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INTERPRETIVE SUMMARY

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Genetic Background of Methane Emission by Dutch Holstein Friesian cows measured with Infrared Sensors in Automatic Milking Systems

Van Engelen et al.

Methane is a greenhouse gas and is produced in the rumen of dairy cows during the digestion of feed. A study was carried out to investigate whether it would be possible to breed for lower methane emission by using methane measurements obtained from infrared sensors during automatic milking. Part of the variation in the sensor measured methane was due to the genetic background of the cow. This indicates that measurements obtained from infrared sensors during automatic milking can be used to select for lower methane emission by dairy cows during breeding.

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13 **Genetic Background of Methane Emission by Dutch Holstein Friesian Cows**
14 **Measured with Infrared Sensors in Automatic Milking Systems**

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ABSTRACT

25

26

27 International environmental agreements have led to the need to reduce methane emission by
28 dairy cows. Reduction could be achieved through selective breeding. The aim of this study
29 was to quantify the genetic variation of methane emission by Dutch Holstein Friesian cows
30 measured using infrared sensors installed in automatic milking systems (AMS).
31 Measurements of methane (CH₄) and carbon dioxide (CO₂) on 1508 Dutch Holstein Friesian
32 cows located on 11 commercial dairy farms were available. Phenotypes per AMS visit were
33 the mean of CH₄ (CH₄mean), mean of CO₂ (CO₂mean), CH₄mean divided by CO₂mean
34 (Ratiomean), and their log₁₀-transformations CH₄log, CO₂log, and Ratiolog. The
35 repeatabilities of the log₁₀-transformed methane phenotypes were 0.27 for CH₄log, 0.31 for
36 CO₂log, and 0.14 for Ratiolog. The heritabilities of these phenotypes were 0.11 for CH₄log,
37 0.12 for CO₂log, and 0.03 for Ratiolog. These results indicate that measurements taken using
38 infrared sensors in AMS are repeatable and heritable and, thus, could be used for selection for
39 lower CH₄ emission. Furthermore, it is important to account for farm, AMS, day of
40 measurement, time of day, and lactation stage when estimating genetic parameters for
41 methane phenotypes. Selection based on CH₄log instead of Ratiolog would be expected to
42 give a greater reduction of CH₄ emission by dairy cows.

43

44 **Keywords:** methane emission, dairy cow, AMS, non-dispersive infrared sensor

45

46

INTRODUCTION

47

48 Agriculture contributes 24% of the total global greenhouse gas emissions (IPCC, 2014). The
49 single largest pollution source within agriculture is enteric fermentation, i.e., the breakdown

50 of feed in the rumen that results in the production of methane (**CH₄**) (Gerber et al., 2013).
51 Approximately 8% of the worldwide agricultural greenhouse gas emissions originate from
52 enteric **CH₄** emissions by dairy cows (FAOSTAT, 2012;FAO, 2014), showing the impact of
53 dairy production on global warming. In December 2015, agreements were made in Paris
54 between 195 countries to tackle climate change and keep global warming firmly below 2°C
55 (UNCCC, 2015). These agreements have further emphasized the importance of the reduction
56 of **CH₄** emission by dairy cows.

57 Reduction of **CH₄** emission by dairy cows can be achieved through a combination of
58 mitigation strategies comprising dietary, microbial, management, and breeding strategies
59 (Cottle et al., 2011;Hristov et al., 2013). Selective breeding has the advantage of giving a
60 cumulative, permanent, and long-term reduction of **CH₄** emission. An example of breeding for
61 reduced **CH₄** is the reduction of 13% of **CH₄** per kg of milk in Dutch dairy cattle that has been
62 realized from 1990 to 2010 by selection for higher milk production (Vellinga et al., 2011). A
63 further reduction of **CH₄** emission through selective breeding is wanted and requires
64 quantification of possible genetic variation in **CH₄** emission.

65 To quantify possible genetic variation in **CH₄** emission, **CH₄** emission needs to be measured
66 on large numbers of individual cows. One of the measurement methods could be infrared
67 sensors installed in automatic milking systems (AMS). The infrared sensor samples the breath
68 of the cows present in the AMS and measures **CH₄** and carbon dioxide (**CO₂**) concentration
69 continuously. An advantage of this system is that cows visit the AMS several times per day
70 and these repeated visits ensure repeated measurements of the same cow over the day and
71 over time (Garnsworthy et al., 2012a). Furthermore, sensors can easily be moved from one
72 AMS to another and, thus, provide the opportunity to measure individual **CH₄** emission on
73 large numbers of cows.

74 Previous studies have shown that CH₄ measurements based on infrared sensors in AMS are
75 repeatable. Lassen et al. (2012) summarized CH₄ measurements per AMS visit by taking the
76 mean of CH₄, the mean of CO₂ and the mean of the ratio between CH₄ and CO₂.
77 Repeatabilities ranged between 0.22 and 0.46 for 50 Holstein cows and 43 Jersey cows. Bell
78 et al. (2014b) found a repeatability of 0.74 for mean of CH₄ per AMS visit for 36 Holstein-
79 Friesian cows. These repeatabilities illustrate that infrared sensors in AMS could provide the
80 repeatable measurements on individual cows that are needed to quantify possible genetic
81 variation in CH₄ emission.

82 These CH₄ phenotypes can be influenced by farm conditions (Bell et al., 2014a), hour of the
83 day (Garnsworthy et al., 2012b), and week of lactation (Lassen et al., 2016); therefore, these
84 effects were studied. Farm conditions can impact CH₄ emission via the differences in feed
85 regimes between farms (Bell et al., 2014a; Hammond et al., 2016). Hour of the day can
86 influence CH₄ emission as cow behavior, time after feeding, and ambient conditions change
87 throughout the day (Garnsworthy et al., 2012b; Lassen et al., 2012; Bell et al., 2014b). Week of
88 lactation can affect CH₄ emission as the amount and composition of feed varies throughout
89 lactation (Garnsworthy et al., 2012b; Bell et al., 2014a; Lassen and Løvendahl, 2016).

90 Repeated measurements obtained from infrared sensors can be used to estimate the variation
91 in CH₄ emission between cows. Lassen and Løvendahl (2016) found genetic variation in CH₄
92 emission that was summarized in several phenotypes. The heritabilities ranged between 0.16
93 and 0.21, providing support for the use of CH₄ concentrations measured using infrared sensors
94 in AMS to decrease CH₄ emission through selective breeding.

95 The aim of this study was to quantify the genetic variation of CH₄ emission by Dutch dairy
96 cows measured using infrared sensors installed in AMS. The dataset comprised of CH₄ and
97 CO₂ measurements taken with infrared sensors on Dutch Holstein Friesian cows located on

98 commercial dairy farms. Measurements were summarized into different CH₄ phenotypes per
99 AMS visit and repeatability and heritability were calculated for these phenotypes.

100

101

MATERIALS AND METHODS

102

Ethical Statement

104 This research was accredited by the animal experimentation committee of Wageningen
105 University and Research and the central committee animal trials under application number
106 2013085 and trial code 2013097.

Methane Sensor

108 Methane phenotypes were measured using sensors. These sensors were tested in climate
109 respiration chambers (CRC) before they were installed on commercial farms. In this test, CH₄
110 emissions of 20 individual Holstein Friesian cows were recorded in CRC for 3 consecutive
111 days and, simultaneously, by the sensor. In the CRC, CH₄ and CO₂ were measured every 12.5
112 min as described by Heetkamp et al. (2015). The sensors were gas analyzers (SenseAir LPL
113 CH₄/CO₂, Rise Acreo, Stockholm, Sweden) that were installed in line with the cow's nostrils
114 when standing and facing forward. Air was drawn through the instrument at 1 l/min; CH₄ and
115 CO₂ concentrations were measured continuously using a non-dispersive infrared (NDIR)
116 technique, and logged twice per second. Phenotypes were defined as follows: CH₄ production
117 (l/d) from CRC; CH₄ concentration (ppm) from sensor; and CH₄:CO₂ ratio from sensor.

Data

119 CH₄ and CO₂ concentrations (ppm) were measured on 1508 primiparous and multiparous
120 dairy cows from 11 commercial farms in the Netherlands. On nine of these farms, cows were
121 fed in the morning, whereas on one farm cows were fed in the evening. In addition, some
122 farms had automatic feed pushers that compiled the feed continuously during the day, and one

123 farm had an automatic feeder that fed the cows freshly mixed feed up to 30 times a day.
124 Furthermore, cows on some farms could graze during the day whereas cows on other farms
125 were kept indoors. More than 85% of the cows were at least 7/8 Holstein Friesian.
126 Measurements were taken during milking in automatic milking systems (AMS; Lely
127 Astronaut A4, Lely Industries NV, Maassluis, the Netherlands) using NDIR sensors. A total
128 of four sensors were used to collect all data by installing them consecutively in different
129 AMS. Measurements were taken in a total of 23 AMS, one to four AMS per farm, between
130 November 2013 and March 2016. The data from these sensors were linked to the data from
131 the AMS to obtain the identification numbers (ID) of the cows and, subsequently, additional
132 animal information, such as week of lactation. The data of the sensors were aligned to the
133 AMS visits as both were recorded on different devices. The alignment between these devices
134 was based on the pattern of AMS visits, i.e., duration and order of AMS visits and the time
135 between the AMS visits. This pattern was aligned in such a way that CH₄ and CO₂
136 concentrations were highest during AMS visits and lowest in between AMS visits. After
137 alignment, the ID of the cows were used to link sensor data to data from the cooperative cattle
138 improvement organization CRV (Arnhem, the Netherlands) to obtain the pedigree. The
139 pedigree was traced back two generations, resulting in 4,214 animals in the pedigree.

140 *Data Editing*

141 Data from the sensors and AMS were edited based on several conditions. The first condition
142 was that only data from days with sensor measurements for at least 30% of the day were kept.
143 Days with less than 30% data were mostly without data or the data present were fragmentary
144 and these days were, therefore, discarded. The number of days with measurements ranged
145 from ten up to 81 per AMS. The second condition was that AMS visits should last at least 90
146 seconds and AMS visits shorter than 90 seconds were removed from the dataset. Removing
147 AMS visits shorter than 90 seconds ensured that most AMS visits that did not result in a

148 milking, and would, thus, not provide a steady measurement, were removed from the dataset.
149 The third condition was that the ID of the cow visiting the AMS should be known. AMS visits
150 without cow ID were removed from the dataset, as these could not be linked to the pedigree.
151 The fourth condition was that week of lactation of the cow visiting the AMS should be known
152 and cows should be between one and 60 weeks in lactation. The fifth condition was that AMS
153 visits with missing CH₄ phenotypes (see phenotypes) or missing model effects (see model 1)
154 were removed from the dataset. The sixth and last condition was that each cow should have at
155 least four AMS visits. After editing, a total of 129,900 AMS visits on 1,508 dairy cows that
156 had on average 86 AMS visits (range: 4-295 AMS visits) were available for analysis. The
157 number of cows ranged between 62 and 224 per farm.

158 AMS visits that had a standardized residual effect >3.5 based on model 1 for CH₄mean,
159 CH₄log, CO₂mean, CO₂log, Ratiomean or Ratiolog were considered outliers and were
160 removed. After removal of the outliers, the dataset consisted of 123,369 AMS visits from
161 1,508 dairy cows. This dataset was used to estimate the variance components and genetic
162 parameters.

163 *Phenotypes*

164 The CH₄ and CO₂ measurements were summarized per AMS visit into six phenotypes. To
165 correct for the background levels of CH₄ and CO₂ in the barn, offsets for CH₄ and CO₂ were
166 calculated per AMS visit. The background levels of CH₄ were assumed to be 0 ppm and the
167 offset for CH₄ was the mean of the 10 lowest values for CH₄ in a specific AMS visit. As the
168 background levels of CO₂ were assumed to be 400 ppm, the offset for CO₂ was the mean of
169 the lowest 10 values for CO₂ minus 400. The CH₄ offset was subtracted from the individual
170 (twice per second) CH₄ measurements during a specific AMS visit and the CO₂ offset was
171 subtracted from the individual CO₂ measurements. After adjustment for the offsets, the
172 methane phenotypes were calculated. The first phenotype is the mean of CH₄ per AMS visit

173 (**CH₄mean**). The second phenotype is the mean of CO₂ per AMS visit (**CO₂mean**). The third
 174 phenotype is based on the ratio (CH₄/CO₂) per AMS visit and is calculated as the mean of
 175 CH₄ divided by the mean CO₂ per AMS visit (**Ratiomean**). The residuals of the traits based
 176 on model 1 were not normally distributed. For example, the residuals of CH₄mean showed a
 177 thicker and longer right tail (Kurtosis=3.24). Therefore, phenotypes were log₁₀-transformed
 178 and after transformation residuals became normally distributed (Kurtosis of log₁₀-transformed
 179 CH₄mean=0.55). These log₁₀-transformations resulted in the fourth phenotype **CH₄log**
 180 (log₁₀(CH₄mean)), fifth phenotype **CO₂log** (log₁₀(CO₂mean)), and sixth phenotype **Ratiolog**
 181 (log₁₀(Ratiomean)). Furthermore, milk yield in kg per AMS visit was included as a general
 182 trait.

183 *Data Analysis*

184 Variance components of the phenotypes were estimated with ASReml 4.1 (Gilmour et al.,
 185 2015) using the following model:

$$186 Y_{ijklm} = \mu + \text{DayAMS}_i + \text{Lactationweek}_j + \text{Hour*Farm}_k + \text{Animal}_l + \text{Permanent}_m + e_{ijklm}$$

187 (model 1)

188 Where Y_{ijklm} is the dependent variable (CH₄mean, CO₂mean, Ratiomean, CH₄log, CO₂log,
 189 Ratiolog or milk yield); μ is the mean; DayAMS_{*i*} is the combined effect of day of
 190 measurement, farm of measurement, AMS of measurement and sensor of measurement (991
 191 levels); Lactationweek_{*j*} is the fixed effect of week of lactation (60 levels); Hour*Farm_{*k*} is the
 192 fixed interaction of hour of the day (24 levels) and farm of measurement (11 levels); Animal_{*l*}
 193 is the random additive genetic effect of animal ($\sim N(0, \mathbf{A}\sigma^2_{\text{Animal}})$) with additive genetic
 194 relationship matrix **A** and additive genetic variance σ^2_{Animal} ; Permanent_{*m*} is the random
 195 permanent environmental effect ($\sim N(0, \mathbf{I}\sigma^2_{\text{Permanent}})$) with identity matrix **I** and permanent
 196 environmental variance $\sigma^2_{\text{Permanent}}$; and e_{ijklm} is the random error effect ($\sim N(0, \mathbf{I}\sigma^2_{\text{Error}})$) with
 197 identity matrix **I** and residual variance σ^2_{Error} .

198 **Measurement Period**

199 For analysis, all data available for each cow were used, ranging from 1 to 81 days per cow. To
200 study the effect of length of measurement period a subset of the data was used consisting of
201 data from one AMS on one farm during 50 consecutive days from December 2015 to
202 February 2016. The measurement period lengths that were tested were 3, 5, 10, 20, and 30
203 consecutive days. For each of these lengths, five individual datasets with that length in
204 consecutive days were created by random sampling from the dataset of 50 days (e.g., five
205 datasets containing 30 consecutive days). Datasets of the same measurement period length
206 were sometimes partially overlapping. Repeatabilities were calculated for each measurement
207 period dataset, and repeatabilities and their standard errors were averaged over the five
208 datasets for each measurement period length.

209 **Genetic Parameters**

210 The repeatability was calculated as follows:

211
$$\text{Repeatability} = \frac{\sigma_{\text{Animal}}^2 + \sigma_{\text{Permanent}}^2}{\sigma_{\text{Animal}}^2 + \sigma_{\text{Permanent}}^2 + \sigma_{\text{Error}}^2}$$

212 with additive genetic variance σ_{Animal}^2 , permanent environmental variance $\sigma_{\text{Permanent}}^2$ and
213 residual variance σ_{Error}^2 . The heritability (h^2) was calculated as follows:

214
$$h^2 = \frac{\sigma_{\text{Animal}}^2}{\sigma_{\text{Animal}}^2 + \sigma_{\text{Permanent}}^2 + \sigma_{\text{Error}}^2}$$

215 We presented the descriptive statistics on all six phenotypes, but the genetic parameters of
216 only the log₁₀-transformed phenotypes were shown. As the residuals of the untransformed
217 phenotypes were not normally distributed, this could affect the results found for these genetic
218 parameters. Effects of the model parameters were presented on the untransformed phenotypes
219 as these effects are then easier to interpret.

220 The accuracy of the breeding value for CH₄ emission for a cow was calculated as: $\sqrt{\frac{mh^2}{(m-1)t+1}}$

221 where m is the number of repeated sensor measurements in an AMS, h^2 is the heritability, and
222 t is the repeatability. The accuracy of breeding value for CH_4 for a bull with half-sib daughters
223 was calculated as: $\sqrt{\frac{nx^2}{(n-1)x^2+4}}$ where n is the number of half-sib daughters and x is the
224 accuracy of the breeding value of the daughters with 25 repeated sensor measurements each.

225

226

RESULTS

227

Methane Sensor Test

228 During the test, the repeatabilities of CH_4 production (l/d), CH_4 concentration (ppm) and
229 $CH_4:CO_2$ ratio were calculated using data averaged per cow per day. Repeatability of CH_4
230 production obtained from CRC measurements was 0.87 (s.e.=0.04), repeatability of CH_4
231 concentration obtained from sensor measurements was 0.90 (s.e.=0.04) and repeatability of
232 $CH_4:CO_2$ ratio obtained from sensor measurements was 0.94 (s.e.=0.02). The correlation
233 between CH_4 production obtained from CRC and CH_4 concentration obtained from sensor
234 was 0.71 (s.e.=0.10). The correlation between CH_4 production obtained from CRC and
235 $CH_4:CO_2$ ratio obtained from sensor was 0.49 (s.e.=0.18).

Descriptive Statistics

238 Descriptive statistics on the six methane phenotypes and on milk production per AMS visit
239 are represented in Table 1. After the \log_{10} -transformation, the means and standard deviations
240 of CH_4 mean and CO_2 mean decreased, whereas the mean and standard deviation increased in
241 an absolute sense for Ratiomean. A milk yield of 0 kg for 619 AMS visits illustrates that
242 during these AMS visits the cows were not milked by the AMS. These AMS visits were still
243 present in the dataset, despite removing AMS visits shorter than 90 seconds. We considered
244 any AMS visit longer than 90 seconds suitable for CH_4 and CO_2 measurements, irrespective if
245 the cow was being milked during that visit.

246 *Effect of Hour of the Day on Methane Emission*

247 In general, the effect of hour of the day on CH₄mean, as obtained from model 1, was lower
248 during the night and higher during the day. This general pattern showed variation between
249 farms, as demonstrated by farms A and B in Figure 1. Farm A showed two distinct peaks in
250 CH₄mean during the day: the first peak around 9 AM and the second peak around 8 PM. Farm
251 B showed a strong increase in CH₄mean during the day compared with the night. Both types
252 of patterns were present in the dataset, but most farms had a pattern similar to that of farm A.

253 *Effect of Week of Lactation on Methane Emission*

254 The effect of week of lactation on CH₄mean, as obtained from model 1, for the first 50 weeks
255 of lactation is presented in Figure 2. The effect of week of lactation on CH₄mean increased
256 rapidly during the first 12 weeks of lactation. After this strong increase, the effect of week of
257 lactation on CH₄mean remained relatively constant until 35 weeks in lactation and decreased
258 gradually thereafter. Between weeks 12 and 35, the effect of lactation week accounted for 3-
259 7% of the variation in the mean of CH₄mean.

260 *Genetic Parameters for Methane Emission*

261 Repeatabilities and heritabilities of the log₁₀-transformed methane phenotypes and milk
262 production are presented in Table 2. The repeatabilities ranged between 0.14 and 0.31 for the
263 methane phenotypes, were similar for CH₄log and CO₂log, and were lower for Ratiolog. The
264 heritabilities were lower than the repeatabilities and ranged between 0.03 and 0.12 for the
265 methane phenotypes. Heritabilities were similar for CH₄log and CO₂log, and were lower for
266 Ratiolog. Milk yield per AMS visit had a higher repeatability (0.45) and heritability (0.17)
267 than the methane phenotypes. Standard errors of the repeatabilities and heritabilities were
268 between 0.005 and 0.03.

269 The accuracy of the breeding value for CH₄ emission expressed as CH₄log for a cow based on
270 25 repeated sensor measurements in an AMS was 0.61. For a bull with 25 daughters, where

271 each daughter has 25 repeated sensor measurements, the accuracy of the breeding value for
272 CH₄ emission was 0.85.

273 *Effect of Measurement Period*

274 The average repeatabilities and standard error of each measurement period length are
275 presented in Table 3. All three methane phenotypes showed higher repeatabilities in
276 measurement periods longer than 5 days compared with shorter measurement periods.
277 Measurement periods longer than 10 days did not lead to further improvements of the
278 repeatabilities. Standard errors decreased with increasing measurement period length, but the
279 largest decrease occurred with the increase in measurement period from 3 to 5 days.
280 Furthermore, repeatabilities of the measurement period of 10 days were not significantly
281 different from the repeatabilities of the dataset with all observations from that specific AMS.

282

283 **DISCUSSION**

284

285 The aim of this study was to quantify the genetic variation in methane phenotypes measured
286 with NDIR sensors in AMS. Methane phenotypes based on sensor measurements of CH₄ and
287 CO₂ on Dutch dairy cows were both repeatable and heritable. The repeatabilities of these
288 phenotypes ranged between 0.14 and 0.31. The heritabilities of these phenotypes ranged
289 between 0.03 and 0.12, indicating that there is genetic variation in these phenotypes.

290 *Methane Sensor Test*

291 High repeatability of CH₄ production obtained from CRC measurements and of CH₄
292 concentration obtained from sensors agree with the literature (Bell et al., 2014b; Donoghue et
293 al., 2016). The high repeatabilities found in the present study demonstrate high consistency
294 between subsequent measurements on the same animal, implying high precision of
295 measurement and suggesting consistent differences between animals. High repeatabilities and

296 moderate correlations demonstrate the potential of the sensor method for the collection of
297 phenotypes on CH₄ emission for large numbers of individual animals. Repeatabilities are
298 expected to be lower when installed in AMS because of more variable conditions on the farm.
299 This limitation is most likely compensated by the ability to perform large-scale collection of
300 data on commercial dairy farms. This facilitates repeated measures on a single animal and
301 recording large numbers of animals, both contributing to the accuracy of estimated breeding
302 values.

303 *Methane Phenotypes*

304 Phenotypes used in this study were measured in parts per million (ppm). In the literature
305 concentration measurements (in ppm) have been transformed to CH₄ production (g/day) using
306 a dilution factor or using CO₂ production (Madsen et al., 2010; Garnsworthy et al.,
307 2012a; Lassen and Løvendahl, 2016). These transformations, however, are based on several
308 assumptions, like a constant CO₂ production of a cow throughout the day, that may not always
309 be met. The CH₄ production that is obtained after transformation is affected by the accuracy
310 of these assumptions. For breeding, absolute values are not needed, as it focusses on the
311 relative differences between animals to select the best animals.

312 Phenotypes similar to those used in our study were also used in other studies (e.g. Madsen et
313 al. 2010; Lassen et al. 2012; Bell et al. 2014b). The absolute values of such similar
314 phenotypes, however, have not been published except for Ratiomean, i.e., the mean of the
315 ratio between CH₄ and CO₂. We included Ratiomean in our study because it was reported in
316 other studies and can be used to quantify methane production (Madsen et al., 2010). The
317 absolute value of Ratiomean in our study was considerably higher than the one reported by
318 Lassen et al. (2012), i.e., 0.17 vs. 0.065. The reason for this difference is unclear as the
319 absolute values of the underlying traits to Ratiomean, i.e., CH₄mean and CO₂mean, were not
320 reported by Lassen et al. (2012). Breeding, however, does not depend on absolute values, and,

321 therefore, it is expected that the difference in absolute value of Ratiolog compared with the
322 literature would not affect the direction of selection if Ratiolog would be used for selection.

323 *Milk Yield*

324 Selective breeding for milk yield has led to substantial genetic progress over time. Heritability
325 for milk yield per AMS visit in our study was 0.17 (s.e.=0.03). This heritability is slightly
326 lower than the heritability of 0.24 reported (Mulder et al., 2004) for milk yield per day
327 recorded in AMS. The heritability of CH₄log was 0.11 (s.e.=0.02), which is comparable with
328 the heritability of milk yield per AMS visit. This indicates that there is potential for a
329 reduction in methane emission through selective breeding when using sensor measurements in
330 AMS.

331 *Effect of DayAMS*

332 The DayAMS effect that was used in the model includes the effects of the day, farm, AMS,
333 and sensor of measurement. These effects could not be disentangled in our study, because
334 most measurements took place on one farm at a time, with a single sensor installed per AMS.
335 To study the impact of the DayAMS effect on the methane phenotypes, an additional analysis
336 was performed in which DayAMS was included in model 1 as a random effect instead of as a
337 fixed effect. This analysis showed that the percentage of total variation that was explained by
338 the DayAMS effect was 56% for CH₄log, 27% for CO₂log, and 82% for Ratiolog. These
339 results indicate that the phenotypes were largely influenced by the DayAMS effect, and
340 illustrates that accounting for the effects of day of measurement, farm, AMS, and sensor is
341 important when analyzing methane phenotypes. Farm conditions that are known to influence
342 CH₄ measurements are season, air flow, and barn management (Wu et al., 2016). The large
343 effect of DayAMS agrees with the other studies that acknowledge the impact of farm of
344 measurement and farm conditions on methane measurements (Bell et al., 2014a; Hammond et
345 al., 2016).

346 ***Effect of Hour of the Day on Methane Emission***

347 Hour of the day had a significant effect in our analysis with a p-value below 0.001 when
348 DayAMS was included in model 1 as random. The size of the effect is relatively small
349 compared with the effect of DayAMS. This is in line with previous studies that reported
350 diurnal variation in methane emission, mainly driven by the time of feeding of the cows
351 (Garnsworthy et al., 2012b; Lassen et al., 2012; Bell et al., 2014b). To deal with the rather
352 different feeding strategies of the 11 farms in our study (see material and methods), a farm by
353 hour of the day interaction was included in the model instead of a single hour of the day
354 effect. Not only the moment of feeding differed between the farms, but also the amount of
355 times the cows were fed and the possibility of grazing. These diverse strategies resulted in
356 hour of the day effects per farm that were different for each farm. Therefore, inclusion of the
357 interaction between hour of the day and farm instead of a single hour of the day effect into the
358 model was preferred to deal with these diverse feeding strategies.

359 ***Effect of Week of Lactation on Methane Emission***

360 Week of lactation had a significant effect in the model with a p-value below 0.001 when
361 DayAMS was included in model 1 as random. The size of the effect is relatively small
362 compared with the effect of DayAMS. As feed composition and intake usually changes
363 throughout lactation, week of lactation can affect CH₄ emission (Garnsworthy et al.,
364 2012b; Bell et al., 2014a; Lassen and Løvendahl, 2016). Previous studies also reported effects
365 of lactation stage on methane emission measured in AMS (Garnsworthy et al., 2012b; Bell et
366 al., 2014a; Lassen and Løvendahl, 2016). Similar to our study, these studies found an increase
367 in methane emissions during the first weeks of lactation. The highest level of methane
368 emission was found at around 10 weeks of lactation by Lassen and Løvendahl (2016), at 20
369 weeks of lactation by Garnsworthy et al. (2012b), and at 12 weeks of lactation in our study.
370 After the initial increase in methane emission per week of lactation, either a stable level of

371 methane emission until 50 weeks in lactation was reported (Bell et al., 2014a), or a decrease
372 in methane emission per week of lactation was reported (Garnsworthy et al., 2012b; Lassen
373 and Løvendahl, 2016). The extent of this decrease varied between 20% of the peak methane
374 emission at 50 weeks of lactation (Garnsworthy et al., 2012b) and 80% of the peak methane
375 emission at 44 weeks of lactation (Lassen and Løvendahl, 2016). In our study, methane
376 emission decreased with about 33% of the peak methane emission at 50 weeks of lactation.
377 The pattern found in our study is comparable with the patterns found in literature and the
378 found differences in patterns might be explained by many different factors such as the used
379 phenotypes. Our study used CH₄mean in ppm as phenotype whereas the other studies used
380 methane in g/day that was either from the integral area under the peaks of methane emission
381 (Garnsworthy et al., 2012b) or by the ratio between CH₄ and CO₂ in relation to heat-
382 producing units (Lassen and Løvendahl, 2016). Based on the results of our study and of the
383 literature, inclusion of lactation stage into the model to analyze methane emission is
384 recommended.

385 *Repeatabilities of Methane Phenotypes*

386 Selective breeding requires a repeatable phenotype for methane emission and methane
387 phenotypes measured in AMS using infrared sensors could be suitable phenotypes. In this
388 study, repeatabilities of the log₁₀-transformed methane phenotypes ranged between 0.14 and
389 0.31. Other studies have reported repeatabilities of CH₄ measured in AMS that ranged
390 between 0.34 for the mean of CH₄ and 0.86 for the mean of CO₂ (Lassen et al., 2012; Bell et
391 al., 2014b). In general, the repeatabilities found in other studies were higher than the
392 repeatabilities found in our study. Both studies of Lassen and Bell used a model that corrects
393 for diet effects and this might explain the higher repeatabilities found in these studies
394 compared with our study. Repeatabilities found in our study and other studies do confirm that
395 methane measurements by infrared sensors in AMS provide repeatable phenotypes.

396 *Effect of Measurement Period*

397 Measurement period has influenced the repeatabilities found in our study, and other studies
398 have chosen different measurement periods. Bell et al. (2014b) measured CH₄ and CO₂ for a
399 35-day period whereas Lassen et al. (2012) measured for a 3-day period. The results obtained
400 from our study indicate that repeatabilities (and their standard errors) remained stable in
401 measurement periods of at least 10 consecutive days. In other words, the value of additional
402 repeated measurements beyond 10 days of measurements on the same individual was close to
403 zero. Although Lassen et al. (2012) used a shorter measurement period than our study, their
404 reported standard errors are small (s.e.=0.003 – 0.006). This indicates that the repeatability
405 reported will likely not be affected by increasing the measurement period.

406 *Genetic Parameters for Methane Emission*

407 The heritabilities of the log₁₀-transformed phenotypes in this study were 0.11 for CH₄log, 0.12
408 for CO₂log, and 0.03 for Ratiolog. Lassen and Løvendahl (2016) measured methane using
409 infrared sensors in AMS on 3,121 Holstein cows and calculated heritabilities of methane
410 emission. Methane emission calculated using the ratio between CH₄ and CO₂ (in ppm) gave a
411 heritability of 0.16 and both CH₄ in g/day and CH₄ in g/kg fat and protein corrected milk gave
412 a heritability of 0.21. These heritabilities were slightly higher compared with the heritabilities
413 of CH₄log and CO₂log in our study. The heritability of Ratiolog of our study is considerably
414 lower compared with the other heritabilities. Lassen et al. (2012) used the ratio between CH₄
415 and CO₂ to create a more stable phenotype that was less influenced by the position of the head
416 of the cow to the sensor. In our study, however, we found that Ratiolog had relatively more
417 total variation and less genetic variation than CH₄log and CO₂log. Therefore, based on the
418 results of our study, the use of CH₄log for selection instead of Ratiolog would be expected to
419 give a greater reduction of methane emission by dairy cows.

420 The heritability of the phenotypes showed that there is genetic variation present in CH₄ and
421 CO₂ measured using infrared sensors in AMS, indicating that these phenotypes could be used
422 in selective breeding. The reduction in methane emission that could be achieved through
423 selective breeding depends on the genetic variance of methane emission, the intensity of
424 selection, the accuracy of selection, and the relationship between methane emission and the
425 other breeding goal traits. The accuracies of breeding values for methane emission for cows
426 and bulls were 0.61 and 0.85, respectively. This illustrates that fairly accurate estimates of
427 breeding values for selective breeding can be obtained based on repeated methane
428 measurements on a limited number of daughters per bull.

430 CONCLUSIONS

431
432 CH₄log, CO₂log, and Ratiolog were all repeatable and heritable, but Ratiolog had a lower
433 repeatability and heritability than the other two traits. It is recommended to measure CH₄ and
434 CO₂ on at least 10 consecutive days to maximize repeatabilities of the methane phenotypes. It
435 is important to account for farm, AMS, day of measurement, time of day, and lactation stage
436 when estimating genetic parameters for methane phenotypes. The use of CH₄log for selection
437 instead of Ratiolog would be expected to give a greater reduction of methane emission by
438 dairy cows.

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441
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444

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514

515 **Table 1.** Descriptive statistics of phenotypes on methane (CH₄) and carbon dioxide (CO₂)
 516 measured with non-dispersive infrared (NDIR) sensors in automatic milking systems (AMS)
 517 on 1,508 Dutch dairy cows (123,369 AMS visits)

Trait	Mean	SD ¹	Minimum	Maximum
CH ₄ mean (ppm)	254	230	11	2073
CH ₄ log (ppm) ²	2.25	0.37	1.04	3.32
CO ₂ mean (ppm)	1443	681	408	9054
CO ₂ log (ppm) ²	3.11	0.20	2.61	3.96
Ratiomean	0.17	0.12	0.01	0.87
Ratiolog ²	-0.87	0.27	-1.92	-0.06
Milk (kg) ³	10.8	3.4	0.00	36.50

518 ¹ Standard deviation.

519 ² Log₁₀-transformed phenotypes.

520 ³ Milk production per AMS visit.

521

522 **Table 2.** Repeatabilities and heritabilities of phenotypes on methane (CH₄) and carbon
 523 dioxide (CO₂) measured with non-dispersive infrared (NDIR) sensors in automatic milking
 524 systems (AMS) on 1,508 Dutch dairy cows (123,369 AMS visits)¹

Trait	repeatability	heritability
CH ₄ log (ppm) ²	0.27 (0.008)	0.11 (0.02)
CO ₂ log (ppm) ²	0.31 (0.009)	0.12 (0.02)
Ratiolog ²	0.14 (0.005)	0.03 (0.01)
Milk (kg) ³	0.45 (0.010)	0.17 (0.03)

525 ¹ This table contains the repeatability ($\text{repeatability} = \frac{\sigma^2_{\text{Animal}} + \sigma^2_{\text{Permanent}}}{\sigma^2_{\text{Animal}} + \sigma^2_{\text{Permanent}} + \sigma^2_{\text{Error}}}$) and the heritability ($\text{heritability} = \frac{\sigma^2_{\text{Animal}}}{\sigma^2_{\text{Animal}} + \sigma^2_{\text{Permanent}} + \sigma^2_{\text{Error}}}$) with their
 526 respective standard errors in parentheses.
 527

528 ² Log₁₀-transformed phenotypes.

529 ³Milk production per AMS visit.

530

531 **Table 3.** The average repeatability of phenotypes on methane (CH₄) and carbon dioxide
 532 (CO₂) measured with non-dispersive infrared (NDIR) sensors in automatic milking systems
 533 (AMS) on Dutch dairy cows over different measurement period lengths^{1,2}

Measurement period	N	CH ₄ log ³	CO ₂ log ³	Ratiolog ³
Total AMS period	8,851	0.19 (0.029)	0.16 (0.026)	0.19 (0.026)
3 days	376	0.12 (0.075)	0.12 (0.062)	0.14 (0.079)
5 days	650	0.15 (0.055)	0.12 (0.044)	0.15 (0.054)
10 days	1,295	0.22 (0.049)	0.17 (0.043)	0.22 (0.048)
20 days	2,567	0.23 (0.040)	0.18 (0.034)	0.23 (0.038)
30 days	3,827	0.23 (0.037)	0.18 (0.031)	0.22 (0.034)
50 days	6,296	0.22 (0.032)	0.16 (0.026)	0.21 (0.030)

534 ¹Per measurement period length 5 random samples were taken from the dataset of 50
 535 consecutive days, and numbers reported are the average over these 5 random samples. Total
 536 AMS period consists of all data from the one AMS (73 days) of which the dataset of 50
 537 consecutive days was obtained.

538 ² The table contains the measurement period in days, the average number of AMS visit per
 539 measurement period (N), and the average repeatability per methane phenotype (repeatability
 540 = $\sigma^2_{\text{Animal}} + \sigma^2_{\text{Permanent}} / \sigma^2_{\text{Animal}} + \sigma^2_{\text{Permanent}} + \sigma^2_{\text{Error}}$) with their respective average standard
 541 error (s.e.) in parentheses.

542 ³Log₁₀-transformed phenotypes.

543

544 **Figure 1.** Effect of hour of the day on CH₄mean (ppm) measured with non-dispersive infrared
545 (NDIR) sensors in automatic milking systems (AMS) on 1,508 Dutch dairy cows. The figure
546 shows two representative farms (A = 5,554 AMS visits; B = 20,458 AMS visits). The effect
547 of hour 4 was set to zero for both farms to enable comparison and the other effects are
548 expressed relative to hour 4.

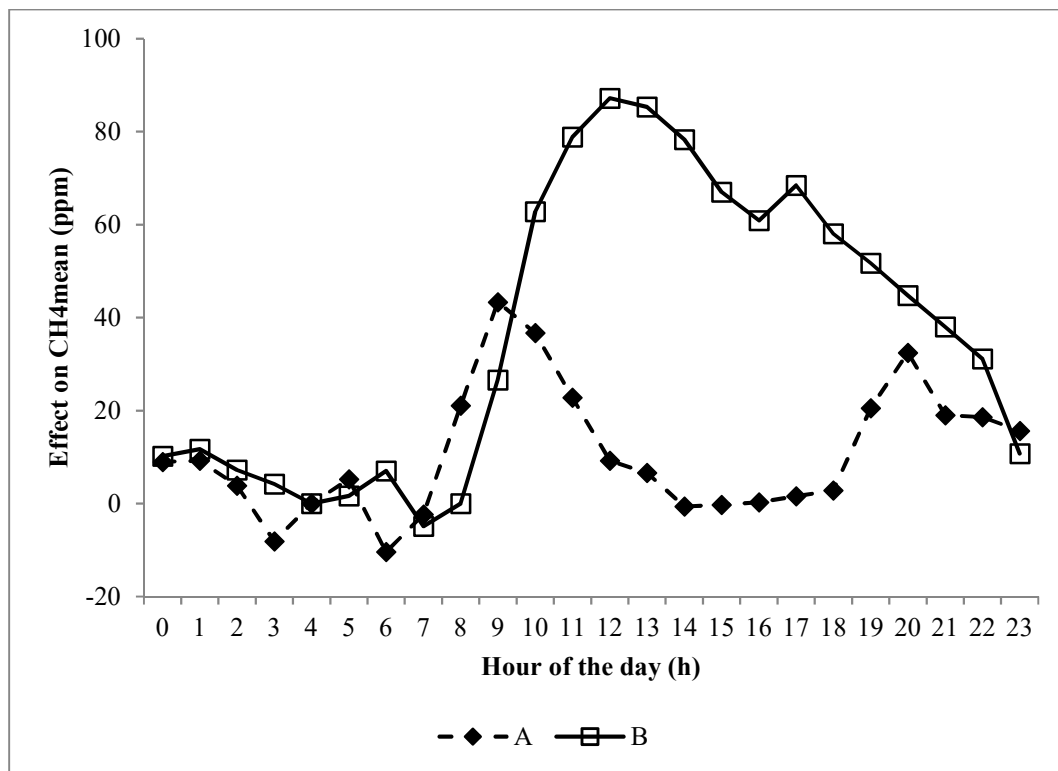
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550 **Figure 2.** Effect of week of lactation on CH₄mean (ppm) measured with non-dispersive
551 infrared (NDIR) sensors in automatic milking systems (AMS) on 1,508 Dutch dairy cows
552 (123,369 AMS visits). The effect of week 4 of lactation was set to zero and the other effects
553 are expressed relative to week 4.

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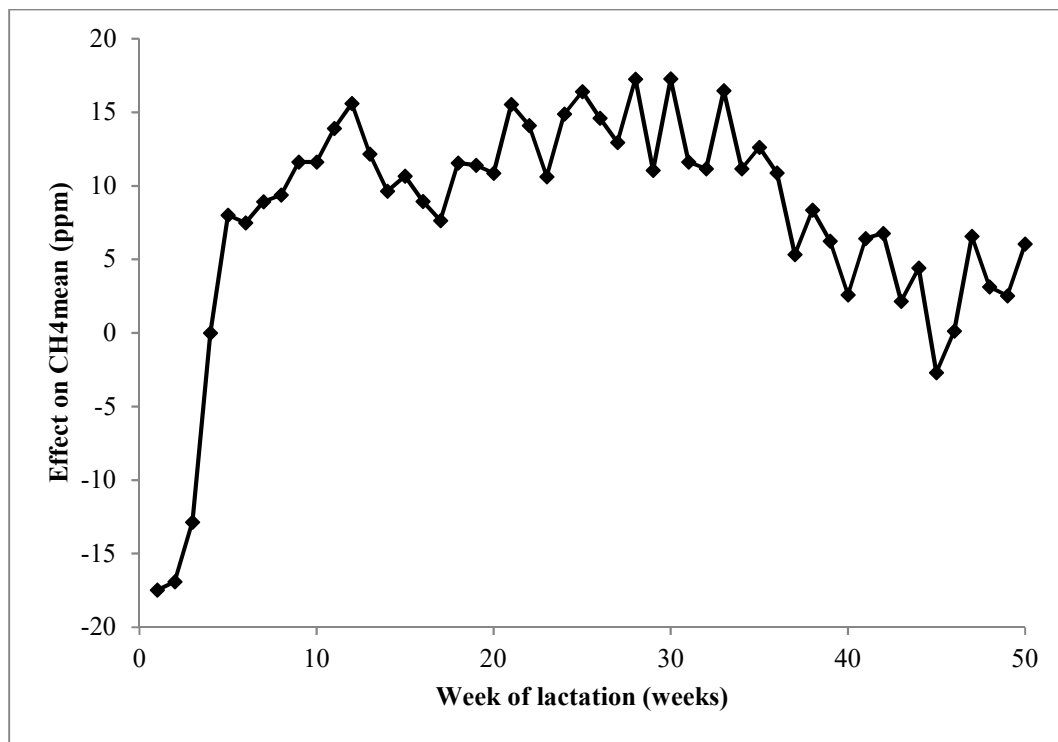


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560 Van Engelen et al., Figure 2

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