Technical note: Evaluation of interval between measurements and calculation method for the quantification of enteric methane emissions measured by respiration chamber

Rong Wang,^{1,2} Min Wang,^{1*} Xiu Min Zhang,¹ Hua Ming Yang,³ Jiang Nan Wen,^{1,2} Zhi Yuan Ma,¹ Bai Lin Feng,⁴ Jin Ping Deng,^{2,5} and Zhi Liang Tan¹

¹CAS Key Laboratory for Agro-Ecological Processes in Subtropical Region, National Engineering Laboratory for Pollution Control and Waste Utilization in Livestock and Poultry Production, South Central Experimental Station of Animal Nutrition and Feed Science in the Ministry of Agriculture, Institute of Subtropical Agriculture, The Chinese Academy of Sciences, Changsha, Hunan 410125, China

²College of Animal Science and Technology, Hunan Agricultural University, Changsha 410128, China

³Jilin Academy of Agricultural Sciences, Changchun 130033, China

⁴Youzhuo Animal Husbandry Co. Ltd., Ningxiang, Hunan 410601, China

⁵Guangdong Provincial Key Laboratory of Animal Nutrition Control, Subtropical Institute of Animal Nutrition and Feed, College of Animal Science, South China Agricultural University, Guangzhou, Guangzhou 510642, China

ABSTRACT

Respiration chambers share one analyzer working in parallel, and methane (CH₄) concentrations have to be measured at certain intervals. The maximum and minimum values in the kinetics of CH₄ emissions can be missed during the interval between measurements, which may influence the quantification of CH₄ emissions. Chambers must be opened for morning feeding and cleaning, which causes a loss of CH₄ data. Calculation methods are needed to estimate the lost CH₄ emission data, which may influence the estimated amount of daily CH₄ emissions. In this study, we measured the CH₄ emissions of 10 growing Chinese Holstein dairy heifers in respiration chambers. Methane concentrations were measured every 0.5 min to obtain the 23-h kinetics of CH₄ emissions, which were further selected at different intervals between measurements (i.e., 5, 30, 60, 120, 180, and 240 min) to evaluate the effects of interval on quantification of CH₄ emissions. The missing 1-h kinetics of CH₄ emissions before feeding were not measured, and 2 calculation methods were used to estimate the missing 1-h kinetics of CH₄ emissions: mean value of measuring period (the mean method) and the nearest value of measurement just before chamber opening (the nearest method). The results showed that the rates of CH₄ emission from 10 heifers varied from 4.56 to 11.42 g/h. The increment of intervals decreased maximum rate of CH₄ emission and increased minimum rate of CH₄ emission. Interval caused less than 5% of

the difference in measuring CH₄ emissions. Although the mean method had greater estimated daily CH₄ emission than the nearest method, the difference was within 3%. The interval between measurements (≤ 3 h) and calculation method had little influence on enteric CH₄ emission measurements.

Key words: methane emission, respiratory chamber, interval, calculation method

Technical Note

Enteric methane (CH₄) is a loss of energy for ruminants and has become a major environmental concern due to its contribution to global warming (Johnson and Johnson, 1995; Reisinger and Clark, 2017; Hristov et al., 2018). Respiration chambers are frequently used for measuring enteric CH₄ emissions in ruminants (Storm et al., 2012; Hammond et al., 2016). The respiration chamber system generally consists of several chambers (Derno et al., 2009) that share one analyzer working in parallel; as a result, it measures gas concentrations at certain intervals (Wang et al., 2017). Therefore, CH₄ emissions are measured at several time points throughout the day. Rate of CH₄ emission is not constant throughout the day (Wang et al., 2015, 2016). Measuring CH₄ concentrations at different intervals can miss maximum and minimum values and thus affect the estimate of total CH₄ emissions, which need verification.

Chambers need to be cleaned and gas analyzers need to be calibrated every day during the period of CH₄ measurement (Derno et al., 2009). Based on our experience, it takes about 1 h to clean the chambers before morning feeding; therefore, 24-h CH₄ emissions are estimated using 23-h CH₄ emission data. Two calculation methods can be used to estimate the missing 1-h

Received January 3, 2019.

Accepted March 13, 2019.

*Corresponding author: mwang@isa.ac.cn

CH₄ emission data: the mean value of the 23-h measuring period (referred to as the mean method), and the nearest value (23 h) of measurement just before chamber opening (referred to as the nearest method). The rate of CH₄ emission tends to be at the lowest level before morning feeding and then increases after feeding. Therefore, the mean values are higher after feeding than before feeding, leading to the overestimation of daily CH₄ emissions. Hristov et al. (2018) reported that the mean method can cause a small overestimation of daily CH₄ emissions. Further studies are still needed to evaluate the effect of calculation method on estimate of daily CH₄ emissions.

All experimental procedures followed institutional guidelines for the care and use of animals and were approved by the Animal Care Committee, Institute of Subtropical Agriculture, the Chinese Academy of Sciences, Changsha, China. The experiment was conducted at Youzhuo Animal Husbandry Co. Ltd. in the Ning-Xiang County of Hunan Province, China. Ten growing Chinese Holstein dairy heifers with a wide range of BW (Table 1) were selected for this experiment. Cows were fed a TMR consisting of 50% corn silage, 25% corn grain, 10% soybean meal, 7.5% cottonseed meal, 5% dried distillers grains with solubles, 0.1% montmorillonite, and 4.9% premix (DM basis). The premix was formulated to provide 1,000,000 IU of vitamin A, 200,000 IU of vitamin D, 1,250 IU of vitamin E, 8,000 mg of Zn, 80 mg of Se, 120 mg of I, 2,000 mg of Fe, 40 mg of Co, 2,500 mg of Mn, and 2,000 mg of Cu (per kilogram of premix). The DM of the TMR was 477 g/kg, and the chemical composition of the TMR was 139 g of CP, 472 g of NDF, 235 g of ADF, and 232 g of starch/kg of TMR (DM basis). Gross energy in TMR was 15.2 MJ/kg of TMR. Cows were fed twice daily at 0900 and 1600 h and had free access to feeds and fresh water.

Enteric CH₄ emissions were measured and calculated according to the protocol of Wang et al. (2015). Briefly, CH₄ concentrations were measured (every 0.5 min) continuously for 23 h after morning feeding in inlet and outlet gas using an FGD F200 sensor (Axetris AG, Kaegiswil, Switzerland). The CH₄ concentration in the remaining 1 h before morning feeding, for chamber cleaning and feeding, was not measured. It took less than 3 min to provide fresh feed during the afternoon feeding, and CH₄ concentrations were continuously measured in this study.

Methane concentrations were further selected at different intervals (i.e., 5, 30, 60, 120, 180, and 240 min) using measured 23-h kinetics of CH₄ emissions. The 23-h CH₄ emissions of different intervals were then recalculated using equations provided by Wang et al. (2015):

$$\Delta V_i = \left[R_g \times \left(\frac{CC_i + CC_{i-1}}{2} - \frac{CA_i + CA_{i-1}}{2} \right) \right] / 10^6 + V_c \times 1,000 \times (CC_i + CC_{i-1})$$

$$R_i = \frac{\Delta V_i}{t_i - t_{i-1}}$$

$$V_h = \sum_{i=1}^{i=n} \Delta V_i,$$

where ΔV_i is the amount of CH₄ emission (L) between time t_i and t_{i-1} ; R_g is the flow rate of gas (L/h) in the duct; CC_i is the concentration of CH₄ (10⁶ L/L) in the outlet air at time t_i ; CA_i is the concentration of CH₄ (10⁶ L/L) in the inlet air at time t_i ; V_c is the volume of the chamber (m³); R_i is the rate of CH₄ emission (L/h) between t_i and t_{i-1} ; i is the number of sampling within

Table 1. Characteristics of the dairy heifers used (n = 10)

Cow	BW (kg)	Age (mo)	CH ₄ emission (g/23 h)	CH ₄ emission rate (g/h)		
				Mean	Maximum	Minimum
1	279	6.9	105	4.6	7.6	2.3
2	330	9.5	194	8.4	12.6	5.4
3	340	10.7	181	7.9	11.1	4.2
4	352	9.7	172	7.5	11.1	4.7
5	368	11.8	200	8.7	12.5	5.6
6	385	8.9	153	6.6	10.4	3.9
7	402	11.8	194	8.4	13.6	5.0
8	419	11.9	263	11.4	17.6	7.0
9	419	11.9	210	9.1	13.8	5.8
10	427	11.5	187	8.1	11.8	5.1
Mean	372	10.5	186	8.1	12.2	4.9
SEM	15.0	0.53	12.8	0.56	0.81	0.40

Figure 1. Relationships of 23-h methane emissions with BW (A) and age (B) in dairy heifers ($n = 10$). The lines in each panel are the best linear regression line. Each point represents the data of 1 cow, with a total of 10 data points.

23 h; n is the total number of samplings; and V_h is the amount of CH_4 emission (L) within 23 h.

Daily (24-h) CH_4 emissions can be calculated by summing measured 23-h CH_4 emissions and 1-h missing CH_4 emissions. The 1-h missing CH_4 emissions were estimated using the mean and nearest methods. Mean method used the mean rate of measured 23-h CH_4 emission, whereas the nearest method used the nearest value (23-h) of measurement just before chamber opening.

Statistical analysis was performed using SPSS 21.0 software (SPSS Inc., Chicago, IL). Effects of interval were analyzed by mixed linear procedure, and the analytic model included interval ($i = 7; 0.5, 5, 30, 60, 120, 180$, and 240 min) as fixed effect and dairy heifer ($j = 10: 1, 2, 3, 4, 5, 6, 7, 8, 9$, and 10) as the random effect and repeated measures, with covariance being compound symmetry structure. Effect of calculation method was analyzed using mixed linear procedure, and the analytic model included calculation method ($i = 2$, mean and nearest methods) as the fixed effects and dairy heifer ($j = 10, 1, 2, 3, 4, 5, 6, 7, 8, 9$, and 10) as the random effect and repeated measures, with covariance being compound symmetry structure. Association between measured 23-h CH_4 emissions and BW or age was evaluated using linear regression model. Statistical significance was declared at $P \leq 0.05$, and a tendency toward significance was declared at $0.05 < P \leq 0.10$.

The 23-h kinetics of CH_4 emissions and rates of CH_4 emission varied widely among 10 cows (Table 1), and the amount of 23-h CH_4 emissions was positively cor-

related with BW ($r = 0.70$; $P = 0.02$) and age ($r = 0.85$; $P = 0.002$) of cows (Figure 1). Animal BW, a key explanatory variable for CH_4 emissions, is always included in the model to predict CH_4 emission (Moraes et al., 2014). Some cows with similar BW can have different CH_4 emissions due to having different genetic backgrounds (Jonker et al., 2016).

The rates of CH_4 emission were not constant throughout the day and ranged between 4.6 and 11.4 g/h (Figure 2). The maximum rate of CH_4 emission occurred about 1 to 2 h after feeding, and then the rate of CH_4 emission gradually declined to the basal level. When animals were fed twice per day, 2 distinct peaks were observed in the diurnal kinetics of CH_4 emissions (Figure 2). This phenomenon is also reported by previous studies (van Zijderveld et al., 2010; Guyader et al., 2015; Wang et al., 2018). However, when measurement frequency was decreased by increasing the interval between measurements, diurnal kinetics of CH_4 emissions changed significantly ($P < 0.001$; Table 2). For example, when the interval was increased from 0.5 to 240 min, apparent diurnal kinetics of CH_4 emissions changed so that instead of 2 peaks, only 1 peak was detected. Furthermore, increased interval significantly ($P \leq 0.05$) decreased maximum rate of CH_4 emission and increased minimum rate of CH_4 emission (Table 2). Our result confirmed that increasing interval between measurements could result in not detecting the actual or true maxima and minima for rates of CH_4 emission.

When the interval was increased from 0.5 to 180 min, the calculated amount of 23-h CH_4 emissions was not

affected. Although the maximum and minimum values in the kinetics of CH_4 emissions were missed when CH_4 concentrations were measured at different intervals, the missed maximum and minimum values might offset each other, leading to the unchanged estimate of total CH_4 emissions. When the interval was extended to 4 h, the calculated amount of 23-h CH_4 emissions was significantly ($P < 0.001$) increased compared with the

estimate made using measurements at 0.5-min intervals. Similar results have been observed using the Green-Feed system. For example, Manafiazar et al. (2017) found that increased intervals between measurements caused an increase in estimate of CH_4 emissions in beef cattle, and these results can be inconsistent if only a small number of samples are measured. However, in the current research, the extent of increased maximum

Figure 2. The kinetics of 23-h rate of methane emission (g/h) from dairy heifers at different intervals (mean value of 10 animals). Black points are the methane data measured every 0.5 min, and red points are the selected methane data at different intervals between measurements (i.e., 10, 30, 60, 120, 180, and 240 min). Arrows show the time when the fresh feeds were provided.

Table 2. Effect of interval between measurements on enteric CH₄ emissions in dairy heifers (n = 10)

Interval (min)	CH ₄ emission (g/23 h)	CH ₄ emission rate (g/h)		
		Mean	Maximum	Minimum
0.5	186 ^a	8.1 ^a	12.2 ^d	4.9 ^a
5	186 ^a	8.1 ^a	11.8 ^{cd}	5.2 ^{ab}
30	186 ^a	8.1 ^a	11.4 ^c	5.6 ^{bc}
60	186 ^a	8.1 ^a	10.8 ^b	5.7 ^{cd}
120	188 ^a	8.2 ^a	10.1 ^a	6.0 ^{de}
180	184 ^a	8.0 ^a	9.8 ^a	6.2 ^e
240	194 ^b	8.5 ^b	10.1 ^a	7.0 ^f
SEM	1.9	0.08	0.26	0.18
<i>P</i> -value				
Interval	<0.001	<0.001	<0.001	<0.001
Animal	<0.001	<0.001	<0.001	<0.001

^{a-f}Means within a column with different superscripts differ ($P \leq 0.05$).

value was about +4.6%, which was smaller than 21.9% obtained using the GreenFeed system (Jonker et al., 2016). We surmise that different measurement systems and intervals between measurements may be the cause of this variation.

The calculated amount of daily 24-h CH₄ emissions was further investigated using the mean method and the nearest method to estimate 1-h missing CH₄ emissions (Table 3). Compared with the nearest method, the mean method resulted in a greater estimate of daily CH₄ emissions ($P < 0.001$) in dairy heifers (Table 3). This result was within our expectations. The rate of CH₄ emission was always lowest before morning feeding because of unavailable or residual feeds for animals. However, calculation method seemed to have little effect on calculating daily CH₄ emissions, as daily CH₄ emissions were just 0.74 to 2.53% greater when estimated using the mean method than when estimated using the nearest method. This finding is consistent with the findings reported by Hristov et al. (2018), who

also estimated that the mean method may overestimate daily CH₄ emissions by approximately 2%.

Increasing the interval between measurements may lead to an underestimation of maxima and an overestimation of minima with respect to the pattern of daily enteric CH₄ emissions but does not affect the estimate of 23-h CH₄ emission if the interval between measurements is ≤ 3 h. The mean method has greater estimated daily CH₄ emission than the nearest method, but the difference is within 3%.

ACKNOWLEDGMENTS

This work was supported by National Key Research and Development Program of China (grant no. 2018YFD0501800 and 2016YFD0500504), National Natural Science Foundation of China (grant no. 31561143009), Hunan Province Science and Technology Plan (grant no. 2017NK1020), China Agriculture Research System (grant no. CARS-36), and Youth

Table 3. Effect of calculation method on daily methane emission (g/24 h) in dairy heifers (n = 10)

Interval (min)	Calculation method ¹		Difference (%)	SEM	<i>P</i> -value
	Mean	Nearest			
0.5	200	198	1.1	<0.001	<0.001
5	199	197	1.0	<0.001	<0.001
30	201	199	0.9	<0.001	<0.001
60	195	193	1.1	<0.001	<0.001
120	203	198	2.3	<0.001	<0.001
180	200	194	2.7	<0.001	<0.001
240	203	197	2.7	<0.001	<0.001

¹Mean method = the missing 1-h data are replaced by the mean value of the 23-h measuring period. Nearest method = the missing 1-h data are the nearest value of measurement just before chamber opening (i.e., emission data at 23 h).

Innovation Promotion Association CAS (grant no. 2016327). The authors declare that they have no conflict of interest.

REFERENCES

- Derno, M., H. G. Elsner, E. A. Paetow, H. Scholze, and M. Schweigel. 2009. Technical note: A new facility for continuous respiration measurements in lactating cows. *J. Dairy Sci.* 92:2804–2808. <https://doi.org/10.3168/jds.2008-1839>.
- Guyader, J., M. Eugene, B. Meunier, M. Doreau, D. P. Morgavi, M. Silberberg, Y. Rochette, C. Gerard, C. Loncke, and C. Martin. 2015. Additive methane-mitigating effect between linseed oil and nitrate fed to cattle. *J. Anim. Sci.* 93:3564–3577. <https://doi.org/10.2527/jas.2014-8196>.
- Hammond, K. J., L. A. Crompton, A. Bannink, J. Dijkstra, D. R. Yáñez-Ruiz, P. O’Kiely, E. Kebreab, M. A. Eugène, Z. Yu, K. J. Shingfield, A. Schwarm, A. N. Hristov, and C. K. Reynolds. 2016. Review of current in vivo measurement techniques for quantifying enteric methane emission from ruminants. *Anim. Feed Sci. Technol.* 219:13–30. <https://doi.org/10.1016/j.anifeedsci.2016.05.018>.
- Hristov, A. N., E. Kebreab, M. Niu, J. Oh, A. Bannink, A. R. Bayat, T. M. Boland, A. F. Brito, D. P. Casper, L. A. Crompton, J. Dijkstra, M. Eugène, P. C. Garnsworthy, N. Haque, A. L. F. Hellwing, P. Huhtanen, M. Kreuzer, B. Kuhla, P. Lund, J. Madsen, C. Martin, P. J. Moate, S. Muetzel, C. Muñoz, N. Peiren, J. M. Powell, C. K. Reynolds, A. Schwarm, K. J. Shingfield, T. M. Storlien, M. R. Weisbjerg, D. R. Yáñez-Ruiz, and Z. Yu. 2018. Symposium review: Uncertainties in enteric methane inventories, measurement techniques, and prediction models. *J. Dairy Sci.* 101:6655–6674. <https://doi.org/10.3168/jds.2017-13536>.
- Johnson, K. A., and D. E. Johnson. 1995. Methane emissions from cattle. *J. Anim. Sci.* 73:2483–2492. <https://doi.org/10.2527/1995.7382483x>.
- Jonker, A., G. Molano, C. Antwi, and G. C. Waghorn. 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. *J. Anim. Sci.* 94:4326–4337. <https://doi.org/10.2527/jas.2016-0646>.
- Manafiazar, G., S. Zimmerman, and J. A. Basarab. 2017. Repeatability and variability of short-term spot measurement of methane and carbon dioxide emissions from beef cattle using GreenFeed emissions monitoring system. *Can. J. Anim. Sci.* 97:118–126. <https://doi.org/10.1139/cjas-2015-0190>.
- Moraes, L. E., A. B. Strathe, J. G. Fadel, D. P. Casper, and E. Kebreab. 2014. Prediction of enteric methane emissions from cattle. *Glob. Chang. Biol.* 20:2140–2148. <https://doi.org/10.1111/gcb.12471>.
- Reisinger, A., and H. Clark. 2017. How much do direct livestock emissions actually contribute to global warming? *Glob. Chang. Biol.* 24:1–13. <https://doi.org/10.1111/gcb.13975>.
- Storm, I. M. L. D., A. L. F. Hellwing, N. I. Nielsen, and J. Madsen. 2012. Methods for measuring and estimating methane emission from ruminants. *Animals (Basel)* 2:160–183. <https://doi.org/10.3390/ani2020160>.
- van Zijderveld, S. M., W. J. Gerrits, J. A. Apajalahti, J. R. Newbold, J. Dijkstra, R. A. Leng, and H. B. Perdok. 2010. Nitrate and sulfate: Effective alternative hydrogen sinks for mitigation of ruminal methane production in sheep. *J. Dairy Sci.* 93:5856–5866. <https://doi.org/10.3168/jds.2010-3281>.
- Wang, M., R. Wang, X. Sun, L. Chen, S. Tang, C. Zhou, X. Han, J. Kang, Z. Tan, and Z. He. 2015. A mathematical model to describe the diurnal pattern of enteric methane emissions from non-lactating dairy cows post-feeding. *Anim. Nutr.* 1:329–338. <https://doi.org/10.1016/j.aninu.2015.11.009>.
- Wang, M., R. Wang, T. Y. Xie, P. H. Janssen, X. Z. Sun, K. A. Beauchemin, Z. L. Tan, and M. Gao. 2016. Shifts in rumen fermentation and microbiota are associated with dissolved ruminal hydrogen concentrations in lactating dairy cows fed different types of carbohydrates. *J. Nutr.* 146:1714–1721. <https://doi.org/10.3945/jn.116.232462>.
- Wang, M., R. Wang, X. Zhang, E. M. Ungerfeld, D. Long, H. Mao, J. Jiao, K. A. Beauchemin, and Z. Tan. 2017. Molecular hydrogen generated by elemental magnesium supplementation alters rumen fermentation and microbiota in goats. *Br. J. Nutr.* 118:401–410. <https://doi.org/10.1017/S0007114517002161>.
- Wang, R., M. Wang, E. M. Ungerfeld, X. M. Zhang, D. L. Long, H. X. Mao, J. P. Deng, A. Bannink, and Z. L. Tan. 2018. Nitrate improves ammonia incorporation into rumen microbial protein in lactating dairy cows fed a low-protein diet. *J. Dairy Sci.* 101:9789–9799. <https://doi.org/10.3168/jds.2018-14904>.