Technical note: In vitro total gas and methane production measurements from closed or vented rumen batch culture systems

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ABSTRACT

This study compared measured gas production (GP) and computed CH4 production values provided by closed or vented bottles connected to gas collection bags. Two forages and 3 concentrates were incubated. Two incubations were conducted, where the 5 feeds were tested in 3 replicates in closed or vented bottles, plus 4 blanks, for a total of 64 bottles. Half of the bottles were not vented, and the others were vented at a fixed pressure (6.8 kPa) and gas was collected into one gas collection bag connected to each bottle. Each bottle (317 mL) was filled with 0.4000 ± 0.0010 g of feed sample and 60 mL of buffered rumen fluid (headspace volume = 257 mL) and incubated at 39.0°C for 24 h. At 24 h, gas samples were collected from the headspace of closed bottles or from headspace and bags of vented bottles and analyzed for CH4 concentration. Volumes of GP at 24 h were corrected for the gas dissolved in the fermentation fluid, according to Henry’s law of gas solubility. Methane concentration (mL/100 mL of GP) was measured and CH4 production (mL/g of incubated DM) was computed using corrected or uncorrected GP values. Data were analyzed for the effect of venting technique (T), feed (F), interaction between venting technique and feed (T × F), and incubation run as a random factor. Closed bottles provided lower uncorrected GP (−18%) compared with vented bottles, especially for concentrates. Correction for dissolved gas reduced but did not remove differences between techniques, and closed bottles (+25 mL of gas/g of incubated DM) had a greater magnitude of variation than did vented bottles (+1 mL of gas/g of incubated DM). Feeds differed in uncorrected and corrected GP, but the ranking was the same for the 2 techniques. The T × F interaction influenced uncorrected GP values, but this effect disappeared after correction. Closed bottles provided uncorrected CH4 concentrations 23% greater than that of vented bottles. Correction reduced but did not remove this difference. Methane concentration was influenced by feed but not by the T × F interaction. Corrected CH4 production was influenced by feed, but not by venting technique or the T × F interaction. Closed bottles provide good measurements of CH4 production but not of GP. Venting of bottles at low pressure permits a reliable evaluation of total GP and CH4 production.

Key words: in vitro gas production, methane, venting technique, rumen fermentation

Technical Note

Several batch culture systems are available to measure gas production (GP) during in vitro rumen fermentation. Many of these systems are equipped with devices for gas venting at fixed interval times (Theodorou et al., 1994) or at a fixed pressure (Cone et al., 1996; Davies et al., 2000; Calabrò et al., 2005; Tagliapietra et al., 2010). Gas venting is recommended to avoid pressure conditions that cause a partial dissolution of CO2 in the fermentation fluid, with a consequent underestimation of total GP (Tagliapietra et al., 2010) and possible disturbance of microbial activity (Theodorou et al., 1994) and thereby CH4 production. Vented GP systems are less frequently used to evaluate the composition of gas produced, because the vented gas must be collected in bags that are leak proof and are not permeable to CO2 or CH4. Closed GP systems are more commonly used for measurements of CH4 production. With a closed system, the gas is not vented and remains in the bottle headspace until the time of collection for analysis (Pell and Schofield, 1993; Getachew et al., 2005; Pellikaan et al., 2011). However, the gas pressure generated in the headspace will cause a partial dissolving of CO2 in the fermentation fluid that can alter the GP composition, so that a correction for solubilized CO2 is required. The difference in CO2 concentration in the headspace and the amount dissolved in the fluid can, in turn, alter the CH4 concentration in the collected gas (Patra and Yu, 2013). The objective of the current study was to
compare, also in term of repeatability, the effects of 2 systems of gas collection, one based on closed fermentation bottles and one based on vented bottles connected to tight plastic bags, on total GP, gas composition and CH$_4$ production computed from GP and gas composition measures.

Two forages (meadow hay and ryegrass hay) and 3 concentrates (corn grain, dry sugar beet pulp, and expeller flaxseed) were incubated. The 5 feeds were selected to cover a large variability in chemical composition and to generate different pressure conditions in the bottle headspace during incubation. All feeds were provided by a dairy farm located in the province of Brescia (Italy). About 1 kg of each feed was ground by a hammer mill with a screen size of 1 mm. From each feed, 15 subsamples were prepared: 12 samples were used for the incubations (6 for each run) and the remaining 3 for chemical analysis. Feeds were analyzed in 3 replicates for proximate composition (AOAC International, 2012). The NDF, inclusive of residual ash, was determined with α-amylase and sodium sulfitre (Mertens, 2002) using the Ankom$^{290}$ Fiber Analyzer (Ankom Technology, Macedon, NY). The ADF, inclusive of residual ash, and sulfuric acid lignin [lignin$_{(sa)}$] contents were sequentially determined according to Robertson and Van Soest (1981). Chemical composition of the 5 feeds is reported in Table 1.

Incubations were conducted following the procedures detailed by Tagliapietra et al. (2012), according to a factorial design where the 5 feeds were simultaneously tested using the 2 techniques, closed or vented bottles, in each of 2 consecutive incubations. In each incubation, each feed was tested with 3 replications (using 3 bottles) plus 2 blanks containing only the buffered rumen fluid, for a total of 32 bottles. Rumen fluid was collected using an esophageal probe (Tagliapietra et al., 2012) from 3 intact, dry Holstein cows ad libitum and 2.5 kg/d of concentrates (0.5 kg of dry sugar beet pulp, 1 kg of corn grain, and 1 kg of sunflower meal) for 3 wk preceding fluid collection. Differences between fluid collected with the probe instead of fluid collected from fistulated cows were not considered relevant for the comparative purposes of this work. The rumen fluid collected from the 3 cows was poured into 2 thermal flasks preheated to 39 ± 0.5°C and immediately transferred to the laboratory. The rumen fluid collected from the 3 cows was mixed, filtered through 3 layers of cheesecloth to eliminate feed particles, and then mixed with the buffer mineral solution in a 1:2 ratio (Menke and Steingass, 1988). All operations were conducted under anaerobic conditions by flushing with CO$_2$, and the time required for all operations was less than 30 min. Each bottle (317 mL) was filled with 0.4000 ± 0.0010 g of feed sample and 60 mL of buffered rumen fluid (Menke and Steingass, 1988), leaving a corresponding headspace volume of 257 mL. Bottles, placed into an incubator at 39.0 ± 0.5°C for 24 h, were not agitated during incubation. All bottles used in this experiment were from the A#RF GP System (Ankom Technology), which was successfully tested for absence of gas pressure variation (70 kPa) over a period of 7 d. For the purpose of the current study, half of the bottles were not vented during incubation, to simulate the closed system technique, and the others were connected with gas-proof Tygon tubing (Saint-Gobain Performance Plastics, Paris, France) to 1-L gas sample bags (standard FlexFoil, SKC Inc., Pennsylvania, PA) and automatically vented by an open-closed valve (model LHLX0502100BC, The Lee Co., Essex, CT) when headspace pressure reached 6.8 kPa. This pressure corresponded to 17 mL for a headspace volume of 257 mL. All gas sample bags were previously vacuum-sealed to eliminate possible traces of air. The closed and vented bottles were equipped with pressure sensors (pressure range: –69 to +3,447 kPa; resolution: 0.27 kPa; accuracy: ±0.01 of measured value) that measured the pressure inside the bottles every 1 min. At the end of incubation (24 h), gas was collected with a 10-mL, gas-tight syringe (Artsana S.p.A., Como, Italy) from the headspace of each closed bottle, and from the headspace and gas-tight plastic bag for each vented bottle. At sampling, the syringe was flushed to ensure the collection of a homogeneous sample, which was immediately injected into a 9-mL Vacuette (Greiner Bio-One

<table>
<thead>
<tr>
<th>Feed</th>
<th>DM</th>
<th>NDF</th>
<th>ADF</th>
<th>Lignin$_{(sa)}$</th>
<th>CP</th>
<th>Ether extract</th>
<th>Ash</th>
<th>NSC$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn grain</td>
<td>900</td>
<td>106</td>
<td>23</td>
<td>—$^3$</td>
<td>93</td>
<td>37</td>
<td>15</td>
<td>749</td>
</tr>
<tr>
<td>Dry sugar beet pulp</td>
<td>935</td>
<td>443</td>
<td>250</td>
<td>29</td>
<td>95</td>
<td>60</td>
<td>51</td>
<td>351</td>
</tr>
<tr>
<td>Flaxseed expeller</td>
<td>923</td>
<td>260</td>
<td>118</td>
<td>40</td>
<td>375</td>
<td>91</td>
<td>59</td>
<td>215</td>
</tr>
<tr>
<td>Meadow hay</td>
<td>893</td>
<td>435</td>
<td>305</td>
<td>60</td>
<td>90</td>
<td>15</td>
<td>86</td>
<td>374</td>
</tr>
<tr>
<td>Ryegrass hay</td>
<td>888</td>
<td>501</td>
<td>347</td>
<td>30</td>
<td>149</td>
<td>15</td>
<td>111</td>
<td>134</td>
</tr>
</tbody>
</table>

$^1$Lignin$_{(sa)}$ = sulfuric acid lignin.

$^2$NSC = nonstructural carbohydrates, computed as (100 – NDF – CP – ether extract – ash).

$^3$Amount not measurable.
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