



## Uncertainty assessment of the breath methane concentration method to determine methane production of dairy cows

Liansun Wu,<sup>\*1</sup> Peter W. G. Groot Koerkamp,<sup>\*†</sup> and Nico Ogink<sup>\*†</sup>

<sup>\*</sup>Farm Technology Group, Wageningen University, PO Box 16, 6700 AA, Wageningen, the Netherlands

<sup>†</sup>Wageningen Livestock Research, PO Box 135, 6700 AC, Wageningen, the Netherlands

### ABSTRACT

The breath methane concentration method uses the methane concentrations in the cow's breath during feed bin visits as a proxy for the methane production rate. The objective of this study was to assess the uncertainty of a breath methane concentration method in a feeder and its capability to measure and rank cows' methane production. A range of controlled methane fluxes from a so-called artificial reference cow were dosed in a feed bin, and its exhaled air was sampled by a tube inside the feeder and analyzed. The artificial reference cow simulates the lungs, respiratory tract, and rumen of a cow and releases a variable methane flux to generate a concentration pattern in the exhaled breath that closely resembles a real cow's pattern. The strength of the relation between the controlled methane release rates of the artificial reference cow and the measured methane concentrations was analyzed by linear regression, using the coefficient of determination ( $R^2$ ) and the residual standard error as performance indicators. The effect of error sources (source-sampling distance, air turbulence, and cow's head movement) on this relation was experimentally investigated, both under laboratory and barn conditions. From the laboratory to the dairy barn at the 30-cm sampling distance, the  $R^2$ -value decreased from 0.97 to 0.37 and the residual standard error increased from 75 to 86 ppm as a result of barn air turbulence, the latter increasing to a theoretical 94 ppm if modeled variability due to cow's head movement was accounted for as well. In practice, the effect of these random errors can be compensated by sampling strategies including repeated measurements on each cow over time, thus increasing the distinctive power between cows. However, systematic errors that may disturb the relation between concentration and production rate, such as cow variation in air exhalation rate and air flow patterns around sampling locations that differ between barns, cannot be compensated by repeated measurements. As a re-

sult, the methane concentrations of breath air will vary between cows with the same methane production. We conclude that the capability of the breath concentration measurement method to adequately measure and rank methane production rates among cows is highly uncertain and requires further investigation into variation sources with a systematic nature.

**Key words:** methane, dairy cow, breath measurement, measurement error

### INTRODUCTION

Enteric methane produced by dairy cows has become a global concern because these emissions account for 4% ( $\pm 26\%$ ) of anthropogenic greenhouse gas emissions (Gerber et al., 2013). Enteric methane from dairy cows can be mitigated through nutritional manipulations or breeding animals with lower methane production (Cottle et al., 2011). To assess the effects of these mitigation strategies, breath methane concentration (BMC) or so-called sniffer methods have been developed to assess the methane production of dairy cows at commercial farms. These methods use a gas sampling tube from the front of a cow's head to a gas analyzer to continuously analyze methane concentrations in the cow's breath when they are milked in a milking robot or visit a feed station (Garnsworthy et al., 2012; Lassen et al., 2012; Bell et al., 2014). Measured BMC is then processed to determine the cow's daily methane production rate. However, it can be questioned if a cow's actual methane production, a flux calculated as the product of concentration and transporting air volume, can be adequately represented by concentration only. Although Garnsworthy et al. (2012) found a good relation between methane production rates measured by the BMC method and in respiration chambers, Huhtanen et al. (2015) observed a weak relation between methane concentration measured by the BMC method and methane flux measured by the GreenFeed. The strength of the relation between measured BMC and the actual methane production depends on 2 different aspects.

The first aspect is animal related and deals with the relation between methane production rate and BMC

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<sup>1</sup>Corresponding author: [liansun.wu@qq.com](mailto:liansun.wu@qq.com)

at the point where it leaves the animal's mouth/nose area (further called exhalation point). If the associated air volume rate that transports the methane out of the animal is constant among cows, then variability in methane production will be directly reflected by the variability of methane concentration at the animal's exhalation point. Variability in air exhalation rates of individual cows, however, can be expected to affect the methane concentration at the exhalation point. The cow's inhalation and exhalation air mixes with methane emitted in the lungs and with the methane eructating from the rumen in the cow's respiratory tract, and the air exhalation volume thus is an important factor in transporting and emitting the methane. A higher air exhalation rate therefore will lead to stronger dilutions of the methane flux from the rumen, causing lower BMC at the exhalation point.

The second aspect is to which extent the animal's breath after exhalation is diluted with barn air at the sampling point. A high dilution variability weakens the relation between measured concentration at the sampling point and the true methane concentration at its exhalation point. The dilution of emitted methane from the exhalation point can be affected by several factors: the airflow pattern around the cow, the distance between exhalation and sampling point, and the cow's head movement in the feeder. Existing variable airflow in dairy barns (Joo et al., 2015; Wu et al., 2016) mixes with and dilutes the cow's emitted methane in breath. Huhtanen et al. (2015) observed a significant decrease and variation of measured concentrations by the BMC method in the laboratory with a model cow head when sampling distance changed from 0 to 30 cm, moving the head, or introducing wind through the feed bin. Therefore, the sampling distances influence the dilution rate and cause variations in measured BMC.

Consequently, the measured BMC is a vulnerable index in determining methane production. The effects of (turbulent) aerial flow conditions, positioning of sampling point, real cow's head movement, and especially the air exhalation rate on measured BMC, still require further research. Therefore, the objective of this study was to (1) assess the uncertainty of a BMC method under laboratory and barn conditions, and (2) its capability to measure and rank cows' methane production.

## MATERIALS AND METHODS

### *Assessment of the Breath Methane Concentration Measurement in the Laboratory*

To assess the performance of the breath methane measurement method, we first tested the method with a so-called artificial reference cow (**ARC**) in the air

quality laboratory of Wageningen Livestock Research. The ARC is a device developed to release a variable methane flux and concentration pattern in time from a nose piece. The changes in methane flux and concentration pattern simulate methane release from lungs and eructation. The ARC precisely controls the released methane flux and concentrations at preset values to closely resemble a real cow's pattern. The ARC is made of a cylinder in which a piston inhales and exhales air with a controlled tidal volume, piston frequency, and temperature, and in which a mass flow controller doses methane from a gas cylinder. The mixed air is transported by the piston movement through a tube to a nose piece. A detailed description of this system and tests of its accuracy is given by Wu et al. (2015). The ARC's nose was placed into a model feed bin with the same dimensions and shape as the actual bins that cows use in a milking robot (Figure 1). The feed bin was made of cardboard (60.5 cm × 46.0 cm × 29.0 cm) and was partially enclosed. The inlet of the sampling tube was positioned 5 and 30 cm away from the ARC's nose. Air from the inlet point in the bin was continuously sampled at a rate of 4 L/min and analyzed by a Fourier transform infrared spectroscopy gas analyzer (GAS-MET DX-4000, Gasmeter Technologies Oy, Helsinki, Finland; calibrated according to factory instructions in the range of 0–3,000 ppm) giving one gas concentration value per 2 or 3 s. In addition, a 3-dimensional anemometer (WindMaster, Gill Instruments, UK) was placed next to the feed bin to measure air velocity outside the bin every second during the experiment.

At the start of the experiment, the ARC was run without injecting methane until the breath air had warmed up to the desired temperature of about 25°C. At the 5-cm sampling distance (Figure 1), the ARC simulated 5 different cows with controlled methane release rates from 200 to 400 g/d, with increments of 50 g/d. Each flux level was sampled during 3.6 min, denoted as one measurement. The measurement for each simulated cow was repeated 5 times in randomized order. The tidal volume and breath frequency of each simulated cow were controlled at 4.4 L and 30 times per minute.

At the 30-cm sampling distance, which was assumed to be the standard distance used in the feed bin of a milking robot, the ARC simulated 11 different cows with controlled methane release rates from 200 to 400 g/d with increments of 20 g/d. The tidal volume and breath frequency of each simulated cow were controlled at 4.4 L and 30 times per minute. Each simulated flux was released during 5 min, a time interval that a cow is assumed to spend in the milking robot, and this interval was denoted as one measurement. The measurement for each simulated cow was repeated 4 times. In addition,

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