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

A. Jayanegara, Ikhsan and T. Toharmat
Department of Nutrition and Feed Technology, Faculty of Animal Science,
Bogor Agricultural University, Jl. Agatis Kampus IPB Dramaga Bogor 16680 - Indonesia
Corresponding E-mail: anu_jayanegara@yahoo.com

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ABSTRACT

Rumen microbes breakdown feed to produce volatile fatty acids (VFA), carbon dioxide, ammonia and methane (CH₄). Metabolic hydrogen in the form of reduced protons is used during CH₄ formation as well as during VFA synthesis. Therefore, VFA concentration in the rumen may stoichiometrically be related to CH₄ emission. The aim of this study was to evaluate methane emission between experimental and model estimates. Two stoichiometrical models for predicting CH₄ 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. Adjustment by considering H₂ recovery lowered the bias significantly. It can be concluded that Moss model had better accuracy in predicting CH₄ 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 (CH₄) 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 CH₄ 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 CH4 formation by rumen microbes. Regarding the individual VFA composition and its relationship with CH4 emission, acetate and butyrate promote CH4 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 H2 in the rumen to be used by methanogens. By this relation, CH4 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 CH4 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 CH4 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 CH4 mission from VFA profiles is expected to be a solution to the problem. Although some stoichiometrical relationships between VFA composition and CH4 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.

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 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 CH4. Profile of individual VFA, i.e. acetate, propionate, butyrate, isobutyrate, valerate and iso-valerate 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 CH4 and VFA were ml/l and mmol/l, respectively. In order to enable a direct stoichiometrical relationship between both variables, therefore, the unit of CH4 (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 CH4 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 CH4 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 Hu 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:
CH₄ after adjustment = CH₄ before adjustment × 100/H₂ 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 significance at P<0.05. As outlined by Alemu et al. (2011), prediction error of estimation was calculated by mean square prediction error (MSPE):

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

where:
- \( n \) = number of observations
- \( O_i \) = CH₄ observed
- \( P_i \) = CH₄ 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 CH₄ emissions by estimated model of Hegarty and Nolan (2007), estimated model of Moss et al. (2000), and CH₄ observed after H₂ 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 CH₄ 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; Bhattacharjee 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 CH₄ observed before adjustment is \( Y = 0.423 \times X - 3.176 \) with \( R^2 = 0.465 \) and the estimated model line equation of Hegarty and Nolan (2007) to CH₄ observed before adjustment is \( Y = 0.374 \times X - 3.296 \) with \( R^2 = 0.478 \). While, the estimated model line equation of Moss et al. (2000) to CH₄ after adjustment is \( Y = 0.845 \times X - 4.672 \) with \( R^2 = 0.662 \) and the estimated model line equation of Hegarty and Nolan (2007) to CH₄ observed after adjustment is \( Y = 0.741 \times X - 4.801 \) 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. CH₄ Estimated, H₂ Recovery and CH₄ After Adjustment

| No. | Species                  | CH₄ Estimated (mmol/l) Hegarty | CH₄ Estimated (mmol/l) Moss | H₂ recovery (%) | CH₄ After Adjustment (mmol/l) |
|-----|--------------------------|-------------------------------|----------------------------|-----------------|-------------------------------|
| 1   | *Acacia mangium*         | 16.4<sup>ab</sup>            | 12.2<sup>ab</sup>          | 40.3<sup>gh</sup> | 8.4<sup>def</sup>             |
| 2   | *Acacia villosa*         | 14.6<sup>a</sup>             | 12.7<sup>a</sup>           | 32.6<sup>bc</sup> | 3.2<sup>a</sup>              |
| 3   | *Albizia falcataria*     | 21.9<sup>efgh</sup>          | 19.1<sup>efg</sup>         | 33.2<sup>bcd</sup> | 9.2<sup>efg</sup>            |
| 4   | *Artocarpus heterophyllus* | 22.3<sup>fgh</sup>          | 19.4<sup>fgh</sup>         | 40.4<sup>gh</sup> | 12.0<sup>lij</sup>           |
| 5   | *Calliandra calothyrsus* | 20.0<sup>cdef</sup>          | 17.5<sup>cdef</sup>        | 34.3<sup>bcd</sup> | 8.9<sup>efg</sup>            |
| 6   | *Canna indica*           | 20.7<sup>cdefgh</sup>        | 18.0<sup>cdefg</sup>       | 38.6<sup>gh</sup> | 9.0<sup>efg</sup>            |
| 7   | *Carica papaya*          | 26.4<sup>j</sup>             | 22.9<sup>j</sup>           | 53.7<sup>l</sup>  | 17.9<sup>n</sup>             |
| 8   | *Cidemia hirta*          | 19.9<sup>cde</sup>           | 17.2<sup>cde</sup>         | 36.5<sup>def</sup> | 9.4<sup>fg</sup>             |
| 9   | *Cycas rumphii*          | 19.8<sup>cde</sup>           | 17.3<sup>cde</sup>         | 38.8<sup>fg</sup> | 10.0<sup>gh</sup>            |
| 10  | *Erythrina orientalis*   | 21.1<sup>cdefgh</sup>        | 18.3<sup>cdefg</sup>       | 46.1<sup>ij</sup> | 12.6<sup>j</sup>             |
| 11  | *Eugenia aquea*          | 16.5<sup>ab</sup>            | 14.4<sup>ab</sup>          | 28.9<sup>a</sup>  | 4.7<sup>b</sup>              |
| 12  | *Hibiscus tiliaceous*    | 18.9<sup>cd</sup>            | 16.5<sup>cd</sup>          | 37.9<sup>efg</sup> | 9.5<sup>fg</sup>             |
| 13  | *Ipomoea batatas*        | 26.2<sup>j</sup>             | 22.8<sup>j</sup>           | 45.1<sup>ij</sup> | 15.9<sup>lm</sup>            |
| 14  | *Lantana camara*         | 23.0<sup>ghi</sup>           | 20.0<sup>ghi</sup>         | 45.6<sup>ij</sup> | 14.0<sup>k</sup>             |
| 15  | *Leucaena diversifolia*  | 21.6<sup>efgh</sup>          | 19.0<sup>efg</sup>         | 41.2<sup>gh</sup> | 11.7<sup>ij</sup>            |
| 16  | *Leucaena leucocephala*  | 22.5<sup>gh</sup>            | 19.5<sup>fgh</sup>         | 43.6<sup>ghi</sup> | 12.4<sup>j</sup>             |
| 17  | *Manihot esculenta*      | 26.7<sup>j</sup>             | 23.2<sup>j</sup>           | 48.1<sup>jk</sup> | 16.9<sup>mn</sup>            |
| 18  | *Melia azadirach*        | 25.1<sup>ij</sup>            | 21.7<sup>ij</sup>          | 50.1<sup>k</sup>  | 15.5<sup>l</sup>             |
| 19  | *Mimosa invisa*          | 19.6<sup>cde</sup>           | 17.1<sup>cde</sup>         | 37.9<sup>efg</sup> | 8.2<sup>de</sup>             |
| 20  | *Morinda citrifolia*     | 24.8<sup>ij</sup>            | 21.4<sup>ij</sup>          | 56.2<sup>l</sup>  | 16.9<sup>mn</sup>            |
| 21  | *Myristica fragrans*     | 20.4<sup>cdefg</sup>         | 17.9<sup>cdefg</sup>       | 31.9<sup>ab</sup> | 8.2<sup>de</sup>             |
| 22  | *Paspalum dilatatum*     | 22.4<sup>fgh</sup>           | 19.5<sup>fgh</sup>         | 46.6<sup>ij</sup> | 14.0<sup>k</sup>             |
| 23  | *Persea americana*       | 21.5<sup>efgh</sup>          | 18.8<sup>efg</sup>         | 37.5<sup>efg</sup> | 10.9<sup>hi</sup>            |
| 24  | *Pithecelobium jirginga* | 21.0<sup>cdefgh</sup>        | 18.3<sup>cdefg</sup>       | 32.7<sup>bc</sup> | 8.0<sup>de</sup>             |
| 25  | *Psidium guajava*        | 18.4<sup>bc</sup>            | 16.0<sup>bc</sup>          | 35.2<sup>cdef</sup> | 7.6<sup>d</sup>              |
| 26  | *Sesbania grandiflora*   | 25.8<sup>j</sup>             | 22.4<sup>j</sup>           | 48.3<sup>jk</sup> | 16.1<sup>lm</sup>            |
| 27  | *Swietenia mahagoni*     | 18.4<sup>bc</sup>            | 16.1<sup>bc</sup>          | 31.7<sup>ab</sup> | 6.5<sup>c</sup>              |

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 CH₄

| CH₄ Model Comparison | MSPE  | RMSPE (%) |
|----------------------|-------|-----------|
| Observed – Hegarty and Nolan (2007) | 115.10 | 10.73     |
| Observed – Moss et al. (2000) | 64.14 | 8.01      |
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$_2$ recovery, 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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