Methane emissions in growing heifers while eating from a feed bin compared with 24-hour emissions and relationship with feeding behavior

Ashraf Biswas,1,2* Ajmal Khan,1 Dongwen Luo,1 and Arjan Jonker1* © 2022, The Authors. Published by Elsevier Inc. and Fass Inc. on behalf of the American Dairy Science Association®. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/). Received November 04, 2021. Accepted March 06, 2022.

Methods

Summary

We determined the relationships between daily CH₄ emissions estimated during mealtime and measured daily CH₄ emissions, and that with feeding behavior in growing heifers (Hereford × Holstein-Friesian; n = 8) fed alfalfa silage in respiration chambers, which were linked to an analyzer that measured CH₄ in each chamber every 3 min. Each 3-min measurement was expressed as grams per day and averaged per 24 h or per time during a meal. We observed a strong correlation (r = 0.88) between CH₄ emissions (g/d) during mealtime (276±12.7 g/d) and measured over 24 h (262±24.0 g/d) was observed.

• Visits to the feed bin (r = -0.45), average meal size (r = -0.57) and average daily eating rate (r = -0.48) were negatively related with CH₄ yield (g/kg dry matter intake).

Conclusion

• CH₄ measured during meals were similar to 24 h measured CH₄ output in growing heifers fed ad libitum alfalfa silage in respiration chambers.

• Feeding behavior parameters, based on feed bin visits, explained some of the variation in CH₄ yield between heifers.

Highlights

• Methane emissions during mealtime, converted to daily emissions, were compared with 24-h methane emissions from heifers fed ad libitum alfalfa silage in respiration chambers.

• Methane measured during meals was similar to 24-h measured CH₄ output.

• Visits to the feed bin, average meal size, and average daily eating rate were natively related with CH₄ per unit of intake.

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Methane emissions in growing heifers while eating from a feed bin compared with 24-hour emissions and relationship with feeding behavior

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Abstract: The objective of the current study was to determine the relationship of daily CH4 emissions estimated during mealtime compared with measured daily CH4 emissions, and determine the relationship with feeding behavior, in growing heifers fed alfalfa silage in respiration chambers. Data from 8 growing cattle (Hereford × Holstein-Friesian) individually housed in 4 respiration chambers and fed ad libitum alfalfa silage delivered in Insentec feed-bins to record feeding behavior and intake were used. The 4 chambers were linked to 1 analyzer, which measures CH4 in each chamber approximately every 3 min. Each 3-min measurement was expressed as grams per day and averaged per 24 h or per time during a meal. A strong correlation (r = 0.88; determined using Deming regression) was observed between CH4 emissions (g/d) during mealtime (276 ± 22.7 g/d) and measured over 24 h (262 ± 24.0 g/d), without apparent systematic bias. Feeding behavior parameters that were correlated with CH4 yield (g/kg dry matter intake) in the current study were a negative correlation with the number of visits to the feed bin (r = −0.45), average meal size (r = −0.57), and average daily eating rate (r = −0.48). In summary, CH4 measured during meals was similar to 24-h measured CH4 output in growing heifers fed ad libitum alfalfa silage in respiration chambers, and some feeding behavior parameters, based on feed bin visits, explained some of the variation in CH4 yield between animals.

Methane emitted by ruminants is a potent greenhouse gas (GHG) that constitutes approximately 15% of global GHG emissions (Gerber et al., 2013) and approximately 33% of total GHG emissions in New Zealand (MfE, 2017). Therefore, a large body of research is in progress to find mitigation options, which has sparked the development of cheaper and more practical methods with higher throughput to measure CH4 from ruminants. Many of these new methods estimate emissions based on multiple short-term (spot-samples) measurements from individual ruminant animals. One spot-sampling strategy developed is based on analyzing breath samples while the animal is visiting a feed bin with forage or TMR (Troy et al., 2016; Flay, 2018). However, the rate of CH4 emissions is not constant during the day and is affected by diet, feed allowance, and feeding pattern (Müller et al., 1980; Jonker et al., 2014), which might affect the predictive power of CH4 spot-sampling methods. Currently, little is known about the accuracy of methane emission estimates based on breath sample analysis during feeding.

The objective of the current study was to determine the relationship of daily CH4 emissions estimated during mealtimes compared with measured daily CH4 emissions and the relationship with feeding behavior in growing heifers fed alfalfa silage in respiration chambers. The hypothesis was that there would be a strong relationship between daily CH4 emissions estimated during mealtimes compared with measured daily CH4 emissions.

The animal experiment reported here was reviewed and approved by the AgResearch Grasslands Animal Ethics Committee (Palmerston North, New Zealand), and heifers were cared for according to the AgResearch Code of Ethical Conduct (AgResearch CEC, 2018). Data from 8 growing heifers (Hereford × Holstein-Friesian; BW = 487 ± 29 kg) were used for the current analysis (Jonker et al., 2014, 2016). Animals were fed ad libitum alfalfa silage, which contained 369 g/kg DM of NDF, 238 g/kg DM of CP, and 10.4 MJ/kg DM of ME calculated according to NRC (2000). During the measurement period, the animals were individually housed in 4 respiration chambers for 3 (first group of 4 over the weekend) or 2 (second group of 4 during the week) consecutive days. The silage was fed in Insentec feed-bins on loadcells (Hokofarm Group BV) inside the chambers with bins refilled at approximately 0800 and 1530 h. Airflow rate was 1.8 m3/min in each chamber and therefore the time required to exchange the chamber air was approximately 9 min. The 4 chambers were linked to a switching unit that directs the air stream of each chamber to one gas analyzer in sequence, which took approximate 3 min per cycle. Every 3-min measured CH4 value was expressed as grams per day as follows: CH4 (g) per measurement time interval/measurement interval (min) × 1,440 min in 24 h.

The Insentec feed bin system recorded entry and exit time and feed weight for each eating event during the day allowing the calculation of feeding time (min), intake (g), and intake rate (g/min) for each visit to the bin. However, during a meal, the animal sometimes takes the head out of the feed bin to chew and then goes back in, resulting in several consecutive recordings that are part of one meal. It was, therefore, necessary to define a meal criterion with start and end times. Here, we define meal criteria as described previously (Tolkamp et al., 2000; von Keyserlingk and Weary, 2010) based on the frequency distribution of intervals (feed bin exit time to next entry time) expressed on a log scale. The bimodal pattern was apparent with 20 min at the intersection between the 2 peaks. Therefore, for the current study, a 20-min interval was used...
as the threshold to define if a visit to the feed bin fell within a meal or if a new meal started. This interval for meal criteria was in a similar range of 17.9 to 29.8 min as previously found in growing heifers (DeVries and von Keyserlingk, 2009a,b).

Then, the start and end time of each meal was aligned with the respiration chamber data to identify the CH4 emissions measurements during each meal. The multiple CH4 values (which were already expressed as g/d as described above) within a meal were averaged to generate the CH4 production within a meal. The 24-h measured CH4 production (g/d) was calculated by averaging all ~3-min CH4 values.

Deming regression was performed to compare daily 24-h measured CH4 and daily CH4 calculated during mealtime (Linnet, 1993). Deming regression allows fitting a straight line to 2-dimensional data where both variables (X and Y) are measured with error. Bland-Altman (Bland and Altman, 1986) mean difference plot was generated to identify potential systematic bias and outliers in the data. Pearson correlations of feeding behavior parameters with daily methane production (CH4p) and yield (CH4y), all measured in respiration chambers with growing beef cattle in 2 experiments fed a range of diets (R2 = 0.64 in experiment 1 and R2 = 0.24 in experiment 2). However, absolute values were much lower (~3×) with the feed bin system than in respiration chambers and including diet fed in the multiple-regression model greatly improved the prediction of daily CH4 using feed bin CH4 data (concordance correlation from 0.55 increased to 0.79). In contrast, Derno et al. (2013) concluded that short-term CH4 measurements during feeding at the feed bin did not reflect average daily CH4 production based on respiration chamber data with dairy cows. However, this conclusion was based on time series analysis cross-correlation (correlation between 2 times series at lags) between feed intake and CH4 emissions, which is different from an analysis where CH4 measured during multiple feeding events in a day is averaged and then compared with 24-h measured CH4 emissions, as in the current study and the study of Troy et al. (2016).

Table 1. Methane production (g/d) and yield (g/kg DMI) estimated from measurements during mealtime at the feed bin and from 24 CH4 measurements in growing heifers fed ad libitum alfalfa silage in respiration chambers

| Item                                      | Mean  | SD   | Maximum | Minimum | CV   |
|-------------------------------------------|-------|------|---------|---------|------|
| 24-h CH4 (g/d)                            | 262   | 24.0 | 319     | 226     | 9.2  |
| 24-h CH4 (g/kg of DMI)                    | 25.3  | 2.15 | 29.0    | 20.8    | 8.5  |
| Mealtime CH4 (g/d)                        | 276   | 22.7 | 313     | 241     | 8.2  |
| Mealtime CH4 (g/kg of DMI)                | 25.8  | 3.07 | 35.3    | 20.8    | 11.9 |

1The 24-h measured CH4 production was calculated by averaging all ~3-min CH4 values recorded in a 24-h period by the chamber system.

Deming regression (Figure 1). The 95% confidence interval of the slope between 24-h measured CH4 and mealtime CH4 estimated using Deming regression was 0.77 to 1.35, which indicates that the slope was not different from 1. There was no trend visible in the Bland-Altman plot, suggesting that there was no systematic bias in CH4 estimates based on simulated mealtime CH4 measurements.

These results are consistent with the findings of Troy et al. (2016), who compared CH4 emissions determined with a custom-built hood system over Insentec feed-bins, with one open side to allow access to the feed by the animal, followed by measurements in respiration chambers with growing beef cattle in 2 experiments fed a range of diets (R2 = 0.64 in experiment 1 and R2 = 0.24 in experiment 2). However, absolute values were much lower (~3×) with the feed bin system than in respiration chambers and including diet fed in the multiple-regression model greatly improved the prediction of daily CH4 using feed bin CH4 data (concordance correlation from 0.55 increased to 0.79). In contrast, Derno et al. (2013) concluded that short-term CH4 measurements during feeding at the feed bin did not reflect average daily CH4 production based on respiration chamber data with dairy cows. However, this conclusion was based on time series analysis cross-correlation (correlation between 2 times series at lags) between feed intake and CH4 emissions, which is different from an analysis where CH4 measured during multiple feeding events in a day is averaged and then compared with 24-h measured CH4 emissions, as in the current study and the study of Troy et al. (2016).

Table 2. Feed intake and feeding behavior parameters of 8 growing heifers fed ad libitum alfalfa silage and Pearson correlation (r) of feeding behavior parameters with daily methane production (CH4p) and yield (CH4y), all measured in respiration chambers

| Item                                      | Mean  | SD   | Maximum | Minimum | CV   |
|-------------------------------------------|-------|------|---------|---------|------|
| DMI (kg/d)                                | 10.8  | 1.56 | 14.2    | 7.5     | 14.5 |
| Feed bin visit frequency (/d)             | 97    | 34   | 169     | 47      | 35.1 |
| Number of meals† (/d)                     | 13    | 2.9  | 22      | 9       | 21.7 |
| Eating time (min/meal)                    | 14.7  | 3.74 | 22.3    | 7.8     | 25.4 |
| Meal duration (min/meal)                  | 34.7  | 13.4 | 71.4    | 19.4    | 38.8 |
| Total eating time (min/d)                 | 186   | 25.3 | 241     | 132     | 13.6 |
| Total mealtime (min/d)                    | 436   | 112.5| 642     | 232     | 25.8 |
| Meal size (kg of DMI)                     | 0.8   | 0.15 | 1.21    | 0.59    | 19.2 |
| Eating time eating rate (g of DM/min)     | 57.4  | 12.7 | 91.4    | 39.6    | 22.1 |
| Mealtime eating rate (g of DM/min)        | 30.4  | 8.87 | 46.7    | 17.2    | 29.2 |
| Interval between meals (min/interval)     | 75.6  | 16.02| 110.8   | 40.3    | 21.2 |

CH4p† CH4y

1The 24-h measured CH4 production was calculated by averaging all ~3-min CH4 values recorded in a 24-h period by the chamber system.

2Meal criteria was defined as described previously (Tolkamp et al., 2000; von Keyserlingk and Weary, 2010) based on the frequency distribution of intervals of the feed bin exit time to next entry time expressed on a log scale (Figure 1).

*P < 0.05.
It is not very useful to compare \( \text{CH}_4 \) production estimates (g/d) to findings of other studies because DMI is the main driver of \( \text{CH}_4 \) production (Charmley et al., 2016; Jonker et al., 2017); however, \( \text{CH}_4 \) emissions per unit of DMI (yield) can be compared when animals are fed similar diets and feeding level. The \( \text{CH}_4 \) yield in the current study averaged 25.3 g/kg DMI (range, 20.8–29.0 g/kg DMI; Table 1), which is in a similar range as for growing, dry, and lactating cattle fed forage-based diets (18.5–25.8 g/kg DMI; Jonker et al., 2020). Other studies measuring \( \text{CH}_4 \) during all feeding events at the feed bin, using GreenFeed systems, also found

**Figure 1.** Deming regression (A) and Bland-Altman plot (mean difference plot; B) of \( \text{CH}_4 \) production during mealtime at the feed bin (expressed as g/d) with 24-h measured \( \text{CH}_4 \) production (g/d) by 8 growing heifers fed ad libitum alfalfa silage in respiration chambers. The shaded area indicates the 95% confidence interval; this was −111.5 to 48.9 for the intercept (−31.32) and 0.77 to 1.35 for the slope (1.06). The 24-h measured \( \text{CH}_4 \) production was calculated by averaging all ~3-min \( \text{CH}_4 \) values recorded in a 24-h period by the chamber system.
CH₄ yields in a similar range for growing heifers fed alfalfa cubes (20.7–22.7 g/kg DMI; Flay et al., 2019) and growing beef cattle fed concentrate-based diets (21.1–23.7 g/kg DMI; Biswas et al., 2018). These suggest that CH₄ yields based on measurement to the feed bin can provide similar estimates to those measured during 24-h periods. However, the number of diets and animal measurements tested using this system are currently limited and the conclusion of Derno et al. (2013) suggested that mealtime CH₄ emissions could not be used to estimate 24-h CH₄ emissions. Therefore, further studies using other cattle categories and feeding different diets should be carried out to come to more robust conclusions about the accuracy of measuring CH₄ during all feeding events only compared with 24-h measured emissions.

The heifers ate their feed on average in 13 meals/d, consuming 800 g of DM/meal, lasting 34.6 min/meal, and at a rate of 30.4 g of DM/min in the current study (Table 2), which was a similar range as previously found in growing dairy heifers who ate their feed in 6.8 to 11.1 meal/d, consuming 520 to 980 g/meal, lasting 26.0 to 62.9 min/meal, and at a rate of 37.7 to 57 g of DM/min (DeVries and von Keyserlingk, 2009a,b). Feeding behavior parameters that were correlated with CH₄ production in the current study were a positive correlation (r = 0.78) with the number of meals per day and a negative correlation (r = −0.50) with average eating time per meal (min/meal). Previously, intake time was found to correlate (concordance correlation) positively with CH₄ production in 2 studies (Muñoz-Tamayo et al., 2019; Ramirez-Agudelo et al., 2019). In the current study, total daily mealtime and total daily eating time had very weak correlations with CH₄ production. There was also only a very weak correlation of daily DMI with daily mealtime and daily eating time (data not shown), which likely explains why intake time was a poor predictor of CH₄ production in the current study.

Feeding behavior parameters that were correlated with CH₄ yield in the current study were a negative correlation with the number of visits to the feed bin (r = −0.45), average meal size (r = −0.57), and average daily eating rate (r = −0.48). Llonch et al. (2018) also found a negative association between the number of visits to the feed bin and CH₄ yield in growing beef cattle, supporting the finding of the current study. Offering less frequent and larger meals to lactating dairy cows and sheep was previously found to result in lower CH₄ yield (Müller et al., 1980; Swainson et al., 2011), suggesting that the relationships of feeding behavior with CH₄ yield in the current study make sense from a biological point of view.

In summary, CH₄ measured during meals was similar to 24-h measured CH₄ output in growing heifers fed ad libitum alfalfa silage in respiration chambers. Some feeding behavior parameters, based on feed bin visits, explained some of the variation in CH₄ production and yield.

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A. Biswas was financially supported by the New Zealand government through the Global Research Alliance Livestock Emissions and Abatement Research Network (LEARN) awards program, and the animal trial was funded by the New Zealand government to support the objectives of the Livestock Research Group of the Global Research Alliance on Agricultural Greenhouse Gases.

The authors have not stated any conflicts of interest.