Analysis of three methods for the estimation of in vitro CH\textsubscript{4} production from vented bottles.

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Summary

In vitro methane production from three experiments incubating 1.0 gm DM of wheat and Lucerne, different amounts (0, 0.5, 1.0 and 1.5 gm DM) of wheat and lucerne and Lucerne, corn, wheat and barley with different degrees of processing, was calculated using [CH\textsubscript{4} concentration in headspace x headspace volume] + [CH\textsubscript{4} concentration in gas bag x GP] as the gold standard method and compared to the five algorithms proposed by Hannah et al. (2016). The algorithms predicted methane production well. Method 1a had the greatest correlation and Lin’s concordance coefficient with the gold standard and can be recommended for estimating methane production when vented gas is not collected in gas bags.

Introduction

Several strategies have been proposed for reducing enteric methane production (MP), and it is therefore important to quantify MP. One method for measuring total gas production (GP) and MP is through an in vitro fermentation technique using the Ankom\textsuperscript{TM} system. The “gold standard” method for estimating MP using the Ankom\textsuperscript{TM} system requires collection and analysis of the headspace and the vented gas (Hannah et al. 2016). However, Hannah et al. (2016) proposed other options to calculate methane production without the need to collect and analyse the vented gas. These options involve algorithms to estimate methane production based on the volumes of vented gas and the final methane concentration in the headspace of the Ankom\textsuperscript{TM} incubation flask. This study aimed to evaluate the five algorithms proposed by Hannah et al. (2016) with the “gold standard” method for estimating MP by the Ankom\textsuperscript{TM} technique under three incubation scenarios.

Materials and Method

The substrates (1.0 gm DM) in the first experiment comprised wheat and lucerne. The substrates in the second experiment comprised different amounts (0, 0.5, 1.0 and 1.5 gm DM) of wheat and Lucerne. The substrates (1.0 gm DM) in the third experiment comprised Lucerne, corn, wheat and barley with different degrees of processing. Each treatment was tested in eight replicates. In all incubations, substrate was incubated in Ankom\textsuperscript{TM} bottles each containing 75 mls of Kansas State buffer solution and 25 mls of ruminal fluid. Cumulative pressure and the ideal gas law were used to estimate GP over the course of the incubation period. As the gold standard method, MP was calculated as: [CH\textsubscript{4} concentration in headspace x headspace volume] + [CH\textsubscript{4} concentration in gas bag x GP].

Results and Discussion

The five algorithms proposed by Hannah et al. (2016) were compared against the gold standard.

All algorithms predicted MP very well with close concordance with the gold standard method. Method 1a had the greatest correlation (R=0.956) and Lin’s concordance coefficient (CC = 0.952) with the gold standard, although only just. In method 1a, MP is calculated as:
\[ MP = \frac{C_n}{K_n} (U + A), \]

Where; 
\[ K_i = r_i K_{i-1} + s_i, \quad r_i = \frac{V_i}{V_i + u_i}, \]

\[ s_i = \frac{u_i + a_i}{V_i + u_i}, \quad K_0 = 0, \quad C_n = \text{CH}_4 \text{ mixing ratio in headspace}, \]

\[ U = \text{gas volume added to headspace (ml)}, \quad A = \text{volume of CO}_2 \text{ absorbed into ruminal fluid (ml)}, \]

\[ V_i = \text{pressure at the start of venting interval (atm)}, \quad u_i = \text{change in pressure during venting interval (atm)}, \]

\[ a_i = \text{negative change in pressure in blank control during the venting interval (atm)}, \quad n = \text{number of ventings}, \]

\[ i = 1 \ldots n \] is venting number.

**Table 1** Lin’s concordance, correlation coefficient and experimental coefficient of variation of the five algorithms proposed by Hannah et al. (2016) with the gold standard for which %CV=9.0.

<table>
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<th>Algorithm</th>
<th>Concordance</th>
<th>Correlation</th>
<th>%CV</th>
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<tr>
<td>1a</td>
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<td>0.956</td>
<td>10.3</td>
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<tr>
<td>1b</td>
<td>0.945</td>
<td>0.951</td>
<td>10.5</td>
</tr>
<tr>
<td>1c</td>
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<td>2b</td>
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<td>0.950</td>
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</table>

It is concluded that if vented gas is not collected during *in vitro* studies with the Ankom™ system, then total methane production can be accurately estimated by using any algorithm listed in Table 1, with small cost to experimental precision.

**Acknowledgments**

This project was supported by the Climate Change Emissions Management Corporation, Alberta, Canada, the University of Melbourne, Agriculture Victoria and the Primary Industries Climate Challenges Centre.

**References**

