (Rosadi et al., 2018). Exploration of forage resources needed to overcome this problem by utilizing the leaves of *Rhizophora apiculata* as feed source. Indonesia has the second largest mangrove forest in the world with an area of 42,550 km^2 (Sridahr, 2004), while the area of West Sumatra's mangrove forest is 39,832 hectares, with the largest density in the Mentawai Islands Regency (Noegroho, 2013). In Indonesia, mangrove forests often serve as partial range-lands. Ruminant's livestock liked Buffaloes, cattle, goats, and sheeps naturally known to feed mangrove leaves (Kusmana and Sukristijiono, 2016). Based on nutritive value, mangrove leaves get the best forage's cathegory (Hamilton and Snedaker 1984). In other hand, tannins of mangrove leaves affect to methanogenic microbes as anti-methanogenic agent (Makkar et al., 2007). The impact of synergy between feed which contain tannin with fiber as carbohydrate source increasing fiber digestibility and nutrient utilization (Arowolo et al., 2018). This study aimed to evaluate the combination of mangrove leaves (*Rhizophora apiculata*) and native grass based on phytochemical contents, nutrient and cells fiber digestibility, rumen fluid characteristic, and gas production using an *in vitro* methodology.

MATERIALS AND METHODS

Materials

The study was conducted in December 2021 until February 2022. Sample of mangrove leaves (*Rhizophora apiculata*) was carried out from Sasak Village, West Pasaman Regency, West Sumatra. Native grass collected from Limau Manis Village, Padang, West Sumatra used plot method with 1 m X 1 m size. Rumen liquid for in vitro was obtained from slaughterhouse from Kacang Goats (a native Indonesian goat) in Sungai Sapih Village, Padang, West Sumatra. Whole rumen is taken directly from the slaughterhouse to a laboratory where in vitro fermentation equipment has been prepared. The whole rumen is tied at one end so that the liquid does not spill, then put in a clean and airtight plastic bag,put in a bucket during the trip so it doesn't shake and maintains the rumen temperature. Analysis of in vitro, proximate analysis, van soest analysis, rumen liquid analysis, gas production already implemented in Animal Nutrition Laboratory, Andalas University. Phytochemical contents analysis was carried out in Biotechnology Laboratory, Andalas University. The mangrove leaves are taken from the top (3-4 top leaves) like pick tea leaves methods. Mangrove leaves was dried as hay using oven with 30° C temperature for 3 days. The sample of hay mangrove leaves and native grass dried using oven with a set on 60° C during 48 h, then the sample was cut and mashed with grinding machine. The chemical composition of *hay mangrove leaves* and native grass is presented in Table 1. The experiment used randomized block design with 4 treatments and 5 replications. The treatments consisting of P1= 60% Hay Mangrove Leaves (HML)+ 40% Native Grass (NG), P2=50% HML+50% NG, P3=40% HML+ 60% NG, and P4=30% HML+70% NG. The chemical composition of each treatment diet is given in Table 2.

Phytochemical

Samples combination HML and NG of each treatments was mashed into powder. Then determined the total phenol using folin ciocalteu reagents (Singleton and Rossi, 1965), as was performed by (Marinova et al., 2005). The standard was made with 1 ml gallic acid with four different concentrations (120, 140, 160 and 180 ppm) and added with 1 ml of 9% folin ciocalteu, 9 ml H_2O and boiled. Incubated the mixture at room temperature for 5 minutes, then 4 ml H_2O and 10 ml of Na_2CO3 7% were added. The mixture was stirred and incubated at room temperature for 90 minutes. The absorbance was measured using a spectrophotometer (UV-1800 Shimadzu A11455008975) with wavelength of 750 nm. Gallic acid was used as a standard and the result was expressed as milligram of gallic acid equivalents (GAE) per gram of defatted sample.

Links Researchers

Table 1: The chemical composition of feed material (% DM)

Composition (%)	Feed Material	
	Hay Mangrove Leaves	Native Grass
Dry Matter	89.80	87.90
Organic Matter	94.81	88.52
Crude Protein	9.10	4.68
Crude Fiber	11.09	24.74
Crude Fat	3.00	1.24
Ash	5.19	11.48
NFE	71.62	57.86
TDN	73.40	49.99
NDF	34.96	57.58
ADF	23.57	36.72
Cellulose	11.45	20.85
Hemicellulose	14.98	30.79
Lignin	7.30	4.25

Table 2: The chemical composition of the treatments (% DM)

Composition (%)	Treatment			
	P1	P2	P3	P4
Dry Matter	89.04	88.85	88.66	88.47
Organic Matter	92.29	91.67	91.04	90.41
Crude Protein	9.06	9.05	9.03	9.02
Crude Fiber	16.55	17.92	19.28	20.65
Crude Fat	2.30	2.12	1.94	1.77
Ash	7.71	8.34	8.96	9.59
NFE	64.39	62.59	60.78	58.97
TDN	68.55	67.17	65.78	64.40
NDF	44.01	46.27	48.53	50.79
ADF	28.83	30.15	31.46	32.78
Cellulose	15.21	16.15	17.09	18.03
Hemicellulose	21.30	22.89	24.47	26.05
Lignin	6.08	5.78	5.47	5.17

Tannin in the dry extracts was determined with hide-powder method that had modified by (Seigler et al., 1986). The number of dry extracts was dissolved in distilled water, filtered and the obtained filtrate then divided into two parts. The first part was dried to see the level of water soluble extract. The second part was treated with the hide powder to withhold the tannin content. The level of tannin was calculated by the formula: Tannin (%) = level of water soluble extract (%) - level of water soluble extract without tannin (%).

In vitro Method

Tilley and Terry method (Tilley and Terry, 1963) used for *in vitro* experiment to determine the feed digestibility, rumen liquid characteristics, and gas production. Fresh rumen liquid was filtered using nylon (100 µm sieve) and filled into pre-warmed (39°C) thermos flasks. Filtered rumen liquid was diluted with the buffer solution suggested by McDougall Fresh rumen liquid was filtered using nylon (100 µm sieve), then filled into pre-warmed 39°C thermos flasks. Rumen liquid was diluted with buffer solution recommended by McDougall (1947), with ratio rumen fluid: buffer solution (1:4). Incubated 2.5 g sample in each Erlenmeyer flask with 250 mL solution (rumen fluid and buffer solution mixed), an aerobical condition by inserting carbon dioxide gas into the flask, then sealed it with a rubber lid. All flask was placed in a shaking incubator with 39° C for the temperature, and 100 rpm for speed during 48 hours. Gas production is measured by withdrawing all the fermented gas accommodated using a syringe and reading the volume scale. Methane gas volume measurement was done used indirect method (Fieves et al., 2005) modified by (Ramaiyulis, 2018). The gas that has been drawn into the syringe for measurement of the total gas sprayed into a vacuum bottle with a rubber cap which already contains 20 ml of 10M NaOH and is plugged in an empty syringe. Methane gas will come out through an empty syringe and read the scale volume, while part of the CO_2 gas will be captured by NaOH.

After incubation, immersing the flask into ice water for stopping microbial activity, then pH was measured. After that, the supernatant was separated by moving the content of each flask in centrifuge tubes at 4° C with 3000 rpm for 5 minute. The result of supernatant was stored inside bottles on freezer with -18° C temperature until NH_3 and total Volatile Fatty Acid (VFA) analysis completed. Conway and O' Malley method (Conway and O'Malley, 1942) used for determined NH_3 levels. The residue was filtered using whatman No. 41 filter paper, then dried in oven at 60° C for 24 hours (AOAC, 2005).

Chemical Analyses

Each treatment's sample combination and residue was analyzed using proximate analysis (AOAC, 2005) to determine the contents of dry matter, organic matter, protein, fat, and crude fiber. Van soest analysis (Van soest et al., 1991) to determine the contents of NDF, ADF, cellulose, hemicellulose, and lignin.

Statistical analysis

The obtained data were analyzed using General Linear Model by IBM SPSS Statistics 26 (Yanti et al., 2021a). Data groups that showed statistical significance ($p<0.05$) were further analyzed using Duncan's Multiple Range Tests (DMRT).

RESULTS AND DISCUSSION

Effect Combination of Hay Mangrove Leaves and Native Grass on Phytochemical

Data of total phenol and tannin of the experimental are summarized in Table 3.

Table 3: Total Phenol and Tannin

Phytochemical	Treatment				S.E
	P1	P2	P3	P4	
Total Phenol (mg GAE/gr)	18.65[a]	15.52[b]	11.52[c]	9.71[d]	0.200
Tannin (%)	5.69[a]	4.83[b]	4.22[c]	3.56[d]	0.033

Description : Different letters ([a,b,c]) in the same rows indicate significant differences ($p<0.05$); S.E : standard error

Combination of hay mangrove leaves and native grass is significantly different ($p<0.05$) on total tannin and total phenol. Tannin of mangrove leaves ranged from 5.69-3.56%, then total phenol ranged from 18.65%-9.71%. The results of statistical analysis showed that total phenol and tannin in P1 was higher than in other treatments ($p<0.05$). Based on the table above, it was found that the higher used of mangroves in the treatment resulted in a high total phenol and tannin combination, this is based on previous research by Sari et al. (2022) anti-nutritional substances contained in mangrove leaves in the form of tannins and another polyphenol compounds. The total phenol and tannin content are related because tannins are included in secondary metabolites synthesized on plants and classified as polyphenolic compounds and had ability to form complex compounds with another macromolecule. In other hand, tannins also interact with proteins derived, so that reducing their availability for rumen microorganism (Egea et al., 2016). Tannin protected protein from the degradation of rumen microorganism, that will make positive effect to the forage digestibility increase. These metabolites substances are mainly found in the post rumen gastrointestinal tract. On abomasum tannin and protein can release from complex binding because the pH condition was low which enables protein degrade by the enzyme, like pepsin. Hence, amino acids it contains are readily available to livestock requirement (Yanti et al., 2021a).

Effect Combination of Hay Mangrove Leaves and Native Grass on Nutrient Digestibility

Data of nutrient digestibility of the experimental combination hay mangrove leaves and native grass are summarized in Table 4.

Table 4: Nutrient Digestibility

Nutrient Digestibility (%)	Treatment				S.E
	P1	P2	P3	P4	
Dry Matter	59.12[b]	58.04[c]	62.82[a]	56.54[d]	0.549
Organic Matter	61.72[b]	60.90[b]	65.69[a]	59.69[c]	0.657
Crude Protein	64.24[b]	63.54[c]	66.17[a]	59.74[d]	0.125

Description : Different letters ([a,b,c]) in the same rows indicate significant differences ($p<0.05$);
S.E : standard error

Dry matter, organic matter, and protein digestibility were also found to be significantly different ($p<0.05$). The dry matter digestibility ranged from 62.82-56.54%, whereas organic matter digestibility ranged from 65.69-59.69%, and the protein digestibility ranged from 66.17-59.74%. The results of statistical analysis showed that the digestibility of dry matter, organic matter, and crude protein in P3 was higher than in other treatments ($p<0.05$). The variations on the feed digestibility show the contribution of the feed for livestock requirements and the synthesis of microbial proteins for producing energy. This is appropriate with the research of Nolan and Dobos (2005) they ferment the ingredients of feed (proteins, sugars, polysacharides) to generate the ATP molecules required to keep the homeostatic and maintaining their growth. The number of microorganisms on rumen may have been increased, therefore the distribution area for feed degradation was present. All of this leading to an increased the TDN values (Zebeli et al., 2007).

The higher organic matter, dry matter and protein digestibility on P3 ($p<0.05$). The higher number of dry matter digestibility affect to the higher chances of good nutrition can be used by livestock for growth. P3 had higher digestibility might be happened because the tannin content at treatment on Table 3 is relatively low. Strong bond between protein and tannins, carbohydrates or another substance will affect the digestibility of fiber. Carbohydrate consist of crude fiber and other polysaccharides are non-soluble substances such as hemicellulose, cellulose, and lignin. The decrease in active tannin levels will binding carbohydrates and can be well degraded by microbe's rumen, the effect of digestibility will also be increased (Yanti et al., 2021b).

The average of dry matter and organic matter in vitro digestibility on this study was lower than in the research of Jamarun et al. (2021) with the ranges from 60.50%-73.36%. Organic matter digestibility consists of feed ingredients digestibility in the form of organic substances like protein, carbohydrate, fat, and vitamin. The organic ingredients present in feed is available in the form of insoluble, therefore a process of breaking down substances is required it becomes easy substances late. High digestibility of organic matter hay mangrove leaves combine with native grass might happened cause rumen microorganism's activity caused in the treatment feed used contained high nutritional content (Suardin, 2014).

In this study organic matter produced ranged from 90.00%-91.11%. The content of this organic material is not too different from research Sari et al. (2022) results of proximate analysis organic matter of Mangrove Leaves (*Rhizhopora apiculata*) soaking with lime water around 91.14%-92.07%. High digestibility of organic matter in this study, it is suspected because dry matter digestibility in the study higher. Based on Sutardi (1980), material degradation organic matter is closely related to dry matter degradation because some ingredients of dry matter consist of organic matter.
The protein digestibility on P3 is higher than other treatments ($p<0.05$). This combination protein source might result a better biological value of protein composition and increased protein synthesis of rumen microbes (Atmojo et al., 2019). Protein protect can also using tannins. Tannins are compounds that can be used to protect proteins from rumen microbial degradation, because tannin are able to bind proteins by forming complex compounds that resistant to protease, resulting in decreased protein degradation in the rumen (Cahyani et al., 2012).

Effect Combination of Hay Mangrove Leaves and Native Grass on Fiber Fraction Digestibility

Data of fiber fraction digestibility of the experimental combination hay mangrove leaves and native grass are summarized in Table 5.

Table 5: Fiber Fraction Digestibility

Fiber Fraction Digestibility (%)	Treatment				S.E
	P1	P2	P3	P4	
ADF	48.82[b]	46.10[c]	50.67[a]	44.87[d]	0.264
NDF	52.60[b]	50.82[c]	57.22[a]	46.77[d]	0.440
Cellulose	50.58[b]	47.72[c]	55.84[a]	45.37[d]	0.352
Hemicellulose	61.31[b]	59.48[c]	63.27[a]	56.52[d]	0.332

Description : Different letters ([a,b,c]) in the same rows indicate significant differences ($p<0.05$); S.E : standard error

Fiber fraction digestibility were found to be significantly different ($p<0.05$) on ADF, NDF, cellulose, and hemicellulose digestibility (Table 5). ADF, NDF, cellulose, hemicellulose digestibility ranged from 50.67-44.87%; 57.22-46.77%; 55.84-45.37%; 63.27-56.52%. The results of statistical analysis showed that digestibility of ADF, NDF, cellulose and hemicellulose in P3 was higher than in other treatments ($p<0.05$). The number of fiber fraction digestibility was affected by many factors, including the composition of fiber fraction, amount fiber in the feed, and microorganism activity (Maynard et al., 2005). Based on Jamarun et al. (2018) and Yanti et al. (2021c) low crude fiber in feed content made easier to digest because the microbes also easily to penetrate the cell walls. Beside of that, the higher number of crude fiber in a feed content will affect to the cell walls thicker and resistant to microbes digesting. That can result in a decrease of feed digestibility. It can be seen from the lowest. Another substance affected the fiber fraction digestibility is anti-nutritional compound. The higher fiber digestibility on P3 ($p<0.05$) was found that tannin content on Table 3 was low. The lowest fiber digestibility on P4 ($p<0.05$), even the tannin content on P4 was lowest, but the crude fiber was higher than other treatments (Table 2), crude fibre had in general the lowest digestibility (De-Oliveira et al., 2011). Tannin might bind with rumen microorganism's cell wall and can inhibit microorganism growth and enzyme activity (Smith et al., 2005). Tannins also the defaunation agent and functions to protect protein, the weakness in their function is the phenol compound. Tannins also have antibacterial properties. Gram-positive bacteria are sensitive to polyphenols certain (Smith et al., 2003) whereas some fiber fraction digesting bacteria including gram bacteria positive. Tannins in high doses will decrease the digestibility of fiber in the rumen.

Effect Combination of Hay Mangrove Leaves and Native Grass on Rumen Liquid Characteristics

Data of rumen liquid characteristics of the experimental combination of hay mangrove leaves and native grass are summarized in Table 6.

Table 6: Rumen Liquid Characteristics

Rumen Liquid Characteristics	Treatment				S.E
	P1	P2	P3	P4	
pH	6.58[a]	6.59[a]	6.56[a]	6.62[a]	0.007
VFA (mM)	142.25[b]	138.70[c]	156.20[a]	129.60[d]	1.017
NH_3 (mg/100ml)	8.94[b]	8.47[b]	9.84[a]	7.38[c]	0.123

Description : Different letters ([a,b,c]) in the same rows indicate significant differences ($p<0.05$);
S.E : standard error

The result show that pH found to be not significantly diferet ($p>0.05$) on Table 6. This study has normal range pH of the rumen fluid for fermenting feed is still within 6.56-6.62. Normal rumen condition has pH for 5.5-6.9, dry material content of 10-13%, and temperature of 38-41°C for optimum rumen microbes (Puniya et al., 2015). Rumen pH is important because plays role in the capability of microbial rumen to degrade feed protein (Buckner et al., 2013).

Total Volatile Fatty Acid (VFA) in this study ranged from 156.20-129.60 mM and significantly different ($p<0.05$). The results of statistical analysis showed that VFA in P3 was higher than in other treatments ($p<0.05$). The higher number of VFA in P3 correlated with higher fiber fraction digestibility of P3 in Table 5. Fiber fraction contained in feed because crude fiber contains carbohydrates which is one of the important factors that can affect VFA production. This is in accordance with the opinion of Pamungkas et al. (2008) which stated that ruminant rations mostly consist of forage containing structural carbohydrates in the form of fiber fraction (cellulose and hemicellulose) and simple carbohydrates that are easily fermentable (sugars, starches), both of which will then be fermented into Volatile Fatty Acids (VFA), methane and CO_2.

The results of statistical analysis showed that NH_3 in P3 was higher than in other treatments ($p<0.05$). The higher number of NH_3 in P3 correlated with higher protein digestibility of P3 in Table 4. Degradation in the rumen will produce amino acids which will then undergo deamination to produce NH_3, VFA and CO_2 (Kozloski et al., 2000). This VFA production was enough for rumen microbial protein synthesis, VFA needs for microbial rumen growth about 80-160 mM (Van Soest, 1994). Increased microbial production correlated with increased of VFA concentration. Quickness of feed degraded by rumen microbes could be show on increased of the VFA number. Anggraeni et al. (2017) VFA production is the energy adequacy for ruminant. The difference of feed composition, physical form, and feed processing can alter the composition of VFA in rumen. NH_3 of all treatments was enough for microbial protein production, based on McDonals et al. (2002) NH_3 6-21 mM production increasing the microbial protein production. Putri et al. (2013) high concentrations of ammonia might be reason of the faster degradation protein feed process than the formation of microbial protein. The rumen fluid ammonia concentration is influenced by the protein consumed and protein degradation process on rumen. Rahmadi et al. (2010) the low of NH_3 on rumen influenced by N availability on feed. NH_3 affects the growth of microorganisms because it is used in protein synthesis.

Effect Combination of Hay Mangrove Leaves and Native Grass on Gas Production

Data of gas production of the experimental combination hay mangrove leaves and native grass are summarized in Table 7.

Total gas production and methane gas found to be significantly different ($p<0.05$) on Table 7. Total gas production ranged from 167.20-133.80 ml/hours, whereas methane gas ranged from 20.57-41.00 ml/gr Dry Matter. The highest total gas production digestibility was found in P3 ($p<0.05$). Gas production is the result of a fermentation process that occurs in the rumen which can show microbial activity in the rumen and describes the amount of organic matter digested (Ella et al., 1997). The higher gas production mean better quality of the feed ingredients, this can be seen in Table 4 digestibility of nutrients and Table 5 digestibility of crude fiber that P3 has the highest digestibility. Methane production is correlated with considerable energy losses, and decreasing energy gain and productivity.

Table 7: Gas Production

Gas Production	Treatment				S.E
	P1	P2	P3	P4	
Total Gas Production (ml/hours)	151.70^{b}	141.80^{c}	167.20^{a}	133.80^{d}	2.350
Methane Gas (ml/gr Dry Matter)	28.75^{c}	38.59^{b}	20.57^{d}	41.00^{a}	0.049

Description : Different letters (^{a,b,c}) in the same row indicate significant differences ($p<0.05$); S.E : standard error

The highest methane gas production on P4 ($p<0.05$). P4 contain higher crude fiber (Table 2) and received at least tannin protection because the use of HML was lowest than the other treatment and. Crude fiber content has a relationship with the production of methane gas, the higher crude fiber content increased potential for methane gas production (Beauchemin et al., 2008).Crude fiber is Tannin decreasing the protozoa's population in the rumen (Patra and Saxena, 2010). The indirectly inhibitation mechanism of methane gas production using tannin via fiber digestion with reduces the production of hydrogen, and directly mechanism via the activity and growth of methanogens (Tavendale et al., 2005). This indicates that tannin of hay mangrove leaves was affecting the ruminal methanogenesis pathway with decreasing protozoa ecology (Goel et al., 2008) and had toxic effect for methanogenic bacteria (Carulla et al., 2005). High methane gas production is not good because methane gas having a negative impact on the environment and also energy losses which should be converted into livestock nutrient absorption.

CONCLUSION

This research concludes that P3 (40% hay mangrove leaves + 60% native grass) had the best resulted effected on nutrient and fiber in-vitro digestibility, rumen fluid characteristic and gas production. Future research can be conducted to determine the effect combination of 40% hay mangrove leaves and 60% native grass on ruminant livestock production.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests

AUTHORS CONTRIBUTIONS

Novirman Jamarun, Suyitman, Khasrad, Elihasridas, and James Hellyward formulated the experimental design and experimental work at the laboratory. Rani Winardi Wulan Sari and Gusri Yanti drafted the manuscript, did experimental work at the laboratory and data analysis. All the authors read and approved the final version of the manuscript.

REFERENCES

AOAC (Association of Official Analytical Chemyst). (2005). Official Method of Analysis of The Association of Official Analytical of Chemist. Arlington, Virginia, USA: Association of Official Analytical Chemist, Inc.

Anggraeni AS, Herdian H, Sakti AA, Sofyan A, Ekaningrum M. (2017). Effect of Urea Treatment of Cocoa Pod on Rumen Fermentation Characteristics *In vitro*. IOP Conf. Ser: Earth Environ. Sci. 101 012031. 1-8. https://iopscience.iop.org/article/10.1088/1755-1315/101/1/012031

Arowolo MA, He J. (2018). Use of Probiotics and Botanical Extracts to Improve Ruminant Production in the Tropics : A Review. Anim. Nutrit. 4: 241-249. https://doi.org/10.1016/j.aninu.2018.04.010

Atmojo FA, Indarto E, Kustantinah. (2019). Nutrients Intake and Fiber Fraction Digestibility of Kacang Goats Supplemented with Different Proportions of Soybean Meal and *Calliandra Calothyrsus* in the Diet. IOP Conf. Series: Earth Environ. Sci. 387 012095. https://doi.org/10.1088/1755-1315/387/1/012095.

Beauchemin KA, Kreuzer M, O'Mara F. McAllister TA. (2008). Nutritional Management for Enteric Methane abatement: a review. Australian J. Experimen. Agric. 48:21-27.

Buckner, CD, Klopfenstein TJ, Rolfe KM, Griffin WA, Lamothe MJ, Watson AK, MacDonald JC, Schacht WH, Schroeder P. (2013) Ruminally Undegradable Protein Content and Digestibility for Forages Using The Mobile Bag *In Situ* Technique. J. Anim. Sci. 91(6): 2812-2822. https://doi.org/10.2527/jas.2012-5982

Cahyani RD, Nuswantara LK, Subrata A. (2012). Pengaruh Proteksi Protein Tepung Kedelai Dengan Tanin Daun Bakau Terhadap Konsentrasi Amonia, *Undegraded Protein* dan Protein Total Secara *In vitro*. Anim. Agric. J. 1: 159-166.

Carulla JE, Kreuzer M, Machmüller A, Hess HD. (2005). Supplementation of *Acacia mearnsii* Tannins Decreases Methanogenesis and Urinary Nitrogen in Forage-Fed Sheep. Aust. J. Agric. Res. 56: 961-970. https://doi.org/10.1071/AR05022

Conway EJ, O'Malley E. (1942) Microdiffusion Methods. Ammonia and Urea using Buffered Absorbents (Revised Methods for Ranges Greater than 10µg. N). Biochem J. 36(7-9): 655-661.

Crampton EE, Harris LE. (1969). Applied Animal Nutrition 2nd Edition. L. H. Freeman and Co, San Francisco.

Dahuri. (2003). Keanekaragaman Hayati Laut Aset Pembangunan Berkelanjutan Indonesia. PT. Gramedia Pustaka Utama, Jakarta.

De-Oliveira L, Takakura FS, Kienzle E, Brunetto MA, Teshima E, Pereira GT, Vasconcellos RS, Carciovi AC. (2011). Fibre Analysis and Fibre Digestibility in Pet Foods - A Comparison Of Total Dietary Fibre, Neutral And Acid Detergent Fibre And Crude Fibre. Journal of Animal Physiology And Animal Nutrition. 96. 895-906. http://dx.doi.org/10.1111/j.1439-0396.2011.01203.x

Egea AV, Allegretti LI, Lama SAP, Grilli D, Fucili M, Guevara JC, Villalba JJ. (2016) Diet Mixing and Condensed Tannins Help Explain Foraging Preferences by Creole Goats Facing The Physical and Chemical Diversity of Native Woody Plants in The Central Monte Desert (Argentina). Anim. Feed Sci. Technol. 215: 47-57.

Ella AS, Hardjosoewignya TR, Wiradaryadan, Winugroho M. (1997). Pengukuran Produksi Gas dari Hasil Proses Fermentasi Beberapa Jenis Leguminosa Pakan. Dalam : Prosiding Sem. Nas II-INMT Ciawi, Bogor.

Fievez V, Babayemi OJ, Demeyer D. (2005). Estimation of Direct And Indirect Gas Production in Syringes: a Tool to Estimate Short Chain Fatty Acid Production that Requires Minimal Laboratory Facilities. Anim. Feed Sci. Technol. 123-124: 197-210. https://doi.org/10.1016/j.anifeedsci.2005.05.001

Goel G, Makkar HPS, Becker K. (2008). Effect of *Sesbania sesban* and *Carduus pycnocephalus* and Fenugreek (*Trigonella foenumgraecum L.*) Seeds and Their Extracts on Partitioning of Nutriens from Roughage and Concentrate Based Feeds to Methane. Anim. Feed Sci. Technol. 147: 72-89. https://doi.org/10.1016/j.anifeedsci.2007.09.010

Hamilton, L.S, Snedaker S.C. (1984). Handbooks for Mangrove Areas Management. IUCN-UNESCO. pp.123.

Jamarun N, Zain M, Arief, Pazla R. (2018). Populations of Rumen Microbes and The *in vitro* Digestibility of Fermented Oil Palm Fronds in Combination With Tithonia *(Tithonia Diversifolia)* And Elephant Grass *(Pennisetum Purpureum)*. Pak. J. Nutr . 17: 39-45. https://doi:10.3923/pjn.2018.39.45

Jamarun, Pazla R, Yanti G. (2021). Effect Of Boiling On In-Vitro Nutrients Digestibility, Rumen Fluid Characteristics, and Tannin Content Of Mangrove (*Avicennia Marina*) Leaves as Animal Feed. International Conference On Green Agro-Industry and Bioeconomy. IOP Conf. Series: Earth Environ. Sci. 733. https://doi.org/10.1088/1755-1315/733/1/012106

Kozloski GV, Ribeiro HMN, Rocha JBT. (2000). Effect of The Substitution of Urea for Soybean Meal on Digestion in Steer. Can. J. Anim. Sci. 80: 713-719.

Kusmana C, Sukristijiono S. 2016. Mangrove Resource uses by Local Community in Indonesia. J. Nat. Resour. Environ. Manag. 6:217-224. http://dx.doi.org/10.19081/jpsl.2016.6.2.217

Makkar HPS, Francis G, Becker K. (2007). Bioactivity of Phytochemicals in Some Lesser-Known Plants and Their Effects and Potential Applications in Livestock and Aquaculture Production Systems. Animal. 1:1371-1391. https://doi.org/10.1017/S1751731107000298
Marinova D, Ribarova F, Atanassova M. (2005). Total Phenolics and Total Flafonoids in Bulgarian Fruits and Vegetables. J. Univ. Chem. Technol. Metallurgy. 40: 3255-260.
Maynard LA, Loosil JK, Hintz HF, Warner RG. (2005). Animal Nutrition. (7th Edition) McGraw-Hill Book Company, New York, USA.
McDonald P, Edwards JFD, Greenhalgh RA, Morgan CA. (2002). Animal Nutrition. 6th ed. Pretice Hall, London.
McDougall EI. (1947). Studies on Ruminant Saliva. 1. The Composition and Output of Sheep's Saliva. Biochem. J. 43(1): 99-109.
Noegroho A. (2013). Profil kelautan dan Perikanan Provinsi Sumatera Barat untuk Mendukung Industrialisasi KP. Jakarta. Pusat Data, Statistik dan Informasi.
Nolan JV, Dobos RC. (2005). Nitrogen Transactions in Ruminants. in: Dijkstra J, Forbes JM, France J. Quantitative Aspects of Ruminant Digestion and Metabolism. 2nd ed. CABI Publishing, Wallingford, UK 2005: 177-206. https://doi.org/10.1079/9780851998145.0000
Pamungkas, D., Anggraeni Y.N., Kusmartono and Krishna N.H. (2008). Produksi Asam Lemak Terbang dan Amonia Rumen Sapi Bali pada Imbangan Daun Lamtoro dan Pakan Lengkap yang Berbeda. Seminar Nasional Teknologi Peternakan dan Veteriner. 197-204.
Patra AK, Saxena J. (2010). A New Perspective on The Use of Plant Secondary Metabolites to Inhibit Methanogenesis in the Rumen. J. Phytochem. 71. https://doi.org/10.1016/j.phytochem.2010.05.010
Pramudji. (2001). Mangrove Forest Ecosystem and Its Role as a Habitat for Various Aquatic Fauna. Ocean. Volume XXVI. 4: 13-23.
Puniya AK, Singh R, Kamra DN. (2015). Rumen Microbiology: From Evolution to Revolution. Springer, New Delhi.
Putri LDNA, Rianto E, Arifin M. (2013). Pengaruh Imbangan Protein dan Energi Pakan Terhadap Produk Fermentasi di dalam Rumen Pada Sapi Madura Jantan. Anim. Agric. J. 2: 94-103.
Rahmadi D, Sunarso J, Achmadi, E. Pangestu, A. Muktiani, M. Christiyanto, Surono, Surahmanto. (2010). Ruminologi Dasar. Universitas Diponegoro Press, Semarang.
Ramaiyulis. (2018). Manipulasi Fermentasi Rumen dengan Suplementasi Ampas Daun Gambir untuk Meningkatkan Efisiensi Ransum dan Performa Sapi Bali. Disertasi. Fakultas Peternakan Universitas Andalas.
Rizki R, Safitri E, Asroen A. (2016). Morfologi *Bruguiera Cylindrica (L.) Blume* yang Tumbuh di Hutan Mangrove Kecamatan Siberut Utara Kabupaten Kepulauan Mentawai. Sainstek: Jurnal Sains dan Teknologi.7: 26-32. http://dx.doi.org/10.31958/js.v7i1.122
Rosadi K, Abdullah L, Kumalasari NR, Yaman MA. (2018). Evaluasi Peforma Benih *Indigofera zollingeriana* dari Tanaman Berbeda Umur. J. Bullet. Makanan Ternak. 105: 1-10.
Rusdin, Ismail M, Mustaring, Purwaningsih S, Andriana A, Dewi SU. (2009). Studi Potensi Kawasan Lore Tengah Untuk Pengembangan Sapi Potong. Media Litbang Sulteng 2 (2) : 94–103, 2009.
Russell JB, Muck RE, Weimer, P. J. (2009). Quantitative Analysis of Cellulose Degradation and Growth of Cellulolytic Bacteria in The Rumen. FEMS Microbiology Ecology. 67: 183–197. https://doi.org/10.1111/j.1574-6941.2008.00633.x
Sari RWW, Jamarun N, Pazla R. (2020). Pengaruh Perebusan Dengan Air Abu Sekam Terhadap Nilai Nutrisi Daun Mangrove (*Avicennia marina*). Prosiding Webinar Nasional Sapi Kerbau IV. ISBN: 978-623-7763-73-4. 158-164.
Sari RWW, Jamarun N, Suyitman, Khasrad, Yanti G. (2021). Mangrove *(Avicennia marina)* Leaves as an alternative feed resources for ruminants. 2nd International Conference on Animal Production for Food Sustainability 2021. IOP Conf. Series: Earth Environ. Sci. 888 012079. https://doi:10.1088/1755-1315/888/1/012079
Sari RWW, Jamarun N, Arief, Pazla R, Yanti G, Ikhlas Z. (2022). Nutritional Analysis of Mangrove Leaves *(Rhizophora apiculata)* Soaking with Lime Water for Ruminants Feed. International Conference on Sustainable Anim. Resour. Environ. https://doi:10.1088/1755-1315/1020/1/012010
Seigler DS, Seilheimer S, Keesy J, Huang HF. (1986). Tannin from Four Common Acacia species of Texas and Northeastern Mexico. Econ. Bot. 40: 2220-232. https://doi.org/10.1007/BF02859146
Shridhar, A, Sridhar M, Sridhar N, Singh ISB, Varghese V. (2004). Probiotic Effects of Lactic Acid Bacteria Against *Vibrio alginolyticus* In Penaeus (*Fenneropenaeus) indicus* (*H. Mime Edwards*). Asian Fish. Sci., 17: 71-80.
Singleton VL, Rossi JA. (1965). Calorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. Amer. J. Enol. Vitic. 16: 144-158.
Smith AH, Imlay JA, Mackie RI. (2003). Increasing the Oxidative Stress Response Allows *Escherichia Coli* to Overcome Inhibitory Effect of Condensed Tannins. Appl. Environ. Microb. 69: 3406-3411. https://doi.org/10.1128/AEM.69.6.3406-3411.2003
Smith AH, Zoetendal E, Mackie RI. (2005). Bacterial Mechanisms to Overcome Inhibitory Effects of Dietary Tannins. Microb. Ecol. 50: 197-205. https://doi.org/10.1007/s00248-004-0180-x
Suardin, Natsir S, Rahim Aka. (2014). Kecernaan Bahan Kering dan Bahan Organik Campuran Rumput Mulato (*Brachiaria Hybrid.Cv.Mulato*) dengan Jenis Legum Berbeda Menggunakan Cairan Rumen Sapi . JITRO. 1:16-22.
Suherman, M. (2019). Cara mengolah hijauan menjadi pakan Hay. BPPSDMP, Kementerian Pertanian.
Sutardi, T. (1980). Landasan Ilmu Nutrisi. Jilid I. Fakultas Peternakan. Institut Pertanian Bogor, Bogor.
Tavendale MH, L.P. Meagher, D. Pacheco, N. Walker, G.T. Attwood, S. Sivakumaran. (2005). Methane Production From In Vitro Rumen Incubation with Lotus pedunculatus and Medicago sativa, and Effects of Extractable Condensed Tannin Fractions on Methanogenesis. Anim. Feed Sci. Technol. 123/124: 403-419. https://doi.org/10.1016/j.anifeedsci.2005.04.037
Tilley JMA, Terry RA (1963). A wo stage technique for the *in vitro* digestion of forage crops. Grass and Forage Sci. 18 : 104 – 111. https://doi.org/10.1111/j.1365-2494.1963.tb00335.x
Van Soest PJ. (1994). Nutritional Ecology of the Ruminant. 2nd ed. Ithaca. Comstock Publishing Associates a Division of Cornell University Press. 253-280.
Van Soest PJ, Robertson JB, Lewis BA. (1991). Methods for Dietary Fiber, Neutral Detergent Fiber, and Nonstarch Polysaccharides in Relation to Animal Nutrition. J. Dairy

Sci. 74: 3583-3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2

Yanti G, Jamarun N, Suyitman S, Satria B, Sari RWW. (2021a). Mineral status of soil, sea water, and mangrove (*Avicennia marina*) forages in several coastal areas of West Sumatra. Vet. World. 14: 1594-1601.

Yanti G, Jamarun N, Elihasridas. (2021b). Effect of Boiling Mangrove (Avicennia marina) Leaves in Husk ash Water on In-Vitro Digestibility of Fiber Fraction (NDF, ADF, Cellulose, and Hemicellulose). J. Peternakan Indonesia. https://doi.org/10.25077/jpi.23.2.168-173.2021

Yanti G, Jamarun N, Elihasridas, Astuti T. (2021c). Quality Improvement of Sugarcane Top as an Animal Feed with Biodelignification by Phanerochaete chrysosporium Fungi on *In-vitro* Digestibility. ICOMSET 2020. https://iopscience.iop.org/article/10.1088/1742-6596/1940/1/012063/

Zebeli Q, Tafaj M, Weber I, Dijkstra J, Steingass H, Drochner, W. (2007). Effects of Varying Dietary Forage Particle Size in Two Concentrate Levels on Chewing Activity, Ruminal Mat Characteristics, and Passage in Dairy Cows. J. Dairy Sci. 90: 1929–1942. https://doi.org/10.3168/jds.2006-354