

1 **Comparison of enteric CH<sub>4</sub> emissions between the respiration chamber and the CO<sub>2</sub>**  
2 **method in Holstein heifers.**

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22

23 **ABSTRACT**

24 We validated the CO<sub>2</sub> method (CO<sub>2</sub>T) for measuring enteric CH<sub>4</sub> emissions using emissions  
25 data derived from the respiration chamber (RC). The study was conducted over a period of  
26 108 day (d) using 10 Holstein heifers with a mean initial body weight (BW) of 178 kg and  
27 average age of 7.7 months. There were 3 consecutive measurement periods, each consisting  
28 of 21 d of premeasurement feeding, followed by 15 d of CH<sub>4</sub> measurement using the two  
29 testing methods. The diet consisted of 55% timothy hay and 45% concentrate, and was  
30 supplied twice daily at 1.2% to 1.5% BW on an as-fed basis. The CO<sub>2</sub>T was employed for a  
31 period of 3 d, immediately followed by RC measurement for a period of 12 d. Measurements  
32 were conducted at morning and afternoon feeding. There were no significant differences in  
33 dry matter intake (DMI, kg/d;  $P = 0.16$ ), CH<sub>4</sub> production (L/d and L/kg BW<sup>0.75</sup>;  $P = 0.49$  and  
34 0.63, respectively), and CH<sub>4</sub> yield (L/kg DMI;  $P = 0.28$ ) between the two methods. Results  
35 obtained using the RC and the CO<sub>2</sub>T were strongly correlated for both CH<sub>4</sub> production (L/d;  $r$   
36 = 0.67,  $P = 0.04$ ) and CH<sub>4</sub> yield (L/kg DMI;  $r = 0.61$ ,  $P = 0.06$ ). Linear regression analysis  
37 revealed moderate positive relationships between the two methods for CH<sub>4</sub> production ( $R^2 =$   
38 0.52;  $P = 0.019$ ) and CH<sub>4</sub> yield ( $R^2 = 0.43$ ;  $P = 0.039$ ). We conclude that the CO<sub>2</sub>T and the  
39 RC produce comparable results, with acceptable accuracy and precision.

40

41 **Keywords:** The CO<sub>2</sub> method; Enteric methane production; Respiration chamber; Holstein  
42 heifer

## 43 1. Introduction

44 Global warming is one of most important issues facing the world today, and shows no  
45 signs of a decline (Karl et al., 2015). The livestock sector is responsible for 14.5% of total  
46 anthropogenic greenhouse gas emissions, with 39% of these emissions derived from CH<sub>4</sub>  
47 produced by enteric fermentation in ruminants (Gerber et al., 2013). The demand for animal  
48 protein has led to an increase in the number of livestock, which may further increase  
49 greenhouse gas production (McLeod, 2011).

50 A variety of strategies have been proposed to mitigate enteric CH<sub>4</sub> emissions from  
51 ruminants, involving feed manipulation, supplementation of lipids and fatty acids, organic  
52 acids, plant secondary metabolites, chemical compounds such as 3-nitrooxypropanol, and  
53 genetic selection (Knapp et al., 2014; Patra et al., 2017). To evaluate the feasibility of such  
54 strategies, it is essential to use appropriate methods to measure in vivo CH<sub>4</sub> production. As  
55 one of the most accurate techniques, the respiration chamber (RC) system is considered the  
56 gold standard. However, this method has several limitations, including high setup costs, the  
57 potential to restrict animal behaviors such as eating and walking, unsuitability for large-scale  
58 experiments, the need for well-trained experts for equipment operation, and intensive labor  
59 requirements (Patra, 2016). The need for more convenient and versatile CH<sub>4</sub> measurement  
60 techniques has led to the development of several alternate methods, including the SF<sub>6</sub> tracer  
61 gas technique, the GreenFeed system, hand laser CH<sub>4</sub> detectors, and the CO<sub>2</sub> method (CO<sub>2</sub>T)  
62 (Okpara, 2018; Storm et al., 2012; Zhao et al., 2020).

63 The CO<sub>2</sub>T was developed by Madsen et al. (2010), who used daily total CO<sub>2</sub> production  
64 and the CH<sub>4</sub>:CO<sub>2</sub> ratio to estimate CH<sub>4</sub> production. Total CO<sub>2</sub> production is calculated using  
65 equations, and the CH<sub>4</sub>:CO<sub>2</sub> ratio is obtained by measuring animal exhalation. Previous  
66 studies have employed this method to estimate CH<sub>4</sub> production by dairy cows in an automatic  
67 milking system (Haque et al., 2014) and to demonstrate the repeatability of CH<sub>4</sub> production in  
68 dairy cows (Haque et al., 2015). Additionally, Haque et al. (2017) demonstrated that CH<sub>4</sub>  
69 estimates derived using this method in metabolic cages are comparable to CH<sub>4</sub> production

70 data calculated using the CH<sub>4</sub>:CO<sub>2</sub> ratio with metabolic cages and CO<sub>2</sub> production measured  
71 using the RC, with reasonable precision. However, no study has directly compared CH<sub>4</sub>  
72 emissions obtained using the CO<sub>2</sub>T and the RC.

73 Therefore, we compared enteric CH<sub>4</sub> emissions from Holstein heifers obtained using  
74 these two methods. Our hypothesis was that these methods would produce comparable  
75 results, with acceptable precision.

76

## 77 **2. Materials and methods**

### 78 **2.1. Animal ethics**

79 All experimental procedures involving animals were approved by the Seoul National  
80 University Institutional Animal Care and Use Committee, Republic of Korea (permission no.  
81 SNU-240117-4-1). The experiment was conducted in accordance with national guidelines  
82 provided by the Committee.

83

### 84 **2.2. Experimental design, animals, and feeding management**

85 This study was conducted over a period of 108 d using 10 Holstein heifers with an average  
86 body weight (BW) of  $178 \pm 24$  kg (mean  $\pm$  SD) and an average age of  $7 \pm 0.7$  months (mean  
87  $\pm$  SD) at the commencement of the trial. The study included 3 consecutive measurement  
88 periods, each consisting of 21 d of premeasurement feeding followed by 15 d of CH<sub>4</sub>  
89 measurement using the CO<sub>2</sub>T and the RC (3 d for CO<sub>2</sub>T, 12 d for RC). Throughout the entire  
90 period, the animals were fed 55% timothy hay and 45% concentrate twice daily at 1.2% to  
91 1.5% BW on an as-fed basis using Calan doors (Table 1). The animals had free access to  
92 water, and daily feed intake was calculated as the difference between the amount of supply  
93 andorts.

94

### 95 **2.3. CH<sub>4</sub> estimation using the CO<sub>2</sub>T**

96 Gas sampling for the CO<sub>2</sub>T was conducted during a period of 3 d, immediately before the  
97 start of the RC CH<sub>4</sub> measurement period. Exhaled breath samples were continuously  
98 analyzed for CH<sub>4</sub> and CO<sub>2</sub> concentrations (ppm) during the morning and afternoon feeding  
99 periods. CH<sub>4</sub> and CO<sub>2</sub> concentration data were collected at 10 s intervals for 10 min per  
100 animal. Breath samples were collected by attaching the sampling inlet to the feeder, which  
101 was sufficiently close to obtain gas samples with minimal dilution by ambient air. Each  
102 individual feeder was covered with flexible transparent plastic sheet to minimize wind  
103 disturbance. A background air sample from the barn was collected for 50 min during each  
104 experimental day to analyze the background CH<sub>4</sub> and CO<sub>2</sub> concentrations.

105 A gas analyzer (VA-3000; Horiba, Kyoto, Japan) equipped with a non-dispersive infrared  
106 CO<sub>2</sub> gas sensor was used to detect the CO<sub>2</sub> concentrations of incoming air samples. Another  
107 gas analyzer (Airwell+7; Kinsco Technology, Seoul, Republic of Korea) equipped with a  
108 tunable diode laser absorption spectroscopy CH<sub>4</sub> gas sensor was used to detect CH<sub>4</sub>  
109 concentrations. The gas sensors were calibrated daily, immediately before sampling, using  
110 standard gas mixtures with concentration of 807 μmol/mol CH<sub>4</sub> and 7,996 μmol/mol CO<sub>2</sub> in  
111 N<sub>2</sub> (Air Korea, Yeosu, Republic of Korea). The recorded CH<sub>4</sub> and CO<sub>2</sub> concentration data for  
112 both breath and background air samples were stored in a data logger in a computer until data  
113 analysis.

114 CH<sub>4</sub> production estimates were obtained through the stepwise calculation of the CH<sub>4</sub>:CO<sub>2</sub>  
115 ratio of breath samples, total CO<sub>2</sub> production per day, and total CH<sub>4</sub> production per day, as  
116 previously described (Madsen et al., 2010).

117 Breath CH<sub>4</sub> and CO<sub>2</sub> concentration data were corrected by subtracting the background  
118 CH<sub>4</sub> and CO<sub>2</sub> concentrations. Any paired CH<sub>4</sub> and CO<sub>2</sub> concentrations containing a negative  
119 value after correction were removed from the data pool to avoid bias in the total CH<sub>4</sub>  
120 estimation. Then the ratio of CH<sub>4</sub> to CO<sub>2</sub> was calculated. To estimate the total CO<sub>2</sub>

121 production, the heat production (HP) of each animal was calculated based on the BW,  
122 average daily gain, energy content of the diet, and days of pregnancy, according to equations  
123 (1), as follows (CIGR, 2002):

$$124 \quad \text{HP (Watt)} = 7.64 \times \text{BW}^{0.69} + Y \left[ \frac{23}{M} - 1 \right] \left[ \frac{57.27 + 0.302 \times \text{BW}}{1 - 0.171 \times Y} \right] + 1.6 \times 10^{-5} \times p^3$$

125 (1)

126 where  $Y$  is the average daily gain of the animal,  $M$  is the energy content of the feed (MJ/kg dry  
127 matter), and  $p$  represents the days of pregnancy (d) of the heifer. Then the total  $\text{CO}_2$  production  
128 of the animal was calculated based on the heat producing unit (HPU = HP/1000), according to  
129 equations (2), as follows (Pedersen et al., 2008):

$$130 \quad \text{CO}_2 \text{ (L/d)} = \text{HPU} \times 180 \times 24 \quad (2)$$

131 The amount of  $\text{CH}_4$  production was estimated as the product of the  $\text{CH}_4:\text{CO}_2$  ratio and the total  
132 daily  $\text{CO}_2$  production, according to equations (3), as follows:

$$133 \quad \text{CH}_4 \text{ (L/d)} = \text{CO}_2 \times \text{CH}_4/\text{CO}_2 \quad (3)$$

134

#### 135 **2.4. $\text{CH}_4$ measurement by the RC**

136 Immediately after the  $\text{CO}_2\text{T}$  measurement,  $\text{CH}_4$  and  $\text{CO}_2$  production were measured using  
137 the RC with 4 open-circuit whole-body respiratory chambers, as previously described  
138 (Bharanidharan et al., 2018). Briefly, the chambers were constructed of steel frames and  
139 covered with detachable, transparent polyvinyl chloride sheets to guarantee visual  
140 communication between the animals. The outer dimensions of the chamber were 137 cm  
141 width  $\times$  356 cm depth  $\times$  200 cm height, for a total volume of 9.7  $\text{m}^3$ . All chambers were  
142 equipped with a feeder, water bowl, and air conditioner (model DK-C-150E; Dryer Korea,  
143 Gwangju, Republic of Korea) to maintain a temperature of 25°C and relative humidity of  
144 50%. An air blower (ALFFIZ-WBCAI-015H; Busung, Gimhae, Republic of Korea) was  
145 installed to facilitate air mixing and circulation.

146 The 10 heifers were randomly assigned to 3 groups, each of which spent 3 d in the RCs.

147 RC adaptation training was performed 3 times (4 d each) prior to the commencement of the  
148 study. CH<sub>4</sub> recovery in each chamber was independently tested for a period of 1 d by  
149 injecting a standard CH<sub>4</sub> gas mixture (25% mol/mol balance N<sub>2</sub>; Air Korea, Yeosu, Republic  
150 of Korea) prior to gas measurements for each group. Differences in gas concentration  
151 between the inlet and outlet gas (DCH<sub>4</sub>, ppm) and the known dry standard temperate and  
152 pressure ventilation rate (Dry STP VR), injected CH<sub>4</sub> concentration (InCH<sub>4</sub>), and CH<sub>4</sub>  
153 injection rate (InjR) were used to calculate theoretical CH<sub>4</sub> (ThCH<sub>4</sub>) and gas recovery rates of  
154 the chamber, according to equations (4) and (5), as follows:

$$155 \quad \text{Theoretical CH}_4 \text{ (ThCH}_4\text{, ppm)} = [\text{InCH}_4 \text{ (\%)/100} \times \text{InjR (mL/min)/Dry STP VR} \\ 156 \text{ (mL/min)} \times 1,000,000 \quad (4)$$

$$157 \quad \text{Gas recovery rate (\%)} = \text{DCH}_4 \text{ (ppm)/ThCH}_4 \text{ (ppm)} \times 100 \quad (5)$$

158 During the experimental period, the same routine was conducted 1 d before the animals  
159 were placed in the chamber, and CH<sub>4</sub> emissions were calculated, according to equatinos (6),  
160 as follows:

$$161 \quad \text{CH}_4 \text{ emissions (L/min)} = [\text{Dry STP VR (mL/min)} \times \text{DCH}_4 \text{ (ppm)/1,000,000}] \times 100/\text{Gas} \\ 162 \text{ recovery rate (\%)} \quad (6)$$

163 The gas analysis system comprised analyzers (VA-3000; Horiba, Kyoto, Japan) containing  
164 non-dispersive infrared CH<sub>4</sub> and CO<sub>2</sub> gas sensors connected to a gas sampling pump  
165 (Columbus Instruments, Columbus, OH, USA). Gas samples were collected at the inlet and  
166 outlet of the chamber by a sealed rotary pump connected to a flow meter (LS-3D; Teledyne  
167 Technologies, Thousand Oaks, CA, USA) that provided a constant wet ventilation rate (wet  
168 VR) of 450 L/min. Based on the stability of the gas concentrations and the number of  
169 chambers in operation, the air was sub-sampled every 14 min at background air and chamber  
170 air sampling periods alternating as 90 and 120 s, respectively, for each chamber. Prior to  
171 sampling, the gas sensors were calibrated using standard gas mixtures with concentrations of

172 807  $\mu\text{mol/mol}$   $\text{CH}_4$  and 7,996  $\mu\text{mol/mol}$   $\text{CO}_2$  in  $\text{N}_2$  (Air Korea, Yeosu, Republic of Korea).

173

## 174 **2.5. Chemical composition analysis of feed**

175 We followed methods established by the Association of Official Agricultural Chemists  
176 (AOAC, 2006) to analyze the dry matter (method 930.15), crude protein (Kjeldahl  $\text{N} \times 6.25$ ,  
177 method 981.10), ether extract (method 920.39), ash (method 942.05), Ca (method 927.02), P  
178 (method 965.17), and acid detergent fiber (method 973.18) of the feed. Neutral detergent  
179 fiber content was determined using the ANKOM200 Fiber Analyzer (Ankom Technology,  
180 Macedon, NY, USA), as previously described (Mertens et al., 2002). The gross energy of the  
181 feed was determined using an automatic isoperibol calorimeter (model 6400; Parr Instrument  
182 Co., Moline, IL, USA).

183

## 184 **2.6. Statistical analyses**

185 All statistical analyses were conducted using R statistical software (v4.0.4; R Core Team.,  
186 2021). Prior to statistical analyses,  $\text{CH}_4$  emissions and DMI data from 3 measurement periods  
187 were arithmetically averaged per heifer. All data were assessed for normality using the  
188 Shapiro–Wilk test. Differences in DMI,  $\text{CH}_4$  production, and  $\text{CH}_4$  yield between the two  
189 methods were evaluated using the Wilcoxon signed-rank test because the data were not  
190 normally distributed. Spearman correlation coefficient analyses were conducted to determine  
191 the relationship between the 2 methods. The repeatability of each method in determining  $\text{CH}_4$   
192 production and yield was calculated using the ‘rptR’ package in R (Stoffel et al., 2017). For  
193 repeatability analysis, we used 2 and 3 d of data obtained by the RC and the  $\text{CO}_2\text{T}$ ,  
194 respectively. Within-animal and between-animal coefficients of variation (CV) were  
195 calculated for each period and then averaged.

196

## 197 **3. Results**

### 198 3.1. Feed intake and CH<sub>4</sub> emissions

199 Table 2 shows the DMI, CH<sub>4</sub> production, and CH<sub>4</sub> yield data. DMI during the RC and the  
200 CO<sub>2</sub>T measurements did not differ ( $P = 0.16$ ). CH<sub>4</sub> production (127 vs. 120 L/d and 2.05 vs.  
201 2.16 L/kg BW<sup>0.75</sup> for the RC and the CO<sub>2</sub>T, respectively) and CH<sub>4</sub> yield (23.5 vs. 21.6 L/kg  
202 DMI, respectively) did not differ ( $P \geq 0.28$ ) between the two methods. A positive relationship  
203 was detected between DMI (kg/d) and CH<sub>4</sub> production (L/d) for both the RC ( $R^2 = 0.46$ ,  $P =$   
204  $0.03$ ; Fig. 1A) and the CO<sub>2</sub>T ( $R^2 = 0.72$ ,  $P = 0.002$ ; Fig. 1B).

205

### 206 3.2. Relationships between the RC and the CO<sub>2</sub>T

207 Regression analyses revealed moderate positive relationships between the RC and the  
208 CO<sub>2</sub>T for both CH<sub>4</sub> production (L/d;  $R^2 = 0.52$ ,  $P = 0.02$ ; Fig. 2A) and CH<sub>4</sub> yield (L/kg DMI;  
209  $R^2 = 0.43$ ,  $P = 0.04$ ; Fig. 2B). Correlation analyses revealed strong relationships between the  
210 RC and the CO<sub>2</sub>T for CH<sub>4</sub> production (L/d) and CH<sub>4</sub> yield (L/kg DMI). The RC and the  
211 CO<sub>2</sub>T showed correlation coefficients of 0.67 ( $P = 0.04$ ) for CH<sub>4</sub> production (L/d) and 0.61  
212 ( $P = 0.06$ ) for CH<sub>4</sub> yield (L/kg DMI) (Table 3). The RC showed similar repeatability (0.66  
213 and 0.58 for CH<sub>4</sub> production and yield, respectively) to the CO<sub>2</sub>T (0.65 and 0.49,  
214 respectively) (Table 3).

215 The CO<sub>2</sub>T showed lower between-animal CV (7.43% and 6.33% for CH<sub>4</sub> production [L/d]  
216 and yield [L/kg DMI], respectively) than the RC (32.9% and 31%, respectively) (Table 3).  
217 The within-animal CV of CH<sub>4</sub> emissions (L/d and L/kg DMI) was lower than the between-  
218 animal CV for both the CO<sub>2</sub>T and the RC. The CO<sub>2</sub>T showed lower within-animal CV  
219 (2.55% and 3.05% for CH<sub>4</sub> production [L/d] and yield [L/kg DMI], respectively) than the RC  
220 (7.77% and 9.91%, respectively).

221

### 222 3.3. Validation of the CO<sub>2</sub>T principle

223 Average CH<sub>4</sub> production (L/d) was also estimated by the CO<sub>2</sub>T calculation method using

224 CO<sub>2</sub> and CH<sub>4</sub> concentration data measured in the respiration chamber (CO<sub>2</sub>RC). No  
225 difference in CH<sub>4</sub> production was found between values estimated by the CO<sub>2</sub>RC (127 L/d)  
226 and the CO<sub>2</sub>T (136 L/d) ( $P = 0.66$ ; Table 4).

227

#### 228 **4. Discussion**

229 In this study, the CO<sub>2</sub>T exhibited no significant differences in CH<sub>4</sub> production and yield  
230 compared to the RC method.

231 Correlation analyses between the RC and the CO<sub>2</sub>T for CH<sub>4</sub> production and yield showed  
232 relatively high correlation coefficients (0.67 and 0.61 for CH<sub>4</sub> production and yield,  
233 respectively). Our results are consistent with those reported for other CH<sub>4</sub> measurement  
234 methods. For example, correlation coefficients between the GreenFeed and the RC for CH<sub>4</sub>  
235 production and CH<sub>4</sub> yield in cattle were 0.85 and 0.58, respectively (Velazco et al., 2016).  
236 Doreau et al. (2018) reported a wide range of correlation coefficients (0.33 to 0.78) for CH<sub>4</sub>  
237 production and yield using the SF<sub>6</sub> technique and the RC. Regression analyses between the  
238 RC and the CO<sub>2</sub>T showed positive relationships, consistent with the results of other study  
239 with the CO<sub>2</sub>T in lactating cows (Hellwing et al., 2013).

240 Repeatability reflects the consistent variation found in repeated measurements of the same  
241 subject under the same conditions (Nakagawa and Schielzeth, 2010). In this study, the  
242 repeatability values for CH<sub>4</sub> production and yield obtained by the CO<sub>2</sub>T were 0.5 and 0.49,  
243 respectively, which are higher than those reported by Haque et al. (2015), who reported  
244 repeatability values of 0.36 and 0.41 for CH<sub>4</sub> production by dairy cows determined using the  
245 CO<sub>2</sub>T. Both Haque et al. (2015) and the present study employed the same CO<sub>2</sub> method to  
246 measure CH<sub>4</sub> production; however, discrepancies in the breath sampling methodology  
247 between the two studies, including the frequency and duration of gas measurements and the  
248 measurement period, may have affected the repeatability of the results. Haque et al. (2015)

249 collected data during the milking process, while the cows were in an automatic milking  
250 system. In such a system, breath sampling must be conducted with the voluntary participation  
251 of the cows, which would have led to variations in the breath sampling conditions that could  
252 have affected the repeatability of the results. In contrast, we used a consistent method of  
253 breath sampling. The repeatability values obtained for the CO<sub>2</sub>T used in this study were  
254 comparable to those obtained for the SF<sub>6</sub> technique and the GreenFeed. For the SF<sub>6</sub>  
255 technique, Garnsworthy et al. (2019) reported a repeatability value of 0.44, while Arbre et al.  
256 (2016) obtained values ranging from 0.59 to 0.87. For the GreenFeed, a range of repeatability  
257 values was reported in studies by Coppa et al. (2021) (0.39 to 0.78) and Huhtanen et al.  
258 (2013) (0.64 to 0.81).

259 CV is a good overall index that represents the degree of precision (Nutter and Schultz,  
260 1995). In this study, the CO<sub>2</sub>T demonstrated comparable or lower CV values (2.55% to  
261 7.43%) for both between- and within-animal variation than those reported by Haque et al.  
262 (2015) (5.88% to 15.7%). Notably, our within-animal CV was lower than that reported by  
263 Haque et al. (2015), possibly due to differences in breath sampling conditions between the  
264 two studies. Moreover, the CV values obtained by the CO<sub>2</sub>T in this study were similar to  
265 those reported for the SF<sub>6</sub> technique by Vlaming et al. (2008) (6.91% and 10.09% for within-  
266 animal, and 6.23% and 27.79% for between-animal CH<sub>4</sub> yield variation) and Grainger et al.  
267 (2007) (6.1% for within-animal and 19.6% for between-animal CH<sub>4</sub> emissions variation).  
268 Similarly, for the GreenFeed, Rischewski et al. (2017) reported 11.5% within-animal CV for  
269 CH<sub>4</sub> production, and Van Wesemael et al. (2016) reported average of 13.1% within-animal  
270 CV and 11.1% between-animal CV. Overall, the CO<sub>2</sub>T showed comparable or better CV  
271 values than both the SF<sub>6</sub> technique and the GreenFeed.

272 The CO<sub>2</sub>T is suitable for large-scale and on-site measurements. In this study, only 20 min  
273 (10 min each for morning and afternoon) were required for daily CH<sub>4</sub> emission measurement

274 per animal, whereas the RC required 24 h. Due to the short sampling time, the CO<sub>2</sub>T can  
275 measure more animals than the RC in the same time period. In addition, the CO<sub>2</sub>T equipment  
276 can be easily transported and adapted to various field conditions, whereas the RC facility  
277 cannot be moved frequently. However, it is a limitation that the CO<sub>2</sub>T is an estimation  
278 method and cannot surpass the accuracy of the RC. Therefore, the CO<sub>2</sub>T is a suitable method  
279 for large scale and on-site field measurements, and it should be used with large sampling  
280 populations.

281

## 282 **5. Conclusion**

283 We compared the CO<sub>2</sub>T and the RC for determining CH<sub>4</sub> production and yield in Holstein  
284 heifers. There were no significant differences in CH<sub>4</sub> emissions between the two methods,  
285 with moderate positive correlations observed between the two. We conclude that the CO<sub>2</sub>T is  
286 a viable alternative to the RC for measuring CH<sub>4</sub> emissions, especially for on-site field  
287 measurements.

288

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294

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386 Tables

387 Table 1. Ingredients and chemical compositions of diets.

Items	Concentrate	Timothy hay
Concentrate ingredients, g/kg DM		
Corn gluten feed	350	
Wheat mill run	170	
Palm kernel meal	133	
Wheat fine	116	
Wheat flour	50	
Molasses	42.1	
Limestone	32.2	
Condensed molasses solubles	24	
Corn fine	23.9	
Copra meal	20	
Porphyry	18.9	
additive fat	10	
Ammonium chloride	2	
Salt	1.9	
Vitamin/Mineral premix <sup>1</sup>	5.9	
Total	1000	
Chemical composition, g/kg DM		
Dry matter (DM)	892	906
Crude protein (CP)	161	152
Ether extract (EE)	28.4	23.6
Neutral detergent fiber (NDF)	303	620
Acid detergent fiber (ADF)	117	351
Non-fiber carbohydrates (NFC) <sup>2</sup>	408	123
Ash	100	81.3
Calcium	15	3.4
Phosphorus	6.4	3.9
Gross Energy (GE), MJ/kg DM	18.1	18.6

388 <sup>1</sup>Mineral and vitamin premix contained vitamin A, 2,650,000 IU; vitamin D3, 530,000 IU;  
389 vitamin E, 1,050 IU; niacin, 10,000 mg; Mn, 4,400 mg; Zn, 4,400 mg; Fe, 13,200 mg; Cu,  
390 2,200 mg; I, 440 mg; Co, 440 mg per kg of additive (Grobc- DC, Bayer Health Care,  
391 Leverkusen, Germany).

392 <sup>2</sup>NFC (g/kg) = 1000 - (CP + EE + ash + NDF).

393 Table 2. Dry matter intake (DMI; kg/day), CH<sub>4</sub> production (L/day, L/kg BW<sup>0.75</sup>), and CH<sub>4</sub>  
394 yield (L/kg DMI) of Holstein heifers measured by the respiration chamber (RC) and CO<sub>2</sub>  
395 method (CO<sub>2</sub>T).

Items	RC	CO <sub>2</sub> T	<i>P</i>
DMI, kg/day	5.34 ± 0.1	5.43 ± 0.2	0.16
CH <sub>4</sub> , L/d	127 ± 34	120 ± 7.9	0.49
CH <sub>4</sub> , L/DMI kg	23.5 ± 5.9	21.6 ± 1.1	0.28
CH <sub>4</sub> , L/kg BW <sup>0.75</sup>	2.05 ± 0.03	2.16 ± 0.5	0.63

396 BW, body weight.

397 Values indicate means and standard deviations (mean ± SD).

398 Table 3. Correlation coefficients, repeatability, and coefficient of variation (CV) of the  
 399 respiration chamber (RC) and CO<sub>2</sub> method (CO<sub>2</sub>T) for CH<sub>4</sub> production and yield.

Items	CH <sub>4</sub> production, L/day		CH <sub>4</sub> yield, L/kg DMI	
	RC	CO <sub>2</sub> T	RC	CO <sub>2</sub> T
Spearman correlation coefficient	0.67*		0.61†	
Repeatability	0.66	0.65	0.58	0.49
SEM	10.9	2.51	1.88	0.35
SD	34.4	7.94	5.95	1.09
Between-animal CV, %	32.9	7.43	31	6.33
Within-animal CV, %	7.77	2.55	9.91	3.05

400 DMI, dry matter intake; SEM, standard error of the mean; SD, standard deviation.

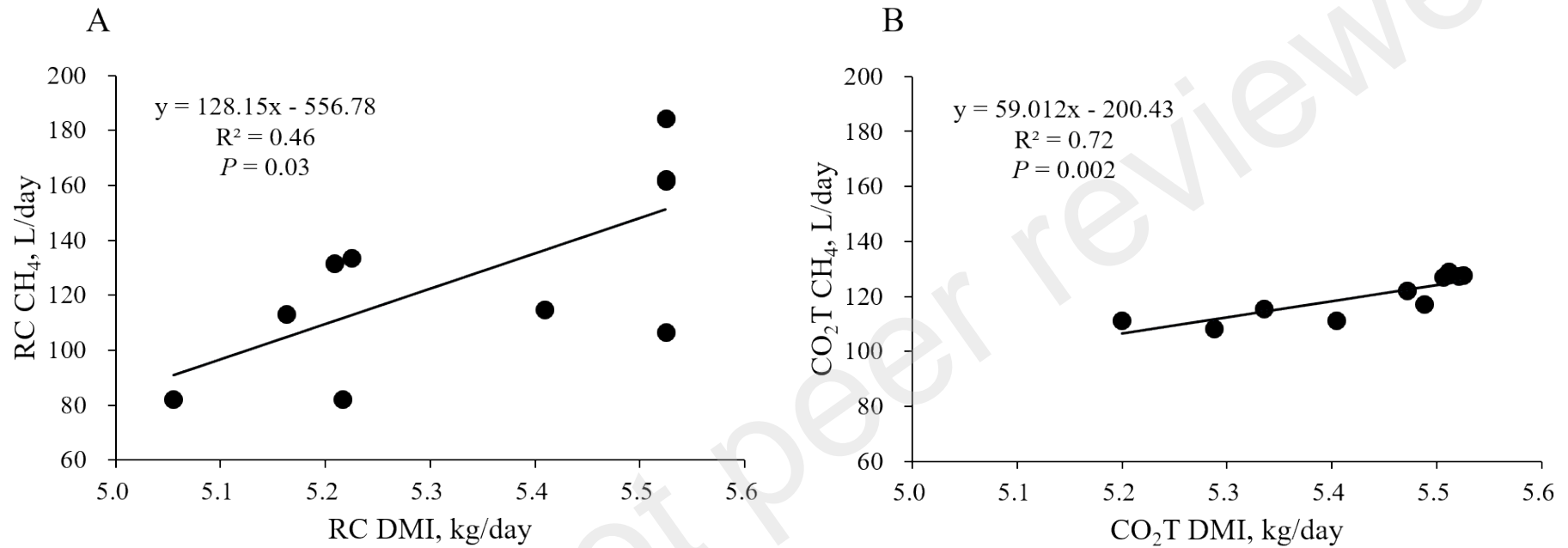
401 \*,  $P < 0.05$ ; †,  $0.05 < P < 0.1$ .

402 Table 4. CH<sub>4</sub> production (L/day) estimated by CO<sub>2</sub>T calculation method using data from  
403 either respiration chamber (CO<sub>2</sub>RC) or CO<sub>2</sub> method (CO<sub>2</sub>T).

Items	CO <sub>2</sub> RC	CO <sub>2</sub> T	<i>P</i>
CH <sub>4</sub> , L/day	127	136	0.66
SD	52.5	49.5	
SEM	9.58	9.03	

404 SD, standard deviation; SEM, standard error of the mean.

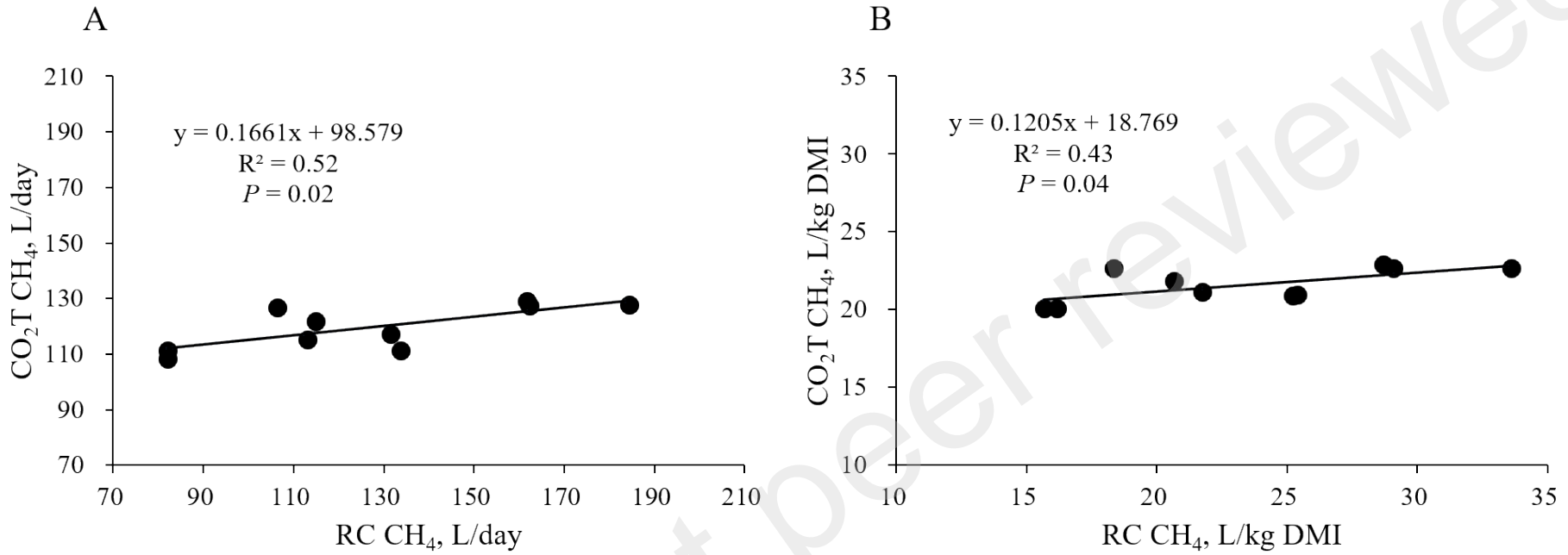
405 **Figures**



406

407 Fig. 1. Linear regressions between dry matter intake (DMI, kg/day) and CH<sub>4</sub> production (L/day) measured by the respiration chamber (RC) (A)

408 and CO<sub>2</sub> method (CO<sub>2</sub>T) (B).



409

410 Fig. 2. Linear regression between the respiration chamber (RC) and CO<sub>2</sub>T method (CO<sub>2</sub>T) for CH<sub>4</sub> production (L/day) (A) and CH<sub>4</sub> yield (L/kg DMI) (B).

411 DMI, dry matter intake.

412