



Feeding Tannins to Goats Increased Strategy Microbial Protein Synthesis and Mitigation of Methane Gas

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Abstract | Manipulation of fermentation in the rumen can be done by giving defaunation agents to protozoa in animal feed using saponins and tannins. *Artocarpus heterophyllus* and *Moringa oleifera*, which are potential sources of tannins that can be used to protect high-quality protein feed so that it can be passed from degradation by rumen microbes. Like a double-edged sword, tannins have both positive and negative biological effects when consumed by goats. The purpose of this study was to determine the best tannin content of *Artocarpus heterophyllus* and *Moringa oleifera* added to goats in a strategy to increase microbial protein synthesis and mitigate methane gas. The experimental design was arranged in a randomized block design, in which 4 treatments and 5 replications consisted of treatment A (40% concentrate + 0% jackfruit leaves + 0% moringa leaves + 60% field grass), ration B (40% concentrate + 5 jackfruit leaves % + moringa leaves 0% + field grass 55%), C ration (40% concentrate + 0% jackfruit leaves + 5% moringa leaves + 55% field grass), D ration (40% concentrate + 2.5% jackfruit leaves + moringa leaves 2.5% + 55% field grass). In results, treatment D has a significant effect ($P < 0.05$) different optimally increasing the digestibility of nutrients, improving the characteristics of rumen fluid NH_3 12.38 mg/100ml, mitigating methane gas (total CH_4) of 26.41%, total tannins 0.0421 mg/l, and increased microbial protein synthesis of 171.22 mg/ml. Thus, treatment D feed can optimize the productivity of ruminants.

Keywords | Goat, Mitigation of methane gas, Tanni, Microbial Protein Synthesis, Protozoa Population

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INTRODUCTION

At this time, the availability of feed in the tropics is generally very dependent on the season, low quality and unstable continuity because in the dry season there is a shortage of feed and vice versa in the rainy season the feed

will be abundant. One way to overcome the problem of feed availability is to use additional feed ingredients from forages such as jackfruit and moringa leaves (Soetanto *et al.*, 2011). In particular, it was found that the addition of jackfruit and moringa leaves had a positive effect on tannins, namely increasing the efficiency of the use of pro-

tein rations, faster livestock growth, and the ability to reduce toxic gas emissions to the environment. The fact that strengthens the ability to reduce methane emissions from tannins is due to its characteristics that bind a number of nutrients thereby reducing rumen fermentation, including suppressing the production of hydrogen gas (Jayanegara *et al.*, 2013). Condensed tannins are chemicals that can preserve proteins, increasing the quantity of protein taken in by the small intestine and reducing NH_3 .

Jackfruit and moringa leaves have these condensed tannins in their chemistry. In the rumen, complex proteins and tannins will not dissolve, but in the acidic abomasum, these components will go through enzymatic digestion, making the protein soluble and usable by livestock (Gebregiorgis *et al.*, 2012). Rumen microbes play a crucial role in ruminant production, especially when it comes to turning waste fibrous feed, that cannot be used by humans into high-quality feed ingredients for cattle (Jayanegara *et al.*, 2015). Reduce methane output via manipulating the rumen's fermentation process. Applying defaunation agents to the protozoa in animal feed using saponins and tannins allows for the manipulation of fermentation in the rumen. Jackfruit and moringa leaves, which can be used as substitute ingredients in feed, can be made from agricultural waste.

Tannins from jackfruit leaves can be utilized to protect proteins. Jackfruit leaves have a combined tannin and condensed tannin concentration of 7.08 and 5.57%, respectively. In a different investigation, petioles and leaves of jackfruit with a condensed tannin content of 130 g/kg were added (Wahyono *et al.*, 2017). One of the factors to be taken into account when using moringa leaves as a blend in very inexpensive feed, in addition to jackfruit leaves, is their availability. According to previous studies, moringa leaves have an EENN of 32.83% and a CP of 26.43% (Harahap *et al.*, 2021).

Based on the research, it was discovered that the addition of jackfruit and moringa leaves had a favorable effect on tannins, including increasing the efficiency of ration protein use, promoting quicker growth in livestock, and lowering the amount of emissions that livestock produce into the environment. Because they contain a variety of antioxidant components such as ascorbic acid, flavonoids, phenolics, and carotenoids, moringa leaves are a good source of natural antioxidants (Ervinatun *et al.*, 2018). Moringa leaves are an excellent feed supplement because to their high levels of ascorbic acid, protein, and important amino acids, particularly methionine, cysteine, tryptophan, and lysine found in the leaves and pods (Kakengi *et al.*, 2002). Therefore, combining low-ammonia jackfruit with moringa leaves may increase the digestion of nutrients and reduce methane gas production. The purpose of this study was to focus on providing the proportions of jackfruit and moringa leaves con-

taining tannins are very interesting constraints on nutrient digestibility, rumen fermentation, mitigation of methane gas, and microbial population.

This study's focus was to identify the amounts of tannins found in jackfruit and moringa leaves as interesting limits on nutrient digestibility, rumen fermentation, microbial protein synthesis, mitigation of methane gas, and microbial population.

MATERIALS AND METHODS

ANIMALS

The livestock used in this study were goats aged about 8-10 months with a weight ranging from 10-15kg, as many as 20 animals. The cages used were wooden cages with a size of 100 × 130cm which were divided into two a place to eat, a place to drink, a means of collecting feces and urine, hanging scales of a capacity of 50kg of livestock, and digital scales for concentrated feed and one unit of cage support equipment.

TREATMENT

This study used a randomized block design with 4 treatments and 5 replication. The treatment is:

Treatment A = 40% concentrate+jackfruit leaves 0%+moringa leaves 0%+field grass 60%.

Treatment B = 40% concentrate+jackfruit leaves 5%+moringa leaves 0%+field Grass 55%.

Treatment C = 40% concentrate+jackfruit leaves 0%+moringa leaves 5%+field grass 55%.

Treatment D = 40% concentrate+2.5% jackfruit leaves+moringa leaves 2.5%+field grass 55%.

The treatment materials and chemical composition of the research ration are presented in Table 1.

IN VITRO ANALYSIS

In-vitro analysis was carried out to determine the pH, ammonia, total volatile fatty acid, acetic, propionic, and butyric of each feed component using the Tilley and Terry method, performed for 48h for forage and 24h for concentrate. Incubation was stopped by immersing the Erlenmeyer flask into ice water to stop the microbial activity, after which pH measurement was carried out using a pH meter. Next, the supernatant was separated from the residue. To do this, the mixture obtained from the *in-vitro* analysis was put into a centrifuge tube and then centrifuged for 30 minutes, 3000 rpm, at 4°C. The supernatant was stored in bottles and then frozen until subsequent analysis was carried out.

CHEMICAL ANALYZES

Each sample (Table 1) was analyzed to determine the dry matter, organic matter, crude protein, crude fiber, extract ether, and ash using proximate analysis (AOAC, 2005).

Table 1: Feed ingredient and nutrient (% dry matter).

Ingredient (% dry matter)	Treatment			
	A	B	C	D
Concentrate	40	40	40	40
Jacfruit leaves	0	5	0	2.5
Moringa leaves	0	0	5	2.5
Field grass	60	55	55	55
Total	100	100	100	100
Nutrient (%)*				
Dry Matter	42.69	46.08	45.90	45.99
Organic Matter	92.96	92.88	92.58	92.73
Crude Protein	12.62	12.86	13.68	13.27
Crude Fiber	25.20	24.60	24.15	24.37
Extract Eter	4.08	4.12	4.22	4.17
Extract Eter Non Nitrogen	51.06	51.30	50.53	50.92
Total Digestible Nutrient	62.27	63.15	63.21	63.18

*Data is provided from the total calculation results of the chemical composition of research ration materials with the arrangement of *in vitro* treatment; A: 40% concentrate+ jackfruit leaves 0%+moringa leaves 0%+field grass 60%; B: 40% concentrate+jackfruit leaves 5%+moringa leaves 0%+Field Grass 55%); C: 40% concentrate+jackfruit leaves 0%+moringa leaves 5%+field grass 55%; D: 40% concentrate+ 2.5% jackfruit leaves+moringa leaves 2.5%+field grass 55%.

Table 2: Determination of total tannin levels in jackfruit and moringa leaves (Harahap et al., 2021).

Samples	Weight Sample (mg)	Absorb- an (Y)	X (mg TAE/L)	Tannin to- tal (mg/L)
Moringa leaves	1000	0.067	19.0325	0.0952
Jackfruit Leaves	1000	0.176	6.6825	0.0334
Treatment				
A	1000	0.757	3.0395	0.0152
B	1000	0.775	3.0575	0.0153
C	1000	1.672	3.9545	0.0198
D	1000	1.684	3.9862	0.0421

Treatment;A:40%concentrate+jackfruitleaves0%+moringa leaves 0%+field grass 60%; B:40% concentrate+jackfruit leaves 5%+moringa leaves 0%+Field Grass 55%); C: 40% concentrate+jackfruit leaves 0%+moringa leaves 5%+field grass 55%; D: 40% concentrate+ 2.5% jackfruit leaves+moringa leaves 2.5%+field grass 55%. Source: Biochemical Laboratory, Animal Science Faculty, Unand, Padang (2021).

DETERMINATION OF TANNIN LEVELS

Wade 1.5 g of tannins, then put into a 100 ml beaker then added 50 ml of water heated at a temperature of 40-60°C for 30 minutes, after cooling the solution was filtered into a 250 ml volumetric flask, then added with water until the

line marks Then 25 ml of the above solution was taken and put into Erlenmeyer added 20 ml of indigocarmin solution then titrated with 0.1N KMnO₄ solution, each time adding 1ml of KMnO₄ until the color changed from blue to green then the titration was carried out drop by drop until the green color turned yellow.

METHANE GAS PRODUCTION

A total of 0.2 g of the ration sample was put into a serum bottle and then filled with 30 ml of a mixture of rumen fluid inoculum and McDougalls buffer using an automatic pipette dispenser, closed with a rubber cover, clamped with aluminum, and incubated in an incubator at 39°C for 48h. Taken using a syringe from a 5 ml serum bottle and put into a 5 ml serum bottle which has been vacuum closed with a rubber cover and clamped with aluminum. Methane gas was measured using Shimadzu (2014), gas chromatography equipped with a thermal conductivity detector, Helium gas was used as a carrier gas with flow rate 10 ml/min, detector and column temperature 250°C and 60°C.

PROTOZOA POPULATION

Protozoa population was calculated based on the method using a hemocytometer chamber, with a dilution of 1:5 (1 ml sample with 5 ml methyl green formaldehyde solution), protozoa were counted under 0.2 mm deep counting chambers and 6 classes were identified based on the method.

STATISTICAL ANALYSIS

All data were subjected to analyse of variance of completely randomized block design using SPSS software. The results were presented as the mean values and standard error of the means. Differences between treatment means were tested by Duncan's multiple range test method and declared significant (P<0.05).

RESULTS AND DISCUSSION

TANNIN CONTENT IN TREATMENT OF JACKFRUIT AND MORINGA LEAVES

Tannins are components of very complex organic substances, composed of difficult to separate and crystallize phenolic compounds, which precipitate proteins out of solution and bind to these proteins. The purpose of the standard curve is to study the relationship between the concentration of the tannic acid solution and the absorbance value to know the concentration of the carnation flower sample. A 1000 ppm standard solution was prepared serially at concentrations of 20, 60, 100, 140 and 160 ppm, standard curve equation derived from values of tannic acid concentration $y = 0.004x - 0.009$ and $r = 0.994$ was used to determine the tannin content and ration composition treatment of jackfruit and moringa leaves. A standard tannin curve is used to determine the tannin content.

The choice of tannic acid is based on the fact that tannic acid is a hydrolyzed group of tannins and can therefore be used as a comparison when measuring total tannin content (Beauchemin *et al.*, 2007). Tannins read by UV-Vis spectrophotometry must be reacted with chromogenic reagents, namely Folin-Dennis and sodium carbonate. Color formation is based on redox reactions in which tannins act as reducing agents. Folin-Dennis acts as an oxidizing agent, oxidized tannin converts Folin-Dennis phosphomolybdate to phosphomolybdenum, which is blue and can absorb light in the visible-ultraviolet wavelength range (Nora *et al.*, 2017). The results obtained show that the moringa leaf extract contains 0.0952 mg/l tannin and the jackfruit leaf contains 0.0334 mg/l tannic acid. In addition, treatments A contained 0.0152 mg/l, B contained 0.0153 mg/l, C contained 0.0198 mg/l, and D contained 0.0421 mg/l.

Table 3: Effect of tannins from jackfruit and moringa leaves in nutrient digestibility.

Parameters	Treatment (%)				SE	P-Value
	A	B	C	D		
Dry matter digestibility	59.93 ^a	59.96 ^a	61.18 ^a	62.94 ^b	0.11	0.61
Organic matter digestibility	76.04 ^a	74.66 ^a	73.60 ^b	75.02 ^a	0.46	4.73
Crude Protein digestibility	68.20 ^a	73.72 ^a	77.77 ^a	74.01 ^a	1.12	210.35
Crude Fiber digestibility	54.54 ^b	57.94 ^b	56.37 ^a	51.87 ^a	1.65	90.62
Ekstrakt eter digestibility	7.14 ^a	7.27 ^b	8.01 ^a	5.78 ^a	1.04	11.68

Values with different superscripts in same row are significantly ($P < 0.05$) different. **Source:** Nutrition laboratory, Faculty of Animal Science, Unand, Padang (2021).

NUTRIENT DIGESTIBILITY

Nutrient digestibility in this experiment Increases at higher protein, and energy. Increased nitrogen availability for microorganisms Increased protein synthesis, microbial activity ability to digest food. Table 3 shows that the treatment with jackfruit and moringa leaves as additional feed in the ration had a significantly different ($P < 0.05$) effect on the digestibility of dry matter (DM), organic matter (OM), crude protein (CP), crude fiber (CF) and extract eter (EE). Further testing with Duncan Multiple Range Test (DMRT) showed digestibility of food substances from the best treatment in the treatment of ration D with DM 62.94%, OM 75.02%, CP 74.01%, CF 51.87%, and EE 5.78% Judging from the results of the highest crude protein digestibility in treatment D directly affect the increase in dry matter and dry matter digestibility organic. Significantly different in crude protein digestibility between treatments was caused by a different ration given the same even though the different levels of giving jackfruit and moringa leaves for each

treatment that can affect protein digestibility in the rumen, this is influenced by the role of moringa leaves (Gebregiorgis *et al.*, 2012). In addition to the type of ingredients that make up the ration, used the same, also the addition of moringa leaves and jackfruit leaves did not have a significant impact on overall rumen microbial activity and total in the rumen, although the cumulative results play a role in the treatment D is able to produce the best digestibility value compared to other treatments to provide the effect on rumen microbial activity in each treatment increased, then with increasing activity (Martin *et al.*, 2010). The increase in the number of microbes also increases the dry matter and digested organic matter. Rumen microbial activity is due to the role rumen microbes play in the fermentation process (Cottle *et al.*, 2011). The activity of the rumen microbes themselves is influenced by the nutrients contained in food ingredients.

Table 4: Effect of tannins from jackfruit leaves and Moringa leaves in rations on rumen fluid characteristics

Parameters	Treatment				SE	P-Value
	A	B	C	D		
pH	6.99 ^a	7.07 ^a	7.33 ^a	7.47 ^a	0.09	5.86
VFA (mM)	130 ^b	138.4 ^a	143.20 ^b	148.80 ^c	1.77	12.98
NH ₃ (mg/100 ml)	8.99 ^b	17.27 ^b	14.25 ^a	12.38 ^a	1.84	3.98

Values with different superscripts in same row are significantly ($P < 0.05$) different. **Source:** Nutrition laboratory, Faculty of Animal Science, Unand, Padang (2021).

RUMEN FERMENTATION CHARACTERISTICS

Table 4 shows that the treatments had a significant effect on the pH of the ruminal fluid in the range 6.99 to 7.47. This means that feeding jackfruit or moringa leaves can help. The effectiveness of feed conversion in ruminants is highly dependent on rumen digestion processes. Rumen maldigestion leads to reduced feed efficiency. A normal rumen pH is in the range of 6.0-7.0 to maintain rumen metabolism. A ruminal pH below 6.0 can reduce fiber digestibility (Warly *et al.*, 2017). Rumen acidity can affect the number of microorganisms active in the fermentation process (Sasongko *et al.*, 2010). Rumen microbes work best at a pH of 6-7, breaking down amino acids to ammonia. An acidic rumen pH atmosphere (low pH) can cause a decrease in ruminal microbial activity. The addition of jackfruit leaf and moringa leaf is highly effective in increasing rumen fluid VFA. The higher the jackfruit and moringa leaf content, the higher the total VFA in the liquid rumen and vice versa. A dose of jackfruit and moringa leaves can increase total VFA by 148.80% with treatment D. Jackfruit and moringa leaves were thought to contain saponins, thus increasing VFA concentrations (Suyitman *et al.*, 2014). Protozoa are predators of ruminal bacteria, and reducing protozoan populations in the rumen affects the increase

of bacterial biomass, thereby increasing the efficiency of microbial protein synthesis. In addition, saponins can also bind ammonia in the rumen at high concentrations and release it again at low concentrations to ensure the availability of ammonia for microbial protein synthesis (Martin *et al.*, 2010), thus giving a more optimal result. Rumen growth is 80–160 μm (Nora *et al.*, 2017). Volatile fatty acids are the final product of the bio-fermentation process in ruminants, providing 70–80% of their needs (Makkar *et al.*, 2014), which is their source of energy.

A process that further catabolizes the products of hydrolytic digestion of fermentation monomers, fermentation of carbohydrates to volatile fatty acids or VFA (Phengvilay-souk and Kaensombath, 2006). Furthermore, providing jackfruit leaves and moringa leaves as supplements in the diet had a significant effect on increasing total NH_3 rumen to 12.93 mm in treatment D (Bhatta *et al.*, 2015). The concentration of NH_3 required for optimal feed digestion by rumen microbes is 5–20 mg/dl (Nooriyan Soroor and Rouzbehan, 2017).

Table 5: Effect of tannins from jackfruit and moringa leaves in rations on fiber fraction

Parameters	Treatment				SE	P-Value
	A	B	C	D		
NDF digestibility	54.35 ^a	55.75 ^a	56.39 ^a	57.66 ^b	2.03	47.36
ADF digestibility	51.12 ^b	52.54 ^a	52.60 ^c	54.65 ^a	2.32	18.43
Cellulosa digestibility	47.38 ^a	41.90 ^b	35.08 ^a	37.45 ^a	2.39	4.53
Hemicellulosa digestibility	64.16 ^a	65.60 ^a	66.90 ^b	69.56 ^b	2.71	18.68

Values with different superscripts in same row are significantly ($P < 0.05$).different. **Source:** Nutrition laboratory, Animal Science Faculty, Unand, Padang (2021).

FIBER FRACTION

Nutrient digestibility is affected by the age of livestock, the extent of feeding, and the chemical composition of food components (Warly *et al.*, 2017). The high digestibility of NDF in treatment D demonstrates that the addition of jackfruit and moringa leaves has important effects due to the ability of rumen microbes to degrade the fiber fraction and the highest protein digestibility. In results, treatments in Table 5 show significantly ($P < 0.05$) different effects on the digestibility of NDF. In this study, NDF digestibility ranged from 54.35 to 57.66%. This study assumes that the feed used has the same chemical composition so that he can digest the same NDF. NDF is a neutral detergent fiber food substance that makes up the majority of wall plants (Warly *et al.*, 2015). This substance is composed of cellulose, hemicellulose, lignin, silica, and some fibrous proteins (Pazla *et al.*, 2021). Rumen microbes use food to generate energy and make the most of these nutrients when needed. Digestibility of ADF is higher than that of ADF, but NDF

has higher digestibility because NDF has several soluble fractions, especially hemicellulose. The highest ADF digestibility was 52.60% for treatment D. ADF (Acid Detergent Fiber) is insoluble in acidic detergents, so it is composed of cellulose, lignin, and silica, so the decomposition of cellulose depends on the number of bacteria growing in the rumen, the ratio of lignin to silica, and crystallization bonding (Metri *et al.*, 2018). The highest cellulose digestibility was found with treatment D, 37.45%. This is because the rumen microbes are not fully equipped to produce sufficient amounts of cellulose enzymes (Suyitman *et al.*, 2021). Ruminants are unable to properly digest and utilize cellulose, the poor digestibility of cellulose in this treatment is due to the poor ability of rumen microbes to digest food material within the rumen (Fariani *et al.*, 2021). Factors affecting cellulose degradation are the number of bacteria growing in the rumen, the proportion of lignin and silica in the diet, and the duration of absorption and degradation of food components by the bacteria in the rumen (Salem *et al.*, 2006). Moreover, hemicellulose digestibility showed significantly ($P < 0.05$) different effects. In this study, the digestibility of hemicellulose ranged from 68.16 to 90.56%. The highest digestibility of hemicellulose was 69.56% for treatment D. This is because rumen microbes were able to use hemicellulose as an energy source, hemicellulose and cellulose are two compounds. As a source, it is very important for ruminants (Putri *et al.*, 2021).

Table 6: Effect of tannins from jackfruit and moringa leaves in rations on methane gas production.

Treatment		Parameters		
		Total Gas Production (ml)	Total CH_4 Production (ml)	Total CH_4 (%)
Without Concentrate	A	94.33	27.67	29.35
	B	108.00	31.00	28.70
	C	87.33	23.17	26.54
	Total	289.66	81.84	84.59
	Average	96.55	27.28	28.20
With Concentrate	A	167.60	43.60	25.96
	B	141.20	39.10	27.69
	C	150.80	39.30	26.06
	D	169.80	44.00	25.92
	Total	629.40	166.00	105.63
	Average	157.35	41.50	26.41

Source: Balai Penelitian Ternak (Balitnak), Ciawi, Bogor (2021).

MITIGATION OF METHANE GAS

Table 6 Sources of ruminal N-NH_3 that do not come from the degradation of dietary proteins also come from the degradation of microbial protoplasm, especially protozoa. Protozoa have the ability to prey on large amounts of protein, carbohydrates and even ruminal bacteria. Thus, proto-

zoa play a role in regulating the migration rate of N within the rumen and supply soluble proteins to sustain bacterial growth. Most protozoa are retained in the rumen and only about 20-40% of protozoan cells reach the intestine (Malik *et al.*, 2017). Methane gas reduction through the addition of jackfruit and moringa leaves is a strategy for manipulating fermentation processes within the rumen, with the aim of increasing microbial protein synthesis and reducing methane gas using feed ingredients (Ji *et al.*, 2016). The presence of tannins may reduce total gas production in in vitro fermentation systems, as the interaction of tannins with feed contributes to total gas production, and the results of the study significantly ($P < 0.05$) different from total methane gas production indicates an effect. Lowest mean or total methane gas in the range of 25.92% obtained from treatment D. This is because the presence of tanning agents in feed ingredients can lead to defaunation. The presence of protein tannin complexes can put pressure on the rumen protozoan population, indirectly affecting protozoan decline.

BIOMASS AND MICROBIAL PROTEIN SYNTHESIS

In present study, treatment ration of jackfruit and moringa leaves significantly increased biomass microbial ($P < 0.05$) different from 7.35 to 20.81 mg/ml. The treatment ration tended to decrease microbial protein synthesis from 77.55 to 228.89 mg/ml (Table 7).

Table 7: Biomassa and microbial protein synthesis.

Parameters (mg/ml)	Treatment				SE
	A	B	C	D	
Microbial Biomass	7.35	17.25	11.25	20.81	3.763642
Microbial Protein	77.55	171.22	105.33	228.89	12.07259

Source : Feed and Nutrition Laboratory, Politeknik Pertanian Payakumbuh (2022).

Microbial protein synthesis occurs through synchronization of protein and energy sources and must be readily biodegradable (Hermon *et al.*, 2008). In this study, the use of jackfruit leaves and moringa leaves in the diet may increase microbial protein synthesis due to the availability of nitrogen from NH_3 and total VFA. Treatment increased microbial protein synthesis from 77.55 to 228.89 mg/ml and microbial biomass from 7.35 to 20.81 mg/ml. This indicates that protein energy synchrony has been achieved. Nutrient utilization and microbial protein synthesis can be achieved when ruminal protein responses and energetic synchronization are optimal (Uddin *et al.*, 2015). Protein is the most important feed source for ruminants because it can stimulate microbial protein synthesis and rumen fermentation, increasing productivity. Enhanced microbial protein synthesis improves NH_3 utilization and fiber digestion efficiency, ensuring optimal nutrient utilization (Hackmann and Firkins 2015).

Table 8: Effect of tannins from jackfruit leaves and moringa leaves in rations on microbial population.

Parameter	Treatment			
	A	B	C	D
Bacteria total (after 48 hrs)	4.21×10^9	4.79×10^9	6.29×10^9	5.49×10^9
Protozoa population (after 48 hrs)	24.33×10^6	36.83×10^6	29.67×10^6	50.67×10^6

Source: Balai Penelitian Ternak (Balitnak), Ciawi, Bogor (2021).

PROTOZOA AND MICROBIAL POPULATION

Table 8 shows the highest total bacterial colony for treatment D of 5.49×10^9 and total protozoan population of 50.67×10^6 . Adding jackfruit and moringa leaves can reduce the total rumen protozoan population. The table shows that treatment C can significantly reduce protozoan populations compared to all protozoa. Treatment C reduced the protozoan population by 29.67 compared to D. Following this result, he also reduced methane gas production by 50%. There is a relationship between protozoan populations and rumen methanogenesis. Between 7 and 37% of methanogens live symbiotically with protozoa in the rumen (Harahap *et al.*, 2020).

CONCLUSION AND RECOMMENDATIONS

Proportion of jackfruit and moringa leaves in overcoming tannins has increased significantly on the evaluation of nutrient digestibility, rumen fluid characteristics, fiber fraction, rumen characteristic, mitigation of methane gas, microbial protein synthesis, and protozoa population. In results, treatment D has a significantly ($P < 0.05$) different optimally increasing the nutrient, improving the characteristics of rumen fluid NH_3 12.38 mg/100 ml, mitigation of methane gas of 26.41%, tannin total 0.0421 mg/l, and increased microbial protein synthesis of 171.22 mg/ml. Thus, treatment D can optimize the productivity of ruminant.

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NOVELTY STATEMENTS

Providing 2.5% tannin from jackfruit leaves and moringa

leaves can increase microbial protein synthesis and mitigate methane gas in goats.

AUTHOR'S CONTRIBUTIONS

Angelia Utari Harahap and Lili Warly supervised the experiment. Hermon and Suyitman, Armina Fariani conducted the experiment and data analysis. A Evitayani and Toga Mahaji prepared tables and finalized the draft. The final version of the manuscript was read and approved by all authors.

GENERATIVE AI OR AI-ASSISTED TECHNOLOGY STATEMENT

The authors declare that no Genrative AI was used in the creation of this manuscript.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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