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Analysis of Methane Emission of Ruminant's Rumen by In Vitro Technique Using Chemical Stoichiometry Method

KEYWORDS: methane emission, ruminant rumen, chemical stoichiometry method

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ABSTRACT *Global warming is a process of increase in average temperature of the Earth's atmosphere, ocean, and land. Global warming is caused by greenhouse gas effect (green house effect). The greenhouse effect is the increase of accumulation of greenhouse gases such as carbon dioxide (CO2) and some kinds of other gases, for example methane gas. This increase is caused by industrial activity, fuel oil burning residue, and also from the agricultural sector including the animal husbandry sector especially those with the ruminants. The studies on the production of methane gas in the last few decades are often brought up by the experts, however, in Indonesia it still has not done much, especially in terms of more effective and efficient methods on the determination of methane gas content. The purpose of this study was to analyze the methane emissions from some types of feed commonly used in Indonesia, the effectiveness of the methane emission test method by using the chemical stoichiometry method, and the environmental management strategies toward the global warming. The research was conducted in October - December 2014 in the Laboratory of Animal Feed Science and Technology, Faculty of Animal Husbandry, Bogor Agricultural University. The method used in this study was a chemical stoichiometry method by measuring the content of volatile fatty acid (VFA). Volatile fatty acid content analysis was performed according to the method Hoeltershinken et al. (1997). The variables that were observed were the nutrient composition of the feed, the content of gross energy (GE), the total gas, the ammonia, the organic matter digestibility (OMD), and the content of VFA. The data obtained were statistically analyzed by analysis of variance ANOVA. The results of analysis of the methane emissions from a variety of feed showed that methane emissions from some of the treatments that were analyzed in this study indicated that the concentrate which with additional 60% elephant grass was the best treatment in reducing the methane emissions by 3.03 mmol / l, the effectiveness of the chemical stoichiometry method was still effective in measuring the methane gas, and the strategy to reduce the methane gas emission through the ruminant's feed could be performed by adding the rice straw and elephant grass to the concentrate.*

1 INTRODUCTION

Global warming is a process of increase in average temperature of the Earth's atmosphere, ocean, and land. The occurrence of the global warming and climate change is a threat to many living creatures, especially those that are fragile to the changes in temperature and climate (Riani 2012). Since the 1980s, the global warming has become a natural phenomenon that is intensively studied. This happening is caused by the effect of greenhouse gases (greenhouse effect), that is the increase of greenhouse gases accumulation such as carbondioxide (CO_2) and other kinds of gases (CH_4, N_2O, CFC) in the layers of atmosphere. This increase is caused by the industrial activity, the existence of fuel oil burning residue, and also from the agricultural sector including the animal husbandry sector, in particular from those with the ruminants.

In the atmospheric layer, there is a methane gas that is the second largest coontributor of greenhouse gases after CO_2. The animal husbandry sector, especially the one with the ruminant animals, is one of the contributors in anthropogenic methane gas accumulation (± 28%) (Beauchemin *et al*. 2008). In addition to cause the global warming, the gas emission of ruminant animals is also a form of energy loss that should also be used to support its productivity. The amount of energy that lost from these ruminant animals is about 8 – 14% of the total energy ingested (Cottle *et al*. 2011).

Methane is also a result of microbial fermentation in the digestive tract of the ruminant animals to the feed component. Methane is a gas that is colorless and odorless, 87% is produced in the rumen and 13% in the large intestine (Murray *et al*. 1976). Methane (CH_4) and nitrogen (N_2O) gases come from different cycles. This CH_4 is usually turned out after the degradation of carbon (C) component during the digestion process toward the feed and the manure, while N_2O is associated with nitrogen (N) cycle with chemical and manure as the main sources (Monteny *et al*. 2006).

Methane gas derived from various sources, both natural and anthropogenic (Rotz *et al*. 2010). More than 70% of methane emission come from the anthropogenic activities (IPCC 2006). Methane is the final product of rumen fermentation during the process of feed digestion, and the animals produce about as much as 7 times amount of CH_4 in sheeps and 9 times in goats.

The emission of methane gas derived from the ruminant animals in the developed countries is different from the methane emission in the developing countries, depending on the factors such as the species of animal, animal reproduction, pH of rumen fluid, ratio of acetate to propionate, methanogen population, composition of feed amd concentration number of feed. The cows is one of the ruminant animals that most contributes to the greenhouse effect through the emission of methane gas, followed by sheeps, goats and buffalos. The estimate of methane emission in cows, buffalos, sheeps and goats in the developed countries are 150,7; 137; 21,9 and 13,7 (g/animal/day), respectively (Sejian *et al.* 2011).

Under the anaerobic condition in the rumen, the oxidation reaction requires energy in the form of ATP that release hydrogen. The amount of hydrogen that is generated is very dependent on the types of feed and the variety of rumen microbes role in microbial fermentation of feed that produce the different final product that is not the same with the hydrogen released. For example, the formation of propionic acid requires the hydrogen, whereas the formation of acetic acid and butyric acid release the hydrogen (Martin *et al.* 2008).

One of the efforts to minimize the emission of methane gas in ruminant animals is through the strategy of feeding which can reduce the methane emission from ruminant animals. It is beneficial in the long term to reduce the accumulation rate of greenhouse gases, and in the short term to reduce the energy loss in ruminant animals. Various efforts have been conducted and shown to be effective in reducing the emission of methane gas from ruminant animals. One of those is by using the antibiotics (Fuller dan Johnson 1981). However, the prohibition on the use of antibiotics as feed additives is expanding (Jayanegara 2009). Therefore, the compound that more natural is needed.

Methane gas is formed in the rumen, it can be inhibited by giving some chemicals. The principle of this inhibition is, among others, based on their toxicity to the methanogenic bacteria, such as halogenated methane compounds, sulfites, nitrates, and trichloroethyl pivalat, or based on the hydrogenation reaction, thus decreasing CO_2 reduction by hydrogen, such as long-chain unsaturated fatty acid compound. Some ionophores like monensin, lasalocid and salinomycin, in addition to increasing the propionic acid content, are also reducing the production of methane gas (Thalib 2008).

The studies on the production of methane gas in the last few decades are often brought up by many experts, however, in Indonesia itself are still not widely carried out, especially in terms of more effective and efficient methods on the determination of methane gas content. It is constrained by several factors, such as the limited equipments, funds, and other supporting facilities. Some methods generally used by the researchers in Indonesia in determining the content of methane gas in the rumen of ruminant animals is, among others, the NaOH method (Yuliana 2014). On the other hand, the method that expected to be more effective and efficient is the chemical stoichiometry method by measuring the content of Volatile Fatty Acid (VFA) (Jayanegara *et al.* 2013), so that the study using the different samples is needed to be done. This study was aimed to analyze the methane emission from several types of feed commonly used in Indonesia, the effectiveness of the methane emission test method by using the chemical stoichiometry method, and the environmental management strategies toward the global warming.

2 MATERIAL AND METHOD

The study was conducted in October – December 2014 in the Laboratory of Animal Feed Science and Technology, Faculty of Animal Husbandry, Bogor Agricultural University. The equipments used in this study were centrifuge, vortex, Erlenmeyer, stirer, mortir or blender, digital scales, tube, plastic tube, pipette, sonicator (ultrasonic water bath), syringe, nylon filter, Conway dish, titration apparatus, furnace, oven with a temperature of 105°C, and waterbath in 60 °C temperature. The materials used in this study were elephant grass (EG), rice straw (RS), cow concentrate (CC), (EG 60% + CC 40%), (RS 60% + CC 40%), methanol, acetone, H_2SO_4, Folin, distilled water, rumen fluid, Vaseline, Buffer solution, boric acid, sulfuric acid and $NaHCO_3$.

The method used was a chemical stoichiometry method by measuring the content of volatile fatty acid (VFA). The analysis of VFA content was done according to the method from Hoeltershinken *et al.* (1997). The variables that were observed in this phase were the nutrient composition of the feed, the content of gross energy (GE), the total gas, the ammonia, the organic matter digestibility (OMD), and the content of VFA.

In this study, the analysis of the nutrient feed composition was done using the proximate analysis and the Van Soest fiber analysis that consisted of Neutral detergent fibre (NDF) and Acid detergent fibre (ADF) levels (Van Soest 1991). The measurement of gross energy content was performed by a *Bomb Calorimeter Parr* 6200, using a reference standard of ASTM D5865, "*Standard Test Method for Gross Calorific Value of Coal and Coke*", whereas the total gas measurement was done based on a method of Menke *et al.* (1979) that was modified by Blümmel *et al.* (1997). The determination of ammonia concentration (Conway 1957), while the measurement of dry matter and organic matter digestibility was done by a method of Tilley dan Terry (1963), and the measurement of *Volatile Fatty Acid* (VFA) was performed by a method cited from Jayanegara *et al.* (2013) by using the equation of Moss *et al.* (2000).

Data Analysis

The data obtained then analyzed statistically by Analysis of Variance (ANOVA). If the analysis result was different significantly, it would be followed by Duncan Test (Steel and Torrie. 1993). The mathematical model of the design used is:

$$Yij = \mu + \tau i + \varepsilon ij$$

Where :

Yij : observation value on the feeding number-i and the repetition number-j

μ : general median
i : number of treatment (A, B, C, D and E)
j : number of repetition (1, 2, and 3)
τi : effect of treatment number-i
εij : effect of treatment excess/ error

3 RESULT AND DISCUSSION

Chemical Composition of Feed

The ration used in this study was the mixture contained elephant grass, rice straw and concentrate with a ratio of 60 : 40. The analysis result of nutrient composition (proximate) of the ration is shown in Table 1.

Table 1 Composition of nutrient of feed in %DM (dry matter)

Nutrient	Concentrate (%)	Elephant Grass (%)	Straw (%)	EG: Concentrate 60%:40%	RS: Concentrate 60%:40%
DM	87,95	86,04	91,86	86,99	90,3
OM	81,76	76,89	74,36	77,68	77,04

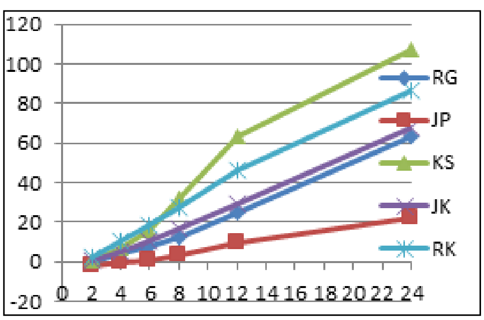
Ash	6,19	9,15	17,5	9,31	13,26
CP	14,22	10,63	6,56	12,1	10,03
CFb	9,76	27,12	36,21	21,15	20,97
CFt	3,46	2,48	1,49	2,9	2,26
Beta-N	54,32	36,66	30,10	41,53	43,78
GE	3601 kal/g	3950 kal/g	3597 kal/g	3807 kal/g	35,98 kal/g
NDF	61,27	77,79	79,29	63,51	69,92
ADF	29,23	68,52	72,76	57,64	63,88

Information: Analysis result obtained from Laboratory of Animal Feed Science and Technology, Faculty of Animal Husbandry, Bogor Agricultural University (2015), DM = dry matter; OM = organic matter; CP = crude protein; CFb = crude fiber; CFt = crude fat; GE = *gros energy;* NDF = *neutral detergent fibre*; ADF = *Acid detergent fibre.*

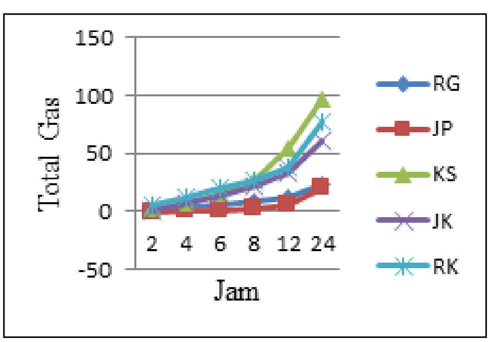
Based on Table 1, it can be seen that the protein content in the concentrate used is 14.22% DM. The making of this concentrate is in accordance with the statement of Blakely and Bade (1994) that said the mixture of dairy cow concentrate consisted of ingredients that contained protein and energy with crude protein content of 14% - 16% DM. The proximate analysis result of elephant grass showed that its protein content was 10.63% DM. Argadyasto *et al*. (2015) reported that the protein content in elephant grass was 7.95% DM. The difference in protein contents obtained can be caused by the difference in harvesting age of the elephant grass. That is because the young elephant grass has a higher protein content. Besides, the other factor that can cause the difference in elephant grass nutrient is the differences in location of harvesting and in environmental condition of places where the elephant grass grow (Jayanegara *et al.* 2009).

Total Gas Production

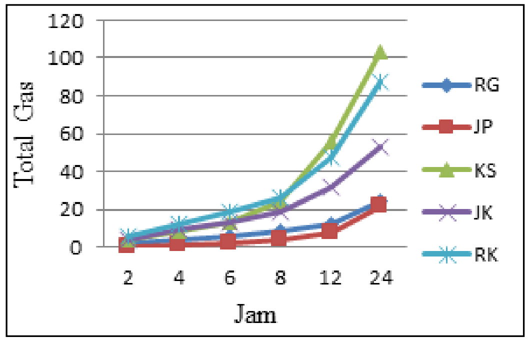
Total gas production as the result of in vitro incubation of each treatment for 24 hours (RS = Rice Straw, EG = Elephant Grass, CS = Concentrate-Straw, CEG = Concentrate-Elephant Grass and CC = Cow Concentrate) is shown in Figure 1.



Repetition 1



Repetition 2



Repetition 3

Figure 1 Total gas of each repetition.

Based on the result of incubation for 24 hours (Figure 1), the highest total gas production was produced by the concentrate by as many as 107,00 ml on the first repetition, while the lowest one was produced by rice straw with 20,00 ml on the third repetition. The difference in result of each treatment is allegedly because of the rumen fluid, incubation condition, and substrate (Jayanegara 2008). The analysis of each treatment showed the different result but the consistency of order in total gas production was stable, that started from the lowest gas production of the samples RS, EG, CS, CEG, and CC.

Dry Matter Digestibility (DMD) and Organic Matter Digestibility (OMD)

Digestibility of dry matter and organic matter are the indicator values for digested nutrients in the ration of ruminant. McDonald *et al.* (2010) stated that the factors affected the value of DMD in the ration were, among others, the proportion of the feed ingredients, the chemical composition, the physical form of ration, the level of feeding, and the internal condition of the livestock. The organic matter digestibility shows the level of nutrient availability in the ration that can be utilized by the ruminant animals. The nutrient digestibility value of a feed material is one of the indicators in determining the quality of this feed material (Tillman *et al.* 1998). The values (%) of DMD and OMD of each treatment is shown in Table 2.

Table 2 Average values of DMD and OMD (%)

Sample	Average of DMD (%)		SD	Average of OMD (%)		SD
RS	24,38	±	4,63	21,85	±	2,74
CS	41,18	±	5,97	37,35	±	5,88
EG	42,81	±	4,54	38,11	±	4,75
CEG	47,77	±	5,09	40,31	±	4,88
CC	60,54	±	4,85	62,81	±	4,79

Information: SD= Standard Deviation.

The result obtained showed that the composition of concentrate added to the rice straw and elephant grass with the level appropriate to the treatment in Table 2 did

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not give effect on DMD and OMD significantly (P>0,05). The result obtained was related to the result of total gas production. This is in line with the opinion of Ella *et al.* (1997) that stated the higher the total gas production that the higher the microorganisms activity in the rumen, thus illustrating the high fermentation process that occurs and the organic matter digested.

Ammonia Concentration (NH3)

Ammonia production in ruminats derived from the microorganisms activity in the rumen that produce the proteolytic enzyme which degrades the protein of ration. The protein that enters the rumen, some will be degraded into ammonia. The concentration of ammonia in each treatment is presented in Table 3.

Table 3 Average of ammonia concentration (NH3)

Sample	N-NH3 (mM)
RS	14,41 ± 0,13
CS	20,55 ± 0,5
EG	12,11 ± 2,69
CEG	17,49 ± 1,45
CC	22,84 ± 0,13

RS = Rice Straw, EG = Elephant Grass, CS = Concentrate-Straw, CEG = Concentrate -Elephant Grass and CC = Cow Concentrate

The concentration of ammonia produced by concentrate-rice straw (CS) and concentrate-elephant grass ranged from 12,11 to 17,49 mM. It was still in an optimal level for the growth of rumen microorganisms. That condition was in accordance with the statement of McDonald *et al.* (2002) that the concentration of ammonia that can optimally support the microorganisms activities for synthesis of microbial protein ranges from 6 to 21 mM. The content of ammonia in concentrate did not support the performance of microorganisms maximally for the synthesis of microbial protein because it was greater than 21 mM.

Methane (CH_4)

The total production of methane gas as a result of in vitro incubation in each treatment for 24 hours of Rice Straw (RS), Elephant Grass (EG), Concentrate-Straw (CS), Concentrate-Elephant Grass (CEG), and Cow Concentrate (CC), is presented in Figure 2.

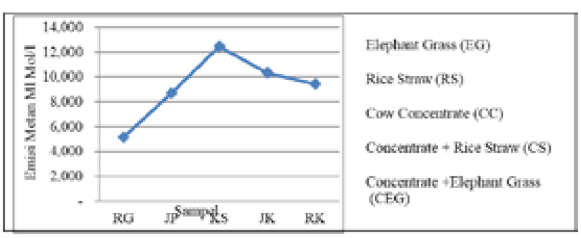

Figure 2 Emission of methane in each sample.

The analysis result using the equation formula according to Moss *et al.* (2000) that was obtained from the analysis result of VFA using gas chromatography showed the data of methane production that the elephant grass gave the lowest emission by 5,13 mmol/l. The largest emissions respectively were generated by rice straw in amount of 8,68 mmol/l, concentrate-elephant grass for 9,42 mmol/l, concentrate-rice straw for 10,32 mmol/l, and the largest was the emission from concentrate that was equal to 12,45 mmol/l. Based on that result, the strategy of feeding that can reduce the methane emission from ruminant livestock will be useful both for the long term in reducing the rate of greenhouse gases, as well as for the short term in reducing the energy loss in livestock (Jayanegara 2008), so it is very correlated with the addition of rice straw for as much as 60% and so also with elephant grass for as much as 60%, which each is mixed by concentrate of 40%.

Correlation Between Gas Production and Content of NH_3

The data obtained from the analysis result showed that the correlation between the gas production and the content of NH_3 of each sample was linier (Figure 3).

Figure 3 Correlation between gas production and NH_3 content

The correlation analysis that was performed on the content of total gas and the concentration of ammonia (NH_3) showed that the coefficient value of determination R^2 = 0,924 It showed that the total gas production of each treatment was proportional to the concentration of ammonia, which was 90,24% was influenced by the total gas to the concentration of ammonia, while the remaining, 9,76% was explained by other factors thar were not observed in this study.

Strategy to Reduce Methane Gas

The researches on the production of methane gas in the last few decades often brought up by the expert because the methane gas is one of the greenhouse gases cause global warming. The result study of the last 10 years gives an information that the ruminants produce 80 million tons of methane/year, that is 28% of anthropogenic emission (Beauchemin *et al.* 2008). Various ways have been made to reduce the production of methane gas. The ruminant livestocks, especially the dairy cows, have been studied, and some strategies in reducing the methane gas have been implemented, such as the addition of ionophores and fats, the use of high-quality forage, and increase the use of grains (concentrate). The reduction of methane gas emission can be done by manipulating the fermentation process in the rumen either by directly inhibiting the methanogens and protozoa, or by diverting the hydrogen molecules from methanogens. Some sources identify the new way to reduce the emission of methane gas, that is with the addition of probiotics, acetogens, bacteriocins, Archaea virus, organic acids, plant extracts (for example, essential oils) for feed, as well as immunization, and genetic selection of the cattle (Boadi *et al.* 2004).

The methane gas is the final product of carbohydrate fermentation in the rumen. To improve the productivity of livestock seems to be the most effective way to reduce the methane gas emission in the short term. It needs to be considered that this method only works if the entire production remains constant. The methods to reach this increase of productivity have been discussed, but nearly all involve the increase in the use of feed containing the higher/lower quality of fiber content (Moss *et al.* 2000). One of the feeding strategies performed is feeding the ruminant livestock with the feed containing tannin, because tannin can reduce the methane gas production (Jayanegara *et al.* 2009).

In this study, the best result of a reduction in methane emission is obtained in a treatment of 60% elephant grass + 40% concentrate (CEG), therefore the strategy to reduce the emission of methane gas by concentrate feeding can

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be conducted effectively and efficiently through the addition of rice straw and elephant grass which is easily found in the community.

Conclusion

From the analysis result of in vitro methane emission of ruminant rumen using the chemical stoichiometry method, it can be concluded that:

The methane emission analysis of several treatments in this study showed that the concentrate added by elephant grass 60% is the best mixture in reducing the methane emission, that is equal to 3,03 mmol/l.

The effectiveness of chemical stoichiometry method is still effective in measuring the methane gas.

The strategy to reduce the methane gas emission through the ruminant's feed can be performed by adding the rice straw and elephant grass to the concentrate.

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