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Concentrations Test of Methane Gas From Swamp Forage Silage with *In Vitro* Method

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ABSTRAK (INDONESIAN)

Penelitian ini bertujuan untuk menguji konsentrasi gas metana dari silase hijauan rawa dengan metode *in vitro*. Penelitian ini menggunakan Rancangan Acak Lengkap (RAL) dengan 3 perlakuan dan setiap perlakuan terdiri dari 5 ulangan. Perlakuan yang digunakan adalah sebagai berikut: P1 (100% Rumput kumpai tembaga (*Hymenachne acutigluma))*, P2 (50% Rumput kumpai tembaga (*Hymenachne acutigluma)* + 50% Kemon air *(Neptunia oleracealour)),* P3 (100% Kemon air (*Neptunia oleracealour)).* Parameter yang diamati meliputi N-Amonia, produksi gas total, konsentrasi gas metana secara *in vitro* and VFA parsial. Hasil sidik ragam menunjukkan bahwa perlakuan berbeda nyata (P<0,05) terhadap konsentasi gas metana secara *in vitro* dengan konsentrasi gas tertinggi pada perlakuan silase yang terbuat dari 50% Rumput kumpai tembaga (*Hymenachne acutigluma)* dan50% Kemon air *(Neptunia oleracealour)*.

Katakunci:Konsentrasi, Metana, Silase Hijauan Rawa, In Vitro.

ABSTRACT

Aims of this study to concentration test of methane gas from swamp forage silage with *in vitro* methods.This study used Completely Randomized Design with 3 treatments and each treatment consisted of 5 replications. The treatments used are as follows: P1 (100% kumpai tembaga grass *(Hymenachne acutigluma)),* P2 (50% kumpai tembaga grass *(Hymenachne acutigluma)* + 50% kemon air *(Neptunia oleracea lour)),* P3 (100% kemon air (*Neptunia oleracea lour*)). The parameters observed N-Ammonia, total gas production, methane gas concentrations *in vitro* and VFA partially. The results of variance showed that treatment significantly different (P <0.05) with methane concentration *in vitro* with highest gas concentration treatment of silage made from 50% kumpai tembaga grass *(Hymenachne acutigluma)* and 50% kemon air *(Neptunia oleracea lour).*

Word key: Concentration, Methane, Silage Forage Swamp, In Vitro.

INTRODUCTION

Rapid population growth in line with rapid industrial growth resulting in increased energy demand and declining environmental quality. Meanwhile, the number of fossil fuels is increasingly limited. Utilization of alternative renewable energy sources and environmentally friendly can be an option. In Europe, energy policies are increasingly promoting energy generation from renewable sources, 27% renewables by 2030 and decrease by 40% ofgreenhouse gas emissions [1]. One renewable energy is biogas or methane fermentation.

The methane gas is usually found in nature in which the destruction of organic material by bacteria occurs without oxygen *(anaerobic),* such as swamps or muddy section on the lake. Therefore, methane gas is often called swamp gas. Indonesia has a wealth of natural resources that are very abundant to produce alternative energy sources.Extensive swamps in South Sumatra Province, about 613 795 hectares consists of 455 949 ha tidal swamp and lowland swamps Ha 157 846 [2]. Forage (grasses) and leguminase are the main feed ingredients commonly used by breeders as rations for ruminant livestock, forage swamps have a high fiber content with low nutritional quality. Natural marsh forage generally contain limitations in protein content, is very limited natural grass protein content which ranges from 4% to the crude fiber content is quite high and is limited to the dry season [3]. Silage is feed fermented in fresh form by the lactic acid bacteria (LAB) with a high water content under*anaerobic* conditions and produce most of its products such as lactic acid, in addition to improving feed quality lactic acid in silage can act as a natural preservative and silage will have long shelf life [5].

The feed consumed by livestock is instrumental in the formation of methane gas. Based on the Jayanegara (2008) report between 6% -10% of the ruminant livestock feed's gross energy is lost as methane [4]. M ethane (CH_4) is produced from acetic acid, hydrogen and carbon dioxide. Methanogenic bacteria use hydrogen, carbon dioxide and despair m acetate to form methane [5]. The concentration of methane gas can be tested by *in vitro* methods where there

Biogas from plants is one of the relatively simple renewable energy sources produced by anaerobic fermentation of organic materials. This precise and inexpensive energy can overcome the people's dependence on fuel oil which is now increasingly rising and its sources getting more and more limited. Information on the production of methane gas from silage from forage swamp so far has not been much studied, therefore it is necessary to conduct research on the potential of methane gas production from the swamp green silage. The results of this study are expected to be used as one of the basic development of biomass utilization of silage forage swamp become source of biogas production as alternative energy to reduce dependency of fossil fuel use.

MATERIAL AND METHOD

This research was conducted in June and September 2017 in the Laboratory of Animal Nutrition and Feed, Faculty of AgricultureSriwijaya University, Livestock and Environment Laboratory, Laboratory of Central Research Institute of Agriculture Pati Central Java and Laboratory Livestock Research Ciawi Bogor. The instruments used are scales, 2 liter plastic derivatives (silos), compressor faucets, rubber caps, masking tape, sprayer, analytical balance, pH meters, gas chromatography (GC).

The materials used consisted of kumpai tembaga grass (*Hymenachne acutigluma*), kemon air (*Neptunia oleracea lour*), Molases, H2SO4 0.3 N, Acetone and CH_4 standard.The research design used was Completely Randomized Design consisting of 3 treatments and 5 replications. Each treatment consists of:

P1 = silage made from kumpai tembaga grass *(Hymenachne acutigluma)*
P2 = silage made from 50% kumpai tembaga grass *(Hymenachne acutigluma)* and 50% kemon air *(Neptuniaoleracea lour)*
P3 = Making silage made Kemon water *(Neptunia oleracea lour)*

The mathematical model research design was unpacking follows [6]:

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

Information :
Y_{ij} : Observation Value
μ : Middle value
τij : The additive effect of the I-th repeat treatment on j-observation
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1.1. Silage Making

Stages of making silage can be done by kumpai copper and water Kemon cut along the 2-5 cm, after cut copper and Kemon kumpai dilayukan water for 24 hours. Each treatment was mixed with molasses that served as an inoculant of 3% then inserted into each derigen-size plastic 2 liter and compacted until no air space, then sealed and stored in a dry place and not exposed to direct sunlight for 21 days, because the process ensilase lasts up to pad a third week [7].

1.2. *In Vitro* Test

Rumen fluid taken from cattle filtered through four layers of gauze then diluted rumen fluid (2: 1) with *Mc Dougall* gassed CO_2 diluted rumen fluid as inoculum. Samples were added to the bottle incubator *in vitro* for the final result 25 ml. The composition of each treatment sample of 1 g was added 25 ml inoculum was then added gas CO_2 incubator bottle closed with butyl rubber stopper. Incubate anaerobically for 24 hours at a temperature of 39 $^{\circ}$ C using a water bath shaker 180 rpm. Observations concentration of methane gas made after incubation for 24 h, 24 h incubation with more practical considerations and reduce the diversity of fermented [10]. Gas first observed at the time of 0-12-18 and 24 hours using a glass syringe, after 24 hours of fermentation gas sample results stored in vacuum tubes venoject to analyze concentrations of methane gas using *gas chromatography* (GC). Laboratory of Central Research Institute of Agriculture Pati Central Java.

1.3. Calculation of the N-ammonia (NH_3)

N-ammonia concentration in rumen fluid is measured by methods mikrodifusi [9]1 ml supernatant samples were placed in one side of the bulkhead *conway* and in the other partition position is placed 1 ml 20% NaOH solution. Position the cup *conway* tilted so that the two solutions do not mix before the cup is closed. At the center is placed 1 ml of boric acid 3% berindikator BCG: MR. On the edge of the cup and the cover is smeared with vaseline to be tightly closed. Then the cup is placed flat so that 20% NaOH solution mixes with the supernatant and in the reaction is released ammonia gas. Ammonia is liberated to be arrested by boric acid. This process will take place is perfect after 24 hours, then boric acid is titrated with 0:01 N HCl until the color changes from blue to red (initial color boric acid). Ammonia levels can be calculated by the formula:

$N\text{-}NH_3$ (mM) = ml titration x 100 x Normality HCl.

1.4. Determination of Partial VFA Levels

VFA measurements performed in the laboratory of the Livestock Research Center Ciawi Bogor using *gas chromatography*. Rumen sample solution in the pipette 1 ml into *eppendorf* tube. Added internal standard. Added approximately 30 mg of sulfo-5- salllisilat acid dihydrate is then shaken, then centrifuged for 10 min at 12,000 rpm with a temperature of 7 $^{\circ}$ C. The solution instances / rumen which has been clear in 1 mL injected into *Gas Chromatography*. Prior to injection of the sample solution or rumen first injected VFA standard solutions that have been added internal standard. Calculation of partial VFA can be calculated with the following formula:

$$\text{VFA (mM)} = \frac{\text{Area of VFA example x Concentration standard VFA}}{\text{Standard area of VFA x BM}}$$

where:

- VFA = Consists of Acetic Acid, propionate, n-butyrate. i so-butyrate, n - valerate and iso-valerate
- BM = Partial VFA Molecular Weight
- The standard VFA concentration is 1mg / ml = 1000 µg / ml

RESULTS AND DISCUSSION

Methane concentration of *In Vitro*

In vitro methods is a metabolic process that occurs outside the body of cattle but the principles and conditions are the same with the processes that occur in the body of livestock.

Total Gas Production

The rate of total gas production of silage forage swamp of rumen fermentation system *in vitro* is presented in Figure 1.

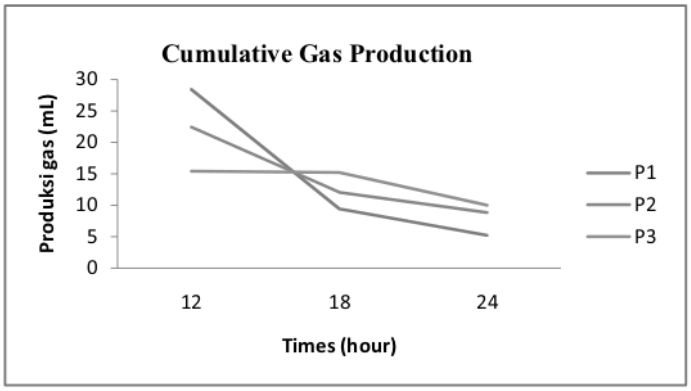

Figure 1. Graph of gas production for 24 hours incubation; P1 (Kumpai Tembaga Grass 100%), P2 (Kumpai Tembaga Grass 50% + 50% Kemon Air), P3 (Kemon Air 100%).

Figure 1 shows that with increasing incubation time of declining gas production in treatment P1 and P2 at the time incubation 18 hours, while the P3 treatment tended to increase in 18 hour incubation and then decreased at 24 h incubation. This is because the amount of substrate fermented diminishing [4].

Table 1. Average cumulative gas production at incubation time of 24 hours

Treatment	Cumulative Gas Production (mL)
P1	$5{,}20^{a}\pm 1{,}09$
P2	$8{,}80^{b}\pm 8{,}80$
P3	$10{,}00^{b}\pm 1{,}41$

Description: P1 (Silage made from kumpaitembaga grass *(Hymenachne acutigluma)),* P2 (Silage made from 50% kumpai tembaga grass *(Hymenachne acutigluma)* and 50% Kemonair *(Neptunia oleracea lour)),* P3 (Silage made Kemonair *(Neptunia oleracealour))* . Different superscript letters within a column indicate significant differences at the level of the test ($p < 0.05$) BNT.

Diversity analysis results showed that the treatment significantly ($P < 0, 05$) against the cumulative gas production. Further test results showed that the treatment P1 BNT significantly different ($P < 0, 05$) to the treatments P2 and P3. According to the table 1 the average production of the highest gas during a 24-hour incubation all contained in P3 treatment at 10 mL while gas production was lowest for the treatment P1 by 5, 2 mL. This is presumably a treatmentkaren b value P3 has a high organic ahan namely 59, 27%, a protein content as high as 15.61%, Karbohidrat in the

diet can increase the protein degradation by microbes which can be used for microbial growth with increased microbial growth is characterized by increased gas production [10].

Table 2. Partial VFA concentration and the concentration of methane gas *in vitro*

Treatment	Acetic Acid (mM)	Propionic Acid (mM)	Iso Butyric Acid (mM)	N Butyric Acid (mM)	Methane Gas (ppm)
P1	48,13 ± 3,72	38,52 ± 5,24	2,24± 0,47	11,44 ± 2,12	$45,80^{a}$± 49,50
P2	51,21 ± 4,15	38,96 ± 5,16	2,15 ± 0,34	11,10 ± 1,95	$16802,71^{b}$± 6525,15
P3	44,01 ± 6,33	32,85 ± 2,37	2,24 ± 0,20	10,71 ± 0,87	$15345,16^{b}$± 10718,98

Description: P1 (Silage made from kumpaitembaga grass *(Hymenachne acutigluma)),* P2 (Silage made from 50% kumpaitembaga grass *(Hymenachne acutigluma)* and 50% Kemonair *(Neptunia oleracea lour)),* P3 (Silage made Kemonair *(Neptunia oleracealour))* . Different superscript letters within a column indicate significant differences at the level of the test (p <0.05) BNT.

Diversity analysis results showed that the treatment had no significant effect (P> 0, 05) against the partial VFA concentrations are acetic acid, propionic acid, butyric acid and iso n butyric acid. The highest concentration of acetic acid contained in the P2 treatment amounting to 51.21 mM while the methane gas concentration was lowest for the treatment amounted to 44.01 mM P3. Propionate concentration highest in P2 treatment amounting to 38.96 mM while the methane gas concentration was lowest for the treatment amounted to 38.52 mM P1. Iso butyrate concentration is highest in the treatment of P1 and P3 in the amount of 2.24 mM while the methane gas concentration was lowest for the treatment of 2.12 mM P2. VFA nyat a partial no effect because the content of dry matter and organic matter were also not berpenga real spirit. Cellulose is an organic material that is important for ruminants because it is used as an energy source. Cellulose, starch and hemicellulose contained in the feed are digested by rumen microbes and produce simple sugars. Simple sugars will undergo glycolysis into pyruvic acid through *anaerobic* glucose *koksidasi.* Pyruvic acid is then converted into VFA such as acetate, propionate, and butyrate, otherwise it produces carbon dioxide ($CO_{2)}$, H_2O and Methane ($CH_{4)}$ [11]. Will monosaccharides glucose and fermented in an anaerobic atmosphere in the rumen to produce volatile fatty acids *(volatile fatty acids)* such as acetate, propionate and butyrate [12]. T otal VFA in the rumen can be used as a benchmark the efficiency of feed in the rumen fermentation process [13].

The formation of methane in the rumen occurs through the reduction of CO_2 by H_2 catalyzed by enzymes produced by *methanogenic archaea.* Diversity analysis results showed that the treatment significantly (P <0, 05) against the concentration of methane gas. Gas concentration highest in P2 treatment amounting to 16 802, 71 ppm while the methane gas concentration was lowest for the treatment P1 at 45.80 ppm.

BNT advanced test results show that P1 treatment were significantly different (P <0, 05) to the treatments P2 and P3. The high concentration of methane gas at P2 treatment is influenced by a high concentration of acetic acid at 51, 21 mM. Improved high acetic acid which can lead to methane gas mbentukan pe [14]. The high concentration of methane gas generated in the treatment of P2 is also due to the high content of nutrients in feed ingredients such as high protein content in P2 treatment with 19, 85%.

CONCLUSION

The concentration of methane *in vitro* highest in the treatment of silage made from 50% kumpai tembaga grass *(Hymenachne acutigluma)* and 50% kemon air *(Neptuniaoleracea lour).* Silage forage swamp with a composition of 50% kumpai tembaga grass

(Hymenachne acutigluma) and 50% kemon air *(Neptuniaoleracealour)* can potentially be a source of energy.

ACKNOWLEDGEMENT

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Katakunci:Konsentrasi, Metana, Silase Hijauan Rawa, In Vitro.

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$$\text{VFA (mM)} = \frac{\text{Area of VFA example x Concentration standard VFA}}{\text{Standard area of VFA x BM}}$$

where:

- VFA = Consists of Acetic Acid, propionate, n-butyrate. i so-butyrate, n - valerate and iso-valerate
- BM = Partial VFA Molecular Weight
- The standard VFA concentration is 1mg / ml = 1000 µg / ml

RESULTS AND DISCUSSION

Methane concentration of *In Vitro*

In vitro methods is a metabolic process that occurs outside the body of cattle but the principles and conditions are the same with the processes that occur in the body of livestock.

Total Gas Production

The rate of total gas production of silage forage swamp of rumen fermentation system *in vitro* is presented in Figure 1.

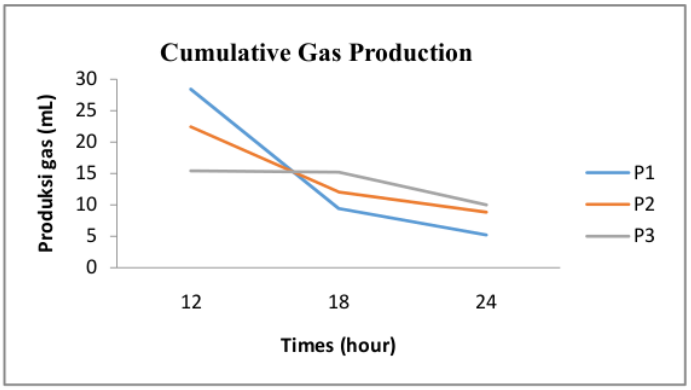

Figure 1. Graph of gas production for 24 hours incubation; P1 (Kumpai Tembaga Grass 100%), P2 (Kumpai Tembaga Grass 50% + 50% Kemon Air), P3 (Kemon Air 100%).

Figure 1 shows that with increasing incubation time of declining gas production in treatment P1 and P2 at the time incubation 18 hours, while the P3 treatment tended to increase in 18 hour incubation and then decreased at 24 h incubation. This is because the amount of substrate fermented diminishing [4].

Table 1. Average cumulative gas production at incubation time of 24 hours

Treatment	Cumulative Gas Production (mL)
P1	$5{,}20^{a} \pm 1{,}09$
P2	$8{,}80^{b} \pm 8{,}80$
P3	$10{,}00^{b} \pm 1{,}41$

Description: P1 (Silage made from kumpaitembaga grass *(Hymenachne acutigluma))*, P2 (Silage made from 50% kumpai tembaga grass *(Hymenachne acutigluma)* and 50% Kemonair *(Neptunia oleracea lour))*, P3 (Silage made Kemonair *(Neptunia oleracealour))* . Different superscript letters within a column indicate significant differences at the level of the test ($p < 0.05$) BNT.

Diversity analysis results showed that the treatment significantly ($P < 0,05$) against the cumulative gas production. Further test results showed that the treatment P1 BNT significantly different ($P < 0,05$) to the treatments P2 and P3. According to the table 1 the average production of the highest gas during a 24-hour incubation all contained in P3 treatment at 10 mL while gas production was lowest for the treatment P1 by 5, 2 mL. This is presumably a treatmentkaren b value P3 has a high organic ahan namely 59, 27%, a protein content as high as 15.61%, Karbohidrat in the

diet can increase the protein degradation by microbes which can be used for microbial growth with increased microbial growth is characterized by increased gas production [10].

Table 2. Partial VFA concentration and the concentration of methane gas *in vitro*

Treatment	Acetic Acid (mM)	Propionic Acid (mM)	Iso Butyric Acid (mM)	N Butyric Acid (mM)	Methane Gas (ppm)
P1	48,13 ± 3,72	38,52 ± 5,24	2,24± 0,47	11,44 ± 2,12	$45,80^{a}$± 49,50
P2	51,21 ± 4,15	38,96 ± 5,16	2,15 ± 0,34	11,10 ± 1,95	$16802,71^{b}$± 6525,15
P3	44,01 ± 6,33	32,85 ± 2,37	2,24 ± 0,20	10,71 ± 0,87	$15345,16^{b}$± 10718,98

Description: P1 (Silage made from kumpaitembaga grass *(Hymenachne acutigluma)),* P2 (Silage made from 50% kumpaitembaga grass *(Hymenachne acutigluma)* and 50% Kemonair *(Neptunia oleracea lour)),* P3 (Silage made Kemonair *(Neptunia oleracealour))* . Different superscript letters within a column indicate significant differences at the level of the test (p <0.05) BNT.

Diversity analysis results showed that the treatment had no significant effect (P> 0, 05) against the partial VFA concentrations are acetic acid, propionic acid, butyric acid and iso n butyric acid. The highest concentration of acetic acid contained in the P2 treatment amounting to 51.21 mM while the methane gas concentration was lowest for the treatment amounted to 44.01 mM P3. Propionate concentration highest in P2 treatment amounting to 38.96 mM while the methane gas concentration was lowest for the treatment amounted to 38.52 mM P1. Iso butyrate concentration is highest in the treatment of P1 and P3 in the amount of 2.24 mM while the methane gas concentration was lowest for the treatment of 2.12 mM P2. VFA nyat a partial no effect because the content of dry matter and organic matter were also not berpenga real spirit. Cellulose is an organic material that is important for ruminants because it is used as an energy source. Cellulose, starch and hemicellulose contained in the feed are digested by rumen microbes and produce simple sugars. Simple sugars will undergo glycolysis into pyruvic acid through *anaerobic* glucose *koksidasi.* Pyruvic acid is then converted into VFA such as acetate, propionate, and butyrate, otherwise it produces carbon dioxide (CO_2), H_2O and Methane (CH_4) [11]. Will monosaccharides glucose and fermented in an anaerobic atmosphere in the rumen to produce volatile fatty acids *(volatile fatty acids)* such as acetate, propionate and butyrate [12]. T otal VFA in the rumen can be used as a benchmark the efficiency of feed in the rumen fermentation process [13].

The formation of methane in the rumen occurs through the reduction of CO_2 by H_2 catalyzed by enzymes produced by *methanogenic archaea.* Diversity analysis results showed that the treatment significantly (P <0, 05) against the concentration of methane gas. Gas concentration highest in P2 treatment amounting to 16 802, 71 ppm while the methane gas concentration was lowest for the treatment P1 at 45.80 ppm.

BNT advanced test results show that P1 treatment were significantly different (P <0, 05) to the treatments P2 and P3. The high concentration of methane gas at P2 treatment is influenced by a high concentration of acetic acid at 51, 21 mM. Improved high acetic acid which can lead to methane gas mbentukan pe [14]. The high concentration of methane gas generated in the treatment of P2 is also due to the high content of nutrients in feed ingredients such as high protein content in P2 treatment with 19, 85%.

CONCLUSION

The concentration of methane *in vitro* highest in the treatment of silage made from 50% kumpai tembaga grass *(Hymenachne acutigluma)* and 50% kemon air *(Neptuniaoleracea lour).* Silage forage swamp with a composition of 50% kumpai tembaga grass

(Hymenachne acutigluma) and 50% kemon air *(Neptuniaoleracealour)* can potentially be a source of energy.

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