AARHUS UNIVERSITY

Coversheet

This is the accepted manuscript (post-print version) of the article.

Contentwise, the accepted manuscript version is identical to the final published version, but there may be differences in typography and layout.

How to cite this publication

Please cite the final published version:

Difford, G. F., Olijhoek, D. W., Hellwing, A. L. F., Lund, P., Bjerring, M. A., de Haas, Y., Løvendahl, P. (2019). Ranking cows' methane emissions under commercial conditions with sniffers versus respiration chambers. *Acta Agriculturae Scandinavica A: Animal Sciences*. DOI: 10.1080/09064702.2019.1572784

You can download the published version at:

<https://doi.org/10.1080/09064702.2019.1572784>

Publication metadata

Title:	Ranking cows' methane emissions under commercial conditions with sniffers versus respiration chambers
Author(s):	Difford, G. F., Olijhoek, D. W., Hellwing, A. L. F., Lund, P., Bjerring, M. A., de Haas, Y., Løvendahl, P.
Journal:	Acta Agriculturae Scandinavica A: Animal Sciences
DOI/Link:	https://doi.org/10.1080/09064702.2019.1572784
Document version:	Accepted manuscript (post-print)

This version is distributed under a non-commercial no derivatives Creative Commons ([CC-BY-NC-ND](#)) user license, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited and not used for commercial purposes. Further, the restriction applies that if you remix, transform, or build upon the material, you may not distribute the modified material.

General Rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognize and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

If the document is published under a Creative Commons license, this applies instead of the general rights.

1 **Ranking cows' methane emissions under commercial conditions with sniffers versus**
2 **respiration chambers**

3 **G.F. Difford** ^{1,2*}, **D. W. Olijhoek**^{1,3}, **A. L. F. Hellwing**³, **P. Lund**³, **M. A. Bjerring**³, **Y. de**
4 **Haas**², **J. Lassen**¹, **P. Løvendahl**¹

5 ¹Center for Quantitative Genetics and Genomics, Department of Molecular Biology and Genetics,
6 Aarhus University, PO Box 50, DK-8830 Tjele, Denmark

7 ²Animal Breeding and Genomics Centre, Wageningen University, P.O. Box 338, 6700 AH
8 Wageningen, Netherlands

9 ³Department of Animal Science, Aarhus University, AU-Foulum, P.O. Box 50, DK-8830 Tjele,
10 Denmark

11

12

13 *Corresponding author: Gareth Difford

14 Email: gareth.difford@mbg.au.dk

15

16

17

18

19 **ABSTRACT:**

20 The objective of the study was to assess the ranking of dairy cows using individual level
21 correlations for methane (CH₄) emission phenotypes on-farm using sniffers and in respiration
22 chambers. In total 20 lactating dairy cows, ten Danish Holstein and ten Danish Jerseys were
23 recorded using sniffers installed in milking robots for three weeks of lactation prior to relocation
24 and acclimation at the respiration chamber (RC) facility where they were each recorded on three
25 occasions within the RC. Pairwise bivariate linear mixed models were used to determine the
26 individual level correlations (r_I) between sniffer phenotypes and RC phenotypes as proxies for
27 genetic correlations. Despite differences in feeding and management, the predicted CH₄ production
28 on farm from sniffers correlated well with CH₄ production in the RC (CH₄_RC) $r_I = 0.77 \pm 0.18$
29 and the direct CH₄ breath concentration (CH₄_C) correlated nearly as well with CH₄_RC $r_I = 0.75$
30 ± 0.20 . The correlations between CH₄ emission phenotypes on-farm from sniffers and CH₄_RC
31 exceeded that of energy corrected milk yield, live weight and dry matter intake demonstrating the
32 potential of sniffers measurements as large-scale indicator traits for CH₄ emissions in dairy cattle.

33 **Keywords:** Methane, sniffers, breath concentration, respiration chambers

34 **Introduction**

35 Methane (CH₄) is a potent greenhouse gas produced by dairy cattle and other ruminants as a natural
36 by-product of fermentation. Research on mitigation strategies such as nutritional additives,
37 vaccines, and genetic improvement, has gained impetus in recent years (Hill et al., 2016). Whilst a
38 reliable and accurate measure of CH₄ emission forms the basis of evaluating all of the
39 aforementioned strategies, they differ in requirements of accuracy and precision as well as the
40 number, frequency and duration of measurement (Hammond et al., 2016). For instance, genetic
41 evaluations require measurements on large numbers of animals under the environmental conditions

42 they are expected to perform in order to obtain accurate estimated breeding values (EBV) (Falconer
43 and Mackay, 1996). The accuracy of EBV is in part conditional on the accuracy and precision of the
44 phenotypes recorded. However, the accuracy of EBVs can be increased through increasing the
45 number of records per animal and/or increasing the sample size of related animals recorded (Mrode,
46 2003), i.e. increasing throughput of less precise phenotypes can still result in accurate EBVs.

47 Indirect calorimetry respiration chambers (RC) are the ‘gold standard’ for CH₄ emission, meaning
48 RC are regarded as the most accurate and precise measurement from which different instruments or
49 techniques are benchmarked (Grainger et al., 2007; Hill et al., 2016). The high accuracy and
50 precision of RC have the benefit of detecting relatively small effects of diets and treatments on CH₄
51 production with small numbers of animals (Patra, 2016). However, the capital investment cost for
52 RC are high and the method is labour intensive, proving to be prohibitive to obtaining
53 measurements on large numbers of animals (Grainger et al., 2007; Madsen et al., 2010).
54 Furthermore, the effects of confinement within the chamber may alter animal behaviour and are not
55 necessarily representative of all production systems like extensive grazing systems (Storm et al.,
56 2012), although promising developments have led to reductions in costs and reduced stress due to
57 confinement (Hellwing et al., 2012). Thus the merit of genetic selection under RC conditions and
58 the expectation of reduced CH₄ emissions under environmental conditions in which animals are
59 expected to perform, have been called into question (Lassen and Løvendahl, 2016).

60 A method that has proven well suited to obtaining large numbers of records on individual animals
61 under commercial conditions is the high-throughput, cost effective, and non-invasive ‘sniffers’
62 installed in the feed bin of automated milking stations (AMS) or concentrate feeders (Garnsworthy
63 et al., 2012; Lassen et al., 2012; Negussie et al., 2016). The non-invasiveness is achieved by
64 limiting the animal-to-instrument interface, so that the animal is not aware it is being measured and
65 measurement does not disrupt farm activities. The disadvantages of this are a loss of precision due

66 to variable barn gas dynamics and added noise if head movement of the cow and background barn
67 gas levels are not accounted for (Huhtanen et al., 2015; Difford et al., 2016; Wu et al., 2018).
68 Furthermore, sniffer methods record the concentration of gases in the captured breath of the cow
69 during milking and are thus a spot measure of gas concentrations and not a full 24 hour mass flux
70 measure (Huhtanen et al., 2015). Rather they utilise the ratio of measures CH₄ to carbon dioxide
71 (CO₂) and predicted CO₂ as a tracer gas to approximate CH₄ production (Madsen et al., 2010).
72 New methods which are cheaper, faster or less invasive are continually under development and are
73 of value when the gold standard proves expensive or prohibitive to largescale recording. For genetic
74 evaluations, a new method can replace a gold standard method if the new method is heritable and
75 their genetic correlation (r_G) exceeds 0.80 (Robertson, 1959). If the r_G is moderate and the new
76 method is heritable, it can be an indicator trait (Negussie et al., 2017). However, the number of
77 related animals with simultaneous measurements from both methods required to accurately estimate
78 r_G with meaningful standard errors, is around 10^3 - 10^4 of animals (Visscher, 1998). If measurements
79 with both methods are taken on different animals or the same animals at different time points, the
80 required numbers for accurate estimation of r_G will be even higher (Bijma and Bastiaansen, 2014).
81 For CH₄ emission, the RC constitutes a separate environment, as other methods cannot be recorded
82 simultaneously within the RC. It is of interest to determine the ranking of animals prior to the
83 investment in thousands of records using both methods, particularly when the gold standard is
84 expensive. To this end, estimating the repeatability of each method and the individual level
85 correlations (r_I) between methods is of value. The repeatability of a method serves as the upper
86 threshold for heritability estimates and r_I serve as proxies for r_G , respectively (Falconer and
87 Mackay, 1996; Wolak et al., 2012). The objective of this study was to assess the consistency in
88 ranking of dairy cows for CH₄ emission using sniffers and respiration chambers.

89 **Materials and Methods:**

90 ***Design and Animals***

91 This experiment was designed to compare CH₄ measurements from the non-invasive sniffer method
92 during AMS milking with the open circuit RC, which is the intensive and traditional method. The
93 link between the two methods was obtained by having 20 cows (ten Holstein and ten Jersey)
94 measured first by the sniffer in a commercial setting and then transferred to the RC facility. All
95 handling of animals was conducted according to a protocol approved by The Animal Experiments
96 Inspectorate, Ministry of Environment and Food of Denmark (Approval number 2016-15-0201-
97 00959).

98 ***Sniffer AMS measurement***

99 Data on CH₄ and CO₂ gas concentrations from the breath of individual Holstein and Jersey cows
100 recorded during milking at Danish Cattle Research Centre (DCRC, Foulum, Denmark), where
101 sniffer sensors were installed in each of the three AMS (DeLaval International AB, Tumba,
102 Sweden). The sniffer instrumentation comprises two sensors, the CH₄ sensor (Guardian NG,
103 Edinburgh Instruments Ltd, Livingston, UK) and the CO₂ sensor (Gascard, Edinburgh Instruments
104 Ltd, Livingston, UK). The equipment installation, and calibration procedures for the sensors are
105 described elsewhere (Difford et al., 2016). The DCRC barn is a free-stall housing system with
106 individual cubicles and cows were offered TMR with an approximate forage to concentrate ratio of
107 (70:30) ad libitum in individualized feeding troughs (RIC-system, Insentec, Marknesse, The
108 Netherlands). Cows were provided up to 3 kg of concentrate per day within the feed bin of the
109 AMS, based on levels of production and thus differences in the forage:concentrate ratio between
110 cows is expected. Cows had free access to AMS and presented on average 2.4 ± 0.86 visits/d (mean
111 $\pm$ SD) during the measurement period. Data on live weight is recorded 10 times every second
112 during AMS milking and processed as described in (Bossen et al., 2009). Milk production from the

AMS and fat, protein and lactose percentage estimated from 48 hour periods each week (Løvendahl and Bjerring, 2006). The estimated milk components were used to correct milk production for fat, protein, and lactose content (ECM) (Sjaunja et al., 1991).

Gas concentrations from the AMS was aligned and merged with the entrance and exit time for each cow visit to the AMS. Data was omitted when the cow's head was predicted to be outside the feed bin using the algorithm described in Difford et al. (2016). The CH₄ and CO₂ gas concentrations for the morning cleaning cycle, when the AMS is empty of any cow, were taken as the ambient barn concentrations for each day, and deducted from the means of each cow visit. The starting time for each visit in the AMS was converted to 24 hour angular radians for modelling of diurnal variation (Lassen et al., 2012). The following model was used on all available DCRC data, within AMS to obtain daily visits corrected for sensor drift, daily variation, and time of day:

$$y_{ijklm} = \mu + d_i + b_j + \sum_{k=1}^3 (f_{1k} \sin \theta + f_{2k} \cos \theta) + C_l + e_{ijklm} \quad (1)$$

where y_{ijklm} is the natural logarithm of background corrected AMS visit means of CH₄ and CO₂; d_i is the effect of test day i ($i = 23$ d); b_j is the effect of the first day after each calibration j ($j = 3$); f_{1k} and f_{2k} are regression coefficients of Fourier series linear covariates of the time of day of measurement, modelled as harmonic pairs. The time of day of visit expressed as 24 hour angular radians is denoted by θ . Term C_l is the random effect parameter for each cow $C_l \sim ND(0, I\sigma^2_c)$, and e_{ijklm} is the residual $\sim ND(0, I\sigma^2_e)$. In order to correct daily AMS visit means, the residuals for each visit are combined with random cow solutions, intercept, calibration day, and the regression coefficients f_{1k} and f_{2k} multiplied by the angular radian corresponding to 12:00:00 a.m. Further, the CH₄ and CO₂ concentration in ppm was averaged per week of lactation, using an average of visits weighted by the duration of visits. The average weekly CH₄ and CO₂ concentrations were natural log transformed, here after defined as CH₄_C and CO₂_C and combined with the weekly

136 performance data from DCRC for ECM (ECM_C), LW (LW_C), and gestation length (GL). The
137 average daily CH₄ production per week of lactation (CH₄_P; L CH₄/d) was calculated using the
138 ratio of CH₄_C to CO₂_C and the equation for CO₂ production from heat production units (HPU)
139 which utilizes ECM_C, LW_C, and GL (CIGR, 2002) and the conversion from HPU to CO₂ to
140 obtain predicted CO₂ production (CO₂_P; L CO₂/d) (Pedersen et al., 2008) as suggested by Madsen
141 et al. (2010). Data for the 10 Holstein and 10 Jersey cows from the last three weeks prior to
142 relocation to the facilities with the RC was retained for further analysis together with RC records.

143 ***Respiration chamber measurements***

144 The 10 lactating Holstein cows and 10 lactating Jersey cows were entered into a trial containing two
145 dietary treatments, a control and a high concentrate diet with respective forage to concentrate ratios
146 of (68:32) and (39:61) in a cross-over design with back-cross (Olijhoek et al., 2018). The trial was
147 divided into three periods with blocking consisting of four cows per block (five blocks in total per
148 period) with the same cows in each block over periods. Each cow was recorded for duration of 3 d
149 in period 1 and 2 d in periods 2 and 3. Methane and CO₂ production in the RC (CH₄_RC and
150 CO₂_RC) was calculated from the product of the total flow of outgoing air at standard temperature
151 and pressure, and the difference between the gas concentrations in the outgoing air and the gas
152 concentrations in the incoming air from the barn (background). Methane and CO₂ concentration
153 readings from when the chambers were opened twice daily for milking were omitted before
154 calculating the average CH₄ and CO₂ production for each cow, during each period. Live weight was
155 recorded when cows entered and left the RC and the average of these two readings taken from the
156 RC recording period (LW_RC) as per Olijhoek et al. (2018). Milk yield was recorded during
157 milking in the RC and composition determined from two subsequent milkings. The average milk
158 yield and milk composition was used to determined average energy corrected milk yield in the RC
159 (ECM_RC) during RC recording periods (Sjaunja et al., 1991; Olijhoek et al., 2018). The records

160 from these cows were retained for comparison with sniffer measures recorded at DCRC for the
161 same 20 cows.

162 *Statistical Analysis*

163 Pairwise bivariate animal repeatability models were used to estimate variance components
164 controlling for fixed effects. All analyses were performed using DMU version 6 (Madsen and
165 Jensen, 2014). The model for the RC traits was as follows:

$$166 \quad y_{ijklmn} = \mu + B_i + P_j + L_k + D_l + C_m + e_{ijklmn} \quad (2)$$

167 Where y_{ijklmn} is the trait of interest (CH4_RC, CO2_RC, LW_RC and ECM_RC), μ is the intercept,
168 B is the i'th breed (I = 2 levels), P is the j'th effect of block nested in period (j = 12 levels), L is the
169 k'th lactation number (k = 3 levels), D is the l'th effect of diet (l = 2 levels), C_m is the random effect
170 of the m'th cow $C_m \sim ND(0, I\sigma^2_c)$, and e is the residual $\sim ND(0, I\sigma^2_e)$.

171 The model for the on-farm traits was as follows:

$$172 \quad y_{ijklm} = \mu + W_i + BR_j + L_k + C_l + e_{ijklm} \quad (3)$$

173 Where y_{ijklm} is the trait of interest (CH4_P, CO2_P, CH4_C, CO2_C, LW_C and ECM_C), μ is the
174 intercept, W is the i'th week of lactation (I = 2 levels), BR is the j'th breed nested within AMS (j = 3
175 levels), L is the k'th lactation number effect (k = 3 levels), C_l is the random effect of the l'th cow C_l
176 $\sim ND(0, I\sigma^2_c)$, and e is the residual $\sim ND(0, I\sigma^2_e)$.

177 For all pairwise comparisons between RC and on-farm traits it was necessary to restrict residual
178 covariance to zero as cows were recorded in different environments. Repeatability estimates (t)
179 were obtained from the variance components by using the equation:

$$180 \quad t = \frac{\sigma_c^2}{(\sigma_c^2 + \sigma_e^2)}$$

181 Individual level correlations (rI), were computed as the correlation between random cow (4)
182 effects using variance components as show in equation 4:

$$183 \quad rI = \frac{\sigma^2_{c1,c2}}{\sqrt{\sigma^2_{c1}} \cdot \sqrt{\sigma^2_{c2}}} \quad (5)$$

184 The standard errors of the individual level correlations and repeatability estimates where derived
185 using Taylor series approximations.

186 **Results and Discussion**

187 The descriptive statistics for sniffer and RC phenotypes can be found in Table 1. The sniffer
188 predicted mass flux CH₄_P and CO₂_P were closer to that of RC mass flux phenotypes CH₄_RC
189 and CO₂_RC albeit with lower means, higher variability and consequently higher coefficients of
190 variation (CV). The sniffer breath concentration phenotypes (CH₄_C and CO₂_C) were more
191 different from predicted and measured mass flux phenotypes, with lower means, higher variability
192 and CV. All CH₄ and CO₂ phenotypes where moderately to highly repeatable ranging from $t = 0.53$
193 for CH₄_C to $t = 0.87$ for CO₂_P. Live weight and ECM were retained as control variables to
194 ensure that the RC environment was not considerably different from that of the on-farm
195 environment for these production traits. The means, SD and CV for LW and ECM were compared
196 across environments with similar descriptive statistics and repeatability estimates for example
197 LW_C and LW_RC had similar means 568.2 vs 564.5 kg and repeatability $t=0.93$ and $t=0.98$,
198 respectively. Recognizing that in the case of the sniffer phenotypes, they are an average of many
199 measurements from the AMS over a full week of lactation whereas RC phenotypes are the average
200 of measurements over a 2 – 3 d period in the RC.

201 The individual level correlations between sniffer and RC phenotypes are reported in Table 2. For
202 ECM and LW the individual level correlations (r_i) between on-farm AMS and RC were close to

203 unity 0.86 ± 0.15 and 0.92 ± 0.04 , respectively. These r_I indicate very similar ranking of cows
204 across environments, suggesting confinement within the RC had limited effects on ranking for these
205 traits. Many studies have reported that confinement within RC alters behavior and can induce stress
206 resulting in a drop in feed intake (Beauchemin and McGinn, 2006; Llonch et al., 2016). However,
207 the RC in the present study were constructed from transparent polycarbonate to reduce costs and
208 increase cow welfare, as supported by a study describing that these RC provoked no drop in DMI,
209 (Hellwing et al., 2012).

210 Sniffer CH4_P showed the highest r_I of any on-farm phenotype with CH4_RC 0.77 ± 0.18 , which
211 approaches the suggestive threshold of 0.80 for no significant re-ranking, however the standard
212 errors thereof are large. This finding agrees with that of Hellwing et al (2013), who compared CH4
213 production calculated from the ratio of CH4 to CO2 measured within the RC and predicted CO2
214 production to CH4_RC and CO2_RC for 157 cow measurements in 8 feeding experiments and
215 found an R^2 of 55% corresponding to a correlations $R = 0.74$. The performance of CH4_P is reliant
216 on the accuracy of CO2_P as a predicted tracer gas, the r_I between CO2_P and actual measured CO2
217 production (CO2_RC) was similarly high $r_I = 0.79 \pm 0.14$ as that of CH4_P and CH4_RC. A
218 criticism of this CO2 prediction equation is that the metabolizable energy (ME) efficiency and
219 mobilization of body tissues is not taken into account, running the risk of over predicting CO2
220 production of efficient cows (increased LW and ECM at a fixed level of intake) and under
221 predicting CO2 production of inefficient cows (Madsen et al., 2010; Huhtanen et al., 2015). It may
222 be possible to improve the r_I between CH4_P and CH4_RC through improving the prediction
223 accuracy of CO2_RC by taking into account ME utilization. For instance, Negussie et al (2016)
224 compared CH4_P in the breath of 20 lactating Nordic Red cattle from concentrate feeders and
225 CO2_P predicted from ME intake and found CH4_P to have a high concordance correlation
226 coefficient 0.70 and phenotypic correlation 0.80 with CH4_RC.

227 Since all prediction equations have some level of inherent error and traits used in the prediction of
228 CO₂_P, e.g. ECM_C, are already in the breeding goal, there is interest in assessing value of directly
229 measured traits like CH₄_C and CO₂_C with RC traits. In this instance CH₄_C ranked animals
230 comparatively well with CH₄_RC $r_1 = 0.75 \pm 0.20$ as compared to CH₄_P and CH₄_RC $r_1 = 0.77 \pm$
231 0.18 and exceeded that of commercial control variables ECM_C, LW_C and DMI_C, which are
232 routinely used to predicted CH₄ production (Ramin and Huhtanen, 2013).

233 A number of authors have labelled breath gas concentration measures as imprecise (Huhtanen et al.,
234 2015; Goopy et al., 2016; Wu et al., 2018), which is congruent with our results as seen by the lower
235 repeatability estimates of CH₄_C and CO₂_C and the increased CV. However, the aforementioned
236 studies often compare mass flux CH₄ production (g/day) to CH₄ breath concentrations using the
237 coefficient of determination (R^2) and its radicand Pearson's correlation coefficient R, with the
238 expectation that a deviation of R^2 or R from unity (1.0) indicates imprecision. Recognizing that
239 CH₄_C is a separate, by likely correlated trait from CH₄_RC, deviations in R^2 from 1.0 are to be
240 expected regardless of imprecision. Moreover, these studies often compute R in the presence of
241 repeated measures per subject without explicitly modelling the random effect of subject, which has
242 the effect of biasing correlations downwards when one or both of the traits has some imprecision
243 (i.e. $t < 0.80$), known as attenuation of error (Spearman 1904; Adolph & Hardin 2007). Conversely,
244 these studies often fail to account for non-genetic between subject variation (for instance parity,
245 lactation stage, breed etc.) which can inflate estimates of R. When repeatability ($t > 0.80$) and
246 precision is high in one or both phenotypes, and between subject non-genetic factors are accounted
247 for and single measurement are taken per subject, then the phenotypic correlation (r_P) can be a good
248 predictor of r_G (Cheverud, 1988; Roff, 1995). In the case of repeated measurements per subject
249 when one or both traits have some imprecision r_P is still biased downwards and it is necessary to
250 partition variation into between subject variation and within subject variation (i.e. residual error or

251 imprecision) and compute r_I which are one step closer to r_G (Adolph and Hardin, 2007; Dingemans
252 and Dochtermann, 2013). Individual level correlations have been used as proxies for r_G in difficult
253 or expensive to measure traits in dairy cattle such as DMI (Veerkamp et al., 2013), CH_4 production
254 (Zetouni et al., 2018) and energy balance (Løvendahl et al., 2010).

255 The r_G remains the most informative correlation metric for assessing how best to incorporate an
256 alternative method into a selection index. This point is illustrated in the development of the portable
257 accumulation chambers (PAC) which are a short-term total CH_4 emission flux method alternative to
258 RC used in sheep. Goopy et al. (2011) compared the two methods in 39 sheep, by measuring for 22
259 hours in the RC and then measuring 1 and 2 hours immediately after, in the PAC and found $r_P =$
260 0.67. In a subsequent genetic study on 3601 lambs with 4733 records in PAC and 8655 in RC,
261 Jonker et al. (2018) found $r_G = 0.67 \pm 0.11$ between the methods. In this case, r_P was a good proxy
262 for r_G but at a fraction of the cost and justified the investment in obtaining the thousands of records
263 required to accurately define r_G . Furthermore, the PAC is not genetically equivalent to the RC, but
264 has promise as a large scale, cost effective indicator trait. Importantly, Jonker et al. (2018) reported
265 $r_P = 0.27 \pm 0.02$ in the presence of repeated measurements and not r_I which was a biased predictor
266 of r_G . Given r_I for sniffer CH_4 traits ranged from 0.75-0.77 and were higher than PAC correlations
267 with RC, should these correlations be validated in a genetic evaluation, sniffers have the potential to
268 cost effectively generate the large scale recording of thousands of dairy cows for routine genetic
269 evaluation of CH_4 emissions.

270 These are the first r_I reported for any CH_4 recording method with the RC method. Method
271 comparisons to date have made inconsistent use of different correlation metrics. Garnsworthy et al.
272 (2012) recorded CH_4 production in 12 lactating Holstein cows for 10 days in the AMS using
273 sniffers and found an $R^2 = 0.78$ and $R = 0.88$ with a single record of CH_4 production from RC.
274 Similarly, Negussie et al. (2016) recorded CH_4_P using sniffer sensor installed in automatic

concentrate feeders on 22 Finnish Ayrshire cows with subsequent records for CH₄ production in RC and found ($R = 0.80$), repeated measures were not taken with the RC method nor were between subject non-genetic effects such as parity or lactation stage accounted for. Conversely, (Muñoz et al., 2004, 2012) compared CH₄ production from the SF₆ technique with the RC and had repeated measures per method per cow and controlled for technical factors such as bolus release rate, recording period etc. but failed to correct for breed, parity or lactation stage or to compute r_1 , instead reporting $R^2 = 0.69$ and $R = 0.83$. A single study comparing multiple alternative methods has reported repeated measures correlations (r_{RP}) taking into account repeated measures per cow per method (i.e. similar to r_1 without accounting for non-genetic between subject effects) (Sorg et al., 2018). Sorg et al. (2018) found an r_{RP} ranging from 0.57 - 0.74 for CH₄ production between the laser measuring device, GreenFeed and two sniffer systems in lactating Holstein cows from northern Europe. Although the different correlations metrics reported maybe be biased predictors of the r_G between methods, the r_1 reported herein for sniffer CH₄ phenotypes and CH₄ production in the RC are promising as indicator traits.

Conclusion

Methane emission traits derived from breath gas measurements during milking correlated the highest with CH₄ production in RC, exceeding that of LW, ECM and DMI. The individual level correlations with CH₄ production in the RC indicate that sniffer CH₄ traits have the potential to serve as large scale indicator traits of CH₄ production in the RC. Genetic correlations between RC phenotypes and breath gas phenotypes are still needed for effective use in genetic selection indices. Given the difficulties in acquiring suitably large numbers of cows in RC, the most feasible current way to obtain accurate genetic correlations between alternative methods and the RC is through international collaborations and incorporation of genomic information. Given the promising

individual level correlations between sniffer phenotypes and RC further research into genetic correlations between sniffers and RC for CH₄ emission is warranted.

Acknowledgements

G.F. Difford benefitted from an Erasmus-Mundus fellowship and a grant by the Innovation Fund Denmark project REMRUM, within the framework of the European Graduate School in Animal Breeding and Genetics. The authors would like to express gratitude to the EU COST-action METHAGENE network for discussions. The authors would like to thank the staff at DCRC for their assistance.

References

- Adolph, S.C., and J.S. Hardin. 2007. Estimating phenotypic correlations: Correcting for bias due to intraindividual variability. *Funct. Ecol.* 21:178–184. doi:10.1111/j.1365-2435.2006.01209.x.
- Beauchemin, K.A., and S.M. McGinn. 2006. Methane emission from beef cattle: effects of fumaric acid, essential oil and canola oil. *J. Anim. Sci.* 84:1489–1496. doi:10.2527/2006.8461489x.
- Bijma, P., and J.W.M. Bastiaansen. 2014. Standard error of the genetic correlation: how much data do we need to estimate a purebred-crossbred genetic correlation?. *Genet. Sel. Evol.* 46:79. doi:10.1186/s12711-014-0079-z.
- Bossen, D., M.R. Weisbjerg, L. Munksgaard, and S. Højsgaard. 2009. Allocation of feed based on individual dairy cow live weight changes. I: Feed intake and live weight changes during lactation. *Livest. Sci.* 126:252–272. doi:10.1016/j.livsci.2009.07.010.
- Cheverud, J.. 1988. A Comparison of Genetic and Phenotypic Correlations. *Evolution* (N. Y). 42:958–968.
- CIGR. 2002. 4th Report of Working Group on Climatization of Animal Houses: Heat and Moisture Production at Animal and House Levels. S. Pedersen and K. Sällvik, ed. Research Centre Bygholm. DIAS, Horsens, Denmark.
- Difford, G.F., J. Lassen, and P. Løvendahl. 2016. Interchangeability between methane measurements in dairy cows assessed by comparing precision and agreement of two non-invasive infrared methods. *Comput. Electron. Agric.* 124:220–226. doi:10.1016/j.compag.2016.04.010.
- Dingemanse, N.J., and N.A. Dochtermann. 2013. Quantifying individual variation in behaviour: mixed- effect modelling approaches. *J. Anim. Ecol.* 39–54. doi:10.1111/1365-2656.12013.
- Falconer, D., and T. Mackay. 1996. Introduction to quantitative genetics (4th edn). *Genet.*
- Garnsworthy, P.C., J. Craigon, J.H. Hernandez-Medrano, and N. Saunders. 2012. On-farm methane measurements during milking correlate with total methane production by individual dairy

331 cows. *J. Dairy Sci.* 95:3166–80. doi:10.3168/jds.2011-4605.

332 Goopy, J.P., R. Woodgate, A. Donaldson, D.L. Robinson, and R.S. Hegarty. 2011. Validation of a
 333 short-term methane measurement using portable static chambers to estimate daily methane
 334 production in sheep. *Anim. Feed Sci. Technol.* 166–167:219–226.
 335 doi:10.1016/j.anifeedsci.2011.04.012.

336 Grainger, C., T. Clarke, S.M. McGinn, M.J. Auldist, K. a Beauchemin, M.C. Hannah, G.C.
 337 Waghorn, H. Clark, and R.J. Eckard. 2007. Methane emissions from dairy cows measured
 338 using the sulfur hexafluoride (SF6) tracer and chamber techniques. *J. Dairy Sci.* 90:2755–
 339 2766. doi:10.3168/jds.2006-697.

340 Hammond, K.J., L.A. Crompton, A. Bannink, J. Dijkstra, D.R. Yáñez-Ruiz, P. O’Kiely, E.
 341 Kebreab, M.A. Eugenè, Z. Yu, K.J. Shingfield, A. Schwarm, A.N. Hristov, and C.K.
 342 Reynolds. 2016. Review of current in vivo measurement techniques for quantifying enteric
 343 methane emission from ruminants. *Anim. Feed Sci. Technol.* 219:13–30.
 344 doi:10.1016/j.anifeedsci.2016.05.018.

345 Hellwing, A.L.F., P. Lund, J. Madsen, and M.R. Weisbjerg. 2013. Comparison of enteric methane
 346 production predicted from the CH4/CO2 ratio and measured in respiration chambers. *Adv.*
 347 *Anim. Biosci.* 4:6077–6085.

348 Hellwing, A.L.F., P. Lund, M.R. Weisbjerg, M. Brask, and T. Hvelplund. 2012. Technical note: test
 349 of a low-cost and animal-friendly system for measuring methane emissions from dairy cows. *J.*
 350 *Dairy Sci.* 95:6077–85. doi:10.3168/jds.2012-5505.

351 Hill, J., C. McSweeney, A.-D.G. Wright, G. Bishop-Hurley, and K. Kalantar-zadeh. 2016.
 352 Measuring methane production from ruminants. *Trends Biotechnol.* 34:26–35.
 353 doi:10.1016/j.tibtech.2015.10.004.

354 Huhtanen, P., E.H. Cabezas-Garcia, S. Utsumi, and S. Zimmerman. 2015. Comparison of methods
 355 to determine methane emissions from dairy cows in farm conditions. *J. Dairy Sci.* 98:3394–
 356 3409. doi:10.3168/jds.2014-9118.

357 Jonker, A., S.. Hickey, S.. Rowe, P.. Janssen, G. Shackell, S. Elmes, W.. Bain, J. Wing, G.. Grees,
 358 B. Bryson, S. Maclean, K.. Dodds, C.. Pinares-Patiño, E.. Young, K. Knowler, N.. Pickering,
 359 and J.. McEwan. 2018. Genetic parameters of methane emissions determined using portable
 360 accumulation chambers in lambs and ewes grazing pasture and genetic correlations with
 361 emissions determined in respiration chambers. *J. Anim. Sci.* 1–29.
 362 doi:10.1093/ntr/nty008/4823697.

363 Lassen, J., and P. Løvendahl. 2016. Heritability estimates for enteric methane emissions from
 364 Holstein cattle measured using noninvasive methods. *J. Dairy Sci.* 99:1959–1967.
 365 doi:10.3168/jds.2015-10012.

366 Lassen, J., P. Løvendahl, and J. Madsen. 2012. Accuracy of noninvasive breath methane
 367 measurements using Fourier transform infrared methods on individual cows. *J. Dairy Sci.*
 368 95:890–8. doi:10.3168/jds.2011-4544.

369 Llonch, P., S.M. Troy, D. Carol-Anne, S. Miguel, R. John, H.M. J., R. Rainer, and T.S. P. 2016.
 370 Changes in feed intake during isolation stress in respiration chambers may impact methane
 371 emissions assessment. *Anim. Prod. Sci.*. doi:https://doi.org/10.1071/AN15563.

372 Løvendahl, P., and M. a Bjerring. 2006. Detection of carryover in automated milk sampling
 373 equipment.. *J. Dairy Sci.* 89:3645–3652. doi:10.3168/jds.S0022-0302(06)72404-3.

374 Løvendahl, P., C. Ridder, and N.C. Friggens. 2010. Limits to prediction of energy balance from
 375 milk composition measures at individual cow level.. *J. Dairy Sci.* 93:1998–2006.
 376 doi:10.3168/jds.2009-2739.

377 Madsen, J., B.S. Bjerg, T. Hvelplund, M.R. Weisbjerg, and P. Lund. 2010. Methane and carbon
 378 dioxide ratio in excreted air for quantification of the methane production from ruminants.
 379 *Livest. Sci.* 129:223–227. doi:10.1016/j.livsci.2010.01.001.

380 Madsen, P., and J. Jensen. 2014. A user's guide to DMU, version 6, release 5.0. Cent. Quant. Genet.
 381 Genomics Dept. Mol. Biol. Genet. Univ. Aarhus Res. Cent. Foulum Box 50, 8830 Tjele
 382 Denmark.

383 Mrode, R.A. 2003. *Linear Models in Animal Breeding*. Cabi Publishing, Cambridge.

384 Muñoz, C., T. Yan, A.W. Gordon, and D.A. Wills. 2004. Validation of the sulphur hexafluoride
 385 tracer technique for estimating methane emissions from dairy cows using respiration
 386 chambers : preliminary data Reproduced with permission of the copyright owner . Further
 387 reproduction prohibited without permission .

388 Muñoz, C., T. Yan, D.A. Wills, S. Murray, and A.W. Gordon. 2012. Comparison of the sulfur
 389 hexafluoride tracer and respiration chamber techniques for estimating methane emissions and
 390 correction for rectum methane output from dairy cows. *J. Dairy Sci.* 95:3139–48.
 391 doi:10.3168/jds.2011-4298.

392 Negussie, E., Y. de Haas, F. Dehareng, R.J. Dewhurst, J. Dijkstra, N. Gengler, D.P. Morgavi, H.
 393 Soyeurt, S. van Gastelen, T. Yan, and F. Biscarini. 2017. Invited review: Large-scale indirect
 394 measurements for enteric methane emissions in dairy cattle: A review of proxies and their
 395 potential for use in management and breeding decisions. *J. Dairy Sci.* 100:2433–2453.
 396 doi:10.3168/jds.2016-12030.

397 Negussie, E., J. Lehtinen, P. Mäntysaari, A.R. Bayat, A.-E. Liinamo, E.A. Mäntysaari, and M.H.
 398 Lidauer. 2016. Non-invasive individual methane measurement in dairy cows. *Animal* 4:1–10.
 399 doi:10.1017/S1751731116002718.

400 Olijhoek, D.W., P. Løvendahl, J. Lassen, A.L.F. Hellwing, J. Höglund, M.R. Weisbjerg, S.J. Noel,
 401 F. McClean, O. Højberg, and P. Lund. 2018. Methane production, rumen fermentation, and
 402 diet digestibility of Holstein and Jersey dairy cows being divergent in residual feed intake and
 403 fed at two forage-to concentrate ratios. *J. Dairy Sci.* Accepted.
 404 doi:https://doi.org/10.3168/jds.2017-14278.

405 Patra, A.K. 2016. Recent advances in measurement and dietary mitigation of enteric methane
 406 emissions in ruminants. *Front. Vet. Sci.* 3:1–17. doi:10.3389/fvets.2016.00039.

407 Pedersen, S., V. Blanes-Vidal, H. Joergensen, A. Chwalibog, A. Haeussermann, M.J.W. Heetkamp,
 408 and A.J.A. Aarnink. 2008. Carbon Dioxide Production in Animal Houses: A literature review.
 409 *Agric. Eng. Int. CIGR Ejournal* X:1–19. doi:10.1017/CBO9781107415324.004.

410 Ramin, M., and P. Huhtanen. 2013. Development of equations for predicting methane emissions
 411 from ruminants. *J. Dairy Sci.* 96:2476–2493. doi:10.3168/jds.2012-6095.

412 Robertson, A. 1959. The Sampling Variance of the Genetic Correlation Coefficient. *Biometrics*

413 15:469–485.

414 Roff, D.A. 1995. The estimation of genetic correlations from phenotypic correlations: A test of
415 cheverud’s conjecture. *Heredity* (Edinb). 74:481–490. doi:10.1038/hdy.1995.68.

416 Sjaunja, L.O., L. Baevre, L. Junkkarinen, J. Pedersen, and J. Setälä. 1991. A Nordic Proposal for an
417 Energy Corrected Milk (ECM) Formula EAAP Publication 50: Performance Recording of
418 Animals. State of the Art 1990, Centre for Agricultural Publishing and Documentation
419 (PUDOC), Wageningen, the Netherlands.

420 Sorg, D., G.F. Difford, S. Mühlbach, B. Kuhla, H.H. Swalve, J. Lassen, T. Strabel, and M.
421 Pszczola. 2018. Comparison of a laser methane detector with the GreenFeed and two breath
422 analysers for on-farm measurements of methane emissions from dairy cows. *Comput. Electron.
423 Agric.* 153:285–294. doi:10.1016/j.compag.2018.08.024.

424 Veerkamp, R.F., J.E. Pryce, D.M. Spurlock, D.P. Berry, M.P. Coffey, P. Løvendahl, R. van der
425 Linde, J. Bryant, F. Miglior, Z. Wang, M. Winters, N. Krattenmacher, N. Charfeddine, J.
426 Pedersen, and Y. de Haas. 2013. Selection on Feed Intake or Feed Efficiency: A Position Paper
427 from gDMI Breeding Goal Discussions. *Interbull Bull.*

428 Visscher, P.M. 1998. On the sampling variance of intraclass correlations and genetic correlations.
429 *Genetics* 149:1605–1614.

430 Wolak, M.E., D.J. Fairbairn, and Y.R. Paulsen. 2012. Guidelines for estimating repeatability.
431 *Methods Ecol. Evol.* 3:129–137. doi:10.1111/j.2041-210X.2011.00125.x.

432 Wu, L., P.W.G.G. Koerkamp, and N. Ogink. 2018. Uncertainty assessment of the breath methane
433 concentration method to determine methane production of dairy cows. *J. Dairy Sci.* 101:1554–
434 1564. doi:10.3168/jds.2017-12710.

435 Zetouni, L., G.F. Difford, J. Lassen, M.V. Byskov, E. Norberg, and P. Løvendahl. 2018. Is
436 rumination time an indicator of methane production in dairy cows?. *J. Dairy Sci.* 1–12.
437 doi:10.3168/jds.2017-14280.

438

439

440

441 **Table 1.** Descriptive statistics for on farm measurements and respiration chamber measurements.

	Unit	Mean	SD	CV (%)	t ¹
On farm ²					
CH4_P	L/d	573	73.9	12.9	0.58 ± 0.11
CO2_P	L/d	6771	578.7	8.5	0.87 ± 0.11
CH4_C	ppm	410	137.0	33.4	0.53 ± 0.11
CO2_C	ppm	5746	1791.7	31.2	0.56 ± 0.12
CH4/CO2_C	ppm/ppm	0.071	0.009	12.7	0.38 ± 0.13
ECM_C	Kg/d	38.1	5.93	18.1	0.71 ± 0.08
LW_C	Kg	568.2	57.3	10.1	0.93 ± 0.02
DMI_C	Kg/d	22.2	4.9	21.5	0.73 ± 0.07
Respiration Chamber ³					

CH4_RC	L/d	521	56	10.7	0.61 ± 0.12
CO2_RC	L/d	6538	702.3	10.7	0.72 ± 0.10
CH4/CO2_RC	L/L	0.081	0.006	7.7	0.57 ± 0.14
ECM_RC	Kg/d	28.3	5.6	19.8	0.65 ± 0.12
LW_RC	Kg	564.5	62.3	11.0	0.98 ± 0.01

¹t = repeatability intraclass correlation coefficient. ²On farm phenotypes: CH4_P = predicted methane production; CO2_P = predicted carbon dioxide production; CH4_C = methane breath concentration; CO2_C = carbon dioxide breath concentration; CH4/CO2_C = ratio of methane to carbon dioxide breath concentration; ECM_C = Energy corrected milk yield; LW_C = live weight; DMI_C = dry matter intake. ³Respiration chamber phenotypes: CH4_RC = methane production; CO2_RC = carbon dioxide production; CH4/CO2_RC = ratio of methane to carbon dioxide production; ECM_RC = energy corrected milk yield; LW_RC = live weight.

Table 2 Individual level correlations between on farm phenotypes and respiration chamber phenotypes

On farm ¹	Respiration chamber ²				
	CH4_RC	CO2_RC	CH4/CO2_RC	ECM_RC	LW_RC
CH4_P	0.77 ± 0.18	0.63 ± 0.10	0.70 ± 0.24	0.68 ± 0.21	-0.09 ± 0.29
CO2_P	0.74 ± 0.13	0.79 ± 0.14	0.41 ± 0.29	0.58 ± 0.22	0.20 ± 0.27
CH4_C	0.75 ± 0.20	0.80 ± 0.16	0.03 ± 0.39	0.21 ± 0.35	0.60 ± 0.22
CO2_C	0.62 ± 0.24	0.76 ± 0.18	-0.35 ± 0.38	0.06 ± 0.40	0.69 ± 0.18
CH4/CO2_C	0.60 ± 0.27	0.29 ± 0.37	0.83 ± 0.23	0.68 ± 0.23	-0.66 ± 0.24
ECM_C	0.66 ± 0.20	0.54 ± 0.23	0.52 ± 0.26	0.86 ± 0.15	-0.14 ± 0.27
LW_C	0.54 ± 0.22	0.68 ± 0.16	-0.32 ± 0.33	-0.24 ± 0.28	0.92 ± 0.04
DMI_C	0.70 ± 0.17	0.64 ± 0.18	0.18 ± 0.33	0.33 ± 0.26	0.39 ± 0.22

¹On farm phenotypes: CH4_P = predicted methane production; CO2_P = predicted carbon dioxide production; CH4_C = methane breath concentration; CO2_C = carbon dioxide breath concentration; CH4/CO2_C = ratio of methane to carbon dioxide breath concentration; ECM_C = Energy corrected milk yield; LW_C = live weight; DMI_C = dry matter intake. ²Respiration chamber phenotypes: CH4_RC = methane production; CO2_RC = carbon dioxide production; CH4/CO2_RC = ratio of methane to carbon dioxide production; ECM_RC = energy corrected milk yield; LW_RC = live weight.