EFFECT OF COMBINING LEUCAENA AND INDIGOFERA LEAVES IN SWAMP GRASS-BASED DIETS ON DIGESTIBILITY, RUMEN FERMENTABILITY AND METHANOGENESIS

Riswandi Lecturer · **A. I. M. Ali** Lecturer · **Muhakka** Lecturer

S. Sandi Lecturer · **A. N. T. Pratama** Lecturer

Department of Animal Science, Faculty of Agricultural, Sriwijaya University, Indonesia

E-mail: riswandi@fp.unsri.ac.id

ABSTRACT

This research was conducted to evaluate the effect of the combination of Leucaena and Indigofera in swamp grass-based rations on the quality or quantity of feed produced. The research was carried out based on a completely randomized design (CRD) consisting of four treatments and four replications. The types of forage used include termite Bento grass (*Leersia hexandra)* from swamp land; legumes Leucaena and Indigofera. The treatments consisted of A = swamp grass and concentrate (70%:30%), B = swamp grass, Leucaena and concentrate (40%:30%:30%), C = swamp grass, Indigofera and concentrate (40%:30%:30%), D = swamp grass, Leucaena, Indigofera and concentrate (40%:15%:15%:30%). The observed variables consisted of dry matter digestibility (DMD), pH, total Volatile Fatty Acid (TVFA), N-ammonia (N-NH_3), partial VFA, methane gas, total bacteria and protozoa. The study showed that the combination of tree legumes increased (P<0.05) the digestibility values of DM, TVFA, propionic acid, butyrate and total bacteria, while the levels of N-NH_3, acetic acid, acetate-propionate ratio, methane gas, methane gas production and protozoa decreased (P<0.05). Based on the results of the study, it can be concluded that the combination of tree legumes in the ration can increase digestibility, improve rumen fermentation characteristics and reduce methane gas production.

Key words: methane, legumes, swamp grass

مجلة العلوم الزراعية العراقية- 2025 :56 (1):391-401 ريسواندي وآخرون

تأثير الجمع بين أوراق الليوكينا والإنديغوفيرا في الوجبات الغذائية القائمة على عشب المستنقعات على قابلية الهضم، وقابلية الكرش للتخمر وتكوين الميثان

ريسواندي محاضر · أ.إ.م.علي محاضر · محقة محاضر

أ.ن.ن.ت. براتاما محاضر · صوفيا ساندي محاضر

قسم التكنولوجيا وصناعة الثروة الحيوانية ، كلية الزراعة ، جامعة سريويجايا ، إندونيسيا

المستخلص

تم إجراء هذا البحث لتقييم تأثير الجمع بين اللوكينا والإنديغوفيرا في حصص الأعلاف المستنقعية، على جودة أو كمية الأعلاف المنتجة. تم إجراء البحث على أساس تصميم عشوائي بالكامل (CRD) يتكون من أربع معالجات وأربع تكرارات. وشملت أنواع الأعلاف المستخدمة عشب بنتو النمل الأبيض (ليرسيا هيكساندرا) من أراضي المستنقعات؛ وبقوليات ليوكينا وإنديغوفيرا. تألفت المعالجات من (أ) = عشب المستنقعات والمركزات (70%:30%)، (ب) = عشب المستنقعات والليوكينا والمركزات (40%:30%:30%)، (ج) = عشب المستنقعات والإنديجوفيرا والمركزات (40%:30%:30%)، (د) = عشب المستنقعات والليوكينا والإنديجوفيرا والمركزات (40%:15%:15%:30%). تألفت المتغيرات التي تمت ملاحظتها من قابلية هضم المادة الجافة (DMD)، والأس الهيدروجيني، والأحماض الدهنية المتطايرة الكلية (TVFA)، والأمونيا النيتروجينية (N-NH_3)، والأحماض الدهنية المتطايرة الجزئية، وغاز الميثان، والبكتيريا الكلية والبروتوزوا. أظهرت الدراسة أن الجمع بين البقوليات الشجرية زاد (P<0.05) من قيم قابلية هضم الحمض الدهني المتطاير (P<0.05) و TVFA وحمض البروبيونيك والزبدات والبكتيريا الكلية، بينما انخفضت مستويات N-NH_3 وحمض الأسيتيك ونسبة الأسيتات إلى البروبيونات وغاز الميثان وإنتاج غاز الميثان والبروتوزوا (P<0.05).

الميثان، والبقوليات، وعشب المستنقعات

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INTRODUCTION

The problem that often occurs in the provision of ruminant feed in the tropics is that the available feed cannot meet the needs of livestock. This is because in general, the livestock rearing system is still traditional, where the forage provided only comes from natural pastures, the availability of forage fluctuates, low quality (high fiber fraction) so that it has the potential to increase methane gas levels, reduce feed efficiency and performance of ruminants (3). Based on these problems, a strategy is needed to overcome this problem, which is by utilizing local feed materials derived from sub-optimal land such as swamp land which has a variety of forage species that can be utilized as animal feed. One type of swamp grass used as the basic feed is Leersia hexandra or better known locally as termite bento grass, this grass has high production but low nutritional value (low crude protein and high fiber fraction), the high content of fiber fraction of this grass causes this grass cannot be given to livestock in a single feed so it must be combined with legumes (2). Tree legumes are widely available local raw materials, rich in protein, minerals and contain secondary compounds (phytogenic) and have the potential to be a source of bypass protein. Tree legume plants that are often given to livestock include Leucaena and Indigofera. By-pass protein is feed protein that is not degraded by rumen microbes, as a result it can directly undergo an enzymatic digestion process in the abomasum thereby increasing the amount of protein and amino acids to be digested and absorbed in the small intestine (13). Magdalene et al. (15) observed that several phytogenic active components including essential oils, flavonoids, saponins and tannins can change the rumen ecology, reducing acetate concentration, acetate-propionate ratio and methane production as well as increasing ration digestibility and livestock performance. Indigofera (Indigofera zollingeriana) has quite high biomass production with high nutrient content. Indigofera contains 28.98% crude protein (CP), 3.30% Ether extract (EE), 8.49% crude fiber (CF), 0.52% calcium (Ca), 0.34% phosphorus (P), tannin 0.29%, and saponin 0.036% (25) Indigofera has the potential to increase propionic acid, reduce acetate, and the A/P ratio. The presence of secondary compounds in this plant, such as tannin and saponin, has an antiprotozoal effect so it can be used as a defaunation agent, thereby potentially reducing methane gas production (6–8). Leucaena (*Leucaena leucocephala*) is a tree legume plant that has great potential to be developed as a producer of forage for livestock throughout the year. This plant can produce 70 tons of fresh forage or around 20 tons of dry matter/ha/year. The chemical composition of the food in dry matter consists of 25.90% crude protein, 20.40% crude fiber and 11% ash (2.30% Ca and 0.23% P), carotene 530.00 mg/kg and tannin 10.15 mg/kg (9). The research results of Tan et al. (40) stated that the addition of pure condensed tannin from leucaena plant extract at a level of 10 - 30 mg in 500 mg samples decreased methane gas production, total VFA, protozoa and methanogenic bacteria populations. Zain et al. (48) stated that the effect of tannins and saponins in the legume Gliricidia sepium can reduce methane gas emissions and increase the efficiency of rumen fermentation and feed digestibility. Furthermore, Riswandi et al. (33) stated that the addition of leucaena leaves at the level of 20% in Kumpai grass silage-based rations could increase the productivity of Bali cattle. This study aims to evaluate the effect of a combination of leucaena and Indigofera leaves in a swamp grass-based diet on digestibility, rumen fermentation characteristics and methane gas concentration.

MATERIAIS AND METHODS

Sample Preparation and Experimental Design : The swamp forage used is Bento grass obtained from swamp land in Tanjung Seteko village, Ogan Ilir district, South Sumatra Regency, while tree legumes consist of leucaena and Indigofera leaves obtained from the garden collection of the Sriwijaya University animal husbandry study program. Chemical and in vitro analyzes were carried out at the Animal Nutrition and Feed Laboratory, Faculty of Agriculture, Sriwijaya University. The design used was a completely randomized design, consisting of four treatments and four replications. Treatment consisted of A = swamp grass and concentrate (70%:30%), B = swamp grass, leucaena and concentrate (40%:30%:30%), C = swamp grass, Indigofera and concentrate (40%:30% :30%), D = swamp grass, leucaena, Indigofera and concentrate (40%:15%:15%:30%).

Table 1. Nutritional composition of Bento Grass (BG), Leucaena leucocephala (LL), Indigofera zollingeriana (IZ) and Concentrate (% DM).

Nutrient	BG	LL	IZ	CON
Dry Matter (DM)	80.31	88.63	87.65	79.82
Organic matter (OM)	75.48	85.52	83.82	71.68
Crude Protein (CP)	9.29	24.45	28.73	18.02
Crude Fiber (CF)	26.53	16.27	14.85	10.20
Ether extract (EE)	2.31	3.12	3.56	7.94
Nitrogen Free Extract (NFE)	38.48	36.26	35.96	35.45
Total Digestible Nutrient (TDN)	59.30	69.47	75.45	67.58
Neutral detergent fiber (NDF)	65.83	49.42	48.85	37.18
Acid detergent fiber (ADF)	45.52	24.51	23.49	20.85
Hemicellulose	20.31	24.91	25.36	16.33
Cellulose	32.62	14.26	12.65	16.62
Lignin	12.88	11.32	10.42	4.31
Tannin	0.25	10,25	0,27	Nd
Saponin	1.23	5.28	4.25	Nd

Table 2. Chemical Compositions of The Experimental Diets (% DM)

Ingredients	Diet			
	A	B	C	D
DM	80.16	82.66	82.36	82.51
OM	74.34	77.35	76.84	77.10
CP	11.91	16.46	17.74	17.09
CF	21.63	18.55	18.13	18.34
EE	3.99	4.24	4.37	4.31
NFE	37.51	36.91	36.82	38.79
TDN	60.77	64.53	63.32	63.93
NDF	57.23	52.37	52.14	52.26
ADF	38.12	32.12	31.51	31.82
Hemicellulose	19.12	20.26	20.63	20.44
Cellulose	27.83	22.32	21.84	22.08
Lignin	10.31	9.84	9.70	9.77
Tannin	0.17	3.18	0.18	1.68
Saponin	0.86	2.08	1.77	1.92

Note : Laboratory analysis of Animal Nutrition and Feed, Faculty of Agriculture, Sriwijaya University; A = swamp grass and concentrate (70%:30%); B = swamp grass, leucaena and concentrate (40%:30%:30%); C = swamp grass, Indigofera and concentrate (40%:30% :30%); D = swamp grass, leucaena, Indigofera and concentrate (40%:15%:15%:30%).

Feed chemical quality analysis

Tree legumes consisting of leucaena and Indigofera leaves are washed with running water. Both ingredients were dried in the oven for 24 hours, then ground and filtered using a sieve size of 0.5 mm. A total of 10 milliliters of methanol solvent was put into a test tube containing 0.5 g of plant material (leucaena and Indigofera leaves). The tube was then kept for 20 minutes at room temperature. Each sample was then centrifuged (Thermo Scientific IEC Centra CL2 Centrifuge, Fisher Scientific Pte Ltd., Singapore) at 3000 g and 4℃ for 10 min. This procedure was repeated twice, and the supernatants were combined and the tannin and saponin concentrations were then measured (12, 16). All extractions were carried out in triplicate. The type of solvent that produced the highest tannin and saponin concentrations was then used for in vitro rumen fermentation experiments. For this purpose, the organic solvent was removed with a rotavapor (Buchi Rotavapor R-200, Germany), followed by freeze-drying for 24 h to obtain the dry extract. The dried extract was then dissolved in distilled water and underwent in vitro rumen incubation, along with the substrate and rumen buffer solution. The rations prepared consist of swamp grass (Bento grass), tree legumes (Leucaena and Indigofera leaves) and concentrate. The composition of the feed ingredients and nutritional content of the rations can be seen in tables 1 and 2. Sample preparation was carried out by grinding the feed ingredients consisting of feed, namely bento grass, Leucaena, Indigofera and concentrate in proportions (according to treatment). Grass flour and concentrate are mixed homogeneously with a 70:30 formulation, then combined with tree legume feed (Leucaena and Indigofera leaves) according to the treatment. Measurement of nutritional content is based on proximate analysis according to procedures (5) and fiber fraction (44); rumen microbial characteristics, rumen fermentability and in vitro digestibility of feed.

Chemical analysis and In Vitro Fermentation : The research was carried out in vitro based on the method (42). Substrate was put into each incubation bottle as much as 0.5 grams according to the treatment. The incubation medium for each bottle is 90 ml of buffer solution (pH 7.9) consisting of 86 ml of basal solution and 4 ml of reducing agent, as well as 10 ml of buffalo rumen fluid. Rumen fluid is taken in the morning at the slaughterhouse, with the help of several tools, namely nylon filter cloth, flask, Erlemeyer tube, and thermometer. The rumen fluid collection was taken to the laboratory, filtered through a 100 μm nylon filter and added to buffer solution (100 ml/ 900 ml buffer solution). The incubation medium was saturated with CO_2 gas until the media condition became anaerobic, then 100 ml was put into each incubation bottle. The incubation bottle was immediately closed and placed in a water bath incubator (39°C) and incubated for 48 hours. After the incubation time is complete, two drops of $HgCl_2$ are added to inhibit microbial activity. The sample and incubation medium were centrifuged in a tube at 4000 rpm for 10 minutes so that the supernatant and residue were separated. The supernatant was taken to further analyze the concentration of NH_3, total Volatile Fatty Acid (TVFA), and partial VFA, gas production, total number of bacteria and protozoa. N-NH_3 concentration modified Conway procedure (9). According to Martin et al. (17) that methane gas production can be estimated from partial VFA (CH_4 =0.45 C2 –0.275 C3 +0.40 C4). Dry matter digestibility was measured after 48 hours of incubation. The sinter glass was dried first in an oven at 105°C for 24 hours and weighed to determine the weight of each piece. The incubation residue was dried in an oven at 105°C for 24 hours to calculate dry matter digestibility.

Data Analysis

The SAS statistical package (PROC GLM) was used to determine the significance of differences between treatments (SAS, 2013). Data obtained during this study were analyzed according to a Completely Randomized Design (CRD). If there are significant differences, further tests will be carried out using the Duncan Multiple Range Test (39).

RESUITS AND DISCUSSION

Rumen Fermentability Characteristics

The results of the analysis of variance showed that the treatment had a significant effect ($P<0.05$) on DMD, N-NH_3 and TVFA, while pH had no significant effect ($P>0.05$), presented in Table 3.The results showed that the DMD values of treatments B, C and D were higher ($P<0.05$) than treatment A (control), while treatments B, C and D were not significantly different ($P>0.05$), this shows that the provision of tree legumes as a functional feed it can increase the digestibility of BK rations. The high DMD value in the tree legume combination was due to the availability of optimal nutritional and phytochemical content from the tree legume combination treatment ration (Table 2) for rumen microbial growth. Chemical compositions such as low fiber fraction content and high crude protein content in the diet combined with tree legumes have an impact on increasing the microbial population and DMD of the diet. McDonald et al. (18) reported that the factors that influence digestibility are the chemical composition of feed, the ratio of feed ingredients, feed treatment and level of feeding. The results of this research are in line with the research of Riswandi et al. (34) stated that a combination of 30% swamp legumes (water mimosa) in a Guinea grass-based ration resulted in the best treatment for increasing the DM and OM digestibility values of the ration. Furthermore, Zain et al. (48) which states that the influence of phytochemicals (tannins and saponins) in the legume Gliricidia sepium can increase feed digestibility and efficiency of rumen fermentation.

Table 3. Effect of the combination of tree legumes in the diet on DMD, N-NH_3 and TVFA

Parameter	Treatment			
	A	B	C	D
DMD (%)	56.35±0.74^a	57.88±0.33^b	58.42±0.98^b	58.04±0.08^b
pH	6.62±0.09	6.81±0.24	6.68±0.09	6.70±0.08
N-NH_3(mM)	7.47±0.04^a	8.01±0.56^b	9.19±0.44^d	8.47±0.43^c
TVFA (mM)	135.34±0.62^a	137.68±0.38^b	139.83±0.88^d	138.46±0.94^c

Note: Different superscripts in the same column indicate significant differences (P<0.05); A = swamp grass and concentrate (70%:30%), B = swamp grass, leucaena and concentrate (40%:30%:30%), C = swamp grass, Indigofera and concentrate (40%:30% :30%), D = swamp grass, leucaena, Indigofera and concentrate (40%:15%:15%:30%).

Rumen pH

The results showed that tree legume treatment had no significant effect (P>0.05) on rumen pH. The rumen pH value obtained is ideal for rumen microbial growth in the range of 6.62-6.81, so it does not interfere with microbial growth in the rumen. This is because fermentation products such as TVFA and N-NH_3 in this study produce optimal levels so that they can maintain normal rumen pH. McDonald et al. (18) reported that there is a balance of fermentation products, TVFA and N-NH_3, which can also maintain normal rumen pH (5.5-6.5). Furthermore, Ranathunga et al. (31) reported that the normal pH that supports the growth of rumen microbes ranges from 5.5-7.00. The results of this research are in line with the research of Sari et al. (37), reported that the use of the legumes Tithonia diversifolia and Leucaena leucocephala in the diet had no significant effect on pH, resulting in rumen pH ranging between 6.90-6.97.

Total Volatile Fatty Acid (TVFA)

Total VFA is an energy source that comes from carbohydrates in food which are digested by microbes in the rumen. The composition of the VFA formed is influenced by the fermented substrate, microbial population and rumen ecology (8, 19). The results showed that all treatments contributed to an increase (P<0.05) in TVFA levels in the rumen, the lowest TVFA content was in A (135.10 mM), and the highest was in treatment C. Treatment (C) was the treatment with the highest VFA production (139 .75 mM) this is due to the high nutrient content in treatment C (Table 2) so that it is able to provide nutrients for the growth of rumen microbes, and contributes to an increase in the dry matter digestibility value of the treatment ration. Increasing the digestibility of dry matter will increase the activity of rumen microbes to ferment the ration into TVFA (36). The VFA content produced in this study was still within the normal range to ensure rumen microbial activity and growth. This TVFA value is in line with the research results of Riswandi et al. (35), who reported that supplementation of different legumes consisting of Water mimosa, Leucaena leaves, and Acacia in fermented kumpai grass-based rations could increase VFA levels compared to controls, with values ranging from 70.02 – 158. 84 mM. According to Wang et al. (45) the optimal TVFA content required to support rumen microbial growth. The results showed that all treatments contributed to an increase in ammonia levels compared to the control treatment, the lowest N-NH_3 content was in treatment A (control) at 7.47 mM, the highest N-NH_3 content was in treatment C at 9.19 mM. N-Ammonia is a representation of protein degradation by rumen microbes into amino acids which then undergo deamination and become NH_3 as an important component of microbial protein synthesis (26). Treatment C had a higher ammonia content than other treatments, this indicates a high CP content (17.72%) and low levels of phytochemicals (tannin and saponin) in Indigofera tree legumes (table 1). The phytochemical effect in the combination treatment of leucaena leaves (B) was more significant in protecting protein levels as seen from the lower ammonia levels of treatments C and D, because the tannin and saponin levels of treatment B were higher than the other treatments (table 2). Leucaena leaves have protective properties against protein and influence rumen protein degradability because of their thick tannin content (23). The mechanisms of tannin and saponin are different in protecting protein in the rumen. Tannins can bind proteins by slowing the rate of deamination and degradation of amino acids into ammonia. The effect of saponin on reducing ammonia levels is mainly due to its direct antimicrobial effect. As an antiprotozoal agent, saponin also inhibits the activity of rumen proteolytic bacteria (11, 12). Ningrat et al. (22) reported Leucaena has low crude fiber, and high tannin content, it prevents excessive protein breakdown in the rumen, allowing small intestinal protein absorption as true protein. The ammonia levels resulting from this research have met the optimal limit of N-NH_3 requirements for microbial protein synthesis. According to McDonald et al. (18) the optimal concentration of N-NH_3 in rumen fluid to support the growth and activity of rumen microbes varies widely, starting in the range of 4.72-16 .67 mM.

Table 4. Effect of the combination of tree legumes in the diet on partial VFA, A-P ratio and methane gas production

Parameter	Treatment A	Treatment B	Treatment C	Treatment D
Acetate (mM)	90.43±0.75[c]	76.51±0.51[a]	78.19±1.10[b]	76.99±1.58[a]
Propionate (mM)	26.45±1.20[a]	39.77±1.16[b]	39.98±1.71[b]	39.94±1.49[b]
Butyrate (mM)	18.39±0.87[a]	21.41±1.57[b]	21.66±0.76[b]	21.53±1.82[b]
A-P Ratio	3.43±0.17[b]	1.93±0.05[a]	1.96±0.09[a]	1.93±0.08[a]
Methane Gas (mM)	40.78±0.74[b]	32.06±0.73[a]	32.61±0.63[a]	32.27±0.62[a]

Note: Different superscripts in the same column indicate significant differences (P<0.05. A = swamp grass and concentrate (70%:30%), B = swamp grass, leucaena and concentrate (40%:30%:30%), C = swamp grass, Indigofera and concentrate (40%:30% :30%), D = swamp grass, leucaena, Indigofera and concentrate (40%:15%:15%:30%).

Partial VFA, A/P Ratio and Methane Gas Concentration

Acetic (C2), propionic (C3) and butyric (C4) acids are the final results of the carbohydrate fermentation process, both structural and non-structural, by microorganisms in the rumen. These partial VFAs are also produced by microbial fermentation of feed proteins. The results showed that the proportion of acetic acid in treatment A was 66.86%, B 55.57%, C 55.92% and D 55.61%, while the proportion of propionic acid produced in A was 19.54%, B 28.89%, C 28.59% and D 28.85%. This indicates that the combined effect of tree legumes contributes to reducing the proportion of acetic acid and increasing the proportion of propionic acid, thereby reducing the acetate-propionate ratio. Acetic acid is the end product of fiber fermentation, while propionic acid is the end product of sugar and starch fermentation. The results of this study show that supplementation with a combination of tree legumes produces a proportion of acetic and propionic acid that is close to the results of research by Arora (6) which states that the proportion of acetate ranges from 50-65% and propionate ranges from 18-24%. Furthermore, Jayanegara et al. (13) reported that supplementation with various proportions of tannins and saponin-rich plant extracts in the diet (70% Napier grass and 30% concentrate) resulted in acetate proportions of 51.40-56.80% and propionate of 26.00-32.50%.

Ratio C2/C3

The results showed that the combination of tree legumes showed a decrease (P<0.05) in the C2/C3 ratio compared to the control. The low C2/C3 ratio in the treatment with the addition of tree legumes is due to the higher nutritional and phytochemical content in the tree legume treatment which stimulates starch-degrading bacteria to produce propionic acid, thus allowing the formation of higher levels of propionic acid than acetic acid. Jayanegara et al. (13) reported that supplementation with varying proportions of tannins and saponin-rich plant extracts in the diet (70% Napier grass and 30% concentrate) resulted in a decrease in the C2/C3 ratio. Riswandi et al. (34) stated that the combination of different types of swamp forage in Bengal grass-based rations can reduce the C2/C3 ratio. The C2/C3 ratio value has an important meaning in ruminology, a low ratio value will stimulate the formation of body fat (fattening). Propionic acid is glucogenic, it is the main precursor for the formation of blood glucose (14, 30). In treatment A (without tree legumes) with a high C2/C3 ratio, the microbes that play a role in fermentation are cellulolytic and hemicellulolytic bacteria, the fermentation pattern leads to the formation of acetic acid (10)

Methane Gas

The results showed that the combination of tree legume feed had an impact ($P<0.05$) on reducing methane gas. Methane gas production can be estimated using a VFA profile consisting of acetic, propionic and butyric acids, this method produces accurate results and high measurement coefficients (11). The C2/C3 ratio value is closely related to methane gas production. The value of the C2/C3 ratio and methane has a positive correlation, which means that the lower the C2/C3 ratio value, the lower the methane gas production (11, 4). Palangi et al. (24) stated that the formation of acetate and butyrate produces H_2, which provides an opportunity for methanogenic bacteria to convert it into methane. Meanwhile, the formation of propionate requires H_2 so that methane formation will be reduced, Diribi Mijena and Aman Getiso (20) reported that reducing methane gas production in the rumen can increase feed energy which ultimately has a positive effect on livestock productivity. The higher the methane gas production indicates the lower the efficient value of a feed. Methane gas productivity can be influenced by secondary metabolic compounds (phytochemicals) such as tannins and saponins. Ribeiro et al. (32) reported that secondary metabolites such as tannins and saponins can reduce CH_4 emissions by inhibiting rumen ciliate protozoa. According to Ramírez-Restrepoa et al. (29) that the various effects of tannins on methane gas may be due to the chemical structure and concentration from which the tannins are obtained. The effect of saponins on methane gas was reported by Valenzuela Grijalva et al. (43) who observed that tea saponins can inhibit protozoan activity by affecting cell membrane integrity. Dey et al. (7) reported that methanogenesis has a positive correlation with protozoa so that the biological properties of saponins can be used to suppress methane production, by reducing the number of rumen protozoa, and modulating rumen fermentation patterns so that they can inhibit methanogenic bacteria from producing methane gas. The research results of addisu et al. (1) showed that tannin has the ability to reduce methane gas emissions by up to 20%. Patra et al. (27), this decrease is likely caused by the inhibitory effect of methane production from methanogens, protozoa and other hydrogen-producing microbes.

Total Bacteria and Protozoa

The results showed that the combination of tree legumes in the diet increased the total population of rumen bacteria ($P<0.05$) compared to the control, this was due to the lower NDF content of tree legumes than grass as shown in Table 1, resulting in a higher proportion of soluble carbohydrates. In addition, the high content of nitrogen (CP) and dissolved carbohydrates in tree legumes stimulates higher microbial biomass production so that fermentation activity is also higher.

Table 5. Effects of tree legume combinations on total bacteria and protozoa

Parameter	Treatment			
	A	B	C	D
Total Bacteria (10^9)	6.46 ± 0.59^a	9.98 ± 0.54^c	8.48 ± 0.53^b	8.13 ± 0.44^b
Protozoa (10^5)	6.30 ± 0.38^b	4.92 ± 0.48^a	5.16 ± 0.60^a	4.99 ± 0.96^a

Note: Different superscripts in the same column indicate significant differences (P<0.05. A = swamp grass and concentrate (70%:30%), B = swamp grass, leucaena and concentrate (40%:30%:30%), C = swamp grass, Indigofera and concentrate (40%:30% :30%), D = swamp grass, leucaena, Indigofera and concentrate (40%:15%:15%:30%).

This can be seen from the high DMD value, NH_3 and TVFA levels are still within optimal limits, so it is sufficient to support an increase in microbial protein synthesis. This increase in digestibility is in line with increased microbial protein synthesis (18). The high total number of bacteria treated with tree legumes due to the activity of phytogenic feed stimulates the rumen system to run optimally thereby increasing the rumen microbial population. Besides that, the high total population of rumen bacteria is also accompanied by a low population of protozoa. This condition shows that there are fewer rumen microbes that are preyed upon by protozoa, thereby contributing to an increase in the total population of rumen microbes. Protozoa contribute 10-40% of total rumen nitrogen, so a reduction in this population means reduced bacterial predation and lysis and, consequently, lower release of protein breakdown products (38, 46). Pazla et al. (28) reported that the addition of 16% of the legume Tithonia diversifolia to fermented palm fronds and elephant grass increased microbial protein synthesis. This microbial protein has the potential to be a source of single cell protein (essential amino acids) for ruminants. The results showed that the protozoa population decreased with the combination of tree legumes. This condition occurs because of the effects of phytochemical compounds (saponins) in tree legumes, saponins which have a defaunation effect which allows protozoan cell lysis. The study by Ramírez-Restrepo et al. (29) supports this finding because it shows that the saponin content of tea leaves has the potential to be used as an anti protozoan. Saponins react with cholesterol from protozoan membranes, which causes an increase in cell wall permeability, thus inhibiting protozoan activity (34, 47) also confirmed these findings, that the combination of different swamp forages as a phytogenic food source in Guinea grass-based diets could reduce protozoan populations. Jayanegara et al. (13) stated that there are secondary compounds in Indigofera plants with tannin and saponin contents of 2.9 g/kg and 2.6 mg/kg DM, respectively. has an anti-protozoan effect and can be used as a defaunation agent so that it has an impact on reducing protozoan populations. The conclusion of this study is that tree leguminosa supplementation in various ratios in swamp grass-based rations can increase dry matter digestibility, total bacteria, and improve rumen fermentation characteristics, as well as reduce protozoa and methane gas production.

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