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METHANE GAS PRODUCTION IN BUFFALO RUMEN FLUID CONTAINING CITRONELLA RESIDUE (*Cymbopogon nardus* L.) BY *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 (*Cymbopogon nardus* L.) residue, which has not been fully consumed as feed. 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 48 hours. The treatments in this research were 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). For the 48-hour measurements, the highest methane gas production was treatment 98.2% (D); followed by 92.06% (E); 17.71% (C); 15.33% (A); and 13.54% (B). It can be concluded that methane gas can be reduced by residue citronella. 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 to reduce the 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 (Cymbopogon nardus L.) yang belum termanfaatkan maksimal sebagai pakan, dapat digunakan sebagai pengganti hijauan. Tujuan dari penelitian adalah mengetahui produksi metana di cairan rumen kerbau yang mengandung residu serai wangi dengan konsentrat secara in vitro setelah diinkubasi selama 48 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-48 adalah 98,2% (D); followed by 92,06% (E); 17,71% (C); 15,33% (A); and 13,54% (B). 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*

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INTRODUCTION

Methane (CH_4) 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) or enteric fermentation (Food and Agriculture Organization (FAO), 2022). The claim made by Scholtz et al. (2020) that enteric fermentation, a natural by-product of anaerobic microbial fermentation and manure storage, is how ruminants manufacture CH_4. Ruminants are the primary source of methane (Getabalew et al., 2019), producing 250–500 L/day (Olijhoek & Lund, 2017). Hence, ruminant farming has a more substantial effect on greenhouse gas emissions than poultry (Edi & Haryuni, 2023). More than 90% of all livestock-related CH_4 emissions come from ruminants, which account for about 80% of greenhouse gas emissions (Opio et al., 2013). In addition to causing greenhouse gas emissions, ruminant CH_4 emissions cost farmers money because of up to 12% loss of feed energy (Food and Agriculture Organization (FAO), 2022). This concerns the food that ruminants eat and may be an environmental issue. Better feeding practices can lower CH_4 emissions, which will lower animal energy losses and delay the rate at which greenhouse gases build up.

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. Concentrates are high protein (Utiah et al., 2021), low in crude fiber, easy to digest, and excellent nutritional quality animal feed ingredients. Concentrates are created from feed materials such as soybean meal, pollard, onggok, bran, soy sauce pulp, mineral lactate, urea, salt, lime, and molasses (Wahyono, 2015). Apart from concentrates it can also utilize agricultural waste or by-products, such as citronella residue as animal feed for programs to increase feed availability. Citronella residue, also known as the by-product of citronella distillation and extraction, may be utilized as a substitute fiber in ruminant feed (Elihasridas et al., 2022). Additionally, tannins, steroids, flavonoids, and other substances remain in citronella residue. 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 given leftovers from citronella distillation have less foul-smelling excrement (Agricultural Research and Development Agency, 2014).

Residue citronella's tannin content can be an attempt to lower methane generation. This is because tannin can reduce methane emissions (Nawab et al., 2020). Tannins can block the enzymes that microorganisms manufacture and result in microbial toxicity (Astuti et al., 2024), reducing the methanogen population in the rumen, while saponins lower the rumen protozoa population (Krisnawan, 2015). Therefore, rumen condition modifiers could be made from natural, eco-friendly ingredients. Furthermore, the chemicals found in citronella residue can lower methane levels without compromising the quality of the animal feed. Another study also demonstrated that adding 0.18% tannin extract from gambir (*Uncaria gambir* Indonesia) to concentrate can reduce the formation of CH_4 gas by 49.7%. According to research by Sari et al. (2017), the percentage of dry matter, organic matter, and neutral detergent fiber degradation of citronella residue was higher than that of fresh citronella using the in-sacco method. 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

The residue citronella was crushed and filtered at a size of 2 mm after being oven-dried at 60 °C. Whereas concentrated are created from soybean meal, pollard, onggok, bran, soy sauce pulp, mineral lactate, urea, salt, lime, and molasses and created by combining different feed ingredients and placing them in a horizontal mixer (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, 1987).

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 1 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_2SH_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 Procedures, 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 × 1,000/5 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 containing the protozoa. After the supernatant was generated, it was transferred to microtubes and centrifuged at 10,000 rpm for 10 minutes. Bacteria are in 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 by 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 and 48 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 with a significance level of 5% 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. The pH value at each observation hour varied (sig <0.05) between all treatments, according to the findings of the ANOVA test. The pH value of all treatments increased after incubation until 24 hours and then decreased at 48 hours. Sample D had the greatest pH at the 24 hours while treatment D had the highest pH value at the 48 hours (Figure 1).

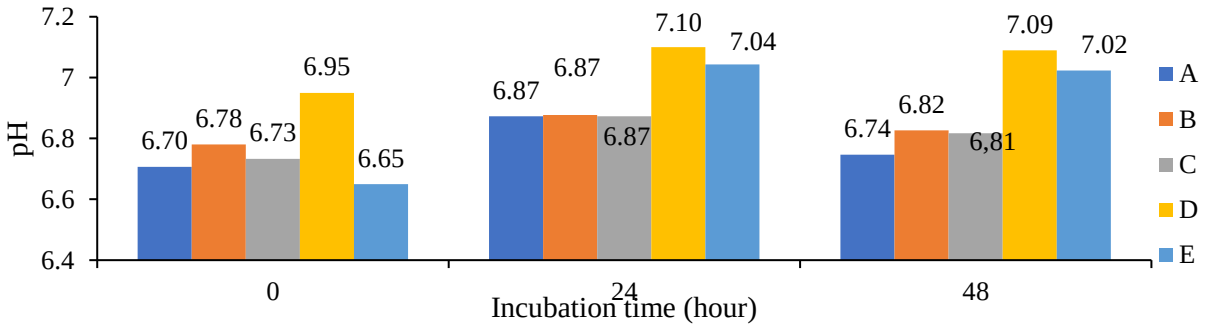

Figure 1. Results of pH values during 48-hour incubation. Note: 0.4 g citronella residue (A); 0.4 g citronella residue + 0.01 g concentrate (B); 0.4 g citronella residue + 0.02 g concentrate (C); 0.01 g concentrate (D); and 0.02 g concentrate (E)

Total Volatile Fatty Acid (VFA) Content

The VFA value at each hour of observation varied (sig 0.05) across all treatments, according to the findings of the Anova test. Treatment E, which included solely concentrate, had the highest VFA value at the 48 hours, as seen in Figure 2.

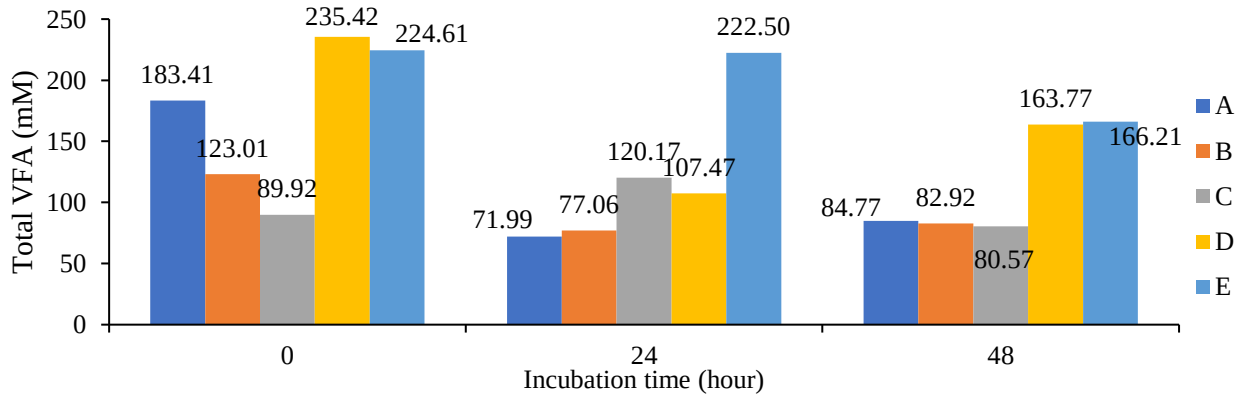

Figure 2. Results of total volatile fatty acids (VFAs) produced during 48 hours. Note: 0.4 g citronella residue (a); 0.4 g citronella residue + 0.01 g concentrate (b); 0.4 g citronella residue + 0.02 g concentrate (c); 0.01 g concentrate (d); and 0.02 g concentrate (e)

Biomass Protozoa and Bacteria

The Protozoa biomass value at each observation hour varied (sig <0.05) among all treatments, according to the ANOVA test results. Treatment E had the highest observation at 24 hours, followed by A, C, D, and B. Sample E had the greatest result, showing a drop in biomass at 48 hours (Figure 3).

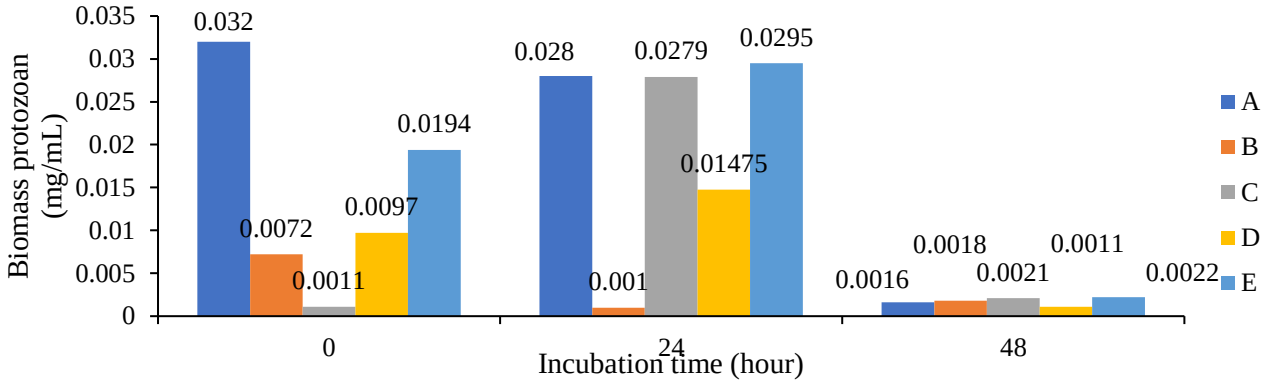

Figure 3. Protozoans biomass measurement result. Note: 0.4 g citronella residue (A); 0.4 g citronella residue + 0.01 g concentrate (B); 0.4 g citronella residue + 0.02 g concentrate (C); 0.01 g concentrate (D); and 0.02 g concentrate (E)

Anova test results show that the value of bacterial biomass at each hour of observation is different (sig <0.05) in all treatments. The bacterial biomass shown in Figure 4 that the range is very low.

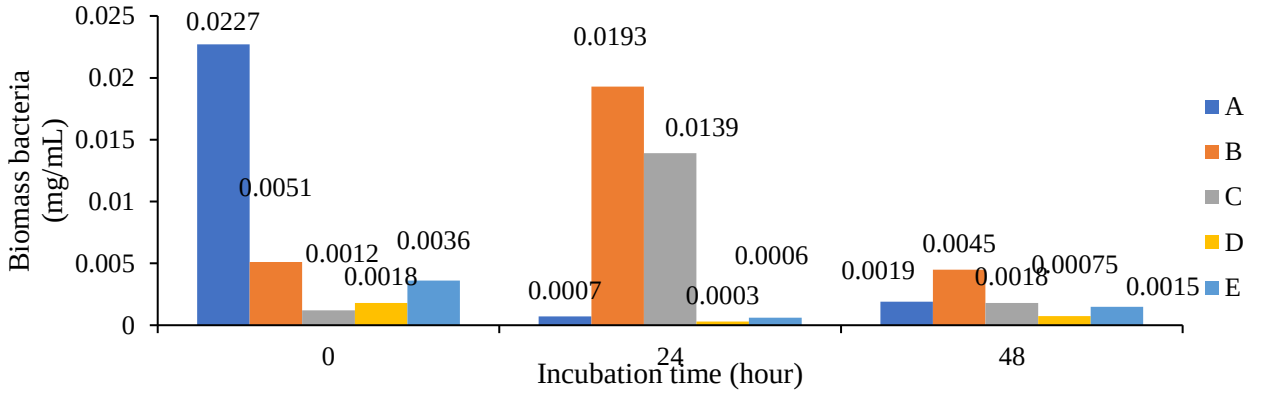

Figure 4. Bacteria biomass measurement results. Note: 0.4 g citronella residue (A); 0.4 g citronella residue + 0.01 g concentrate (B); 0.4 g citronella residue + 0.02 g concentrate (C); 0.01 g concentrate (D); and 0.02 g concentrate (E)

Methane Gas Production

The methane production value at each observation hour varied (sig <0.05) among all treatments, according to the findings of the ANOVA test. After 48 hours of incubation, all samples produced more methane than at the 24 hours. Sample D displayed the highest methane production readings at 24 and 48 hours (Figure 5).

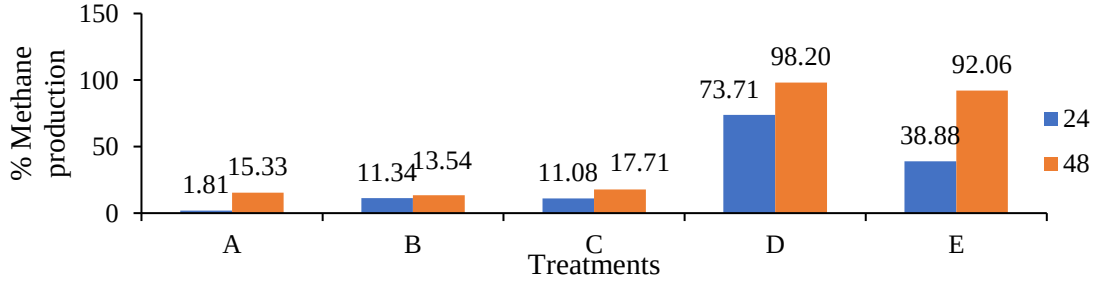

Figure 5. Methane gas measurement results, 0.4 g citronella residue (A); 0.4 g citronella residue + 0.01 g concentrate (B); 0.4 g citronella residue + 0.02 g concentrate (C); and 0.01 g concentrate (D); and 0.02 g concentrate (E)

DISCUSSION

In the in vitro test, the buffalo rumen fluid's pH began at 6.65 (Figure 1). This number is still within the 5.5–7.5 pH range which is ideal for rumen fermentation (Bayne & Edmindson, 2021). It demonstrates that the microorganisms in the rumen fluid are still alive. The pH value increased to 7.05 after adding McDougall's solution to replace saliva. According to Mao et al. (2017), the solution contains sodium bicarbonate ($NaHCO_3$), which can raise the final pH and concentration of total VFAs during in vitro rumen fermentation.

The different initial pH values are due to the different treatments. Sample D has the highest starting pH. Followed by treatments B, C, A, and E (Figure 1). Given that there are still a lot of fatty acids present, this suggests that citronella residue is acidic. Fatty acids are still present in 80% of the residue after distilling citronella. Furthermore, the varying starting pH findings will have an impact on the activity of rumen fluid bacteria and the pH value following incubation.

The pH value increased at 24 hours, due to the degradation of proteins present in citronella residue and concentrate. Crude protein makes up 5.82% of citronella residue and 12% of concentrate. The pH of the sample rises in comparison to the beginning because urea, which is converted to ammonia (NH_3), is alkaline. After 48 hours of incubation, the pH findings were lower than those obtained after 24 hours. Rumen bacteria use ammonia from the breakdown of urea as a source of nitrogen (N) for protein synthesis (Febriyani, 2019). Feed containing protein in concentrates is easier for microorganisms to digest than cellulose in citronella residue.

Ruminants use volatile fatty acids (VFA), which are the byproduct of fermenting carbohydrates, as an energy source (Ranja et al., 2020). Acetic (C2), propionic (C3), and butyric (C4) acids are the primary constituents of volatile fatty acids, which are produced by the anaerobic breakdown of organic matter (Fatawy, 2016). Sample C has a decreased VFA value at 48 hours compared to 24 hours. This is because the rumen microbes' energy source, organic matter, has started to deplete. Energy sufficiency may be indicated by high rumen VFA production, and vice versa (Jayanegara et al., 2006; Hapsari et al., 2018).

Treatment E, which included solely concentrate, had the highest VFA value at 48 hours, as shown in Figure 2. The development of microorganisms increases with the VFA value. This is because high VFA directly increases the activity of microorganisms that break down feed and increase the digestibility of dry matter. Furthermore, rumen microbes may more readily break down and use sample E (concentrate only) to produce pyruvic acid than VFA. Alwi et al. (2013) explained that the crude fiber present in the sample consists of cellulose and hemicellulose. After cellulose and hemicellulose break down, glucose and oligosaccharides are produced, which rumen microbes use, and then be pyruvic acid and VFA (acetic, propionic, and butyric acids). Figures 1 and 2 illustrate how pH might impact the value of VFA. Production of VFA consists of acidic compounds, making the pH value more acidic (Nuswantara, 2009). Acidic substances such as molasses, pollard, soybean meal, onggok, bran, and soy sauce pulp are also found in concentrates.

After 48 hours, there was a decline in biomass, with sample E showing the best results, followed by C, B, A, and D (Figure 3). The amount of microorganisms in the rumen fluid must be ascertained by measuring the biomass since these microorganisms are involved in the fermentation process of feed. According to Castillo-Gonzălez et al. (2014), microbes break down between 50–70% of proteins and polysaccharides in the rumen. The host's feed has an impact on protozoan biomass. Protozoa make up about 25–33% of the microbial biomass in hosts that are fed fibrous feed; ciliated protozoa are thought to make up 25–50% of the total (Purbowati et al. 2014; Firkins et al., 2020). This can be seen in sample A, which had high protozoan measurements at hour 0 (0.032 mg/mL), 24 (0.028 mg/mL), and 48 (0.0016 mg/mL). In addition, high numbers were seen for sample E since the initial observation (0,0194 mg/mL), 24 hours (0.0295 mg/mL), and 48 hours (0.0022 mg/mL). According to Purbowati et al. (2014), protozoa degrade feed with high protein when tested in vitro experiments. Sample D, which also consisted of concentrated, but with a weight of 0.01 g, showed fewer protozoa than treatment E. This indicates that the amount of protein content in the feed also affects protozoa biomass.

Protozoa biomass was seen to decrease at the 48 hours of observation compared to 0 and 24 hours. Tannins in citronella residue may be the source of this. By deactivation microbial enzymes, adhesins, or protein transport, tannins can decrease the number of protozoa, enhance rumen fermentation, boost ruminant production, and lower CH_4 emissions (Sugoro & Yunianto 2006; Hassan et al., 2020). In addition, concentrates also have an acidic composition. Protozoa are sensitive to acids and their numbers will decrease if the pH is low (Karsa, 2016) and die in vitro test at a pH below 5.4. This is appropriate because the pH of the rumen fluid used is not below that scale, so the protozoa are still alive even though the number is reduced.

Although it had decreased at 24 hours, the bacterial biomass in treatments A and B given citronella residue increased at 48 hours (Figure 4). Furthermore, Makkar (2003) and Hidayah (2016) claim that tannins can be utilized to lower protozoan populations. Since protozoa are predators that feed on bacteria to meet their protein needs, a decline in the protozoan population will have an impact on the rise in the bacterial population.

Fermentation of feed in the rumen will produce methane. Methane production for all samples increased after 48 hours of incubation compared to 24 hours (Figure 5). The kind and quality of feed have an impact on producing methane (Jayanegara et al., 2009; Romli, 2022). Figure 5 shows that not only feed type affects methane production, but also feed weight. The percentage shows that methane produced by samples D (73.71%; 98.29%) and E (38.88; 92.06%) was higher than B (11.34; 13.54%), C (11.08; 17.71), and A (1.81; 15.33%), after 48 hours incubation. Methane produced by treatment E was 34.83% lower than D. According to the research of Gustiar et al. (2014), feeding concentrates with a high percentage, of low methane will be produced.

The gas produced indicates the fermentation of feed by microorganisms in the rumen, namely the hydrolysis of carbohydrates into monosaccharides and disaccharides, the fermented into flying fatty acids (VFA), especially acetic, propionic, and butyric acids and in the form of gas, namely methane gas. Only 17.71%, of the methane generated by samples A, B, and C were maximized until 48 hours of incubation. This suggests that the tannins in citronella residue can lower the methane content. Suppling forage that contains tannins can lower methane emissions from in vitro fermentation. For example, Terranova et al. (2018) found that supplying tannin-containing chestnut leaves can reduce methane output in vitro rumen fermentation by 28%.

Since protozoa are hosts for methanogens, the population of protozoa is directly proportional to the amount of methane produced. Since a significant amount of methane is produced in response to the large biomass of protozoa, this is consistent with Figure 3 for treatment E; however, this conclusion does not apply to other samples. The existence of methanogens that can proliferate successfully even in the absence of protozoan symbiosis may be the cause of this (Masruroh et al. 2013).

CONCLUSION

Treatment B (0.4 g + 0.01 g concentrate had the best feed composition for lowering methane levels. Methane gas can be reduced by citronella residues. Future research should look into various ways to lower methane gas in ruminants without sacrificing feed nutrition by varying the amount of citronella residues.

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