The Professional Animal Scientist 34:390–396
https://doi.org/10.15232/pas.2018-01745

Technical Note: The use of total gas collection for measuring methane production in vented in vitro systems

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ABSTRACT

In previous studies using Ankom GP vented in vitro systems (Ankom Technology, Macedon, NY), methane production was estimated from gas production (GP) and a single gas sample from the incubation vessel's headspace at the end of the incubation. An accurate method requires measurement of methane in the incubation vessel's headspace and in the gases vented over the incubation period. This research aimed to determine whether the act of collecting the gas vented from the Ankom GP system influenced the estimation by the Ankom GP system of the volume of gas produced or the composition of the gas produced. A method involving collecting gas through a long (304 cm), narrow (1 mm i.d.) gas line estimated greater GP than a control method not involving collection of gas. A method involving collecting gas through a short (22 cm), wide (4 mm i.d.) gas line did not affect headspace methane percentage or estimates of GP. It is concluded that in vitro methane production can be accurately estimated by using the Ankom GP system together with collection of vented gases into gas collection bags, but only if the Ankom GP system is connected to the gas collection bag via a short, wide gas line.

Key words: Ankom gas production system, gas collection technique, ruminant, methane

INTRODUCTION

Methane production (**MP**) from ruminants may be estimated using in vitro methods (Getachew et al., 1998). Previous work using vented Ankom GP in vitro systems (Ankom Technology, Macedon, NY) involved venting gas from the module when the pressure increased above a set limit, taking a gas sample from the module's headspace at the conclusion of the incubation period, and multiplying the methane percentage (**CH_4%**) in that sample by the gas production (**GP**) to estimate MP (Xu et al., 2010; Dubois et al., 2013; Machado et al., 2014). However, simple multiplication of the final headspace CH_4% by the total gas volume vented does not account for the changing proportion of CH_4% in the vented gas during the course of the incubation and can lead to errors when estimating MP (Hannah et al., 2016).

Collecting the vented gas in a gas collection bag would allow sampling of all the gas produced. This approach would appear by definition to be the gold standard method for quantifying MP when using the Ankom GP system. However, when this approach was first implemented, the researchers did not independently measure the volume of gas vented into the gas collection bags, but instead, they used the volume of gas produced as estimated by the pressure recordings of the Ankom GP system (Cattani et al., 2014). The validity of this approach depends on 2 assumptions: (1) that the act of collecting vented gas into gas collection bags does not affect the accuracy of the Ankom GP system for measuring the volume of gas produced during fermentation; and (2) that the act of collecting vented gas into gas collection bags does not affect the fermentation process and influence headspace methane percentage (**HSCH_4%**). Therefore, this research aimed to validate the use of the Ankom GP system together with collection of vented gases in gas collection bags as a means for measuring in vitro MP.

We hypothesized that connecting a gas line to the system's venting tube would (1) affect the estimations by the Ankom GP system of the volume of GP and (2) lead to a change in the HSCH_4% in the incubation vessel.

MATERIALS AND METHODS

In this study, cows were cared for in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (NHMRC, 2013). Animal use was approved by the Animal Ethics Committee of the Department of Economic Development, Jobs, Transport and Resources, Victoria.

The authors declare no conflict of interest.

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Exp. 1

This initial experiment studied the effect of 3 methods for managing the vented gas and 3 different venting thresholds. The experiment was conducted over 2 in vitro runs; in each run, each combination of vented gas method and venting pressure was tested in quadruplicate.

The experiment involved yeast fermentation and aimed to determine whether collection of vented gases in gas collection bags influenced the volume of gas produced as estimated by the Ankom GP system. Yeast (300 mg), sugar (0.5 g), and distilled water (100 mL) were placed in 310-mL incubation bottles, following the standard Ankom validation test procedure in which yeast and sugar react under anaerobic conditions to produce ethanol plus CO_2 (Shiotani and Yamane, 1981; Ankom, 2016). In the normal operation of the Ankom GP system, the Ankom GP module is placed on the top of the incubation bottle, and gas is vented through a silicon exhaust port on the side of the module. To collect vented gases, the module's silicon exhaust port was connected to a gas collection bag via a gas line, a 3-way stopcock (Model MGT110000; Vitalmed, Kingsgrove, NSW, Australia), and a 16-gauge needle (1.194 mm i.d.; Vitalmed). All connections were sealed with high-vacuum silicon grease, and the modules and bottles were purged with CO_2 before being placed in a water bath maintained at 39°C. The gas collection bags had a capacity of 400 mL and were manufactured in house using a 3-layered gas-impervious foil (Code 1 MAC-15UM Nylon/9UM Foil/80UM LLDPE; Wests Packaging Services P/L, Carrum Downs, Vic, Australia). A small quantity of plumber's silicon (Selleys Roof and Gutter Speed Seal Silicone; Selleys, Padstow, NSW, Australia) was placed on 2 corners of each gas collection bag to act as septa, enabling needle access to each gas collection bag (after Moate et al., 2013).

Samples were incubated for 24 h using 3 methods for managing the vented gas: (1) the Ankom standard operating procedure in which vented gas was not collected (**SOP**); (2) a procedure similar to SOP except gas was vented through a short gas line (22 cm) with a wide i.d. (4 mm) and the gas was collected in a gas collection bag (**CSW**); and (3) a procedure similar to SOP except gas was vented through a long gas line (304 cm) with a narrow i.d. (1.0 mm) and the gas was collected in a gas collection bag (**CLN**). Each combination of method for managing the vented gas and treatment was replicated in 8 bottles over 2 in vitro runs.

The gas lines selected were chosen because of their low cost, availability, and practicality for use with the Ankom GP system. The short, wide gas lines were made of polythene, had an internal volume of 2.8 mL (Extension Line Type Heidelberger; Braun, Bella Vista, NSW, Australia), and placed the gas collection bags as close as possible to the incubation vessels. The long, narrow gas lines were made of polythene, had an internal volume of 2.4 mL (CO_2 Sampling Line CoExPE/PVC M/M Luers; Westmed, Tucson, AZ), and were used to place the gas collection bags away from the incubation baths.

Three different venting thresholds (4.13, 5.51, and 6.89 kPa above the starting pressure) were used to test each method of managing vented gas. When the pressure in the vessel headspace reached the threshold pressure, an internal valve opened for 1 s to release the gas produced. The Ankom GP system has been used in previous studies with the venting threshold pressure set at 6.89 kPa (Russo et al., 2017); the other pressures, 5.51 and 4.13 kPa, were chosen because they represent a gradual decrease from 6.89 kPa. Venting threshold pressures below 4.13 kPa were not used because the identification of each venting becomes difficult.

Cumulative pressure measured by the Ankom GP modules was converted to units of volume using the ideal gas law and Avogadro's law to calculate GP for each module (Ankom, 2016).

$$PV = nRT, \quad [1]$$

where P = pressure in psi (1 psi = 6.8948 kPa), V = gas volume (mL), n = quantity of gas in moles, R = gas constant (8.1345 L kPa/K·mol), and T = temperature in Kelvin. Using Avogadro's law where 1 mol of gas will occupy 25.6 L at 39°C (312 Kelvin) with pressure converted to kilopascals (1 psi = 6.8948 kPa), gas measured in moles can be converted to gas measured in milliliters as follows:

$$\text{GP} = V_j \times P \times 6.8948/8.3145 \times 312 \times 25.6. \quad [2]$$

Thus, shortening Equation 2 gives

$$\text{GP} = V_j \times P \times 0.06641, \quad [3]$$

where GP = gas production (mL), V_j = headspace volume (mL), and P = cumulative pressure recorded by Ankom gas monitor system software.

Exp. 2

A second experiment involved the fermentation of 2 feeds with ruminal fluid and was principally concerned with determining whether collection of vented gases in gas collection bags influenced the CH_4% present in the headspace of the Ankom GP system at the end of a 24-h incubation. Each combination of substrate and method (SOP or CSW) was replicated 4 times in each of the 2 in vitro runs.

Ruminal fluid was collected from 2 nonlactating, rumen-cannulated (www.rumencannula.com), Holstein-Friesian cows that grazed perennial ryegrass (*Lolium perenne* L.) dominant pasture. Collection occurred 1 h before morning feeding. The ruminal fluid was collected from multiple sites in the rumen using a copper pipe and a 100-mL syringe. One liter of ruminal fluid was collected from each cow, and ruminal fluids from both cows were mixed in 2-L