ASSESSMENT OF METHANE ESTIMATION FROM VOLATILE FATTY ACID STOICHIOMETRY IN THE RUMEN *IN VITRO*

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ABSTRAK

Mikroba rumen mendegradasi komponen pakan dan menghasilkan sejumlah produk metabolit seperti asam lemak terbang (volatile fatty acids, VFA), karbon dioksida, amonia dan metana (CH_4). Hidrogen metabolik dalam bentuk proton tereduksi digunakan dalam pembentukan CH_4 dan juga dalam sintesis VFA. Dengan demikian, konsentrasi VFA di rumen berhubungan secara stoikiometri dengan emisi CH_4. Tujuan penelitian ini adalah untuk mengevaluasi kesesuaian emisi CH_4 antara hasil eksperimen dengan prediksi dari komposisi VFA. Dua buah model stoikiometri yang memprediksi CH_4 dari VFA dievaluasi, yakni model Moss *et al.* (2000) dan model Hegarty dan Nolan (2007). Data yang digunakan merupakan data mentah dari sebuah publikasi yang menggunakan sampel berupa 27 hijauan pakan tropis. Prediksi galat dilakukan dengan cara menghitung *root mean square prediction error* (RMSPE). Hasil menunjukkan bahwa model estimasi Moss *et al.* (2000) memiliki RMSPE yang lebih rendah dibandingkan dengan model Hegarty dan Nolan (2007), yakni 8.01% vs 10.73%. Variasi tinggi rendahnya nilai emisi CH_4 dapat diestimasi secara cukup akurat menggunakan kedua model stoikiometri. Penyesuaian nilai estimasi dengan mempertimbangkan H_2 *recovery* dapat mengurangi bias secara signifikan. Dari penelitian ini dapat disimpulkan bahwa model Moss memiliki akurasi yang lebih baik dalam memprediksi emisi CH_4 dari komposisi VFA dibandingkan dengan model Hegarty dan Nolan.

Kata kunci: Metana, VFA, stoikiometri, estimasi

ABSTRACT

Rumen microbes breakdown feed to produce volatile fatty acids (VFA), carbon dioxide, ammonia and methane (CH_4). Metabolic hydrogen in the form of reduced protons is used during CH_4 formation as well as during VFA synthesis. Therefore, VFA concentration in the rumen may stoichiometrically be related to CH_4 emission. The aim of this study was to evaluate methane emission between experimental and model estimates. Two stoichiometrical models for predicting CH_4 from VFA were assessed, i.e. Moss *et al*. (2000) and Hegarty and Nolan (2007) models. The data sets were obtained from a published literature. Samples used were leaves from 27 tropical plant species. Prediction error was conducted by computing root mean square prediction error (RMSPE). Results showed that estimation model of Moss *et al.* (2000) had lower RMSPE value, i.e. 8.01%, than that of Hegarty and Nolan (2007) model, i.e. 10.73%. Variation of methane emission, i.e. the low or high methane can be estimated by VFA composition with a sufficient accuracy. Adjusment by considering H_2 recovery lowered the bias significantly. It can be concluded that Moss model had better accuracy in predicting CH_4 emission from VFA composition than that of Hegarty and Nolan model.

Keywords: Methane, VFA, stoichiometry, estimation

INTRODUCTION

Apart from its contribution to global warming, methane (CH_4) emission from ruminant animals represents energy losses emitted to the atmosphere and may therefore reduce net energy gain for the respective animals (Moss *et al.*, 2000; Cottle *et al.*, 2011). Such CH_4 formation or methanogenesis takes place in the rumen where various microbes are symbiotically living together

in the compartment including the agent of methanogenesis, i.e. methanogenic archaea (Moissl-Eichinger and Huber, 2011; St-Pierre and Wright, 2013). Metabolic hydrogen in the form of reduced protons is utilized during the synthesis of volatile fatty acids (VFA) as well as during CH_4 formation by rumen microbes. Regarding the individual VFA composition and its relationship with CH_4 emission, acetate and butyrate promote CH_4 production while propionate formation can be considered as a competitive pathway for hydrogen use in the rumen (McAllister and Newbold, 2008). Therefore, the proportions of acetate, butyrate and propionate determine the amounts of available H_2 in the rumen to be used by methanogens. By this relation, CH_4 emission can stoichiometrically be calculated from the respective VFA (Moss *et al.*, 2000; Hegarty and Nolan, 2007).

On the other hand, setting up facilities for measuring CH_4 from ruminants either *in vivo* or *in vitro* is unfortunately very costly and such facilities may not be available especially in the developing countries like Indonesia. Currently, in practice, measurement of CH_4 emission is usually conducted by using a respiratory chamber (*in vivo*) or by using gas chromatography technique (*in vitro*) (Bhatta *et al.*, 2007), although other techniques are also available (Sejian *et al.*, 2011). Therefore, estimation of CH_4 mission from VFA profiles is expected to be a solution to the problem. Although some stoichiometrical relationships between VFA composition and CH_4 emission have been previously proposed (Moss *et al.*, 2000; Hegarty and Nolan, 2007), none of the equations have been assessed for their accuracies against empirical data derived from experiments. Accordingly, the aim of this study was to evaluate the accuracy between methane emission as estimated stoichiometrically from VFA and methane emission measured in an *in vitro* system by gas chromatography technique

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MATERIALS AND METHODS

Raw data obtained from previous published study of Jayanegara *et al.* (2011) were used in this research. A total of 27 tropical plant species collected from the area of Bogor were incubated *in vitro* in buffered-rumen fluid for 24 h by following the procedure of Menke and Steingass (1988). Incubation was conducted in eight replicates, represented by a syringe per replicate. In each syringe, 200 mg dry matter (DM) of plant sample was mixed with 30 ml buffered-rumen fluid (rumen:buffer = 1:2 v/v). Prior to use, rumen fluid was strained through four layer of gauze. After 24 h incubation, fermentation gas was sampled (0.15 ml) from each syringe and injected into a gas chromatography (GC) for measuring gas composition including CH_4. Profile of individual VFA, i.e. acetate, propionate, butyrate, isobutyrate, valerate and isovalerate was analyzed from the fermentation fluid by using a high performance liquid chromatography (HPLC) equipped with an UV-Vis detector at 210 nm. The respective VFA analysis was conducted according to Ehrlich *et al.* (1981).

Units of measurements for CH_4 and VFA were ml/l and mmol/l, respectively. In order to enable a direct stoichiometrical relationship between both variables, therefore, the unit of CH_4 (ml/l) was converted to mmol/l using the ideal gas equation as follows:

$$PV = nRT$$

Where:

P = pressure of the gas (atm)
V = volume of the gas (L)
n = number of moles (mol)
R = gas constant (0.08206 L atm/ mol K)
T = temperature of the gas (K)

Stoichiometrical models used for estimating CH_4 from VFA composition were as follow:

1. Hegarty and Nolan (2007), considering the hydrogen recovery of 100% (default):

   $$CH_4 = 0.5\ C_2 + 0.5\ C_4 - 0.25\ C_3 - 0.25\ C_5$$

2. Moss *et al.* (2000), considering the hydrogen recovery of 90% (default):

   $$CH_4 = 0.45\ C_2 - 0.275\ C_3 + 0.40\ C_4$$

Where:

C_2 = acetate
C_3 = propionate
C^4 = butyrate
C_5 = valerate

Hydrogen recovery (%) for observed CH_4 was obtained by an equation from Demeyer and Van Nevel (1979), i.e. Hrec = 2Hp/2Hu × 100, where Hrec is hydrogen recovery, Hp is hydrogen utilized, and Hp is hydrogen produced, with 2Hu = 2 propionate + 2 butyrate + 4 methane + valerate, and 2Hp = 2 acetate + propionate + 4 butyrate + 2 iso-valerate + 2 valerate.

Methane emission after adjustment by the hydrogen recovery was calculated as follows:

CH_4 after adjustment = CH_4 before adjustment × 100/H_2 recovery

Data were analyzed by analysis of variance (ANOVA) and followed by a posthoc test, i.e. Duncan's multiple range test (DMRT) when ANOVA result showed significancy at $P<0.05$. As outlined by Alemu *et al.* (2011), prediction error of estimation was calculated by mean square prediction error (MSPE):

$$MSPE = \sum_{i=1}^{n} \frac{(O_i - P_i)^2}{n}$$

where:

n = number of observations

O_i = CH_4 observed

P_i = CH_4 predicted

Root mean square prediction error (RMSPE) was obtained by square-rooting the MSPE value. The RMSPE value indicates how accurate the model is; lower RMSE value shows better accuracy and *vice versa*. All data analyses were performed by using SPSS software version 16.0.

RESULTS AND DISCUSSION

The values of CH_4 emissions by estimated model of Hegarty and Nolan (2007), estimated model of Moss *et al.* (2000), and CH_4 observed after H_2 recovery adjustment are presented in Table 1. Methane emission resulted from the estimated models of Hegarty and Nolan (2007) and Moss *et al.* (2000) showed that the lowest CH_4 was obtained from the incubation of *Acacia villosa* plant. The plant also contained the highest total tannin among all plants investigated, i.e. 220 g/kg dry matter (Jayanegara *et al.*, 2011). The relationship between total tannin and methane emission generally showed a negative correlation (Jayanegara *et al.*, 2012); Plants contained high tannin levels generated low methane emissions and, *vice versa*, plants contained low tannin levels generated high methane emissions (Jayanegara *et al.*, 2011; Bhatta *et al.*, 2013). Patra and Saxena (2010) stated that tannin may inhibit methanogenesis directly through inhibition on the growth or activity of methanogens, and also indirectly via inhibition of protozoal population. Further, Jayanegara *et al.* (2009) reported that tannin decreased methane production and, among the tannin assays, tannin bioassay (a reflection of tannin activity) was the best predictor of the methane production reduction potential of a plant. Total phenol and total tannin were also good predictors of methane production potential.

Estimated model of Hegarty and Nolan (2007) as well as Moss *et al.* (2000), based on the values on Table 1 resulted in an overestimation of the measured methane production. This was probably due to the much lower of the actual hydrogen recovery, i.e. between 28.9-56.2% than those assumed by both models, i.e. 100% and 90% for Hegarty and Nolan (2007) and Moss *et al*. (2000), respectively. Such lower actual hydrogen recovery may occur since there are different hydrogen pathways other than methanogenesis, such as in the synthesis of the microbial polymers and in other reactions (Morgavi *et al.*, 2010). The importance of these unspecified reactions is difficult to measure and may depend on the mix of species of bacteria and other microbes present. The effect may be greater when inhibitors of methane production have been included in the animal's diet (Hegarty and Nolan, 2007). In real life, production of methane will be lower than the equations because these assumptions are not totally correct. Some NADH or 2(H) is oxidized to provide energy for synthesis of cell polymers (e.g. lipids, amino acids and nucleic acids) during growth of cells, and in various other redox reactions (Czerkawski and Breckenridge, 1975).

Prior to adjustment, the observed methane production was far away from the ideal line where the estimated value is equal to the observed value (Figure 1). Adjustment of the observed methane value by considering its hydrogen recovery led to a closer regression line to the ideal line (Figure 2). This may suggest that the consideration of hydrogen recovery is vital to obtain a more accurate methane prediction. The estimated model line equation of Moss *et al.* (2000) to CH_4 observed before adjustment is $Y = 0.423 X – 3.176$ with $R^2 = 0.465$ and the estimated model line equation of Hegarty and Nolan (2007) to CH_4 observed before adjutment is $Y = 0.374 X – 3.296$ with $R^2 = 0.478$. While, the estimated model line equation of Moss *et al.* (2000) to CH_4 after adjustment is $Y = 0.845 X – 4.672$ with $R^2 = 0.662$ and the estimated model line equation of Hegarty and Nolan (2007) to CH_4 observed after adjustment is $Y = 0.741 X – 4.801$ with $R^2 = 0.671$.

It can be clearly observed in Figure 1 and Figure 2 that the estimated model line of Moss *et al.* (2000) was constantly closer to the ideal line than the estimated model of Hegarty and Nolan (2007). Further, the model showed a quite

Table 1. CH_4 Estimated, H_2 Recovery and CH_4 After Adjustment

No.	Species	CH_4 Estimated (mmol/l)		H_2 recovery (%)	CH_4 After Adjustment (mmol/l)
		Hegarty	Moss		
1	*Acacia mangium*	16.4^{ab}	12.2^{ab}	40.3^{gh}	8.4^{def}
2	*Acacia villosa*	14.6^{a}	12.7^{a}	32.6^{bc}	3.2^{a}
3	*Albizzia falcataria*	21.9^{efgh}	19.1^{efg}	33.2^{bcd}	9.2^{efg}
4	*Artocarpus heterophyllus*	22.3^{fgh}	19.4^{fgh}	40.4^{gh}	12.0^{ij}
5	*Calliandra calothyrsus*	20.0^{cdef}	17.5^{cdef}	34.3^{bcde}	8.9^{efg}
6	*Canna indica*	20.7^{cdefgh}	18.0^{cdefg}	38.6^{gh}	9.0^{efg}
7	*Carica papaya*	26.4^{j}	22.9^{j}	53.7^{l}	17.9^{n}
8	*Clidemia hirta*	19.8^{cde}	17.2^{cde}	36.5^{def}	9.4^{fg}
9	*Cycas rumphii*	19.8^{cde}	17.3^{cde}	38.8^{fg}	10.0^{gh}
10	*Erythrina orientalis*	21.1^{defgh}	18.3^{defg}	46.1^{ij}	12.6^{j}
11	*Eugenia aquea*	16.5^{ab}	14.4^{ab}	28.9^{a}	4.7^{b}
12	*Hibiscus tiliaceous*	18.9^{cd}	16.5^{cd}	37.9^{efg}	9.5^{fg}
13	*Ipomoea batatas*	26.2^{j}	22.8^{j}	45.1^{ij}	15.9^{lm}
14	*Lantana camara*	23.0^{hi}	20.0^{ghi}	45.6^{ij}	14.0^{k}
15	*Leucaena diversifolia*	21.6^{efgh}	19.0^{efg}	41.2^{gh}	11.7^{ij}
16	*Leucaena leucocephala*	22.5^{gh}	19.5^{fgh}	43.6^{hi}	12.4^{j}
17	*Manihot esculenta*	26.7^{j}	23.2^{j}	48.1^{jk}	16.9^{mn}
18	*Melia azadirach*	25.1^{ij}	21.7^{ij}	50.1^{k}	15.5^{l}
19	*Mimosa invisa*	19.6^{cde}	17.1^{cde}	37.9^{efg}	8.2^{de}
20	*Morinda citrifolia*	24.8^{ij}	21.4^{hij}	56.2^{l}	16.9^{mn}
21	*Myristica fragran*	20.4^{cdefg}	17.9^{cdefg}	31.9^{ab}	8.2^{de}
22	*Paspalum dilatatum*	22.4^{fgh}	19.5^{fgh}	46.6^{ij}	14.0^{k}
23	*Persea americana*	21.5^{efgh}	18.8^{efg}	37.5^{efg}	10.9^{hi}
24	*Pithecelobium jiringa*	21.0^{defgh}	18.3^{defg}	32.7^{bc}	8.0^{de}
25	*Psidium guajava*	18.4^{bc}	16.0^{bc}	35.7^{cdef}	7.6^{d}
26	*Sesbania grandiflora*	25.8^{j}	22.4^{j}	48.3^{jk}	16.1^{lm}
27	*Switenia mahagoni*	18.4^{bc}	16.1^{bc}	31.7^{ab}	6.5^{c}

Different superscripts within the same column showed differences at $P<0.05$

Tabel 2. Mean Square Prediction Error (MSPE) and Root Mean Square Prediction Error (RMSPE) between Observed and Estimated CH_4

CH_4 Model Comparison	MSPE	RMSPE (%)
Observed – Hegarty and Nolan (2007)	115.10	10.73
Observed – Moss *et al.* (2000)	64.14	8.01

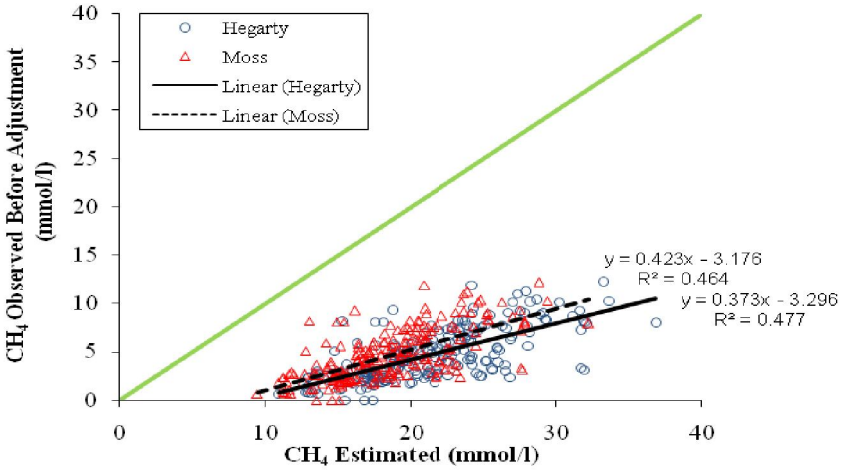

Figure 1. CH_4 Observed before adjustment versus CH_4 estimated

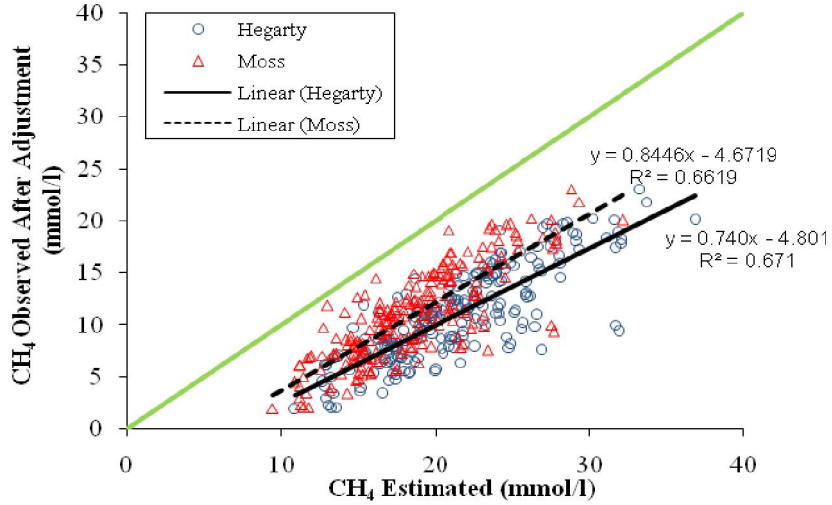

Figure 2. CH_4 Observed after adjustment versus CH_4 estimated

accurate result to explain the variation (low or high) of methane emission. However, there was a substantial bias between CH_4 estimated and CH_4 observed. After considering H_2 ecovery, the bias could be reduced significantly as shown in Figure 2.

Table 2 showed RMSPE values and described how far the estimated model of Hegarty and Nolan (2007) and Moss *et al.* (2000) deviate from the actual values of CH_4 observed in a relative measurement (%). The results of model validation showed that the estimated model of Moss *et al.* (2000) had lower RMSPE value, i.e. 8.01% than the estimated model of Hegarty and Nolan (2007), i.e. 10.73%.

CONCLUSION

Low or high methane emission could be explained quite accurately by volatile fatty acids compositions. However, there was a substantial bias between CH_4 estimated and CH_4 observed. Adjustment by considering hydrogen H_2 recovery decreased the bias significantly. The estimated model of Moss *et al.* (2000) was closer to CH_4 observed than that of Hegarty and Nolan (2007).

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