J. Dairy Sci. 105:4256–4271
https://doi.org/10.3168/jds.2021-21420

# Genetic parameters for repeatedly recorded enteric methane concentrations of dairy cows

**A. E. van Breukelen,* M. A. Aldridge, R. F. Veerkamp, and Y. de Haas**
Wageningen University & Research Animal Breeding and Genomics, 6700 AH Wageningen, the Netherlands

## ABSTRACT

Animal breeding techniques offer potential to reduce enteric emissions of ruminants to lower the environmental impact of dairy farming. The aim of this study was to estimate the heritability and repeatability of methane ($CH_4$) concentrations, using the largest data set from long-term repeatedly recorded $CH_4$ on cows to date, and to evaluate (1) the accuracy of breeding values for different $CH_4$ traits, including using visits or weekly means, and (2) recording strategies (with varying numbers of records and recorded daughters per sire). The data comprised of long-term recording of $CH_4$ and carbon dioxide ($CO_2$), from 1,746 Holstein Friesian cows, on 14 commercial dairy farms throughout the Netherlands. Emissions were recorded in 10- to 35-s intervals, between 64 and 436 d, depending on farms. From each robot visit, $CH_4$ and $CO_2$ concentrations were summarized into various traits, averaged per visit and per week: mean, median, mean log, and mean $CH_4/CO_2$ ratio. Genetic parameters were estimated with animal repeatability models, using a restricted maximum likelihood procedure, and a relationship matrix based on genotypes and pedigree. The heritability was equal for mean and median $CH_4$ per visit (0.13) but lower for log$CH_4$ and $CH_4/CO_2$ (0.07 and 0.01, respectively). Phenotypic and genetic correlations were high ($\geq$0.78) between the $CH_4$ traits, apart from the genetic correlations with the $CH_4/CO_2$ trait, which were negative. To achieve a minimum reliability of 50% for the estimated breeding value of a bull, 25 records on mean $CH_4$, measured on 10 different daughters, were sufficient. Although the heritability and repeatability were higher for weekly (0.32 and 0.68, respectively) than for visit mean $CH_4$ (0.13 and 0.30, respectively), the reliabilities of estimated breeding values from visit or weekly means were equal; thus, we found no advantage in averaging records to weekly means for genetic evaluations.

**Key words:** methane emissions, genetics, dairy cows

## INTRODUCTION

Ruminants produce methane ($CH_4$) by anaerobic fermentation in the rumen, which is emitted in the air mostly through breathing and belching (Herrero et al., 2016). Reducing these emissions can help to lower the environmental impact of dairy farming. Although enteric $CH_4$ emissions have been hypothesized to be carbon neutral (Mitloehner et al., 2020), mitigation still contributes to reducing the total emissions of the sector. Animal breeding offers an opportunity to achieve a permanent, cost-effective, and cumulative reduction in enteric $CH_4$ emissions, which can be implemented in addition to changes in nutrition and manure management (Knapp et al., 2014). To apply breeding techniques, large-scale recording of individual enteric $CH_4$ emissions is essential (de Haas et al., 2017).

Large-scale and cost-effective recording of individual cows is possible by using "sniffers" (Madsen et al., 2010; Garnsworthy et al., 2019). Sniffers use infrared spectroscopy to measure gas concentrations from the breath and belching of cows. The devices are installed in the feed bin of automated milking stations (**AMS**), where continuous measurements of the $CH_4$ concentration (ppm) in the air are taken. Sniffers do not use an air flux measurement, and therefore they cannot measure the $CH_4$ production in grams per cow per day. However, studies have shown high correlations (0.75 ± 0.20 and 0.89 ± 0.07) between on-farm sniffer measurements in parts per million and respiration chamber measurements, in which the exact $CH_4$ emission of an individual cow was measured (Difford et al., 2019; Garnsworthy et al., 2019). This suggested potential in using sniffers to quantify the variation in enteric $CH_4$ emissions between cows, and that the measurements could be used to rank cows from low to high emitters for animal breeding practices.

From $CH_4$ concentrations measured by sniffers, genetic parameters for several traits have previously been

---

Received October 13, 2021.
Accepted February 8, 2022.
*Corresponding author: anouk.vanbreukelen@wur.nl

estimated. Many studies have used averages of measured $CH_4$ concentrations (ppm), for which the heritability ranged between 0.11 ± 0.02 and 0.26 ± 0.11 (van Engelen et al., 2018; Difford et al., 2020; Saborío-Montero et al., 2020). Other studies have predicted $CH_4$ production (g/d) from $CH_4$ concentrations (ppm) by using $CO_2$ as tracer gas, combined with the $CH_4/CO_2$ ratio, as described in Madsen et al. (2010). The heritability for this predicted $CH_4$ production ranged between 0.12 ± 0.16 and 0.45 ± 0.11 (Lassen and Lovendahl, 2016; Pszczola et al., 2017; Difford et al., 2018; Zetouni et al., 2018; Breider et al., 2019). Some studies have discussed $CH_4$ traits in relation to other relevant breeding goal traits, such as milk production ($CH_4$ intensity), DMI ($CH_4$ yield), or residual $CH_4$ (de Haas et al., 2017). Of those 3 traits, only $CH_4$ intensity has a published heritability for dairy cows, estimated as 0.21 ± 0.06 (Lassen and Lovendahl, 2016). Trait definition affects estimates of heritabilities and genetic correlations, although in the literature differences between estimated genetic parameters have also been large when the same trait definition was used. Large differences in parameter estimates might also be due to different recording strategies and circumstances between studies. Furthermore, many currently published estimates are associated with large uncertainties shown by high standard errors. Most initial studies have either a small number of records per cow, a small number of recorded cows, or a small number of recorded farms. However, accurate estimates are needed to derive the expected accuracy of breeding values for different trait definitions and recording strategies, important aspects of setting up a breeding program (Falconer and Mackay, 1996).

In the Netherlands, a breeding goal will be developed to reduce enteric $CH_4$ emissions of dairy cows, for which phenotypes are being collected in AMS by sniffers. The aim of this study was to estimate the heritability and repeatability of $CH_4$ concentrations, using the largest data set from long-term repeatedly recorded cows to date, and to use the data and the corresponding estimates to evaluate (1) the accuracy of breeding values for different traits (and visits or weekly means) and (2) recording strategies (with varying number of records and recorded daughters per sire). The heritability and genetic correlation of alternative $CH_4$ and $CO_2$ traits were also estimated. Data were available for continuous and repeated recording of $CH_4$ and $CO_2$ concentrations by sniffers between 64 and 436 d, on 14 commercial dairy farms throughout the Netherlands, with a total of 1,746 dairy cows. The heritabilities and genetic correlations between different $CH_4$ traits and the described recording strategies provide tools to aid discussions that are needed to construct new breeding goals aiming to reduce enteric $CH_4$ emissions of dairy cows.

## MATERIALS AND METHODS

### Data

Enteric $CH_4$ emissions were recorded in AMS on 14 commercial dairy farms located throughout the Netherlands, between March 2019 and September 2020. On these farms, a total of 475,555 AMS visits from 2,414 dairy cows were recorded. Emissions were recorded by sniffers (WD-WUR version 1.0, Carltech BV). On each farm, a unique device was installed in close proximity to the AMS, with an air inlet leading from the feed bin of the AMS. Various types of AMS systems were used in the study, manufactured by Lely Industries NV, GEA Group, DeLaval BV, and Fullwood Packo BV. On each farm, at most 1 AMS was equipped with a sniffer, even if multiple AMS were used within a herd. Before installation in the AMS, sniffers were calibrated using flacons of $CH_4$, $CO_2$, and nitrogen. The sniffers could measure $CH_4$ concentrations in a range of 0 to 2,000 ppm and $CO_2$ concentrations in a range of 0 to 10,000 ppm.

On each farm, air was sampled continuously, and every 10 to 35 s (varying between devices) a mean was uploaded to the cloud using Arduinos (SODAQ SARA SFF R410M developer board). A data check was performed twice a week to ensure that the sniffers had no sudden change in the mean or variation in emissions. Genotype data, pedigree data, and other phenotypic data were made available by the cooperative cattle improvement organization CRV (Arnhem, the Netherlands). Animals were genotyped with the Eurogenomics 10K chip. The genotype data comprised 1,817 animals with 76,438 SNPs (imputation was routinely performed by CRV), of which 1,611 cows were phenotyped for $CH_4$ emissions. The additional 206 cows that were genotyped but not phenotyped were included to maintain linkage between the small number of herds and the large number of bulls with few daughters. Phenotypic data provided by CRV included test-day milk yield, breed composition, and calving dates.

### Matching Records to Cow Identification Numbers

Sniffers are not able to record cow identification numbers. Therefore, to match a sniffer measurement with an identification number, the $CH_4$ measurements were aligned with identification numbers recorded by the AMS. The records could not be merged based merely on timestamps, because the times of sniffers were set manually, whereby inaccuracies may have occurred, and changes to and from daylight saving time were not registered. Therefore, we used an algorithm that matches the sniffer and AMS records, based on the AMS entry time and peaks of $CH_4$ emissions in the sniffer data, which were located with the function "findpeaks" from the package "pracma" in R version 3.6.1 (R Foundation for Statistical Computing; Borchers, 2019). This method is similar to that described by Garnsworthy et al. (2012). A peak was detected based on an increase of 500 ppm of $CH_4$, with at least 3 increasing datapoints before the peak, followed by 3 decreasing datapoints. A match was defined as a peak that occurred within 30 s before or after the AMS entry time. A 30-s timeframe around the AMS entry was necessary (±time), because the AMS only recorded entry time to the minute, whereas sniffers recorded time at the level of seconds. The time difference with the most matches between sniffer $CH_4$ peaks and AMS entry times was considered to be the true time difference. Time differences were confirmed by visual inspection before matching the data set. When data were not matched correctly, many milkings would not result in increases of $CH_4$, whereas for correctly matched data it could clearly be observed that $CH_4$ concentrations peaked at the start of milkings and would be low and stable when the robot was empty.

## Data Editing

The continuous $CH_4$ measurements within an AMS visit were used to define various $CH_4$ and $CO_2$ traits, of which details are described subsequently in the section "Methane Traits." Milking robot visits that did not result in a milking were discarded, as these cows would not receive pellets and would therefore not put the nose in the feed bin close to the air inlet for a longer period of time. Within AMS visits, records taken during the first minute of milking were discarded. This was to ensure that the cow had reached the feed bin and to account for a delay in the air sample entering the air inlet and reaching the sensor. The $CH_4$ recording period was defined to last at least 2.5 min, to capture not only breathing but also the belching of cows (van Soest, 1994). Last, records after 5 min of milking were discarded, because after 6 min a decrease in the mean $CH_4$ emission of all visits combined was observed. This is most likely because, on average, after the first 6 min of milking, cows have finished eating the pellets and would move the nose away from the air inlet, resulting in a decrease of mean emissions.

Background emissions were filtered by subtracting the lowest 1% quantile of records during milking on the day of the measurement from all individual $CH_4$ and $CO_2$ measurements of that day. Records from cows up to 305 DIM were analyzed, to correctly match the $CH_4$ records to calving dates and the corresponding parity. Cows whose breeds were less than 75% Holstein were removed from the data set. The final data set comprised 308,968 visits from 1,746 individual cows, which all had pedigree data, and 1,611 of these cows also had genotype information.

## Methane Traits

For each AMS visit, a mean, a median, a mean of the log of $CH_4$ and $CO_2$ emissions, and a mean $CH_4/CO_2$ ratio were estimated from the concentrations (ppm) measured every 10 to 35 s (Table 1). Furthermore, a second data set was created, from which a mean per week trait was calculated from all visit traits. The mean per week trait included only weeks with a minimum of 7 robot visits per cow recorded. The data set with weekly means comprised 17,320 records on 1,579 cows. The traits (mean, median, log, and $CH_4/CO_2$ ratio) were selected based on what has been used previously in the literature (Difford et al., 2016; Lassen and Lovendahl, 2016; van Engelen et al., 2018), which typically include 1 or 2 of these traits but never all 4 as a mean per visit and per week. Traits based on predictions of $CH_4$ production were not included in the analyses. Recently it has been shown that the method commonly used to predict $CH_4$ production from $CH_4$ and $CO_2$ concentrations (as described by Madsen et al., 2010, is likely to favor inefficient cows over efficient cows in ranking them from low to high $CH_4$ emitting (Huhtanen et al., 2020). This is most likely a result of biased estimates of $CO_2$ production, due to differences between cows in their efficiency of energy utilization for maintenance or milk production. Additionally, $CH_4$ traits defined as a ratio to other relevant breeding goal traits, such as milk production ($CH_4$ intensity) and DMI ($CH_4$ yield), or as a residual $CH_4$ trait, were not included in the analyses, because information on milk yield and DMI were not available.

## Genetic Parameter Estimation

Genetic parameters were estimated with univariate animal models, which included repeated records, using the REML procedure in ASReml version 4.2 (Gilmour et al., 2015). From the pedigree and genotype data, a combined genetic relationship matrix ($\mathbf{H}^{-1}$ matrix) was constructed following the method of Aguilar et al. (2010) and Christensen and Lund (2010), using calc_grm version r1.143 (Calus and Vandenplas, 2016). Before constructing the $\mathbf{H}^{-1}$ matrix, the pedigree was pruned to include only phenotyped animals and their ancestors, using the statistical programming software R version 3.6.1 and the R package "optiSel" (Wellmann, 2020). The pruned pedigree comprised 34,394 animals, of which 1,746 were phenotyped for $CH_4$ emissions. The final $\mathbf{H}^{-1}$ matrix comprised all 34,394 pedigreed animals.

The significance of fixed effects on the defined $CH_4$ traits was analyzed in ASReml. The random effects included were the additive genetic, parity by permanent environmental, and residual effect. The following model was defined:

$$y_{ijklmno} = \mu + HYW_i + Farm_j \cdot Hour_k + Dur + \sum_{l=0}^{3} DIM_l \beta_l$$
$$+ Par_m + a_n + Parity_o \cdot PE_n + e_{ijklnmo},$$

where $y$ is the phenotype for a $CH_4$ trait; $\mu$ is the mean; $HYW$ is the fixed effect of herd × year × week where the measurement was taken ($i$ = 1–1,120); $Farm{\cdot}Hour$ is the fixed interaction between farm ($j$ = 1–14) and hour of the day ($k$ = 1–24) and was only used on the visit data; $Dur$ is the fixed effect for duration of the visit and was only used on the visit data (excluded for weekly); $DIM$ is the fixed regression coefficient for days in milk; $\beta_l$ is the term of the third-order Legendre polynomial for DIM; $Par$ is the fixed effect of parity ($m$ = 1–4, where 4 is parity 4 or higher); $a$ is the random additive genetic effect of the $n$th animal, $a_n \sim N\left(0, \mathbf{H}\sigma_{\mathrm{a}}^2\right)$, where $\mathbf{H}$ is the combined relationship matrix, and $\sigma_{\mathrm{a}}^2$ is the additive genetic variance; $Parity{\cdot}PE$ is the permanent environmental effect of the cows per parity ($o$ = 1–11), $Parity_o \cdot PE_n \sim N\left(0, \mathbf{I}\sigma_{pe}^2\right)$,where $\mathbf{I}$ is the incidence matrix and $\sigma_{pe}^2$ is the permanent environmental variance per parity; and $e$ is the residual error, $e_{ijklmno} \sim N\left(0, \mathbf{I}\sigma_e^2\right)$, where $\sigma_e^2$ is the error variance.

To estimate phenotypic and genetic correlations between the various $CH_4$ and $CO_2$ traits, a series of bivariate analyses were carried out. The same animal model as described previously, including covariances between the residual, genetic, and permanent environmental effects, was used to carry out the analysis for each pair of traits.

From the variance estimates, the heritability and repeatability of the various $CH_4$ and $CO_2$ traits were estimated (Mrode, 2005). Heritability was defined as follows:

$$h^2 = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_{pe}^2 + \sigma_e^2};$$

and repeatability was defined thus:

$$t = \frac{\sigma_a^2 + \sigma_{pe}^2}{\sigma_a^2 + \sigma_{pe}^2 + \sigma_e^2},$$

where $\sigma_a^2$ is the additive genetic variance, $\sigma_{pe}^2$ is the permanent environmental variance, and $\sigma_e^2$ is the error variance.

## Reliability of Estimated Breeding Values

Two types of reliabilities were compared in this study. The model reliabilities of EBV were obtained directly from the previous model, and were defined as follows (Gilmour et al., 2015):

$$r^2 = 1 - \frac{SE^2}{\sigma_a^2},$$

where SE is the ASReml standard error of the EBV, and $\sigma_a^2$ is the estimated additive genetic variance.

These model reliabilities, derived directly from the data, take into account the true relationships that exist between all animals. Furthermore, predicted reliabilities of EBV were derived from quantitative genetics theory, based on the number of repeated records or the number of daughters per sire (Table 2; Mrode, 2005). These reliabilities assume that records are only available for an individual cow or records are only available on half-sib daughters of a bull, and no other relationships exist

**Table 1.** Mean (±SD), minimum (Min), and maximum (Max) of the traits defined for enteric methane ($CH_4$) and carbon dioxide ($CO_2$) emissions as visit means (308,968 records on 1,746 cows) or weekly means (17,320 records on 1,579 cows)

| | Visit emissions | | | Weekly emissions | | |
|---|---|---|---|---|---|---|
| Item | Mean ± SD | Min | Max | Mean ± SD | Min | Max |
| Mean $CH_4$ (ppm) | 328 ± 269 | 0 | 1,999 | 367 ± 216 | 1 | 1,587 |
| Median $CH_4$ (ppm) | 315 ± 278 | 0 | 2,000 | 357 ± 221 | 1 | 1,856 |
| Mean log $CH_4$ | 2.1 ± 0.9 | −1.3 | 3.3 | 2.2 ± 0.6 | −0.3 | 3.1 |
| Mean $CO_2$ (ppm) | 3,802 ± 1,981 | 3 | 9,692 | 3,820 ± 1,660 | 143 | 9,239 |
| Median $CO_2$ (ppm) | 3,853 ± 2,131 | 2 | 9,804 | 3,867 ± 1,762 | 140 | 9,505 |
| Mean log $CO_2$ | 3.5 ± 0.4 | 0.3 | 4.0 | 3.4 ± 0.3 | 2.1 | 4.0 |
| Mean $CH_4/CO_2$ ratio | 0.10 ± 0.11 | 0 | 1 | 0.13 ± 0.12 | 0 | 1 |

**Table 2.** Number of bulls having 1 to 130 daughters in the data set, in classes of 10

| Number of daughters | Number of bulls |
|---|---|
| 1–10 | 382 |
| 11–20 | 18 |
| 21–30 | 5 |
| 31–40 | 4 |
| 41–50 | 1 |
| 61–70 | 2 |
| 121–130 | 1 |

in the data. The predicted reliabilities for phenotyped cows with repeated records were calculated as

$$r_{cow}^2 = \frac{nh^2}{1+(n-1)t},$$

where $n$ is the number of records, $h^2$ is the heritability, and $t$ is the repeatability.

The predicted reliabilities for sires of phenotyped cows with a predefined number of daughters were then calculated thus:

$$r_{sire}^2 = \frac{\frac{1}{4}d\,r_{cow}^2}{1+\frac{1}{4}(d-1)\,r_{cow}^2},$$

where $d$ is the number of daughters, and $r_{cow}^2$ is the derived reliability for the breeding value of the phenotyped daughters; that is, equivalent to $h^2$ when only one visit or weekly record is available per cow.

## RESULTS

### Average Methane Emissions

The overall mean $CH_4$ emission per visit was 367 ppm, with a standard deviation of 216 ppm (CV = 59%; Table 1, Appendix Figures A1 and A2). The number of days with records ranged from 64 to 436 d for different farms, and depended on the moment of installation and downtime due to maintenance on the sniffers. Mean $CH_4$ and $CO_2$ emissions varied between farms and ranged from 99 to 562 ppm, and from 1,161 to 5,186 ppm, respectively (Table 3).

On average, the mean $CH_4$ emissions peaked at the start of the lactation, after which the emissions gradually decreased, and so the $CH_4$ emissions follow the standard lactation curve of milk yield (Figure 1). By visual inspection of the phenotypic data, diurnal patters were observed on most farms. In general, the mean $CH_4$ emissions were observed to decline during the night, increase in the morning, and stay high throughout the day. On 4 farms, a diurnal pattern could not be observed, for which the $CH_4$ emissions remained constant throughout the day.

### Genetic Parameter Estimation

The estimated heritability and repeatability from mean visit emissions were 0.13 ± 0.01 and 0.30 ± 0.01, respectively (Table 4). After averaging the records per week, the heritability and repeatability for mean $CH_4$ significantly increased for all traits and became moderate (0.32 ± 0.03 and 0.68 ± 0.01, respectively; Table 4). Averaging the recorded visits per week, resulted in a large decrease of the residual variance (Table 5).

**Table 3.** Descriptive statistics per farm for number of cows, total number of days with records (there can be gaps in the recording period), total number of visits recorded, and mean methane ($CH_4$, ppm) and carbon dioxide ($CO_2$, ppm) emissions (±SD)

| Farm | No. of cows | No. of days of recording | No. of visits | Mean ± SD per visit (ppm) $CH_4$ | Mean ± SD per visit (ppm) $CO_2$ |
|---|---|---|---|---|---|
| 1 | 19 | 339 | 2,357 | 133 ± 151 | 2,480 ± 1,180 |
| 2 | 118 | 245 | 16,978 | 314 ± 210 | 3,399 ± 2,198 |
| 3 | 181 | 261 | 31,348 | 99 ± 140 | 2,289 ± 1,172 |
| 4 | 111 | 225 | 18,397 | 562 ± 315 | 5,186 ± 1,821 |
| 5 | 193 | 316 | 36,598 | 501 ± 284 | 4,527 ± 2,255 |
| 6 | 27 | 64 | 756 | 536 ± 248 | 1,161 ± 598 |
| 7 | 93 | 347 | 28,916 | 279 ± 197 | 4,025 ± 1,196 |
| 8 | 156 | 177 | 21,132 | 348 ± 295 | 5,039 ± 2,266 |
| 9 | 293 | 436 | 60,508 | 421 ± 257 | 3,355 ± 2,002 |
| 10 | 188 | 319 | 16,234 | 347 ± 225 | 4,079 ± 1,320 |
| 11 | 40 | 104 | 6,843 | 150 ± 156 | 4,601 ± 1,709 |
| 12 | 98 | 276 | 31,019 | 139 ± 168 | 4,117 ± 1,275 |
| 13 | 145 | 115 | 10,885 | 507 ± 237 | 5,417 ± 1,878 |
| 14 | 84 | 413 | 26,997 | 220 ± 103 | 2,491 ± 1,217 |

**Table 4.** Heritability ($h^2$) and repeatability (t) for mean methane ($CH_4$) emissions, median $CH_4$ emissions, log $CH_4$ emissions, mean carbon dioxide ($CO_2$) emissions, median $CO_2$ emissions, log $CO_2$ emissions, and $CH_4/CO_2$ ratio, averaged per visit and per week (±SE)

| | Visit | | Week | |
|---|---|---|---|---|
| Item | $h^2$ | t | $h^2$ | t |
| Mean $CH_4$ (ppm) | 0.13 ± 0.01 | 0.30 ± 0.01 | 0.32 ± 0.03 | 0.68 ± 0.01 |
| Median $CH_4$ (ppm) | 0.13 ± 0.01 | 0.29 ± 0.01 | 0.32 ± 0.03 | 0.68 ± 0.01 |
| Log $CH_4$ | 0.09 ± 0.01 | 0.18 ± 0.01 | 0.23 ± 0.03 | 0.65 ± 0.01 |
| Mean $CO_2$ (ppm) | 0.16 ± 0.02 | 0.36 ± 0.01 | 0.33 ± 0.03 | 0.71 ± 0.01 |
| Median $CO_2$ (ppm) | 0.16 ± 0.01 | 0.35 ± 0.01 | 0.34 ± 0.03 | 0.71 ± 0.01 |
| Log $CO_2$ | 0.07 ± 0.01 | 0.20 ± 0.01 | 0.20 ± 0.03 | 0.57 ± 0.01 |
| $CH_4/CO_2$ | 0.01 ± <0.01 | 0.08 ± <0.01 | 0.02 ± 0.01 | 0.15 ± 0.01 |

Mean and median $CH_4$ emissions had the highest heritability and repeatability. The trait log-transformed $CH_4$ emissions had a lower heritability than the mean $CH_4$ emissions (0.23 ± 0.03 for weekly log$CH_4$ emissions). The ratio trait had the lowest heritability and a low repeatability (0.02 ± 0.01 and 0.15 ± 0.01, respectively, for weekly $CH_4/CO_2$ emissions).

## Genetic Correlations Between Methane Traits

Phenotypic and genetic correlations were estimated between the weekly emission traits. The estimated genetic correlations between the mean, median, and log$CH_4$ traits were high (0.78–1.00; Table 6). On the contrary, the phenotypic and genetic correlations between the $CH_4/CO_2$ ratio trait and all other traits were negative (−0.08 to −0.45 and −0.27 to −0.99, respectively), and the genetic correlations had high standard errors (0.16–0.29). Furthermore, the phenotypic correlations between $CH_4$ and $CO_2$ emission traits were high (0.70–0.85).

## Reliability of Estimated Breeding Values

Predicted reliabilities for EBV were estimated based on the heritability and repeatability estimates for mean visit and weekly emissions. The predicted reliability for EBV of phenotyped cows with repeated records increased steeply for the first 10 weekly records of mean $CH_4$ emissions, after which the gain per additional recording per cow became smaller (Figure 2). For visits, the increase in reliability was slightly less steep, and the gain per additional recording became smaller after around 25 recorded visits. For phenotyped cows, one weekly mean gave a higher reliability than one visit, which is a result of the higher heritability for weekly means (Figure 2 and Table 7). However, when considering an average of 7 visits per week, the 2 reliabilities

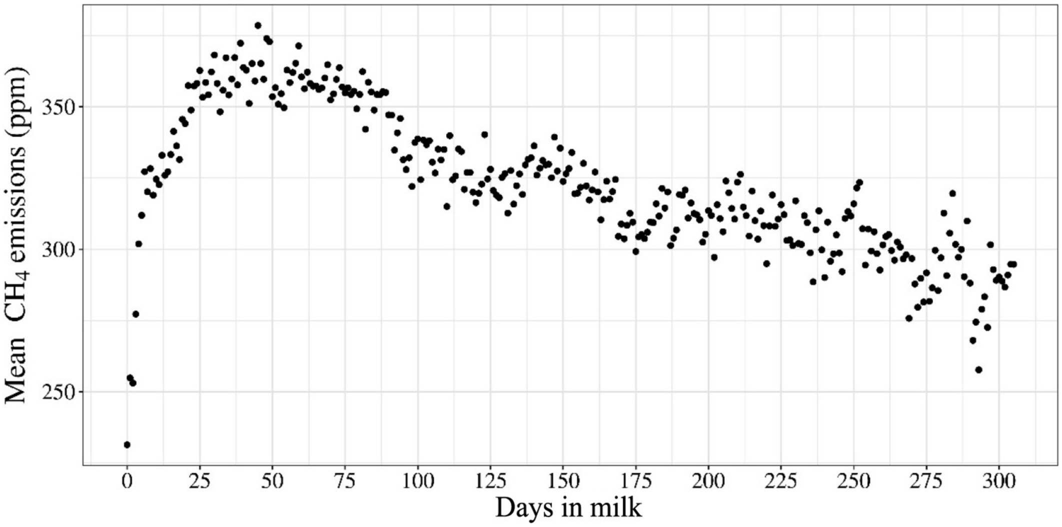

**Figure 1.** Mean methane ($CH_4$) emissions (ppm) per automated milking station visit on each day in milk.

**Table 5.** Genetic $\left(\sigma_a^2\right)$, permanent environmental $\left(\sigma_{pe}^2\right)$, error $\left(\sigma_e^2\right)$, and phenotypic $\left(\sigma_p^2\right)$ variance of mean methane ($CH_4$) and carbon dioxide ($CO_2$) emissions per robot visit and per week (±SE)

| Item | $\sigma_a^2$ | $\sigma_{pe}^2$ | $\sigma_e^2$ | $\sigma_p^2$ |
|---|---|---|---|---|
| Visit $CH_4$ | 5,308 ± 543 | 6,429 ± 335 | 28,024 ± 72 | 39,760 ± 457 |
| Weekly $CH_4$ | 5,371 ± 610 | 6,115 ± 388 | 5,381 ± 62 | 16,867 ± 493 |
| Visit $CO_2$ | 411,526 ± 42,557 | 505,494 ± 25,976 | 1,607,350 ± 4,111 | 2,524,400 ± 35,279 |
| Weekly $CO_2$ | 416,517 ± 47,117 | 471,873 ± 29,585 | 357,251 ± 4,149 | 1,245,600 ± 37,896 |

were approximately equal (7 visits per week versus 1 weekly). This was calculated from the parameter estimates and applying a sensitivity analysis based on their standard errors.

The predicted reliability of EBV for sires increased when a larger number of daughters were recorded per sire, and the level of reliability depended on the number of records for mean $CH_4$ emission per daughter (Figure 3). With a larger number of records per daughter, the reliability of sires was higher, although after 10 recorded weeks or 25 recorded visits, the gain in adding extra records became negligible. For sires with a large number of recorded daughters that all have a large number of records, the difference between the reliability for visit emissions and weekly emissions decreased (Table 7).

For cows with own performance information, including repeated records, the realized reliability for mean $CH_4$ emission was higher than that expected based on the predicted reliability (Figure 4A). For sires, the realized reliability, which included all records, followed the expected pattern from predicted reliabilities (Figure 4B). Figure 4A confirms that the largest increase in reliability came from the inclusion of approximately the first 25 repeated records. By including more repeated records, the reliability for cows only marginally increased.

## DISCUSSION

The aim of this study was to estimate the heritability and repeatability of $CH_4$ concentrations, using the largest data set from long-term repeatedly recorded cows to date, and to use the data and corresponding estimates to evaluate (1) the accuracy of breeding values for different traits (visits or weekly means) and (2) recording strategies (with varying numbers of records and recorded daughters per sire). Data were available on continuous and repeated recording of $CH_4$ and $CO_2$ emissions, from 1,746 dairy cows, on 14 commercial dairy farms throughout the Netherlands. From the data, genetic parameters were estimated with univariate and bivariate animal models, using a restricted maximum likelihood procedure. The results show that mean $CH_4$ emissions had moderate heritability and repeatability, but that there was no advantage in averaging the mean emissions per week to estimate breeding values for sires. From the mean emissions per visit, 25 records measured on 10 different daughters gave reliabilities of breeding values above the Dutch publication threshold of 50%.

### *Phenotypic Analysis*

The mean $CH_4$ emissions recorded in this study was 367 ppm, which is within the low range of previously estimated means from similar sniffer devices (between 254 and 1,288 ppm; van Engelen et al., 2018; Bell et al., 2019; Difford et al., 2019; López-Paredes et al., 2020; Saborío-Montero et al., 2020). Low records can be a result of variations in measurement conditions and drifting of sensors toward zero. This may also have caused the coefficient of variation (CV) to increase to higher levels than expected and caused differences in the CV between farms. By correcting for differences between farms and between measuring weeks within farms in the genetic analysis, we assume that these factors have minimal influence on the estimates of the genetic parameters. Another concern that is often raised is that $CH_4$ measurements by sniffers are inaccurate or biased and are influenced by systematic environmental effects, random errors, and systematic errors (Huhtanen and Hristov, 2018). Systematic environmental effects, such as conditions during the day or farm, are not problematic for genetic analysis and can be separated from genetic effects by the separation of environmental effects in mixed model analysis. Similarly, random errors, which can be a result of movement of the cow's nose and the position of the nose in the feed bin (Wu et al., 2018), do not have to be problematic and can be reduced by taking multiple repeated measurements of each cow. Furthermore, the effect of the position of the nose and head lifting was expected to be reduced by only considering the first 5 min of milking, where we expect most cows were eating concentrates. Systematic errors, such as behavior during milking, are more serious if the errors are also partly genetic in nature. For example, cows that are restless in nature might look around in the milking robot more frequently, causing the measured

**Table 6.** Phenotypic (above the diagonal) and genetic correlations (below the diagonal) between weekly $CH_4$ and $CO_2$ traits (±SE)

| Item | Mean $CH_4$ (ppm) | Median $CH_4$ (ppm) | Log $CH_4$ | Mean $CO_2$ (ppm) | Median $CO_2$ (ppm) | Log $CO_2$ | $CH_4/CO_2$ |
|---|---|---|---|---|---|---|---|
| Mean $CH_4$ (ppm) | | 0.99 ± <0.01 | 0.72 ± 0.01 | 0.85 ± <0.01 | 0.84 ± <0.01 | 0.72 ± 0.01 | −0.09 ± 0.01 |
| Median $CH_4$ (ppm) | 1.00 ± <0.01 | | 0.70 ± 0.01 | 0.83 ± 0.01 | 0.83 ± 0.01 | 0.70 ± 0.01 | −0.08 ± 0.01 |
| Log $CH_4$ | 0.80 ± 0.02 | 0.78 ± 0.02 | | 0.71 ± 0.01 | 0.70 ± 0.01 | 0.70 ± 0.01 | −0.09 ± 0.01 |
| Mean $CO_2$ (ppm) | 0.90 ± 0.01 | 0.88 ± 0.01 | 0.76 ± 0.02 | | 0.99 ± <0.01 | 0.88 ± <0.01 | −0.22 ± 0.01 |
| Median $CO_2$ (ppm) | 0.89 ± 0.01 | 0.88 ± 0.01 | 0.75 ± 0.02 | 1.00 ± <0.01 | | 0.87 ± <0.01 | −0.21 ± 0.01 |
| Log $CO_2$ | 0.89 ± 0.02 | 0.88 ± 0.02 | 0.80 ± 0.02 | 0.99 ± 0.01 | 0.99 ± 0.01 | | −0.45 ± 0.01 |
| $CH_4/CO_2$ | −0.56 ± 0.22 | −0.55 ± 0.22 | −0.27 ± 0.16 | −0.99 ± 0.30 | −0.95 ± 0.29 | 0 ± 0 | |

concentrations to decline. It should be further studied whether this behavior is repeatable and heritable, as has also been pointed out by Wu et al. (2018). Nonetheless, even if sniffer measurements party reflect differences in other traits, $CH_4$ measured by sniffers could still serve as in indicator for true $CH_4$ emissions (Bovenhuis et al., 2018).

The pattern of $CH_4$ emissions over DIM (Figure 1) and the diurnal pattern indicate that sniffers are able to detect variation in emissions. Diurnal patterns have been reported previously (Bell et al., 2014b; Pszczola et al., 2017; van Engelen et al., 2018), and in these studies the $CH_4$ emissions increased during the day and decreased during the night, which is similar to patterns observed in this study. Crompton et al. (2011) showed that these diurnal patterns in $CH_4$ emissions can be caused by changes in feed intake during the day. In our study, the relationship between $CH_4$ emissions and feed intake was not further investigated, as data on feed intake was not available.

For DIM, earlier studies have reported a steep increase of $CH_4$ emissions in the first weeks of lactation, with emissions remaining stable or gradually decreasing thereafter (Garnsworthy et al., 2012; Bell et al., 2014a; Pszczola et al., 2017). This is in agreement with the pattern for DIM observed in this study (Figure 1). Also, feed intake may play a role; daily DMI is typically lower in early lactation compared with mid and late lactation (Krattenmacher et al., 2019).

### Methane Traits

In this study we have analyzed average $CH_4$ concentrations (ppm) measured in the feed bin of milking robots. High individual-level correlations have been reported (0.75 ± 0.20) between sniffer $CH_4$ breath concentration (ppm) measurements and respiration chamber $CH_4$ production (g/d) measurements; this was published by a study that installed sniffers in milking robots for 3 wk of lactation and subsequently in respiration chambers (Difford et al., 2019). Therefore, we assumed that analyzing concentrations measured in parts per million gives sufficient information about relative differences between cows, which is required to select the best-performing animals for breeding practices. Additionally, we analyzed $CH_4/CO_2$ as a ratio trait that is generally used in quantifying $CH_4$ production (Madsen et al., 2010). Nonetheless, to gain confidence in using $CH_4$ concentration measurements from sniffers for genetic evaluations, the relationship with measurements of emissions in grams per day, as is done with the GreenFeed (C-Lock Inc.; Hammond et al., 2016), should be investigated further. This is needed to confirm that the total emissions in grams per day will be

**Table 7.** Predicted reliabilities for cows with own performance information and sires with phenotyped daughters[1]

| No. of records per cow | Predicted reliability, cow | | No. of daughters per sire | Predicted reliability, sire | |
|---|---|---|---|---|---|
| | Visit | Weekly | | Visit | Weekly |
| 1 | 0.13 | 0.32 | 5 | 0.14 | 0.30 |
| | | | 10 | 0.25 | 0.47 |
| | | | 100 | 0.77 | 0.90 |
| 5 | 0.30 | 0.43 | 5 | 0.29 | 0.38 |
| | | | 10 | 0.44 | 0.55 |
| | | | 100 | 0.89 | 0.92 |
| 10 | 0.35 | 0.45 | 5 | 0.33 | 0.39 |
| | | | 10 | 0.49 | 0.56 |
| | | | 100 | 0.91 | 0.93 |
| 25 | 0.40 | 0.46 | 5 | 0.35 | 0.39 |
| | | | 10 | 0.52 | 0.57 |
| | | | 100 | 0.92 | 0.93 |
| 50 | 0.41 | 0.47 | 5 | 0.37 | 0.40 |
| | | | 10 | 0.54 | 0.57 |
| | | | 100 | 0.92 | 0.93 |
| 100 | 0.42 | 0.47 | 5 | 0.37 | 0.40 |
| | | | 10 | 0.54 | 0.57 |
| | | | 100 | 0.92 | 0.93 |

[1] Reliabilities for cows are derived from the numbers of visit or weekly records and heritability and repeatability for visit or weekly means. Reliabilities for sires are derived from the cows' reliability from the number of records and the number of daughters.

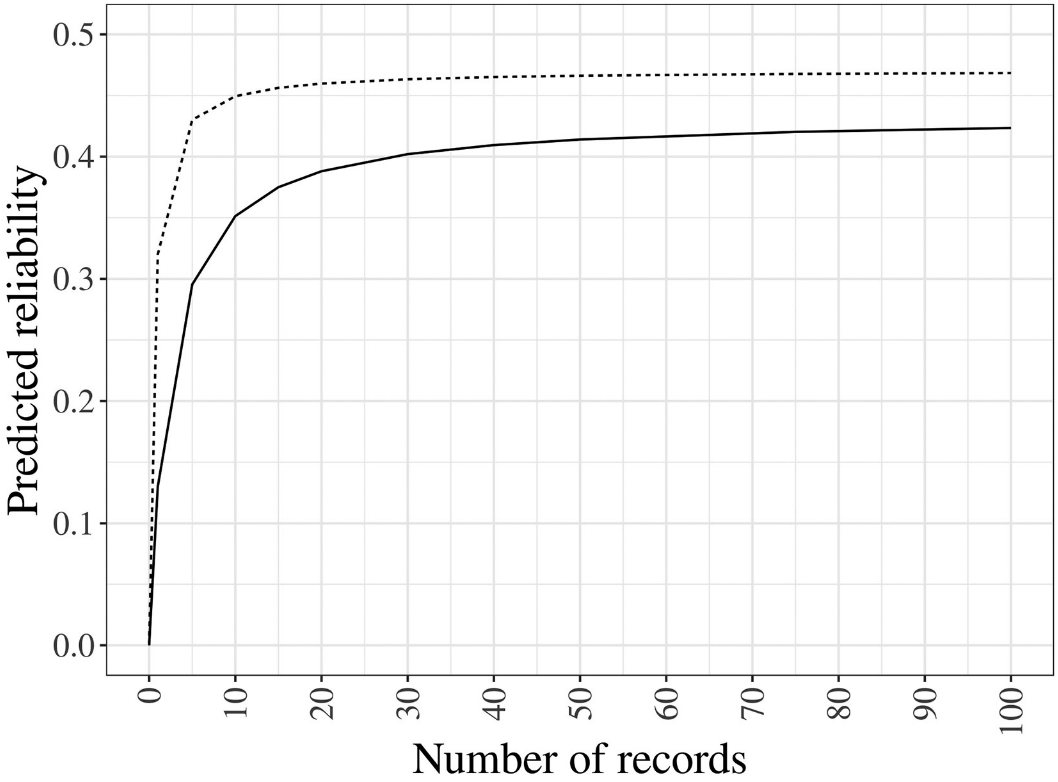

**Figure 2.** Predicted reliability for the mean $CH_4$ emission for cows with own performance information, based on the number of phenotypic records per cow. Calculated for the number of recorded visits (solid line) and the number of recorded weeks (dotted line). Lines were derived from the corresponding estimates of heritability and repeatability (0.13 and 0.30 for visits, and 0.32 and 0.68 for weeks, respectively).

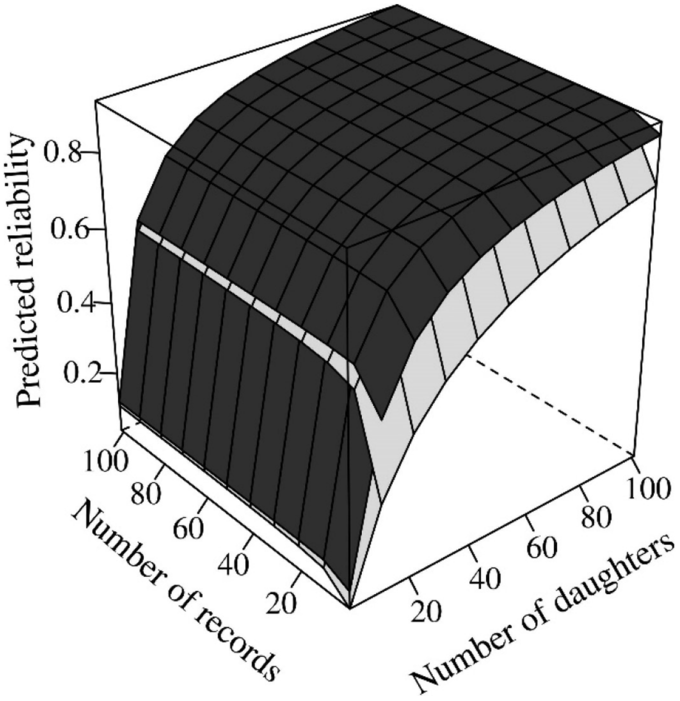

**Figure 3.** Predicted reliability for sires with phenotyped daughters, based on the number of records on mean $CH_4$ emission per daughter and the number of daughters per sire. The reliability was derived for both visit means (light gray) and weekly means (dark gray) from the corresponding estimates of heritability and repeatability (0.13 and 0.30 for visits, and 0.32 and 0.68 for weeks, respectively).

reduced by breeding for reduced $CH_4$ concentrations measured in parts per million.

In the literature, $CH_4$ measurements in parts per million are often converted to grams per day to approximate a cow's total emission. To predict emissions in grams per day from concentration measurements by sniffers, $CO_2$ emissions are used as a tracer gas through a formula that assumes a constant efficiency of energy utilization for different metabolic functions (Madsen et al., 2010). This assumption is not always met and can result in $CH_4$ emissions to be overestimated, on average by 17% for efficient compared with inefficient cows, favoring the inefficient cows (Huhtanen et al., 2020). Other traits that have received interest in the literature are $CH_4$ intensity (g of $CH_4$/kg of milk), $CH_4$ yield (g of $CH_4$/kg of DMI), and residual $CH_4$ (observed $CH_4$ minus predicted, from, e.g., milk yield, $CH_4$; de Haas et al., 2017). These traits would account for the highly conserved relationships between $CH_4$, milk yield, and feed intake, and thus rank cows from low to high emitting, regardless of their level of production or feed intake. However, these relationships can also be addressed by including correlation structures between $CH_4$, milk yield, and feed intake in the selection index. In a simulation study, Zetouni et al. (2017) showed that a multitrait approach resulted in higher genetic gain than by selecting for ratio traits. Additionally, responses to selection for a multitrait index, compared with including a ratio trait, will be easier to interpret, which makes the index more approachable for farmers. Because the interest was in analyzing traits that would be suitable to add to a breeding goal, we did not include analyses on $CH_4$ as a ratio to other breeding goal traits, although more research is needed to verify the effect of using a ratio trait for $CH_4$ in breeding goals.

Many traits had minimum records of 0 ppm $CH_4$ (rounded to zero), which is below what is biologically expected (Table 1, Appendix Figures A1 and A2). The low records were most likely a result of drifting of the sensor calibration that occurred during the study, where the sensor calibration drifted toward zero. As a

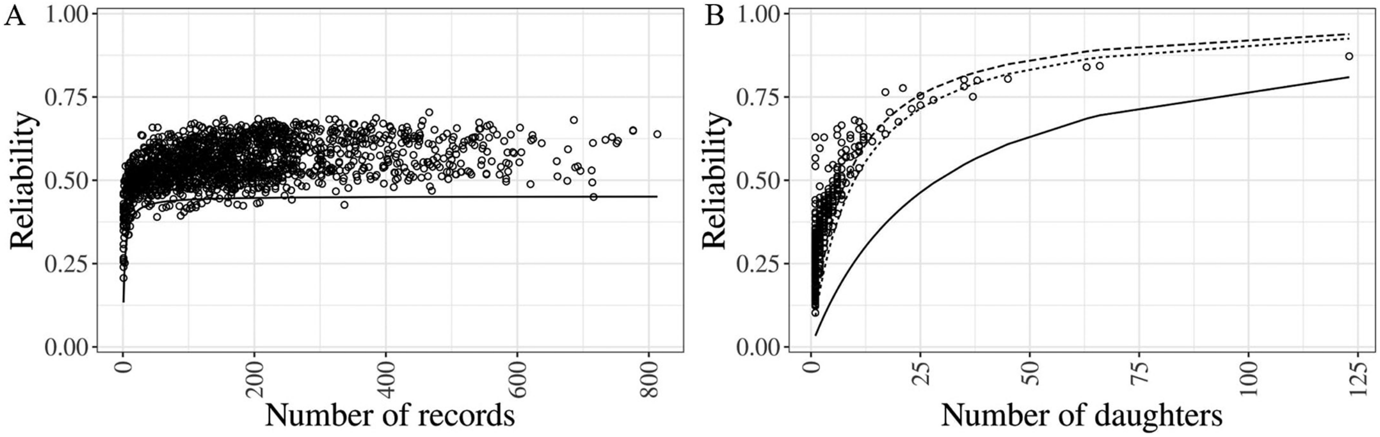

**Figure 4.** Realized reliability of EBV (dots) and expected predicted reliability (lines) for cows with own performance information on the mean $CH_4$ emissions per robot visit, by their number of recorded visits (A), and for sires of phenotyped cows by their number of daughters, which assumes each cow has 1 (solid line), 10 (small-dashed line), or 100 (large-dashed line) repeated records (B).

result, the $CH_4$ data were not normally distributed, and therefore a log-transformation on the $CH_4$ records was performed. Nonetheless, because $CH_4$ emissions are expected to be normally distributed by nature and moved toward normality after averaging the records to means per week (Appendix Figure A2), we do not recommend using log-transformations of a $CH_4$ trait in the breeding objective. Furthermore, log-transformation did not solve non-normality of $CH_4$ in this data set. Therefore, we recommend investigating other options to correct for drift of sensors in future studies; for example, by using censored models or multiple imputation, or by standardization of the data based on the week of the measurement.

Other traits derived from sniffer measurements that are often mentioned in the literature are $CH_4$ emissions estimated from eructation peak traits (Garnsworthy et al., 2012; Bell et al., 2019; López-Paredes et al., 2020). Peak traits were not included in this study, because the recording interval of up to 35 s does not provide sufficiently detailed information. However, estimations of the $CH_4$ emissions from peaks have been shown to be moderately to highly correlated with the average $CH_4$ concentration during milking (0.62 and 0.86, on different diets; Bell et al., 2014b). Therefore, peak traits may still be of interest for breeding when using different recording practices, where concentrations are measured, for example, every second.

### Genetic Parameters

The estimated heritabilities were highest for mean and median $CH_4$ emissions averaged per week (both were 0.32 ± 0.03; Table 4). As expected, averaging the recorded visits to records per week resulted in an increased heritability. Earlier studies reported heritabilities for the mean $CH_4$ concentration (ppm) and mean log$CH_4$ concentration per week, measured by sniffers, ranging from 0.11 ± 0.03 to 0.26 ± 0.11 (Difford et al., 2020; López-Paredes et al., 2020), and for mean $CH_4$ production per week of 0.12 ± 0.16 in a repeatability model (Breider et al., 2019), and of 0.25 ± 0.07 from one weekly record (Zetouni et al., 2018). Thus, the heritability estimated for weekly mean emissions in this study is somewhat higher than what has been observed in literature. This could have been a result of the large quantity of data used and the inclusion of genomic information, as well as of the requirement of a minimum number of 7 records per weekly mean to come to a more reliable average. The heritability for mean $CH_4$ concentration per visit (0.13 ± 0.01 for mean $CH_4$ and 0.09 ± 0.01 for log$CH_4$) is similar to the heritability reported by van Engelen et al. (2018) for log$CH_4$ concentration (0.11 ± 0.02) and by Saborío-Montero et al. (2020) for average $CH_4$ concentration over a period of 2 to 3 wk (0.12 ± 0.01). Estimates that have been reported in the literature for the heritability of mean $CH_4$ production per visit were somewhat higher, at 0.21 ± 0.06 and 0.19 ± 0.09 (Lassen and Lovendahl, 2016; Difford et al., 2018). Residual variances may differ between farms; therefore it is of interest to estimate intra-herd variances. However, we were not able to estimate genetic parameters within farms, because most farms had a limited number of cows recorded, with only one farm having recorded over 200 cows (n = 293; Table 3).

The repeatabilities estimated in this study for mean and median $CH_4$ emissions were moderate and, again, higher for weekly than for visit mean emissions (0.68 ± 0.01 and 0.30 ± 0.01, respectively; Table 4). Repeatability estimates for $CH_4$ concentrations recorded by sniffers have been published previously. In the literature, repeatabilities of mean $CH_4$ concentrations per visit were 0.42 and 0.45 ± 0.07 (Rey et al., 2019; Sypniewski et al., 2019), and 0.27 ± <0.01 and 0.33 for log$CH_4$ (Difford et al., 2016; van Engelen et al., 2018). Thus, the repeatabilities estimated in this study for mean $CH_4$ emissions per visit were lower than previously published estimates (0.30 and 0.18 for mean $CH_4$ and log$CH_4$, respectively). For $CH_4$ production per visit or per day, repeatability estimates have been reported that ranged from 0.36 to 0.66 ± 0.11 (Haque et al., 2015; Negussie et al., 2017; Sorg et al., 2018), which is in the same range as or higher than the repeatabilities reported for $CH_4$ concentrations. Estimates of the repeatability for log $CH_4$ concentrations averaged per week ranged from 0.47 to 0.84 (Difford et al., 2016; Difford et al., 2019; Difford et al., 2020). The repeatability estimated in this study for weekly log$CH_4$ emissions (0.65 ± 0.01) falls within this range.

In this study, the $CH_4/CO_2$ ratio trait had a low heritability (0.01 ± <0.01 to 0.02 ± 0.01) and a low repeatability (0.08 ± <0.01 to 0.15 ± 0.01). Low estimates have also been reported in the study by van Engelen et al. (2018), where the heritability was 0.03 ± 0.01 and the repeatability was 0.14 ± <0.01. However, for the $CH_4/CO_2$ ratio trait, Lassen and Lovendahl (2016) reported a higher estimate of heritability of 0.16 ± 0.04, although in that study emissions were measured for 7 d and averaged over the full recording period. A low heritability indicates that a larger number of records per cow is needed for the $CH_4/CO_2$ ratio trait to accurately estimate EBV, compared with direct measurements of $CH_4$ concentration, which were shown to be moderately heritable in this study. Using $CH_4$ in a ratio trait has other disadvantages. First, as has been addressed previously, direct selection on the trait of interest is more advantageous, as it realizes a higher genetic response than indirect selection on a ratio trait (Zetouni et al., 2017). Second, interpretation would be more difficult, as the level of feed intake, efficiency of energy utilization, and body energy balance can also influence the gas ratio (Huhtanen et al., 2015). Finally, Huhtanen et al. (2015) suggest that air-mixing conditions, caused by the geometry of the feed bin, might influence the $CH_4/CO_2$ ratio.

Genetic correlations between the mean, median, and log$CH_4$ traits were high (0.78–1.00), indicating that these traits can be used interchangeably after standardization (Table 6). In contrast, the phenotypic and genetic correlations between the $CH_4/CO_2$ ratio trait and all other traits were negative. Again, this shows that the $CH_4/CO_2$ ratio is most likely less suitable for application in selection indices and not a good indicator of a cow's $CH_4$ emission. However, in our study the ratio may have been influenced by the drift of $CH_4$ sensors while the $CO_2$ sensor remained stable, making the relationship between the ratio and its component traits nonlinear, and therefore could be more informative when sensors are calibrated regularly.

The phenotypic and genetic correlations between $CH_4$ and $CO_2$ concentrations were positive. The phenotypic correlations ranged from $0.70 \pm 0.01$ to $0.85 \pm <0.01$, and the genetic correlations ranged from $0.75 \pm 0.02$ to $0.90 \pm 0.01$. This is in agreement with what has been reported in an earlier study, where the phenotypic correlations ranged from $0.87 \pm <0.01$ to $0.96 \pm <0.01$, and the genetic correlations ranged from $0.96 \pm 0.03$ to $0.97 \pm 0.03$ (Difford et al., 2020). Furthermore, a study that used respiration chamber measurements also reported a high phenotypic correlation between $CH_4$ and $CO_2$ production (0.93; Aubry and Yan, 2015). The high correlations suggest that a strong relationship exists between the quantity of emitted $CH_4$ and $CO_2$.

### Recording Strategies

For cows with own performance information, the predicted reliability appears higher when estimated from the mean $CH_4$ emission per week compared with the reliability estimated from visits, for the same number of records per cow (0.46 and 0.40, for 25 recorded weeks or visits respectively). However, in this scenario a weekly mean is expected to be calculated from at least 7 recorded visits, and, when comparing the reliability from 7 visits to one week, no gain in reliability is detectable for either scenario. Therefore, to estimate reliable EBV, we found no gain in averaging the recorded visits per week. Similarly, for sires the reliabilities of EBV were approximately equal for one weekly record versus 7 recorded visits. This also illustrates that heritability should always be interpreted in context of the trait definition.

The reliability of EBV estimated from own performance information is constrained by the large difference between the heritability and the repeatability, which is the permanent environmental effect (Falconer and Mackay, 1996). This effect can also be observed in Figure 4A, where the predicted reliabilities, derived from quantitative genetics theory, are plotted based on the number of repeated measurements. By taking measurements further apart in time, the permanent environmental variance could possibly be reduced. When the time gap between records increases, the environmental correlation between records may decay faster than the genetic correlation, which could result in a higher reliability for cows. Although this effect was not investigated in this study, it might be useful in optimizing recording strategies and for efficient use of recording equipment, but should be investigated further. Given that $CH_4$ emissions have been shown to have a different genetic background over DIM (Pszczola et al., 2017), it is important to consider lactation stages. Nonetheless, for a large number of animals, the realized reliabilities by ASReml were higher expected based on the predicted reliabilities (Figure 4). The higher reliability can be explained by the inclusion of extra information on relatives. The predicted reliabilities assume that only $CH_4$ records on the individual animal are available, whereas in the data daughters, half-sibs and full sibs may also have phenotypic records, which provide additional information about the individual animal.

From Figure 4B, we can derive the number of daughters that should be phenotyped in practice to be able to reliably estimate breeding values for the mean $CH_4$ emissions for bulls. The Dutch cattle improvement organization CRV publishes breeding values for milk production traits when the reliability is above 50%, and for all other traits when the reliability is above 25% (CRV, 2020). Using these numbers, in Figure 4 we can observe that around 10 daughters per sire, with at least 10 visits recorded on mean $CH_4$ emissions per daughter, would need to be recorded to reach an accuracy of 50% for sires. However, only a handful of daughters, with at least 10 repeated records per daughter, would need to be recorded to achieve a reliability of 25%. These numbers of records are determined by the estimated heritability and repeatability and assume that these are estimated without error. However, in our study the standard errors (SE) were between <0.01 and 0.04, and therefore a sensitivity analysis was performed, which assumed that the heritability or the reliability (or both) were ± 2 SE. This analysis showed that the required minimum number of records did not change within the expected range of error of the heritability and repeatability estimates.

From the results, we suggest that at least 25 visits should be recorded on cows to accurately calculate EBV for mean visit $CH_4$ emissions for the phenotyped cows or their sires in a repeatability model (Figures 2 and 3). A study using the GreenFeed system, showing the computation of a cow's $CH_4$ or $CO_2$ production rate, required a minimum of 30 recorded visits, each lasting more than 3 min, to obtain a reliable average of multiple short-term breath measurements (Arthur et al., 2017). This indicates that, despite the GreenFeed system's more accurate recording, by correcting the measurements for areal conditions and movement of the head of the cow (Huhtanen et al., 2015), the minimum recording period of the system is similar to the minimum recording period of the sniffer. Additionally, sniffers have the advantage of the ability to record a larger number of cows per recording period, equal to the capacity of the AMS, which is a prerequisite for genetic evaluations for which hundreds to thousands of animals need to be phenotyped. Nonetheless, it is important to realize that these recommendations relate to mean $CH_4$ concentrations with heritability and repeatability in the magnitude of what has been estimated in this study. When new traits are defined, with different parameter estimates, or different models are applied, the recording strategies have to be re-evaluated.

### *Implications*

This study confirms that there is promise in using $CH_4$ emissions measured by sniffers in genetic evaluations. Mean $CH_4$ emissions (ppm) per visit and per week have moderate to high heritability and repeatability, are easy to record and easy to interpret as absolute differences between cows, and could most likely serve as an indicator for total $CH_4$ emissions (g/d), as is suggested by the high correlations between sniffer and respiration chamber measurements (Difford et al., 2019; Garnsworthy et al., 2019). Methane emissions are currently not included in dairy selection indices around the world. Large-scale phenotyping is required first, to investigate the relationships between $CH_4$ emissions and other selection index traits. However, currently not enough measurements were available to derive the relationships between $CH_4$ emissions and other breeding goal traits, which are required before inclusion in a selection index.

It has to be further investigated which trait or traits defined from $CH_4$ emissions measured by sniffers should be added to a breeding goal that aims to reduce enteric $CH_4$ emissions per animal. Previous studies have shown that the heritability of $CH_4$ emissions changes over a lactation, and that the genetic correlation between different DIM was on average 0.74 and could decrease to 0 for DIM further apart (5 vs. 305 DIM; Pszczola et al., 2017; Breider et al., 2019; Sypniewski et al., 2021). This shows that it is important to consider the period in which $CH_4$ was recorded, and not simply to assume a genetic correlation of one between different DIM, as was done with the repeatability model in this study. Random regression models have the advantage of allowing for heterogeneous genetic and residual variances between lactation stages, and can model underlying genetic correlations, similar to models that are used for milk yield in the Netherlands (CRV, 2018). Additionally, correction for heterogeneity of variances might be required to adjust for different variances across herds. Better modeling of the underlying genetic structure will most likely improve the reliability of breeding value estimations and reduce the need for recording in different lactation periods. Random regression models will be investigated with this data set when more data has been collected. Nonetheless, the current estimates of heritability clearly indicate that, also by using a simpler repeatability model, genetic progress can be made.

## CONCLUSIONS

In this study, genetic parameters were estimated for $CH_4$ concentrations continuously measured in the feed bin of milking robots. Moderate heritability and repeatability were estimated for mean and median $CH_4$ emissions. Low heritability was estimated for the ratio trait of $CH_4/CO_2$. Phenotypic and genetic correlations were high between the mean, median, and log$CH_4$ traits, excluding the $CH_4/CO_2$ ratio trait, which was negative. From the mean $CH_4$ emissions per visit, 25 records on mean $CH_4$, measured on 10 different daughters, gave reliabilities of breeding values above the Dutch breeding value publication threshold of 50%. Although the heritability and repeatability for the mean emissions per week were higher than for the mean emissions per robot visit, the reliabilities of estimated breeding values derived from the 2 recording strategies are equal.

## ACKNOWLEDGMENTS

The research leading to these results was part of the Climate Approach Project, funded by the Dutch Ministry for Agriculture, Nature and Food Quality (LNV, The Hague; project number BO-53-003). This work was supported by the TKI Agri & Food project LWV19155 (Wageningen, the Netherlands) and the partners CRV (Arnhem, the Netherlands) and FrieslandCampina (Amersfoort, the Netherlands). The authors gratefully acknowledge the farmers for participating in this research, Johan Ploegaert (Wageningen Livestock Research, Wageningen, the Netherlands) for maintaining the sniffers, Hendrik Jan van Dooren (Wageningen Livestock Research) for setting up data collection, and CRV for providing the pedigree and genotype data. The authors have not stated any conflicts of interest.

## REFERENCES

Aguilar, I., I. Misztal, D. L. Johnson, A. Legarra, S. Tsuruta, and T. J. Lawlor. 2010. Hot topic: A unified approach to utilize phenotypic, full pedigree, and genomic information for genetic evaluation of Holstein final score. J. Dairy Sci. 93:743–752. https://doi.org/10.3168/jds.2009-2730.

Arthur, P. F., I. M. Barchia, C. Weber, T. Bird-Gardiner, K. A. Donoghue, R. M. Herd, and R. S. Hegarty. 2017. Optimizing test procedures for estimating daily methane and carbon dioxide emissions in cattle using short-term breath measures. J. Anim. Sci. 95:645–656. https://doi.org/10.2527/jas.2016.0700.

Aubry, A., and T. Yan. 2015. Meta-analysis of calorimeter data to establish relationships between methane and carbon dioxide emissions or oxygen consumption for dairy cattle. Anim. Nutr. 1:128–134. https://doi.org/10.1016/j.aninu.2015.08.015.

Bell, M. J., P. C. Garnsworthy, D. Mallis, R. J. Eckard, P. J. Moate, and T. Yan. 2019. Modified approach to estimating daily methane emissions of dairy cows by measuring filtered eructations during milking. J. Sustainable Organic Agric. Syst. 69:47–56. https://doi.org/10.3220/LBF15841062570004.

Bell, M. J., S. L. Potterton, J. Craigon, N. Saunders, R. H. Wilcox, M. Hunter, J. R. Goodman, and P. C. Garnsworthy. 2014a. Variation in enteric methane emissions among cows on commercial dairy farms. Animal 8:1540–1546. https://doi.org/10.1017/S1751731114001530.

Bell, M. J., N. Saunders, R. H. Wilcox, E. M. Homer, J. R. Goodman, J. Craigon, and P. C. Garnsworthy. 2014b. Methane emissions among individual dairy cows during milking quantified by eructation peaks or ratio with carbon dioxide. J. Dairy Sci. 97:6536–6546. https://doi.org/10.3168/jds.2013-7889.

Borchers, H. W. 2019. pracma: Practical Numerical Math Functions (Version 2.2.9.). Accessed Sep. 10, 2021. https://CRAN.R-project.org/package=pracma.

Bovenhuis, H., S. van Engelen, and M. Visker. 2018. Letter to the Editor: A response to Huhtanen and Hristov (2018). J. Dairy Sci. 101:9621–9622. https://doi.org/10.3168/jds.2018-15606.

Breider, I. S., E. Wall, and P. C. Garnsworthy. 2019. Short communication: Heritability of methane production and genetic correlations with milk yield and body weight in Holstein-Friesian dairy cows. J. Dairy Sci. 102:7277–7281. https://doi.org/10.3168/jds.2018-15909.

Calus, M. P. L., and J. Vandenplas. 2016. Calc_grm—A program to compute pedigree, genomic, and combined relationship matrices. Animal Breeding and Genomics, Wageningen UR Livestock Research.

Christensen, O. F., and M. S. Lund. 2010. Genomic prediction when some animals are not genotyped. Genet. Sel. Evol. 42:2. https://doi.org/10.1186/1297-9686-42-2.

Crompton, L. A., J. A. N. Mills, C. K. Reynolds, and J. France. 2011. Fluctuations in methane emission in response to feeding pattern in lactating dairy cows. Pages 176–180 in Modelling Nutrient Digestion and Utilisation in Farm Animals. D. Sauvant, J. van Milgen, P. Faverdin, and N. Friggens, ed. Wageningen Academic Publishers.

CRV. 2018. Breeding value estimation of milk production traits with test-day model. Accessed Dec. 12, 2021. https://cooperatiecrv-be6.kxcdn.com/wp-content/uploads/2020/04/E_07-Melkproductie-April-2020.pdf.

CRV. 2020. Publicatieregels stierindexen [Publication rules: Sire indexes]. Accessed Jul. 19, 2021. https://cooperatiecrv-be6.kxcdn.com/wp-content/uploads/2020/12/E_26-Publicatieregels-Stieren-Augustus-2020-1.pdf.

de Haas, Y., M. Pszczola, H. Soyeurt, E. Wall, and J. Lassen. 2017. Invited review: Phenotypes to genetically reduce greenhouse gas emissions in dairying. J. Dairy Sci. 100:855–870. https://doi.org/10.3168/jds.2016-11246.

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. https://doi.org/10.1016/j.compag.2016.04.010.

Difford, G. F., P. Lovendahl, R. F. Veerkamp, H. Bovenhuis, M. Visker, J. Lassen, and Y. de Haas. 2020. Can greenhouse gases in breath be used to genetically improve feed efficiency of dairy cows? J. Dairy Sci. 103:2442–2459. https://doi.org/10.3168/jds.2019-16966.

Difford, G. F., D. W. Olijhoek, A. L. F. Hellwing, P. Lund, M. A. Bjerring, Y. de Haas, J. Lassen, and P. Løvendahl. 2019. Ranking cows' methane emissions under commercial conditions with sniffers versus respiration chambers. Acta Agric. Scand. A Anim. Sci. 68:25–32. https://doi.org/10.1080/09064702.2019.1572784.

Difford, G. F., D. R. Plichta, P. Lovendahl, J. Lassen, S. J. Noel, O. Højberg, A.-D. G. Wright, Z. Zhu, L. Kristensen, H. B. Nielsen, B. Guldbrandtsen, and G. Sahana. 2018. Host genetics and the rumen microbiome jointly associate with methane emissions in dairy cows. PLoS Genet. 14:e1007580. https://doi.org/10.1371/journal.pgen.1007580.

Falconer, D. S., and T. F. C. Mackay. 1996. Introduction to Quantitative Genetics. Longman.

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 cows. J. Dairy Sci. 95:3166–3180. https://doi.org/10.3168/jds.2011-4605.

Garnsworthy, P. C., G. F. Difford, M. J. Bell, A. R. Bayat, P. Huhtanen, B. Kuhla, J. Lassen, N. Peiren, M. Pszczola, D. Sorg, M. H. P. W. Visker, and T. Yan. 2019. Comparison of methods to measure methane for use in genetic evaluation of dairy cattle. Animals (Basel) 9:837. https://doi.org/10.3390/ani9100837.

Gilmour, A., B. Gogel, B. Cullis, S. Welham, and R. Thompson. 2015. ASReml User Guide: Release 4.1: Structural Specification. VSN International Ltd. Accessed Jun. 15, 2021. https://asreml.kb.vsni.co.uk/wp-content/uploads/sites/3/2018/02/ASReml-4.1-Functional-Specification.pdf.

Hammond, K. J., G. C. Waghorn, and R. S. Hegarty. 2016. The GreenFeed system for measurement of enteric methane emission from cattle. Anim. Prod. Sci. 56:181. https://doi.org/10.1071/AN15631.

Haque, M. N., C. Cornou, and J. Madsen. 2015. Individual variation and repeatability of methane production from dairy cows estimated by the $CO_2$ method in automatic milking system. Animal 9:1567–1576. https://doi.org/10.1017/S1751731115000646.

Herrero, M., B. Henderson, P. Havlík, P. K. Thornton, R. T. Conant, P. Smith, S. Wirsenius, A. N. Hristov, P. Gerber, M. Gill, K. Butterbach-Bahl, H. Valin, T. Garnett, and E. Stehfest. 2016. Greenhouse gas mitigation potentials in the livestock sector. Nat. Clim. Chang. 6:452–461. https://doi.org/10.1038/nclimate2925.

Huhtanen, P., A. R. Bayat, P. Lund, A. L. F. Hellwing, and M. R. Weisbjerg. 2020. Short communication: Variation in feed efficiency hampers use of carbon dioxide as a tracer gas in measuring methane emissions in on-farm conditions. J. Dairy Sci. 103:9090–9095. https://doi.org/10.3168/jds.2020-18559.

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

Huhtanen, P., and A. N. Hristov. 2018. Letter to the Editor: Challenging one sensor method for screening dairy cows for reduced methane emissions. J. Dairy Sci. 101:9619–9620. https://doi.org/10.3168/jds.2018-14704.

Knapp, J. R., G. L. Laur, P. A. Vadas, W. P. Weiss, and J. M. Tricarico. 2014. Invited review: Enteric methane in dairy cattle production: Quantifying the opportunities and impact of reducing emissions. J. Dairy Sci. 97:3231–3261. https://doi.org/10.3168/jds.2013-7234.

Krattenmacher, N., G. Thaller, and J. Tetens. 2019. Analysis of the genetic architecture of energy balance and its major determinants dry matter intake and energy-corrected milk yield in primiparous

Holstein cows. J. Dairy Sci. 102:3241–3253. https://doi.org/10.3168/jds.2018-15480.

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

López-Paredes, J., I. Goiri, R. Atxaerandio, A. García-Rodriguez, E. Ugarte, J. A. Jiménez-Montero, R. Alenda, and O. González-Recio. 2020. Mitigation of greenhouse gases in dairy cattle via genetic selection: 1. Genetic parameters of direct methane using noninvasive methods and proxies of methane. J. Dairy Sci. 103:7199–7209. https://doi.org/10.3168/jds.2019-17597.

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

Mitloehner, F., E. Kebreab, and M. Boccadoro. 2020. Methane, cows, and climate change: California dairy's path to climate neutrality. Accessed Oct. 6, 2021. https://clear.ucdavis.edu/sites/g/files/dgvnsk7876/files/inline-files/CLEAR-Center-Methane-Cows-Climate-Change-Sep-2-20_6.pdf.

Mrode, R. A. 2005. Linear Models for the Prediction of Animal Breeding Values. 2nd ed. CABI.

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

Pszczola, M., K. Rzewuska, S. Mucha, and T. Strabel. 2017. Heritability of methane emissions from dairy cows over a lactation measured on commercial farms. J. Anim. Sci. 95:4813–4819. https://doi.org/10.2527/jas2017.1842.

Rey, J., R. Atxaerandio, R. Ruiz, E. Ugarte, O. Gonzalez-Recio, Garcia-Rodriguez, and Goiri.. 2019. Comparison between non-invasive methane measurement techniques in cattle. Animals (Basel) 9:563. https://doi.org/10.3390/ani9080563.

Saborío-Montero, A., M. Gutiérrez-Rivas, A. García-Rodríguez, R. Atxaerandio, I. Goiri, E. López de Maturana, J. A. Jiménez-Montero, R. Alenda, and O. González-Recio. 2020. Structural equation models to disentangle the biological relationship between microbiota and complex traits: Methane production in dairy cattle as a case of study. J. Anim. Breed. Genet. 137:36–48. https://doi.org/10.1111/jbg.12444.

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

Sypniewski, M., T. Strabel, A. Cieslak, M. Szumacher-Strabel, and M. Pszczola. 2019. Technical note: Interchangeability and comparison of methane measurements in dairy cows with 2 noninvasive infrared systems. J. Dairy Sci. 102:9512–9517. https://doi.org/10.3168/jds.2019-16258.

Sypniewski, M., T. Strabel, and M. Pszczola. 2021. Genetic variability of methane production and concentration measured in the breath of Polish Holstein-Friesian cattle. Animals (Basel) 11:3175. https://doi.org/10.3390/ani11113175.

van Engelen, S., H. Bovenhuis, P. P. J. van der Tol, and M. H. P. W. Visker. 2018. Genetic background of methane emission by Dutch Holstein Friesian cows measured with infrared sensors in automatic milking systems. J. Dairy Sci. 101:2226–2234. https://doi.org/10.3168/jds.2017-13441.

van Soest, P. J. 1994. Nutritional Ecology of the Ruminant. Cornell University Press.

Wellmann, R. 2020. optiSel: Optimum Contribution Selection and Population Genetics. Version 2.0.3. Accessed May 4, 2021. https://CRAN.R-project.org/package=optiSel.

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

Zetouni, L., M. Henryon, M. Kargo, and J. Lassen. 2017. Direct multitrait selection realizes the highest genetic response for ratio traits. J. Anim. Sci. 95:1921. https://doi.org/10.2527/jas.2016.1324.

Zetouni, L., M. Kargo, E. Norberg, and J. Lassen. 2018. Genetic correlations between methane production and fertility, health, and body type traits in Danish Holstein cows. J. Dairy Sci. 101:2273–2280. https://doi.org/10.3168/jds.2017-13402.

## ORCIDS

A. E. van Breukelen https://orcid.org/0000-0003-1576-4153
M. A. Aldridge https://orcid.org/0000-0002-9033-3081
R. F. Veerkamp https://orcid.org/0000-0002-5240-6534
Y. de Haas https://orcid.org/0000-0002-4331-4101

## APPENDIX

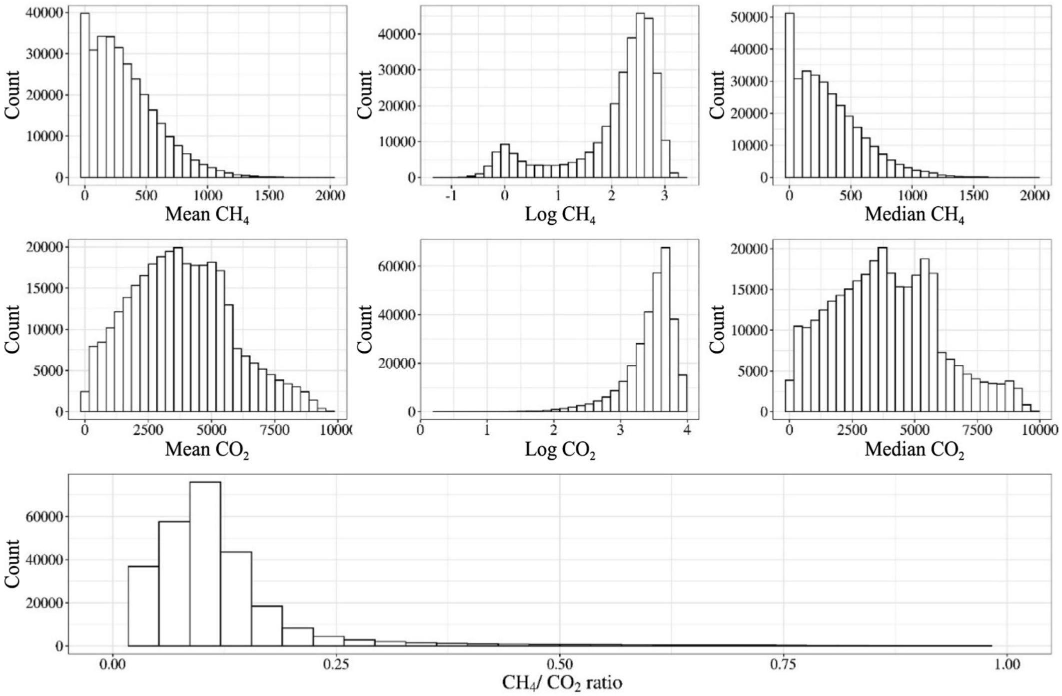

**Figure A1.** Histograms for the methane traits per robot visit: mean $CH_4$, log $CH_4$, median $CH_4$, mean $CO_2$, log $CO_2$, median $CO_2$, and $CH_4$/$CO_2$ ratio.

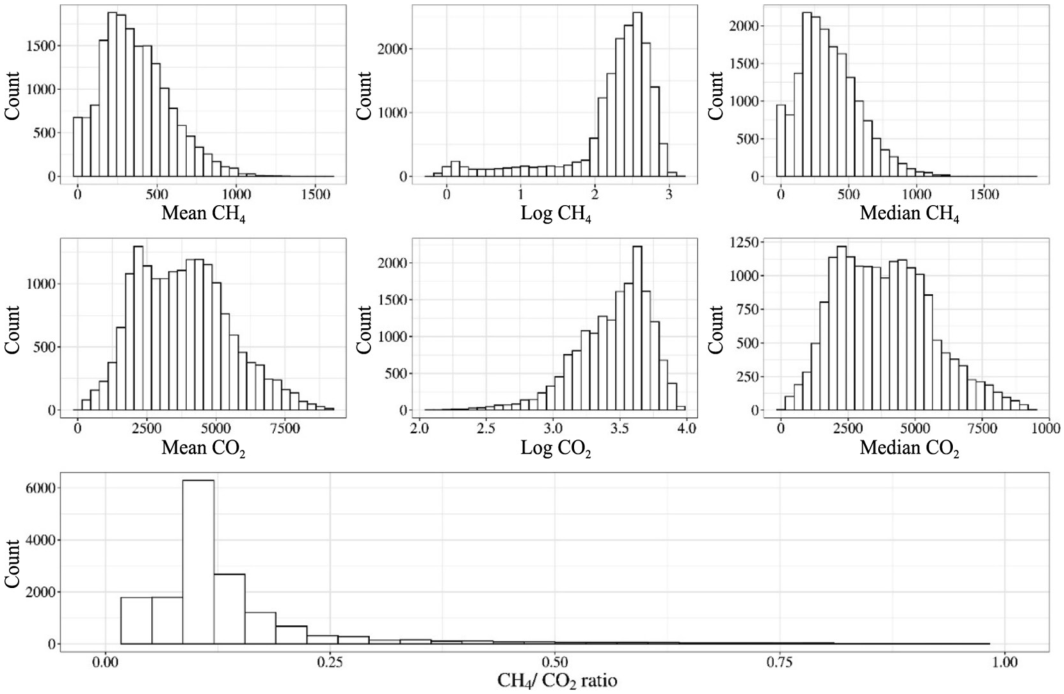

**Figure A2.** Histograms for the methane traits as average per week: mean $CH_4$, log $CH_4$, median $CH_4$, mean $CO_2$, log $CO_2$, median $CO_2$, and $CH_4$/$CO_2$ ratio.