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METHANE GAS PRODUCTION IN BUFFALO RUMEN FLUID CONTAINING CITRONELLA RESIDUE (*Cymbopogon nardus* L.) WITH IN VITRO METHOD

PRODUKSI GAS METANA CAIRAN RUMEN KERBAU YANG MENGANDUNG RESIDUAL SERAI WANGI (*Cymbopogon nardus* L.) SECARA IN VITRO

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Abstract

Methane is one of the greenhouse gases produced by ruminants. One way to reduce methane is by feeding development strategies. Concentrates or forage are two possible forms of animal feed. Forage can be replaced with citronella residue, which has not been fully consumed as feed. Citronella is a plant citronella oil producer, with major components citronella and geraniol. The purpose of the study was to decide the production of methane gas that has residual citronella to concentrate in buffalo rumen fluid in vitro, for 24 hours. The treatments in this research were A (0.4 g citronella residue); B (0.4 g citronella residue + 0.01 g concentrate); C (0.4 g citronella residue + 0.02 g concentrate); D (0.01 g concentrate); and E (0.02 g concentrate). For the 24 hour measurements, the methane gas production findings were A (1.81%), B (11.34%), C (11.08%), D (73.71%), and E (38.88%). This shows that citronella residue can lower methane gas. The study's findings are anticipated to be among the references for using agricultural product residues, particularly citronella as animal feed in an effort to lower the amount of methane produced by the livestock industry.

***Keywords**:* Animal feed; Citronella residue; *Cymbopogon nardus* L.; *In vitro*

Abstrak

Gas rumah kaca yang berasal dari ternak ruminansia, salah satunya adalah metana. Proses reduksi metana dapat dilakukan dengan strategi pengembangan pemberian pakan. Pakan ternak dapat berupa hijauan ataupun konsentrat. Residu serai wangi yang belum termanfaatkan maksimal sebagai pakan, dapat digunakan sebagai pengganti hijauan. Serai wangi (Cymbopogon nardus L.) adalah tanaman penghasil minyak serai wangi yang memiliki komponen utama sitronella dan garniol. Tujuan dari penelitian adalah mengetahui produksi metana di cairan kerbau yang mengandung residual serai wangi dengan konsentrat secara in vitro setelah diinkubasi selama 24 jam. Perlakuan pada penelitian ini adalah 0,4 g residu serai wangi (A); 0,4 g residu serai wangi + 0,01 g konsentrat (B); 0,4 g residu serai wangi + 0,02 g konsentrat (C); 0,01 g konsentrat (D); dan 0,02 g konsentrat (E). Hasil penelitian menunjukkan produksi gas metana untuk pengukuran jam ke-24 adalah A (1,81%), B (11,34%), C (11,08%), D (73,71%), dan E (38,88%). Hal tersebut menunjukkan, gas metana dapat direduksi oleh residu serai wangi. Hasil penelitian diharapkan dapat menjadi salah satu referensi dalam pemanfaatan residu hasil pertanian, khususnya serai wangi sebagai pakan ternak untuk upaya penurunan produksi metana dari sektor peternakan.

Kata kunci : *Cymbopogon nardus* L.; *In vitro*; Pakan ternak; Residu serai wangi

INTRODUCTION

Methane is produced from animal husbandry from two sources, namely digestion and feces (Gustiar et al., 2014). Livestock with rumens can create CH_4 because the digestive process in the rumen produces enteric methane gas (Aldrian et al., 2019). Methane emissions from ruminants are higher than those from pigs (5.90%) and poultry (3%) (Directorate General

of Animal Husbandy and Animal Health, 2007). Hence, ruminant farming has a stronger effect on greenhouse gas emissions than poultry (Edi & Haryuni, 2023). The contribution of ruminants to CH_4 production is about 29% of the total CH_4 emissions from livestock and humans, which only accounts for about 18% of the total greenhouse gases in the atmosphere (Jayanegara et al., 2009a). Methane is released from feed eaten by ruminants when it contains between 12 and 15% energy that cannot be used (Haryanto & Thalib, 2009). This has to do with both environmental concerns and the energy content of the diet that ruminants eat. As a result, feeding enhancement techniques can lower ruminant methane emissions. Such an approach can be used to decrease animal energy losses in the short term, and it can also slow down the rate at which greenhouse gases accumulate over time (Jayanegara et al., 2009b).

Forages or concentrates are fed to animals (Gustiar et al., 2014). For attempts to boost cattle growth and production to be successful, feed must always be available. Agricultural waste or by-products can be best utilized for initiatives to boost feed availability (Directorate General of Animal Husbandry, 2011). This is advantageous since it allows for using agricultural waste, a source of pollution as feed, so that citronella waste from the distillation of citronella oil can be used as a pasture substitute for animal feed (Sukamto et al., 2011). According to the Research Centre for Medicinal and Aromatic Plants (2011), straw waste has lower protein content (3.9%) than citronella, which has a protein content (7%). Citronella's crude fiber content was likewise lower (better), at 25.73% than that of straw (32.9%) and elephant grass (34.15%). Furthermore, animals that are given leftovers from citronella distillation have less foul-smelling excrement (Agricultural Research and Development Agency, 2014).

Citronella's tannin content can be an attempts to lower methane generation. This is because tannin or polyphenol chemicals can reduce methane emissions (Jayanegara et al., 2008). Natural products that are benign to the environment, easily embraced by the public concerning food safety concerns, and have the potential to be used as modifiers of rumen conditions (Bhatta et al., 2012). Furthermore, tannins can lower methane production, and the quality of feed fed to animals is not diminished (Hess et al., 2006). Another study also showed that CH_4 gas production can be mitigated by 49.7% with supplementation of 0.18% tannin extract from gambir (*Uncaria gambir* Indonesia) in concentrate (Ramaiyulis et al., 2022). Efforts to utilize citronella residue combined with concentrate as animal feed must be tested first so that the right composition of citronella residue and concentrate can be known. The study aimed to examine the concentration of methane produced by microorganisms in various feed compositions of concentrate feed combined with citronella residue in buffalo rumen fluid in vitro.

MATERIALS AND METHODS

Citronella was prepared in the laboratory. The leaves and stems of the citronella plant were used. The residue of citronella that had been distilled in a desiccator served as the sample. The material was crushed and filtered at a size of 2 mm after being oven-dried at 60 °C. Additional samples were concentrated and created by combining different feed ingredients and placing them in a horizontal mixer (adapted from Wahyono, 2015). The treatments in this study were A (0.4 g citronella residue); B (0.4 g citronella residue + 0.01 g concentrate); C (0.4 g citronella residue + 0.02 g concentrate); D (0.01 g concentrate); and E (0.02 g concentrate).

Buffalo Rumen Fluid Collection

Two liters of rumen fluid were collected in the morning before the buffaloes were fed, so that the condition of the rumen would not be affected by the feed. A thermos is filled with hot water to create an anaerobic environment by replacing the air within with water vapor. Then, the thermos's hot water is thrown out, and the buffalo rumen fluid is taken and added

right away. After that, four-layer gauze is used to filter the rumen fluid while CO_2 gas is present (Preston, 1995).

Preparation of Mc Dougall's Solution (Artificial Saliva)

Mc Dougall's solution consists of buffer solution, micromineral, macromineral, reducing solution, and resazurin. The buffer solution is mixed with 35 g $NaHCO_3$ and 4 g NH_4HCO_3 in 1,000 mL of distilled water. Micromineral consists of 13.2 g $CaCl_2.2H_2O$, 10 g $MnCl_2.4H_2O$, 1 g $CoCl_2.6H_2O$, and 8 g $FeCl_3.6H_2O$ in 100 mL of distilled water. Macromineral solution was prepared by mixing 5.7 g Na_2HPO_4, 6.2 g KH_2PO_4 and $MgSO_4.7H_2O$ in 1,000 mL of distilled water. The reducing solution was prepared from 1.119 mg $Na_2S.H_2O$, 7.8 mL NaOH 1 N, and 180 mL distilled water. The resazurin solution was prepared by putting 100 mg resazurin into 100 mL of distilled water (Krishnamoorthy 2001).

pH Value

Standard pH values (4, 7, and 9) were used to calibrate the pH meter. The pH meter electrode was inserted into samples, each of which consisted of 5 mL, the pH value was recorded after the pH value on the screen stabilized. After removing the electrode, it was cleaned with distilled water and put back in for the subsequent sample. After completion, the electrode was immersed again in distilled water (Plummer 1971).

Total Volatile Fatty Acid (VFA) Content

Measurement of total volatile fatty acid (VFA) content by steam distillation method (General Laboratory Procedure, 1966). A 5 mL sample was obtained, added 1 mL of 15% H_2SO_4, and the sample was centrifuged for 10 minutes at 3,000 rpm. After that, 5 mL of supernatant was distilled. The distillation results were collected up to 300 mL in Erlenmeyer which previously contained 5 mL of 0.5 N NaOH. Two drops of 0.1 N phenolphthalein (PP) indicator were added, and the mixture was titrated with 0.5 N HCl until there was a color change from pink to colorless. The calculation of total VFA is VFA (mM) = (a-b) × N HCl × 1000/5 mM; a= volume of blank (mL), b= used volume (mL), N HCl= HCl concentration.

Biomass of Bacteria and Protozoa

Microtubes were placed in a 105 °C oven for one hour, then placed in a desiccator for 15 minutes and the initial weight (B0) was weighed. A 1.5 mL sample was added to the microtubes. The sample was centrifuged at 1,500 rpm for 10 minutes, until supernatant and pellet were formed. The supernatant was transferred to a new microtube. The microtubes were centrifuged for 10 minutes at 3,500 rpm until a precipitate formed. The precipitate was the protozoa. After the supernatant was generated, it was transferred to microtubes and centrifuged at 10,000 rpm for 10 minutes. Bacteria are the precipitate. Protozoa and bacteria-containing microtubes were first heated to 60 °C for 24 hours, then transferred to an oven at 105 °C for one hour. After 15 minutes in a desiccator, the microtubes were taken out and their final weight (Bt) was measured. The formula microbial biomass (g/mL)= Bt-B0 was used to calculate the biomass of bacteria and protozoa (Blummel et al. 1999).

Gas Production

The Hohenheim gas test method Menke et al. (1979) was used to determine gas production at 0, 2, 4, 6, 12, 24, and 48 hours. The samples were mashed and weighed 0.4 g based on the treatment after being dried for 24 hours at 60 °C in the oven. After that, the sample was placed into the syringe's bottom. Samples were incubated in triplicate. Rumen fluid was added with Mc Dougall solution, then stirred with a magnetic stirrer and supplied with CO_2

gas. Up to 40 mL of rumen fluids were placed into the syringe. It was then incubated at 39 °C in a water bath, and the amount of gas was measured.

CH_4 Gas Production

Measurement of CH_4 gas production was carried out after determining the concentration of CH_4 gas in the total fermentation gas in each syringe. The CH_4 concentration was measured using the MRU VarioPlus gas analyzer. To determine the incubation outcomes after 24 hours, the concentration of CH_4 was measured. The percentage of CH_4 gas in the syringe is shown by the number on the gas analyzer. The equation CH_4 gas production at hour (mL)= CH_4 gas concentration (%) × total gas production at the hour (mL) (Wahyono 2015).

Data Analysis

Measurement data were statistically analyzed using an ANOVA computation in a completely randomized design with three repetitions of each treatment.

RESULTS

pH Value

Adding citronella residue and concentrate, among other treatment variations, caused disparities in the starting pH levels. Sample D exhibits the highest initial pH, followed by treatments B, C, A, and E (Figure 1). The high lipid content in citronella residue implies its acidity. Fatty acids remain in the S residue left behind after citronella is distilled. Moreover, the initial pH variations will affect both rumen fluid bacteria activity and the pH during incubation. All treatments showed an increase in pH following a 24-hour incubation period. At the 24-hour mark, sample D had the highest pH value, while samples E, A, B, and C exhibited identical values (Figure 1).

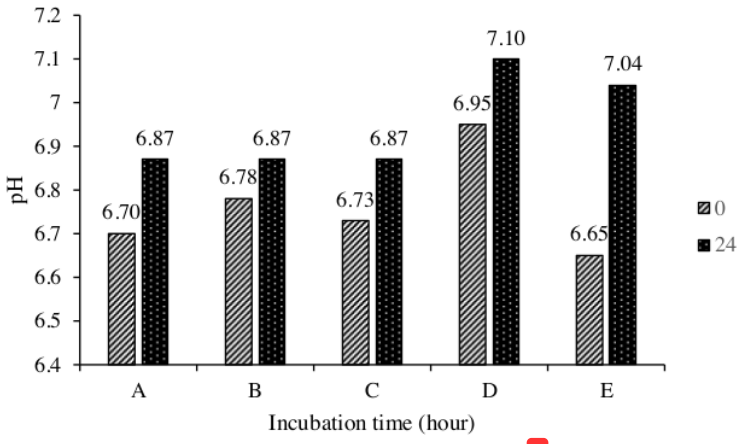

Figure 1. Results of pH values during 24-hour incubation 0.4 g citronella residue (a); 0.4 g citronella residue + 0.01 g concentrate (b); 0.4 g citronella residue + 0.02 g concentrate (c); 0.01 g concentrate (d); and 0.02 g concentrate (e)

Total Volatile Fatty Acid (VFA) Content

Samples A, B, D, and E's VFA test results showed a decline from 0 hours to 24 hours. This due to the depletin of organic materials, that rumen microbes rely on for energy. At 0-

hours, sample D exhibited the highest VFAs value, followed by E, A, B, and C. After 24 hours, the highest VFAs value were observed in samples E (Figure 2).

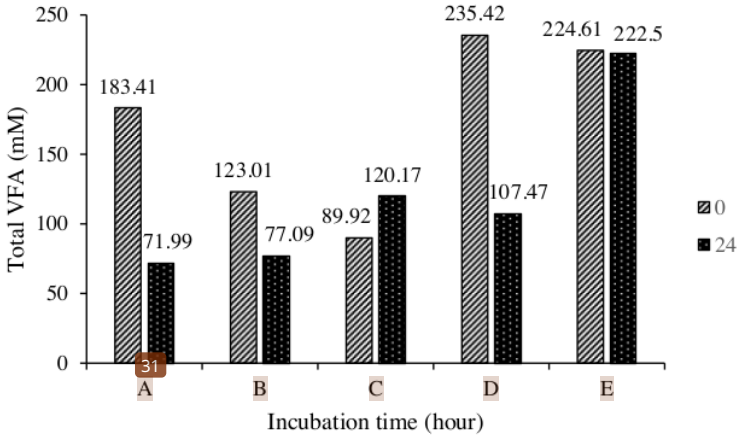

Figure 2. Results of total volatile fatty acids (VFAs) produced during 24 hours, 0.4 g citronella residue (a); 0.4 g citronella residue + 0.01 g concentrate (b); 0.4 g citronella residue + 0.02 g concentrate (c); 0.01 g concentrate (d); and 0.02 g concentrate (e)

Sample A had the highest protozoan biomass according to the initial measurement results, followed by samples E, D, B, and C. Treatment E had the greatest 24-hour observation followed by A, C, D, and B (Figure 3). The range of the bacterial biomass, which is displayed in Figure 4, is extremely small, ranging from 0.0015–0.3026 mg/mL. Sample A exhibits the highest initial biomass concentration at 0.0227 mg/mL. Samples B, E, D, and C came next, at 0.0051; 0.0036; 0.0018; and 0.0012, respectively. After 24 hours, treatment B exhibited the the highest value (0.0193 mg/mL), followed by C, A, E, and D at 0.0139; 0.0007; 0.0006; 0.0003 mg/mL, respectively.

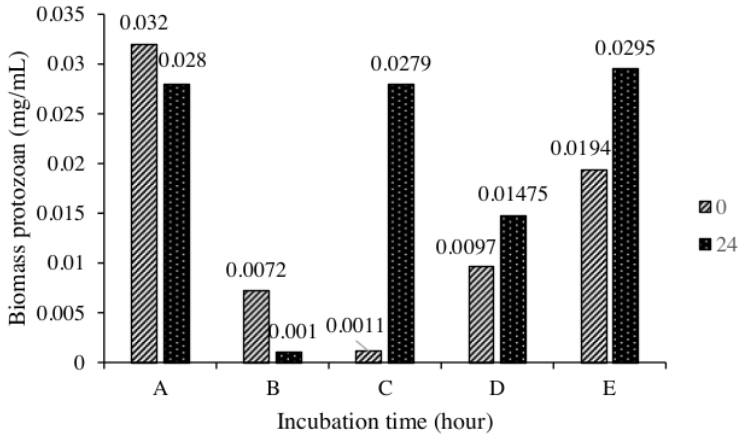

Figure 3. Protozoans biomass measurement results, 0.4 g citronella residue (a); 0.4 g citronella residue + 0.01 g concentrate (b); 0.4 g citronella residue + 0.02 g concentrate (c); 0.01 g concentrate (d); and 0.02 g concentrate (e)

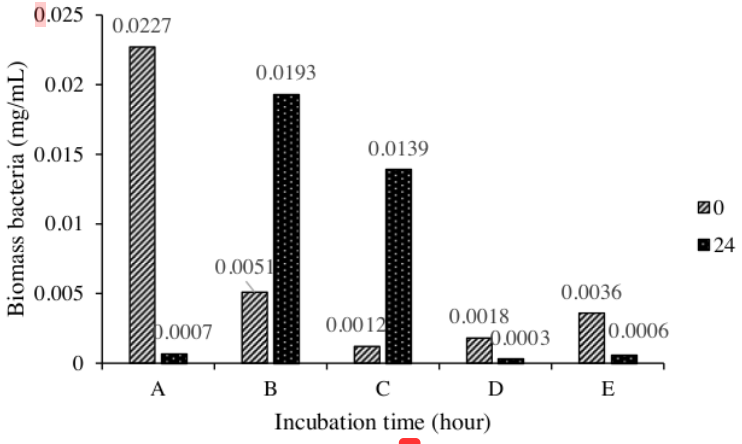

Figure 4. Bacteria biomass measurement results, 0.4 g citronella residue (a); 0.4 g citronella residue + 0.01 g concentrate (b); 0.4 g citronella residue + 0.02 g concentrate (c); 0.01 g concentrate (d); and 0.02 g concentrate (e)

Sample D had the greatest measurement of methane production over 24 hours, followed by samples E, B, C, and A. In comparison to samples A, B, and C, which contained citronella residue, Figure 5 demonstrates that the concentrate in samples D and E created the amount of methane.

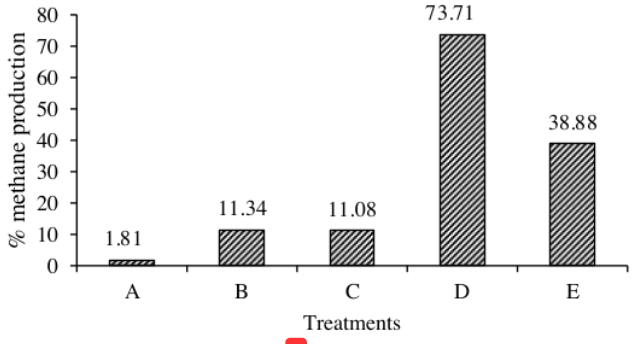

Figure 5. Methane gas measurement results, 0.4 g citronella residue (a); 0.4 g citronella residue + 0.01 g concentrate (a); 0.4 g citronella residue + 0.02 g concentrate (c); and 0.01 g concentrate (d); and 0.02 g concentrate (e)

DISCUSSION

The pH value directly influences the growth of microorganisms involved in the fermentation process and serves as a straightforward indicator of fermentation. During rapid fermentation, organis acids accumulate, leading to decrease in pH (Sugoro, 2010). Maintaining stable pH levels is crucial for microbial stability during their active phases (Franzolin &

Dehority, 2010). The buffalo rumen fluid used in the in vitro experiment had a pH of 6.65. Falling within the typical rumen pH range of 5.5-7.5 (Franzolin & Dehority, 2010). This suggests that there are living rumen microbes. When Mc Dougall solution was used to replace saliva, the pH value became 7.05. The solution contains sodium bicarbonate which is important for maintaining the pH value, and as a buffer solution against the flying fatty acids (VFAs), which are produced from bacterial fermentation (Tillman et al., 1998).

Protein breakdown in the citronella residue and concentrate resulted in a pH increase after 24 hours. Crude protein makes up 5.82% of the residue from citronella, whereas concentrate has 12%. Urea, one of the protein-containing components in the concentrate, generates alkaline ammonia during breakdown. Consequently, the sample's pH increases compared to its initial state. Microorganisms have exploited ammonia from the breakdown of urea as a nitrogen source for protein synthesis (Indriani et al., 2013). Furthermore, feed containing protein in the concentrate is easier for microorganisms to digest than feed containing cellulose in the citronella residue.

The primary effect of rumen pH levels below 6 is that there may be a sharp decline in the digestion of fiber. This occurs because bacteria responsible for producing enzymes that break down crude fiber operate less efficiently at this pH. Additionally, a low rumen pH can indicate the feed degradation process (Syahrir et al., 2009), and bacterial cells struggle to maintain stability under such conditions (Rode et al., 2010).

Limited VFAs are a sign of limited energy available to rumen microbes. The breakdown process of digesting carbohydrates in ruminants rumen produces volatile fatty acids (VFAs) (Indriani et al., 2013). According to Siedlecka et al. (2008), the presence of VFAs can suggest bacterial activity. Acetic (C_2), propionic (C_3), and butyric (C_4) acids are the primary constituents of volatile fatty acids, which are produced by the anaerobic breakdown of organic matter (Pamungkas et al., 2008). Volatile fatty acids typically accompany the generation of hydrogen in anaerobic fermentation tests (de Să et al., 2011). Ruminants get a large portion of their energy from the fermentation of microorganisms in the rumen, which produces volatile fatty acids.

Treatment E, which included concentrate, exhibited the highest value of volatile fatty acids VFAs at 24 hours according to Figure 2. Microorganisms tend to proliferate more when VFAs are present in larger quantities. Elevated VFAs directly stimulate microbial activity, leading to improved feed digestion and increased dry matter digestibility (Alwi et al. 2013). Rumen bacteria can more efficiently break down and utilize sample E (concentrate only) to produce pyruvic acid rather than volatile fatty acids (VFAs). According to Alwi et al. (2013), cellulose and hemicellulose make up the crude fiber found in the samples. As cellulose and hemicellulose break down, glucose and oligosaccharides are produced, which rumen microbes use. Pyruvic acid is the next product, followed by VFAs, which are butyric, propionic, and acetic acids.

Sugoro (2010) asserts that the effects of VFAs are directly correlated with pH because the bacteria that generate the VFAs consume them as energy sources right away, meaning that the VFAs do not alter the pH of the rumen fluid. Nevertheless, certain treatments (Figure 1 & 2) do not support the claim above. This is because the pH value becomes more acidic due to the creation of VFAs, which are composed of acidic chemicals (Nuswantara, 2009). Acidic substances such as molasses, pollard, soybean flour, onggok, bran, and soy sauce pulp are also found in concentrates. Moreover, VFAs and ammonia are related. Microbes use volatile fatty acids (VFAs), which are the by-product of fermentation of carbohydrates, as a source of energy and to produce proteins. Consequently, the higher the ammonia level, the greater the amount of VFAs utilized to form proteins in microbes (Suherman et al., 2013).

Since bacteria and protozoa are involved in the feed fermentation process, measurements of biomass are required to ascertain the quantity of these microorganisms in the

rumen fluid. According to Castillo-Gonzălez et al. (2014), microbes break down between 50–70% of proteins and polysaccharides in the rumen. Furthermore, because proteolytic microorganisms like bacteria and protozoa produce protease, peptidase, and deaminase enzymes that break down proteins into amino acids, peptides, and ultimately ammonia, feed protein can also be fermented by these microbes (Pamungkas et al., 2008).

The diet of the host affects the biomass of protozoans. Protozoa account for 25–33% of the rumen biomass when the host consumes a fibrous diet (Purbowati et al. 2014). This was evident in sample A, which exhibited high protozoan levels at hour 0 (0.032 mg/mL) and hour 24 (0.028 mg/mL). Similarly, sample E showed elevated protozoan values after 24 hours (0.0295 mg/mL) and the first observation (0.0194 mg/mL). According to Purbowati et al. (2014), in vitro studies indicate that protozoa are capable of digesting high-protein diets. In contrast treatment E, which also contained a concentrate but weighed 1 g, had a lower protozoan count compared D. This suggests that the protozoa biomass is influenced by the protein present content in the diet.

Tannins in citronella residue may be the source of this since can stop protozoan growth by deactivating adhesins, microbial enzymes, or protein transport (Sugoro & Yunianto, 2006). Additionally, the concentrate has an acidic composition. Purbowati et al. (2014), protozoa are sensitive to acid and low pH can lead to a decrease population. In a study by Dehority (2003), protozoa in vitro die at pH values lower than 5.4. Despite the lower protozoan counts, protozoa remain viable because the pH of the rumen used was not below this range.

Bacterial biomass increased at 24 hours in treatments B and C, which contained citronella residue. This is consistent with the finding of Wahyuni et al. (2014), that feed containing tannins causes an increase in the bacterial population. Furthermore, Makkar (2003) that tannins can reduce protozoan numbers. Since protozoa are predators that feed on bacteria to meet protein requirements, a decline in the protozoan population can lead to an increase in bacterial population.

According to Jayanegara et al. (2009b) research, the type of feed has an impact on methane production even when it is incubated at the same period. Figure 5, however, demonstrates that feed weight has an impact on methane generation in addition to feed type. After 24 hours of incubation, samples D (73.71%) produced more methane than other samples. Furthermore, treatment E produced 38.88% less methane than treatment D. It showed that the weight of the concentrate had an impact on methane generation. Gustiar et al. (2014) research found that a high percentage of feeding concentrate results in low methane production.

After 24 hours of incubation, samples A, B, and C could only produce a maximum of 11.34% methane. This suggests that the tannins in citronella residue can lower the methane content. According to Jayanegara et al. (2009b), providing forage containing tannins can lower methane emissions from fermentation systems in vitro. Furthermore, tannins can shield feed protein from rumen microbes, allowing it to transit through the reticulum-rumen intact as amino acids (Haryanto, 2012).

Because protozoa serve as hosts for methanogens, methane production is strongly correlated with protozoa numbers (Thalib, 2008). Since significant methane production followed high protozoan biomass, this was consistent with treatment E. However, this assertion did not apply to the other samples. The occurrence is possible because certain methanogens can survive without symbiotic relationships with protozoa and still reproduce well (Masruroh et al., 2013).

Morvay et al. (2011) demonstrated the relationship between VFA concentration and methane production. Hydrogen is released during the formation of acetic and butyric acids, which can then be used to produce methane. This is not the same as propionic acid production, which necessitates hydrogen (McAllister & Newbold, 2008). Accordingly, the amount of acetic, butyric, and propionic acids that methanogens will utilize will affect how much

hydrogen is available (Jayanegara et al., 2013). This is due to Mathius et al. (2004) findings that methane generation is regulated by H_2 and CO_2, which bacteria convert to methane, in addition to VFA.

CONCLUSION

The highest methane production was treatment D, which was 73.71%, followed by E (38.88%), B (11.34%), C (11.08%), and A (1.81%). Treatment A's feed composition 0.4 g citronella residue was the most effective for reducing methane. Citronella residues have the ability to decrease methane gas. Other plant leftovers that can be used as animal feed to reduce methane gas without compromising feed nutrition are suggested for future study.

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