# Assessment of the relation between methane concentrations and the methane flux of an artificial reference cow

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## ABSTRACT

Methane produced by dairy cows accounts for about 15% of the global methane emission budget. To mitigate methane emission from dairy cows, one of the primary issues is to evaluate the efficacy of measures to reduce methane emission, like breeding and feeding strategies. Therefore, a technique needs to be developed to quantify the actual methane emission from individual cows under commercial farming conditions. A non-invasive on-farm methane measurement for individual cows was developed recently. The method includes the placement of an air sampling tube in the feed bin of the milking robot, where cows eat concentrates in during milking. The methane concentration is continuously analysed in the sampled breath air. The objective of this study was to validate whether variation of methane production rates between cows and within time can be assessed accurately with only methane concentration results from feed bins. We used an artificial reference cow with exactly known methane emission rates, which simulated methane concentration patterns and methane production rates similar to values measured from real cows in practice. Daily methane production rates of five cows were simulated by the artificial cow at 200, 250, 300, 350 and 400 g/day, respectively. The nose of the artificial cow was fixed into a mimic feed bin with the same dimensions as used in the milking robot. Breath air from the artificial cow was continuously sampled with a sampling point that was installed 5 cm away from the nose. Methane concentrations were measured with a Fourier transform infrared spectroscopy gas analyser. The experiment was carried out under controlled conditions in an air quality laboratory. For each simulation cow the measurement was operated for about 4 minutes and repeated 5 times. The results showed that the average methane concentration measured in the breath air varied from 594 ppm to 1135 ppm. The average dilution rate was 17.1 (s.d. 1.7) % under different imposed daily methane production at the sampling point. Imposed daily methane production rate had a strong positive relation with measured mean, peak and valley methane concentration during eructations in the feeder. It is concluded that methane concentration results analysed from breath methane measurement can present the variation of daily methane production between cows under steady laboratory condition.

**Keywords:** Breath methane measurement, artificial cow, validation

## 1. INTRODUCTION

Methane produced by dairy cows accounts for about 15% of the global methane emission budget. On average, a dairy cow produces 250 to 400 gram methane per day (Bannink et al., 2011), which is mainly released through the mouth and nose by eructation (Murray et al., 1999; Lassey, 2007; Sejian et al., 2010). The release of methane by dairy cows also represents 2-12% loss of the gross energy intake (Blaxter et al., 1972; Johnson and Johnson, 1995). In short, mitigating methane emission from dairy cows will benefit not only the environment but also the energy balance of the cows.

Several strategies have been developed to mitigate methane emission from dairy cows (Boadi et al., 2004). To evaluate the efficacy of these strategies, the respiration chamber method (Blaxter et al., 1972; Hellwing et al., 2012) is currently used as the reference method to measure methane emission rates from individual or pairs of cows. The sulphur-hexafluoride tracer method (Johnson et al., 1994; Grainger et al., 2007) is widely used to measure methane emission from individual cows in pasture. Both methods, however, have drawbacks that limit acquisition of large representative data-sets and applicability under commercial operation; they limit the normal behaviour of cows, give short time period results, or are invasive, costly or labour intensive.

In contrast, a non-invasive on-farm methane measurement for individual cows was developed recently (Garnsworthy et al., 2012; Lassen et al., 2012). The method includes the placement of a tube with sampling point in the feed bin, where the cow is eating concentrates in the milking robot during milking. Methane concentration is continuously analysed in the sampled breath air. Then the measured average methane concentration from each cow is used to determine the methane production levels. Methane concentrations measured at the sampling point, however, depend on how and to what extent the breath air of cows is diluted and transferred to the sampling point, which will be affected by the airflow patterns in the environment.

The objective of this study was to validate whether variation of methane production rates between cows can be assessed accurately with only methane concentration measurements from feed bins. An artificial reference cow that can simulate different methane production rates was used to validate this method in the lab. The relation between measured methane concentrations during patterns of eructation and methane production rates was analysed.

## 2. MATERIALS AND METHODS

### 2.1 The artificial reference cow

The artificial reference cow was designed and constructed by Wu et al., (2014). The artificial reference cow can precisely control and simulate methane concentration and production as real cows. Therefore, different daily methane production rates, representing different cows, can be simulated and used to validate the breath methane concentration measurement method.

### 2.2 Experiment setup

The measurement set-up is shown in figure 1 and the experiment was conducted in the air quality laboratory of Wageningen Livestock Research. The nose of the artificial cow was put into a mimic feed bin with the same dimensions and shapes as the real cow used in the milking robot. The mimic feed bin made of cardboard was 60.5 × 46.0 × 29.0 cm (length, width, height) with partially enclosed. The inlet of the sampling tube was positioned 5 cm away from the nose. The artificial cow’s breath was continually sampled and analysed by a fourier transform infrared spectroscopy (FTIR) analyser one value per two or three seconds.

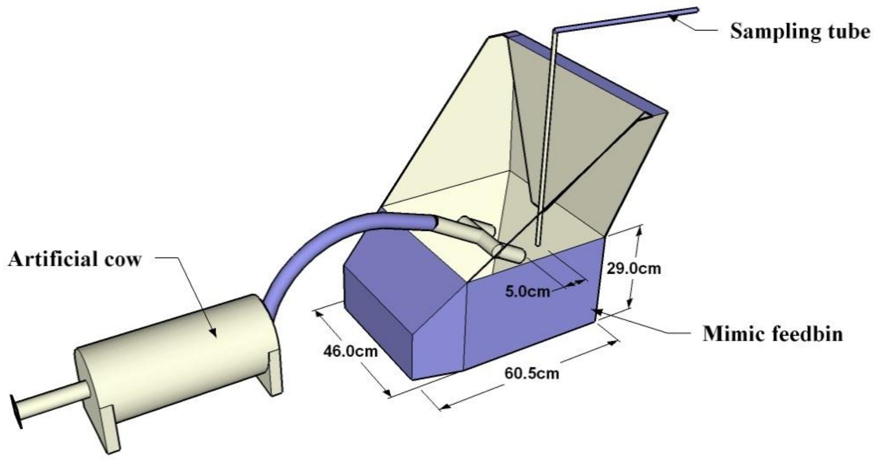


Figure 1. Schematic overview of the experimental set-up to assess breath methane concentration measurements with the artificial cow in the lab.

The artificial reference cow simulated five cows with different daily methane production. Daily methane production rates of these five simulated cows were controlled at 200, 250, 300, 350 and 400 g/day, respectively. Combined with these different overall production rates, also eructation patterns were simulated, which were based on measurements of methane concentrations in breath air of real cows. Each eructation lasted 36 s and by means of different methane injection rates a sinusoidal curve of methane concentration was produced.

At the beginning, the artificial reference cow was running without injecting methane until the breath air was warmed to the desired temperature (about 30 °C). Then, each simulated cow was performed for 6 eructations of 36 s, being 216 s in total, and denoted as one measurement. There was at least a 10 minutes interval between two measurements to ensure methane emitted into the feed bin from the previous measurement was completely cleaned. Five measurements were performed for each simulated cow.

Data was analysed using Genstat software (version16). The relation between daily methane production rates and measured methane concentration (peak, mean and valley values) was investigated with a linear regression model.

A physical model of the artificial cow was used to estimate the methane concentration in the breath air of the cow at the outlet of the nose. The mean difference between this concentration and the concentration measured at the sampling point was calculated for each 216 s measurement. Then the dilution rate at 5 cm away from the sampling point can be analysed.

## 3. RESULTS AND DISCUSSIONS

### 3.1 Measured methane concentration pattern

The five simulated cows produced similar methane concentration patterns under the laboratory conditions (Figure 2): methane concentration fluctuated during the measurement period. It took about 25 s to have the first methane peak. Then the next four fluctuations from different simulation cow had the same time interval (about 36 s), which corresponded to the eructation as the real cows. When the artificial reference cow stopped (216 s), it took about 50 s that the methane concentration decreased to the environmental level.

Both peak and valley methane concentration increased from cow-A to cow-E, when the imposed daily methane production increased from 200 g/day to 400 g/day. Methane concentration during the first eructation was lower than during the following ones. When the breath air introduced into the environment, the sampling point needed time to reach at a stable dilution and mixing. Also the gas analyser (FTIR) needed time to response from low methane concentration to high methane concentration in the breath air. The results measured at the beginning, therefore, should be carefully analysed when applying this method in the field. Certain variations were observed on peak and valley methane concentration in the next four methane fluctuations, especially in cow-D. Theses variations could be caused by the operation of the artificial reference cow, or dilution rates of breath air in the environment.

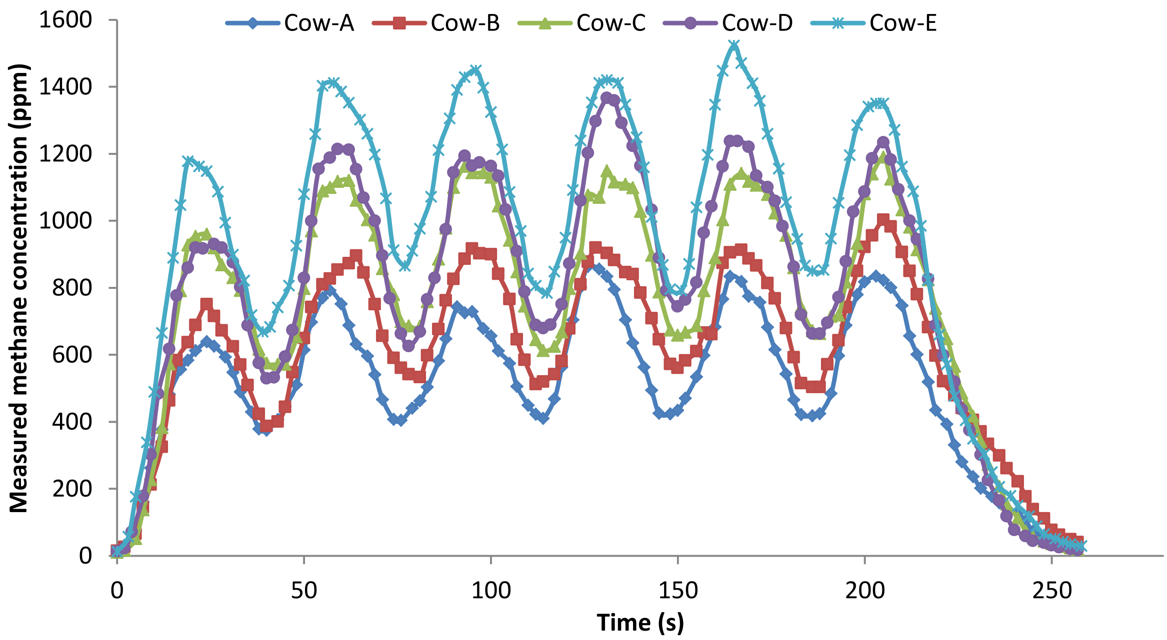


Figure 2. Typical example of the measured methane concentration (ppm) during one measurement period (216 s) for five simulated cows. The six eructations for each simulated cow are visible as sinusoidal patterns. Methane production rates of cow A to E: 200, 250, 300, 350 and 400 g/day.

### 3.2 Measured mean, peak and valley methane concentration versus methane production rates

Measured mean, peak and valley methane concentration were calculated excluding the first eructation in the measurement results, because of the relative low methane concentration level at the first fluctuation. The results in figure 3 show that measured mean, peak and valley methane concentration were strongly linearly related to the imposed daily methane production rates ($R^2 \geqslant 0.90$, $P < 0.001$). The cows with high methane production, the measured methane concentration at the sampling point was also higher. The mean methane concentration had lower measurement variations than peak and valley results. Standard errors of measured mean, valley and peak methane concentration were estimated to be 34.3 ppm, 47.2 ppm and 71.9 ppm, respectively.

The breath methane measurement can present the variation of daily methane production between cows under steady laboratory condition. With higher coefficient of determination and lower estimated standard error, mean methane concentration is a better indication index than peak or valley results.

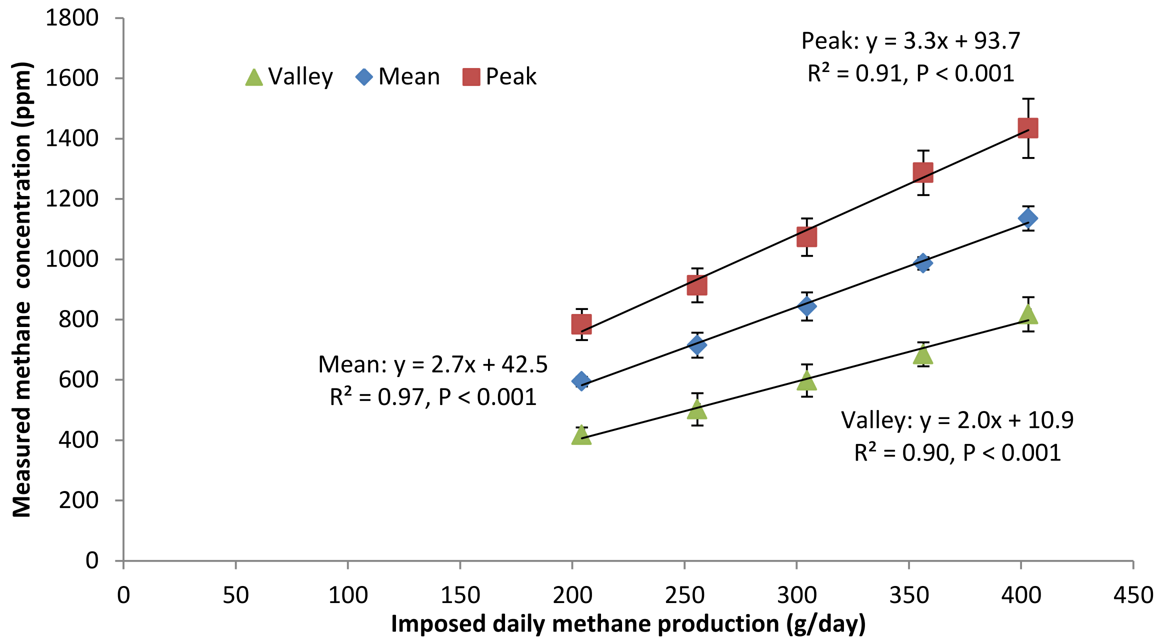


Figure 3. Imposed daily methane production rate (g/day) versus measured mean, peak and valley methane concentration (ppm).

### 3.3 Measured methane concentration versus predicted results

The sampling point during the experiment was 5 cm away from the artificial cow's nose. To analyse the dilution effect of the breath air in this short distance, methane concentration emitted by the artificial cow was modelled with a physical model in each 216 s measurement. The results in table 1 show that measured methane concentration at 5 cm away already were 100~228 ppm lower for different imposed daily methane production rates, compared to the estimated methane concentration produced by the artificial cow. Modelled methane concentration emitted from the cow had smaller standard deviation compared to the measured results. Because physical model only include the variations caused by the mass flow controller between the testing, the measured variations could also be caused by other factors of the artificial cow (e.g. the movement of piston). The mean dilution rate is 17.1% (s.d. 1.7). Therefore, the dilution rate under different imposed daily methane production rates at the same sampling point is quite stable under laboratory conditions.

Table 2. Measured methane concentration at 5 cm away from the nose, predicted methane concentration at the outlet of the nose of an artificial reference cow, and dilution rates under different imposed daily methane production rates

| Imposed daily methane production rate (g/day) | Methane conc. Measured at 5 cm from the nose (ppm) | Methane conc. Predicted at outlet of the nose (ppm) | Dilution rate[b] (%) |
|---|---|---|---|
| | Mean (SD[a]) | Mean (SD[a]) | |
| 200 | 594.1 (15.7) | 695.4 (1.7) | 14.6 |
| 250 | 714.9 (41.6) | 859.5 (2.4) | 16.8 |
| 300 | 843.3 (47.0) | 1036.9 (0.7) | 18.7 |
| 350 | 986.3 (20.3) | 1214.2 (1.6) | 18.8 |
| 400 | 1135.3 (40.7) | 1363.0 (2.0) | 16.7 |

[a]SD: Standard deviation; [b]Dilution rate: 100% × (Predicted – Measured)/Predicted;

The distance between the artificial cow's nose and the sampling point was fixed in the experiment. In reality the cow's head, however, is not fixed in one position when milked in the milking robot. The variable distance between the sampling point and the cow's head is expected to have an effect on the dilution rate and consequently will introduce variations into the measured methane concentration results.

## 4. CONCLUSIONS

It is concluded that results from breath methane concentration measurements can present the variation of daily methane production rates between cows under steady laboratory conditions.

More validation works with the artificial reference cow should be done under more disturbed and varied circumstances with respect to airflow patterns and air velocity. The effect of the cow's head movement on results should also be further investigated.

## 5. ACKNOWLEDGEMENTS

This study was funded by Wageningen UR Livestock Research, the Ministry of Economic Affairs (through KB Sustainable Agriculture) and the China Scholarship Council.

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