Effect of simulated spot-sampling duration and timing on the precision of methane estimation from cattle using respiration chambers

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Abstract

The objective of the current study was to determine the precision of CH_4 emission estimates from simulated spot-samples of variable time length (0.25 to 3.0 h) and over time compared to 24-h measured CH_4 emissions, both based on respiration chamber data of the same day within cattle. Respiration chamber methane data, recorded approximately every 3 min, from two experiments were used, each with 12 growing beef cattle measured over two or three consecutive days. The ~3 min CH_4 emissions during 24 h per animal per day were averaged in time intervals (bins; i.e. simulated spot-sample duration) of 0.25, 0.5, 1.0 and 3.0 h (expressed as g/d) from morning and from afternoon feeding. Precision of each time bin was evaluated by determining between-animal coefficient of variation (CV) and Pearson correlation (r) with 24-h measured emissions. In general, the average CV increased and the CV became more variable over time when the bin duration decreased from 3 h to 0.25 h. The correlation was low (r of approximately 0.1 to 0.5) in the first 0.5 to 2 h after the start of a feeding, after which the correlations increased (r of approximately 0.5 to 0.9) and remained relatively constant up to about 8 h after feeding. The overall trends observed suggest that the precision of a CH_4 measurement improved with increasing simulated spot-sample (bin) duration from 0.25 to 3 h and the best time to take a spot-sample (based on any of the bin durations) would be between approximately 1 to 6-8 h after feeding. However, the best timing to perform spot-sampling was not fully consistent between the experiments and between emissions after morning and afternoon feeding, which warrants further investigation.

Keywords: Spot-sampling; enteric methane; precision; time bin; variation

Introduction

Several methods to estimate methane (CH₄) emitted by cattle based on short-term sampling have been developed in the last decade (Hammond et al. 2016). These methods estimate daily CH₄ emissions by averaging CH₄ emissions from multiple breath spot-samples (two to hundreds per animal) lasting from 3 to 60 minutes, collected over multiple days. These methods have some advantages over the 'gold standard' method, i.e., respiration chambers for measuring CH₄ emissions from ruminants, being that they are generally cheaper, less laborious, have a higher throughput and can be used on-farm (Garnsworthy et al., 2019). However, enteric emissions by cattle during the day are not constant and can vary two to six fold (Biswas & Jonker, 2019; Jonker et al. 2014). Therefore, timing and duration of a spot-sample might affect precision of the CH, emission estimate from cattle. Several studies compared emissions from cattle estimated using spotsampling methods with those of respiration chambers, but measurements cannot be performed simultaneously (e.g., Difford et al. 2018; Jonker et al. 2016). Measurements have, therefore, been performed sequentially with different methods on different days. The actual emissions on those specific days might be quite different, which complicates comparison of methods. Furthermore, a 24-h CH₄ emission estimate (g/d) by multiple spot-samples can be based on as little as 60 min of measurement time per animal. The objective of the current study was to determine the precision of CH₄ emission estimates from simulated spot-samples of variable time length (0.25 to 3.0 h) and over time compared

to 24-h measured CH_4 emissions, both based on respiration chamber data of the same day within cattle.

Materials and methods

Data of two respiration chamber experiments with growing beef cattle (Hereford × Holstein-Friesian; aged 1.3 and 1.8 years with an average body weight of 327 and 442 kg, respectively) fed cut pasture (twice daily) were used. The two experiments were selected from 15 experiments carried out at AgResearch (Palmerston North) on the basis of having a very small or vary large diurnal variation in emissions (2.5 and 4.4 fold range; Biswas & Jonker, 2019). Both experiments were carried out with 12 animals to measure methane emission over two or three days.

The measurements were performed in four opencircuit respiration chambers (volume of 15.4 m³) which are linked to one CH₄ analyser via a switching unit that directs the air stream of each chamber to the gas analyser in sequence, which takes approximately three minutes per cycle. Air flow rate through each chamber was 1.8 m³/ min. Doors of chambers were opened twice daily (approx. 20 min) for feed refusal collection, feeding and cleaning, between approximately 0800 and 0900 h in the morning and between 1500 and 1600 h in the afternoon. There was no data recording during these events.

Data processing and analysis

Each respiration chamber recorded data at approximately 3-min intervals. Each \sim 3-min emissions measurement was expressed as g/d calculated as: CH₄

 $(g/d) = [CH_4 \text{ per } \sim 3 \text{ min } (g) / \text{ time } (min) \times 60 \times 24].$ Then all emission measurements within 24 h per animal were averaged to get one daily CH_4 value (g/d). In addition, the ~3 min CH₄ emissions during 24-h per animal per day were averaged at time intervals (bins, i.e. simulated spot-sample duration) of 0.25 (15 min), 0.5 (30 min), 1.0 and 3.0-h from the start of a CH₄ recording period, which started immediately when closing the respiration chamber doors after the morning and the afternoon feeding (separately). Only data of up to 6-h after morning feeding and up to 15-h after afternoon feeding were included to generate time bins. Then the coefficient of variation (CV) was calculated for each time bin (0.25, 0.5, 1 and 3-h) over time within animal measurement day. In addition, Pearson correlations (precision of a relationship) of each time bin over time with 24-h measured CH₄ was generated using the "cor.test" function in R version 3.6.1 (R Core Team, 2019) and 95% confidence intervals extracted for the correlations between total and periodic methane emissions.

Results

Methane production over 24 h averaged 107 and 148 g/d in Exp. 1 and 2, respectively, and the betweenanimal CV was 7.9% and 7.3%, respectively. The 24-h CH₄ production was previously reported to be (mean \pm CV) 106 \pm 6.6 and 147 \pm 7.5 g/d for Exp. 1 and 2, respectively (Jonker et al. 2017), which includes interpolation of data when the chamber doors were open. This is very similar

Table 1 Average and range of between-animal coefficient of variation (CV) and Pearson correlation of each time bin over time (after morning and after afternoon feeding) with 24-h measured emissions in two experiments with growing beef cattle fed ryegrass-based pasture in respiration chambers

| | Exp. 1 | | Exp. 2 | |
|---------------|-----------------|---------------------------|----------------|-------------|
| | AM | PM | AM | PM |
| CV, average | (min-max) | | | |
| 24-h | 7.9 | | 7.3 | |
| 3-h bins | 13.3 | 9.2 | 10.2 | 10.8 |
| | (12.8-13.8) | (8.5-10.3) | (9.8-10.7) | (7.5-13.6) |
| 1-h bins | 15.0 | 10.1 | 11.2 | 12.2 |
| | (10.7-19.1) | (7.6-12.8) | (10.5-12.6) | (7.7-17.9) |
| 0.5-h bins | 15.5 | 11.1 | 11.8 | 13.1 |
| | (9.9-19.8) | (8.4-13.7) | (9.9-13.7) | (7.1-19.4) |
| 0.25-h bins | 16.7 | 12.5 | 12.5 | 14.4 |
| | (11.6-21.7) | (9.2-18.1) | (10.0 – 16.4) | (7.1-22.8) |
| Correlation (| (r) of time bin | with 24 h CH ₄ | (lower – upper | 95% |
| confidence in | nterval) | | | |
| 3-h bins | 0.78 | 0.78 | 0.67 | 0.75 |
| | (0.60-0.95) | (0.61-0.95) | (0.45-0.89) | (0.57-0.93) |
| 1-h bins | 0.68 | 0.71 | 0.61 | 0.68 |
| | (0.45-0.91) | (0.50-0.92) | (0.36-0.86) | (0.47-0.89) |
| 0.5-h bins | 0.65 | 0.63 | 0.58 | 0.62 |
| | (0.41-0.89) | (0.38-0.88) | (0.33-0.83) | (0.38-0.86) |
| 0.25-h bins | 0.61 | 0.57 | 0.56 | 0.55 |
| | (0.35-0.87) | (0.29-0.85) | (0.30-0.82) | (0.28-0.82) |

to the values in the current analysis where 24-h emissions were defined by averaging all \sim 3-min measurements during the day (each first converted to g/d).

In general, CV increased (on average 9.2-13.3 to 12.5-16.7; Table 1) and became more variable over time (range, 7.5-13.8 to 7.1-22.8; Fig. 1) when the binning duration decreased from 3-h bins to 0.25-h bins, which was more pronounced after the morning feeding in Exp.1 and after the afternoon feeding in Exp. 2 (Table 1). The CV was relatively constant over time after afternoon feeding in Exp. 1 and after morning feeding in Exp. 2, while in Exp. 1 the CV increased between about 1.5 and 4.5 h after morning feeding and in Exp. 2 the CV steadily increased from about 5 h after the afternoon feeding (Fig. 1).

Pearson correlation of a CH₄ time bin with 24-h measured CH₄ decreased (on average 0.67-0.78 to 0.55-0.61) and became more variable (range, 0.62-0.81 to 0.55-0.61; Fig. 2) when the binning duration decreased from 3-h bins to 0.25-h bins (Table 1). In general, the correlation was low in the first 0.5 to 2-h after the start of a feeding (r of approximately 0.1 to 0.5), after which the correlations increased to a plateau (r of approximately 0.5 to 0.9), which remained relatively constant after the morning feeding of both experiments. However, the correlation apparently decreased after about 8 h after feeding in the afternoon of Exp. 1. In Exp. 2, the correlation increased and decreased three times from 2 to 14 h after the afternoon feeding (Fig. 2). In the latter case, 3-h bins resulted in more-constant correlations over time compared to the 0.25-1.0-h bins.

Discussion

The main findings of the current analysis were that the precision (both CV and correlation) of a CH. measurement improved with increasing simulated spot-sampling duration (time bin) from 0.25 to 3 h and the best time to take a spot-sample would be between approximately 1 to 6-8 h after feeding. However, the best timing to perform spot-sampling was not fully consistent between the experiments and between emissions after morning and afternoon feeding. The two experiments (selected from 15 experiments; Biswas & Jonker, 2019) used for the current analysis were selected on the basis of having a very small or large diurnal variation in emissions (2.5 and 4.4 fold range for Exp. 1 and 2, respectively). Our thought was that this might affect the precision of a spot-sampling method at different times in the day, but it did not appear that there was a clear difference in precision for estimating CH. emissions between Exp. 1 and 2.

Unlike in the current study, Robinson et al. (2019) observed relatively little variation in CV for 40-min CH_4 spot samples over time (0930 to 1400-h; new feed offered at 0800 h) for sheep immediately off feed (CV of 22 to 27%) or taken off feed at one hour before the CH_4 measurement (CV of 23 to 28%). Gunter and Bradford (2015) also found

Figure 1 Between-animal coefficient of variation (CV) of diurnal methane emissions averaged in 0.25-, 0.5-, 1.0- and 3.0-h time bins, which started immediately after closing the respiration chamber doors after the morning (at approximately 0800 to 0900 h) and the afternoon (at approximately 1500 to 1600 h) feeding, in two experiments with growing beef cattle fed ryegrass-based pasture in respiration chambers. The symbols indicate the mid-point of each time bin.

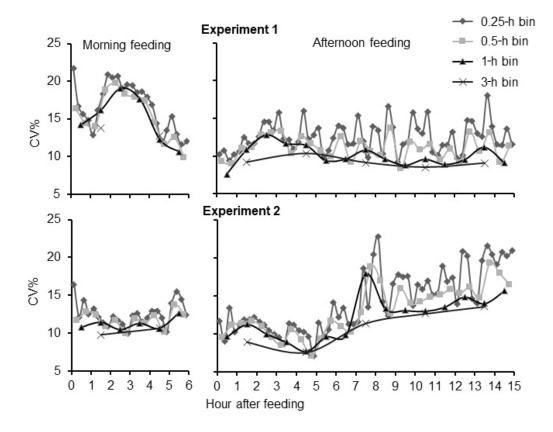
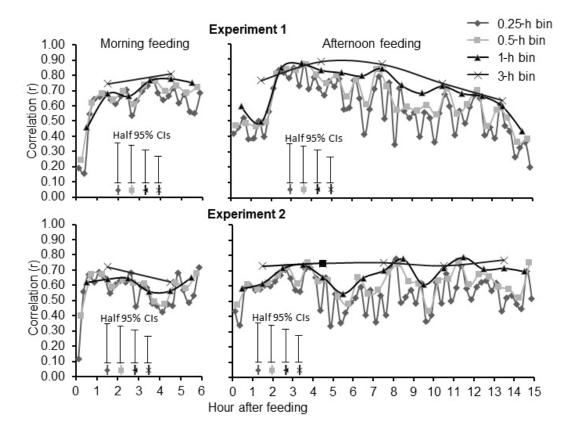


Figure 2 Correlation of each time bin over time (0.25-, 0.5-, 1.0- and 3.0-h time bins) after the morning (at approximately 0800 to 0900 h) and the afternoon (at approximately 1500 to 1600 h) feeding with 24-h measured methane in two experiments with growing beef cattle fed ryegrass-based pasture in respiration chambers. The symbols indicate the mid-point of each time bin and error bars indicate half 95% confidence interval.



little variation in standard error (SE) when 24 h CH_4 data of growing cattle was summarised in six-hour time bins. Therefore, timing of taking a CH_4 spot-sample did not appear to be very important in the studies of Robinson et al. (2019) and Gunter and Bradford (2015).

As in the current analysis, McEwan et al. (2012) also observed that correlation was lower for CH_4 measurements in approximately the first 0.5 h compared to daily CH_4 in sheep in respiration chambers, after which it increased and stabilised. This knowledge was implemented in the measurement protocol with 1-h CH_4 measurements of sheep in portable accumulation chambers (PAC) with sheep being taken off feed at least 0.5 h before the first measurements began (Jonker et al. 2018).

To our knowledge, little information is available in the literature on the effect of spot-sampling duration on the precision of a CH₄ estimate from ruminants. The precision of a CH4 measurement improved with increasing simulated spot-sampling duration (time bin) from 0.25 to 3 h in the current study. However, an evaluation in Australia found that 1- and 2-h CH₄ measurements using PAC resulted in similar precision (Goopy et al. 2011). Furthermore, Robinson et al. (2015) reported similar precision of CH₄ estimates from 1-h and 40-min PAC measurements. In the current analysis, however, 3-h bins appeared more precise than did 1-h bins, which in turn were more precise than 0.5h bins and 0.25-h bins. In general, methane data of PAC had a greater CV than of respiration chamber data (Jonker et al. 2018; Robinson et al. 2019) and this greater variance will make it less likely that different sampling durations can be distinguished. On the other hand, using GreenFeed automated emissions monitoring systems, Arthur et al. (2017) found that precision of ranking beef cattle for CH. improved when spot-sample measurements shorter than three min were excluded from their analysis. However, in the latter case, many more spot samples were taken, randomly spread over the day, which would also be an effective strategy to improve the measurement precision.

Altogether, the current analysis indicates that the precision of a CH_4 measurement improved with increasing simulated spot-sampling duration from 0.25- to 3-h bins, while the best timing to perform spot sampling was not fully consistent between the experiments and between emissions after morning and afternoon feeding, which warrants further investigation.

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References

- Arthur PF, Barchia IM, Weber C, Bird-Gardiner T, Donoghue KA, Herd RM, Hegarty RS 2017. Optimizing test procedures for estimating daily methane and carbon dioxide emissions in cattle using short-term breath measures. Journal of Animal Science 95: 645-656.
- Biswas AA, Jonker A 2019. Circadian variation in methane emissions by cattle and correlation with level and composition of ryegrass-based pasture eaten. New Zealand Journal of Animal Science and Production 79: 61-64.
- Difford GF, Olijhoek DW, Hellwing ALF, Lund P, Bjerring MA, De Haas Y, Lassen J, Løvendahl P 2018.
 Ranking cows' methane emissions under commercial conditions with sniffers versus respiration chambers.
 Acta Agriculturae Scandinavica, Section A — Animal Science 68: 25-32.
- Garnsworthy P, Difford G, Bell M, Bayat A, Huhtanen P, Kuhla B, Lassen J, Peiren N, Pszczola M, Sorg D, Viskers M, Yan T 2019. Comparison of methods to measure methane for use in genetic evaluation of dairy cattle. Animals 9: 837.
- Goopy JP, Hegarty RS, Robinson DL 2009 Two hour chamber measurements provide a reliable estimate of daily methane production in sheep. In Ed. Chilliard Y. Proceedings of the 11th International Symposium on Ruminant Physiology Clermont-Ferrand, France 6-9 September 2009. Pg. 190-191.
- Gunter SA, Bradford JA 2015. Influence of sampling time on carbon dioxide and methane emissions by grazing cattle. American Society of Animal Science Proceedings 66: 201-203.
- Hammond KJ, Crompton LA, Bannink A, Dijkstra J, Yáñez-Ruiz DR, O'kiely P, Kebreab E, Eugène MA, Yu Z, Shingfield KJ, Schwarm A, Hristov AN, Reynolds CK 2016. Review of current in vivo measurement techniques for quantifying enteric methane emission from ruminants. Animal Feed Science and Technology 219: 13-30.
- Jonker A, Molano G, Antwi C, Waghorn GC 2014. Feeding lucerne silage to beef cattle at three allowances and four feeding frequencies affects circadian patterns of methane emissions, but not emissions per unit of intake. Animal Production Science 54: 1350-1353.
- Jonker A, Molano G, Antwi C, Waghorn GC 2016. Enteric methane and carbon dioxide emissions measured using respiration chambers, the sulfur hexafluoride tracer technique, and a GreenFeed head-chamber system from beef heifers fed alfalfa silage at three allowances and four feeding frequencies. Journal of Animal Science 94: 4326-4337.
- Jonker A, Molano G, Koolaard J, Muetzel S 2017. Methane emissions from lactating and non-lactating dairy cows and growing cattle fed fresh pasture. Animal Production Science 57: 643-648.

- Jonker A, Hickey SM, Rowe SJ, Janssen PH, Shackell GH, Elmes S, Bain WE, Wing J, Greer GJ, Bryson B, Maclean S, Dodds KG, Pinares-Patiño CS, Young EA, Knowler K, Pickering NK, Mcewan JC 2018. Genetic parameters of methane emissions determined using portable accumulation chambers in lambs and ewes grazing pasture and genetic correlations with emissions determined in respiration chambers. Journal of Animal Science 96: 3031-3042.
- McEwan J, Hickey SM, Young EA, Dodds KG, Mclean S, Molano G, Sandoval E, Kjestrup H, Hunt C, Pinares-Patino CS 2012. Heritability estimates for hourly measures of methane emissions. 33rd International Society for Animal Genetics Conference. Cairns, Australia 15-20 July 2012.
- R Core Team 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL: http://www.Rproject.org/.
- Robinson DL, Goopy JP, Hegarty RS, Oddy VH 2015. Comparison of repeated measurements of methane production in sheep over 5 years and a range of measurement protocols. Journal of Animal Science 93: 4637-4650.
- Robinson DL, Dominik S, Donaldson A, Oddy H 2019. Repeatabilities, heritabilities and correlations of methane and feed intake of sheep in respiration and portable chambers Animal Production Science https://doi.org/10.1071/AN18383.