Methane Gas Mitigation Strategies to Increase The Productivity of Ruminants by Moringa Leaves and Jackfruit Leaves as Additional Feed

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ABSTRACT
Global warming has become an environmental problem that has received much attention. The Intergovernmental Panel on Climate Change (IPCC) reports that the increase in the earth's surface temperature increases where global warming is related to the high accumulation of greenhouse gases in the atmosphere. The increase in greenhouse gases such as carbon dioxide (CO2), methane (CH4), nitrogen oxides (N2O), and Chlorofluorocarbons (CFC) is a result of the high variety of human activities and about 28% of anthropogenic methane gas emissions come from ruminants. It is due to the formation of methane gas or methanogenesis by methanogenic archaea in the digestive tract of ruminants, especially in the rumen. High fiber feed reduces the efficiency of feed use and increases the production of methane gas (CH4). Reducing methane production in the rumen will increase the energy supply to livestock, thereby increasing the efficient use of feed ingredients. Many strategies have been carried out to manipulate the fermentation process in the rumen to increase microbial protein synthesis and methane gas mitigation. Manipulation of fermentation in the rumen can be done by applying defaunation agents to protozoa in animal feed using saponins and tannins. The alternative feed ingredients used are derived from jackfruit leaves (Artocarpus heterophyllus) and Moringa oleifera leaves are potential sources of tannins used as high-quality feed protein protection to bypass their degradation rumen microbes. Like a double-edged sword, tannins have both positive and harmful biological effects when consumed by livestock. One of the effects is increasing the efficiency of ration protein, faster livestock growth, and reduced environmental emissions caused by livestock. This study aimed to determine the best level of addition of jackfruit and moringa leaves in the ration, which can provide the highest productivity in beef goats. The results of this study were obtained from the phytochemical content of phase 1 of jackfruit and moringa leaves which showed a positive effect from the tests for alkaloids, flavonoids, phenolics, saponins, triterpenoids, steroids, total tannins, and condensed tannins. Proximate content parameters consisted of BK, BO, SK, LK content based on statistical analysis showing that treatments A, B, and C showed an interaction and significant effect ($P < 0.05$). Furthermore, the in vitro digestibility of the DM, BO, SK, LK digestibility based on statistical analysis showed that treatment A, B, and C showed an interaction at a significant level. However, the duration of fermentation had a significant effect ($P < 0.05$) (on BK content, BO, SK, LK.

Keywords: *Jackfruit leaves, methane gas, mitigate, moringa leaves, ruminant.*

1. INTRODUCTION

Global warming has become an environmental problem that has received much attention. The Intergovernmental Panel on Climate Change (IPCC) reports that the earth's surface temperature has increased to 0.74 ± 0.18^{o} C, where the increase in temperature is the most significant temperature increase in the last few thousand years. Global warming is related to the high rate of accumulation of greenhouse gases in the atmosphere,

and about 28% of anthropogenic methane emissions come from ruminants. This is because the process of methane gas formation or methanogenesis by methanogenic archaea in the digestive tract of ruminants, especially in the rumen, where the energy lost as methane from ruminants is quite significant, ranging from 8-14% of the total digested energy. Reducing methane production in the rumen will increase the energy supply to livestock, thereby increasing the efficient use of feed ingredients.

Currently, many strategies have been carried out to manipulate the fermentation process in the rumen to reduce methane production. Manipulation of fermentation in the rumen can be done by applying defaunation agents to the protozoa in animal feed using saponins and tannins. Alternative feed ingredients can be derived from agricultural waste, which are jackfruit leaves and Moringa leaves. Jackfruit leaves (Artocarpus heterophyllus) are a source of tannins that can be used as protein protection. The total content of tannins and condensed tannins in jackfruit leaves is 7.08 and 5.57%. Another study obtained the addition of jackfruit leaves (leaves + petiole) containing 130 g / kg of condensed tannins. Apart from jackfruit leaves, the availability of Moringa oleifera (*Moringa oleifera*) leaves is also relatively abundant and available all year round is one of the considerations to be used as a mixture in relatively cheap feed.

Based on other research, it was found that Moringa leaves contain BETN of 32.83% and PK of 26.43%. Based on the research, it was found that the addition of jackfruit and moringa leaves had a positive effect on tannins, including increasing the efficiency of ration protein use, faster livestock growth, and its ability to reduce environmental emissions caused by livestock. Moringa leaves are a good source of natural antioxidants because they contain various antioxidant compounds such as ascorbic acid, flavonoids, phenolics, and carotenoids [1].

It has high concentrations of ascorbic acid, protein, and essential amino acids, especially methionine, cysteine, tryptophan, and the lysine found in the leaves and pods, making Moringa leaves an ideal feed supplement [2]. Therefore, the combination of jackfruit leaves with low ammonia (NH3) levels is 279 [3] with Moringa leaves will have the potential to improve nutrient digestibility and control methane gas production.

Starting from the study above, the strategy of increasing the efficiency of microbial protein synthesis and methane gas mitigation to increase productivity in beef goats through research that focuses on providing jackfruit and moringa leaves as feed additives is a very interesting constraint.

2. MATERIALS AND METHODS

2.1. Materials

Feed ingredients consist of field grass, jackfruit leaves, and moringa leaves. The object research used rumen goat *in vitro.*

2.2. Methods

Utilization of jackfruit leaves and Moringa leaves as additional feed. The research objective of this phase I study was to determine the tannin content of jackfruit leaves, and Moringa leaves as additional feed ingredients used as goat feed. Were treatment A (40% Jackfruit Leaves + 60% Field Grass), B treatment (40% Moringa Leaves + 60% Field Grass), C treatment (20% Jackfruit Leaves + 20% Moringa Leaves + 20% Field Grass). The study used a randomized block design with three treatments and five replications. The analyzes performed were proximate analysis, Van Soest analysis, digestibility of food substances, phytochemical, and tannin content.

2.3. Determination of parameters

The termination of parameters is nutrient digestibility, *Van Soest* analysis, production characteristic rumen conditions, tannin content (total tannins), and methane gas production.

2.4. Dry matter and organic matter analysis

The dry matter measurement was carried out by heating the feed residue in an oven at 105 ° C for 24 hours, while the organic matter measurement was carried out by heating the residue in a furnace at 600 ° C for 4 hours. The calculation of the digestibility of dry matter and organic matter was based on the method carried out [4].

2.5. Characteristics of the rumen fluid

Measurement of the pH of the rumen fluid was carried out using [5] method, turn on the pH meter and let it stabilize for 15-30 minutes, standardize it with a standard pH 7 buffer solution, rinse with distilled water then dry with a tissue, put the electrode into the fermenter tube, pH value set by looking at the numbers on the monitor screen, VFA levels is the determination of VFA production was carried out utilizing steam distillation.

The supernatant in the tube was pipette 5 ml and put into the distillation tube, add 1 ml of 15% H2SO4, and the distillation tube was immediately closed with a rubber connected to a Liebig cooler. Immediately enter the distillation tube into the distillation flask containing distilled water. During the distillation process, the distillation flask must be heated with the aim that water vapor can force VFA, which will then be condensed in

the Leinbig cooler, the water formed by the distillation is stored in an erlemeyer containing 5 ml 0, 5 N NaOH until the volume is 250-300 ml, after finishing distillation, add 2-3 drops of the phenolphthalein indicator then titrate with N HCl until the color changes from pink to colorless.

2.6. Crude protein analysis

Digestion: the sample is weighed as much as 1 gram, put in a Kjeldahl flask, add 1 gram of selenium catalyst, give 25 ml of technical H¬2SO4, and then digest it in a fume hood until the solution becomes clear and yellowish green then cool. Dilution, the yellowish green sample is transferred to a 250 ml flask that already contains ± 100 ml distilled water. Distillation, the sample that has been diluted is pipette 25 ml (with a hyacinth pipette), then put into a distillation flask and shaken from time to time until the green solution is clear. After the solution is transparent green, diluted from the Kjedhal flask into a 250 ml distilled flask, Titration: The distillate is titrated with 0.1 N H2SO4 (standard solution) until blue-green color changes to pink, which indicates the endpoint of the titration, The determination of NH3 production was carried out according using a Conway cup, 1 ml of supernatant was dropped onto the right side of the Conway plate and 1 ml of 40% NaOH to the left side of the Conway plate, Drop 1 ml of H2BO3 into the center of the cup. Conway then covers the cup with a lid, grease Vaseline on the edge of the cup, and keep it for 24 hours. After 24 hours, titrate with 0.005 N H2SO4 until the color turns reddish green.

2.7. Van Soest analysis

The NDF content is determined by adding a sample of + 1 gram (a) into a 300 ml beaker and adding 80 ml of Neutral Detergent Solution (NDS). After that, extracting (heating) for 1 hour (after boiling), the results of the extraction are filtered using filter paper whose weight is known (b) with the help of a vacuum pump, the residue from the filter is rinsed with hot water and finally, with acetone, the residue is then dried in an oven at 1350 c for 2 hours, then put into a desiccator and weighed (cg).

The ADF content is determined by inserting a ± 1 gram (a) sample into a 300 ml beaker, then adding the ADS (Acid detergent solution) solution. The material is extracted (heated) for 1 hour then filtered with a known weight filter (b) with the help of a vacuum pump, the residue from the filter is washed with hot water and finally with acetone. The result of filtering was put into the oven at 130ºC for 2 hours. After that, the material was put into a desiccator and weighed (c gram). The cellulose content is a continuation of the ADF analysis, where ADF residue (c) is given 72% H2SO4 as much as 25 ml while shaking it occasionally so that the absorption is evenly distributed throughout the sample. Then filtering with a vacuum pump, the residue is rinsed with hot water as much as 300 ml so that the acid content is lost. Finally, rinse with 25 ml acetone. The residue is put in an oven 1350C for 24 hours, then cooled in a desiccator and weighed (d).

2.8. Tanin total

Weighed 1.5 grams of tannin, put it in a 100 ml beaker, and then added 50 ml of water heated at a temperature of 40-60ºC for 30 minutes. After cooling, the solution was filtered into a 250 ml volumetric flask. Then added with water to mark the line, then 25 ml is taken, put into Erlenmeyer, 20 ml of indigocarmin solution is added then titrated with 0.1 N KMnO4 solution, each time adding 1 ml of KMnO4 until the color changes from blue to green then the titration is carried out dropwise until the green color becomes golden yellow, for example, volume is required. Titrant A ml, the determination of the blank is carried out by piping 20 ml of indigocarmin solution into the erlenmeyer and adding water, and then titrating as the example above. For example, a titrant volume of B ml is required.

2.9. Gas Methan Total

A serum bottle containing 0.2 g of the ration sample is filled with 30 ml of a mixture of rumen fluid inoculum and McDougall's buffer using an automatic dispenser pipette, covered with a rubber cap, clamped with aluminum, and incubated in an incubator at 39oC for 24 hours. From a 5 ml serum bottle and put into a 5 ml serum bottle that has been vacuum closed with a rubber cap and clamped with aluminum, Methane gas is measured using a 2014 Shimadzu gas chromatography equipped with a thermal conductivity detector. Helium gas is a carrier gas with a 10 ml/minute flow rate, detector, and column temperature 250oc and 60oc. Methane gas production is calculated from incubation by looking at the gas volume and composition. The formula calculates methane gas: CH4 = (GV + HS) x concentration, where GV = gas volume (ml), HS = headspace volume (ml) from the serum vial and concentration =% methane gas in the analyzed sample [6].

3. RESULTS AND DISCUSSION

The research results showed that addition functional feed of jackfruit and Moringa leaves the showed a significant effect ($P < 0.05$) on digestibility of food substances, fiber fraction, rumen fluid characteristics,

3.1. Phytochemical Test of Moringa Leaves and Jackfruit Leaves

Extraction is a chemical and physical separation of the contents of the simplicial substance using a suitable solvent. The purpose of extraction is to attract chemical components found in natural materials. This extraction is based on the principle of mass transfer of the components of the substance into the solvent, that is, the displacement begins to occur in the interface layer and then diffuses into the solvent. The extraction technique used in this research is maceration because in addition to easier processing, the equipment used is simple. The maceration process is very beneficial in the extraction of natural compounds because immersing plant samples will break down the cell walls and membranes due to the difference in pressure between inside and outside the cell, so that the secondary metabolites in the cytoplasm will dissolve in organic solvents and the compound extraction will be perfect**.**

Table 1. Phytochemical test results of jackfruit leaves and Moringa leaves

Parameter	*Sample*	
	Jackfruit Leaf	Moringa Leaf
Alkaloid	+	+
Flavonoid	+	+
Fenolic/tannin	+	+
Saponin	-	-
Steroid	+	+
Triterpenoid	+	+

Note: Organic Chemistry Laboratory of Natural Materials FMIPA Unand (2020)

Phytochemical Screening After obtaining the thick extract, the extract was then tested for the chemical compounds contained in it using a phytochemical screening test. At this stage, five types of examinations are carried out, namely the examination of alkaloids, flavonoids, tannins, saponins, steroids, and triterpenoids. The results of the phytochemical screening test are presented in Table 1.

Phytochemical test results show that jackfruit and moringa leaves contain various types of secondary metabolites such as phenolics, saponins, flavonoids, tannins, triterpenoids, and alkaloids. The discoloration supports this due to the supplied reagent for extracting jackfruit and moringa leaves. The taller plants have flavonoids that are good in the vegetative section, especially in flowers.

Tabel 2. Tanin Total

No	Sample	Tanin Total
1	Jacfruit Leaf	0,072355 mg/L
2	Moringa Leaf	0,208625 mg/L

Note: Organic Chemistry Laboratory of Natural Materials FMIPA Unand (2020)

The total tannin content in jackfruit leaves is 7.23%, and Moringa leaves 20.86%, so there is still a second mechanism to reduce methane gas production. Tannin compounds can reduce the population of the main actors in the methanogenesis process in the rumen, namely protozoa. Methane gas production has a strong relationship with the protozoan population. Methane gas is produced by archaea bacteria which consume hydrogen. These bacteria are in symbiosis with protozoa. Tannins will directly reduce the growth of methanogenic microbial populations or indirectly reduce the availability of nutrients for rumen microbes.

Flavonoids as flower pigments play an important role. Another function of flavonoids is absorbing ultraviolet light to direct insects, regulating plants, regulating photosynthesis, and working anti-microbial and anti-virus so that it can work on insects. The effects of flavonoids on many organisms may explain why plants containing flavonoids are widely used in traditional medicine. In addition, the content of flavonoids can work as a solid respiratory barrier, inhibiting enzyme and non-enzyme oxidation reactions. The chemical extracts of Moringa leaves and jackfruit leaves are obtained from alkaloids, flannovoid, phenolic, saponins, triterpenoid and steroids which have high substance content. Steroids are compounds derived from triterpenoids and their structure is a multiple of the 6 isoprene units commonly found in plants. The importance of the use of steroids is

Tabel 3. the results of digestibility of food substances from the provision of jackfruit and Moringa leaves

Treatment	Parameters (%)				
	KCBK	KCBO	KCPK	KCSK	KCLK
A	81,93^{a}	82,39^{a}	89,60^{c}	63,37^{b}	65,56^{a}
B	81,70^{b}	81,16^{b}	91,19^{b}	54,09^{b}	65,12^{b}
C	80,02^{c}	73,32^{c}	94,54^{a}	68,56^{a}	45,64^{c}
SE	0,77	0,76	0,79	2,83	9,93

Note: Means within a column with different superscripts are significantly ($P < 0.05$)

Tabel 4. Results of rumen fluid characteristics

Treatment	Parameters (%)			
	pH	VFA	NH_3	Gas Metan (CH_4) Total
A	7,00[a]	151,67[a]	12,04[b]	29,16[a]
B	6,97[b]	126,67[a]	9,07[c]	29,06[b]
C	6,93[c]	156,67[a]	12,89[a]	26,52[c]
SE	0	0,67	0	0,29

Note: Means within a column with different superscripts are significantly (P < 0.05)

physiologically highly active compounds involved in life processes that can affect the hormonal system, such as adrenal hormones (cortisone), sex hormones (estrogen and testosterone) [6]. Before becoming a steroid hormone, the biosynthetic pathway is wholly derived from acetic acid and converted to mevalonic acid.

The results in Table 4 show that the diversity analysis was significantly different (P> 0.05) on the digestibility of dry matter, organic matter, and crude protein from the addition of jackfruit and moringa leaves. The average dry matter digestibility ranged from 72.94 to 73.93%. The high DM digestibility in each treatment was due to the increase in the digestibility of dry matter directly proportional to the increase in the digestibility of organic matter. This means digesting dry matter and ration organic matter as an additional feed from good quality jackfruit and moringa leaves. The higher the digestibility rate of a food ingredient, the food material is of good quality for livestock consumption and is used for its metabolic processes. This is because in general, feed with high digestible nutrients will have a high nutritional value [7]. Anggorodi [8] added that the amount of digestible food substances measures the nutritional value of food, while the quality of a food ingredient is reflected in the number of dry matter consumption.

Provision of field grass and additional feed for jackfruit and Moringa leaves on rumen fermentability, and methane gas production can be seen in the Table 4.

The result shows that the treatment with jackfruit and moringa leaves had a significant effect on the pH of the rumen fluid. The pH of the rumen fluid is neutral in the range of 6.93 - 7. It means that the provision of jackfruit and moringa leaves affects the effectiveness of feed use in ruminants depending on the digestive process in the rumen. Low levels of digestion in the rumen resulting in feed inefficiency. Normal rumen pH to maintain normal rumen metabolism ranges from 6.0 to 7.0. If the rumen pH is lowered to 6.0, it can reduce fiber digestibility. Effect Variance analysis showed that the treatment had a significant effect (P <0.05) on the concentration of rumen fluid characteristics. Jackfruit leaves and moringa leaves are high fiber feed, reducing the efficiency of feed use and increasing the production of methane gas (CH4). The release of methane causes an increase in the concentration of CH4 in the air and causes a loss of 6-13% energy from the feed [9]. The production of methane gas shows that a lot of feed energy is wasted, so that a decrease in methane gas production can reduce the loss of wasted feed energy. The increasing the rate of adding Moringa leaf flour, the more efficient the feed will be. Tannins have two mechanisms to decrease methane gas production: directly by inhibiting the activity and growth of methanogenic bacteria and indirectly by inhibiting fiber digestion, thereby reducing H2 production.

Tabel 5. the results of the fiber fraction in vitro

Treatment	Parameters (%)			
	NDF	ADF	Cellulose	Hemicellulose
A	88,75[b]	88,38[b]	82,29[a]	90,72[a]
B	89,39[a]	90,87[a]	81,65[b]	77,81[c]
C	88,48[c]	87,67[c]	81,01[c]	89,63[b]
SE	0,41	0,67	7,39	4,92

Note: Means within a column with different superscripts are significantly (P < 0.05)

In this study, the digestibility of the fiber fraction of treatment C was 88.48%, ADF 87.67%, cellulose 81.01%, and hemicellulose 89.63%. It is suspected that in this study, the ration used had the same isoprotein and isoenergy composition, so that it would provide the same level of NDF digestibility. According to [10] the digestibility of feed is influenced by the age of the livestock, the level of feeding, and the chemical composition of the food ingredients.

4. CONCLUSION

The results of this study were obtained from the phytochemical content of phase 1 of jackfruit and moringa leaves which showed a positive effect from the tests for alkaloids, flavonoids, phenolics, saponins, triterpenoids, steroids, total tannins, and condensed tannins. Proximate content parameters consisted of BK, BO, SK, LK content based on statistical analysis showing that treatments A, B, and C showed an interaction and significant effect (P <0.05). Furthermore, the in vitro digestibility of the DM, BO, SK, LK digestibility based on statistical analysis showed that treatment A, B, and C showed an interaction at a significant level. However, the duration of fermentation had a significant effect (P <0.05) (on BK content, BO, SK, LK.

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