Genetic background of methane emission by Dutch Holstein Friesian cows measured with infrared sensors in automatic milking systems

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ABSTRACT

International environmental agreements have led to the need to reduce methane emission by dairy cows. Reduction could be achieved through selective breeding. The aim of this study was to quantify the genetic variation of methane emission by Dutch Holstein Friesian cows measured using infrared sensors installed in automatic milking systems (AMS). Measurements of CH₄ and CO₂ on 1,508 Dutch Holstein Friesian cows located on 11 commercial dairy farms were available. Phenotypes per AMS visit were the mean of CH₄, mean of CO₂, mean of CH₄ divided by mean of CO₂, and their log₁₀-transformations. The repeatabilities of the log₁₀-transformed methane phenotypes were 0.27 for CH₄, 0.31 for CO₂, and 0.14 for the ratio. The log₁₀-transformed heritabilities of these phenotypes were 0.11 for CH₄, 0.12 for CO₂, and 0.03 for the ratio. These results indicate that measurements taken using infrared sensors in AMS are repeatable and heritable and, thus, could be used for selection for lower CH₄ emission. Furthermore, it is important to account for farm, AMS, day of measurement, time of day, and lactation stage when estimating genetic parameters for methane phenotypes. Selection based on log₁₀-transformed CH₄ instead of the ratio would be expected to give a greater reduction of CH₄ emission by dairy cows.

Key words: methane emission, dairy cow, AMS, nondispersive infrared sensor

INTRODUCTION

Agriculture contributes 24% of the total global greenhouse gas emissions (IPCC, 2014). The single largest pollution source within agriculture is enteric fermentation (i.e., the breakdown of feed in the rumen that results in the production of CH₄; Gerber et al.,

2013). Approximately 8% of the worldwide agricultural greenhouse gas emissions originate from enteric CH₄ emissions by dairy cows (FAOSTAT, 2012; FAO, 2014), showing the impact of dairy production on global warming. In December 2015, agreements were made in Paris between 195 countries to tackle climate change and keep global warming firmly below 2°C (UNCCC, 2015). These agreements have further emphasized the importance of the reduction of CH₄ emission by dairy cows.

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

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

Previous studies have shown that CH₄ measurements based on infrared sensors in AMS are repeatable. Lassen et al. (2012) summarized CH₄ measurements per AMS visit by taking the mean of CH₄, the mean of CO₂, and the mean of the ratio between CH₄ and CO₂;

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repeatabilities ranged between 0.22 and 0.46 for 50 Holstein cows and 43 Jersey cows. Bell et al. (2014b) found a repeatability of 0.74 for mean of CH₄ per AMS visit for 36 Holstein-Friesian cows. These repeatabilities illustrate that infrared sensors in AMS could provide the repeatable measurements on individual cows that are needed to quantify possible genetic variation in CH₄ emission.

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

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

The aim of our study was to quantify the genetic variation of CH₄ emission by Dutch dairy cows measured using infrared sensors installed in AMS. The data set comprised CH₄ and CO₂ measurements taken with infrared sensors on Dutch Holstein Friesian cows located on commercial dairy farms. Measurements were summarized into different CH₄ phenotypes per AMS visit and repeatability and heritability were calculated for these phenotypes.

MATERIALS AND METHODS

Ethical Statement

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

Methane Sensor

Methane phenotypes were measured using sensors. These sensors were tested in climate respiration cham-

bers (**CRC**) before they were installed on commercial farms. In this test, CH₄ emissions of 20 individual Holstein Friesian cows were recorded in CRC for 3 consecutive days and, simultaneously, by the sensor. In the CRC, CH₄ and CO₂ were measured every 12.5 min as described by Heetkamp et al. (2015). The sensors were gas analyzers (SenseAir LPL CH₄/CO₂, Rise Acreo, Stockholm, Sweden) that were installed in line with the cow's nostrils when standing and facing forward. Air was drawn through the instrument at 1 L/min; CH₄ and CO₂ concentrations were measured continuously using a nondispersive infrared (**NDIR**) technique and logged twice per second. Phenotypes were defined as CH₄ production (L/d) from CRC; CH₄ concentration (ppm) from sensor; and CH₄:CO₂ ratio from sensor.

Data

Methane and CO₂ concentrations (ppm) were measured on 1,508 primiparous and multiparous dairy cows from 11 commercial farms in the Netherlands. On 9 of these farms, cows were fed in the morning, whereas on 1 farm cows were fed in the evening. In addition, some farms had automatic feed pushers that compiled the feed continuously during the day, and 1 farm had an automatic feeder that fed the cows freshly mixed feed up to 30 times a day. Furthermore, cows on some farms could graze during the day whereas cows on other farms were kept indoors. More than 85% of the cows were at least 7/8 Holstein Friesian. Measurements were taken during milking in AMS (Lely Astronaut A4, Lely Industries NV, Maassluis, the Netherlands) using NDIR sensors. A total of 4 sensors were used to collect all data by installing them consecutively in different AMS. Measurements were taken in a total of 23 AMS, 1 to 4 AMS per farm, between November 2013 and March 2016. The data from these sensors were linked to the data from the AMS to obtain the identification numbers (**ID**) of the cows and, subsequently, additional animal information, such as week of lactation. The data of the sensors were aligned to the AMS visits as both were recorded on different devices. The alignment between these devices was based on the pattern of AMS visits (i.e., duration and order of AMS visits and the time between the AMS visits). This pattern was aligned in such a way that CH₄ and CO₂ concentrations were highest during AMS visits and lowest in between AMS visits. After alignment, the ID of the cows were used to link sensor data to data from the cooperative cattle improvement organization CRV (Arnhem, the Netherlands) to obtain the pedigree. The pedigree was traced back 2 generations, resulting in 4,214 animals in the pedigree.

Data Editing

Data from the sensors and AMS were edited based on several conditions. The first condition was that only data from days with sensor measurements for at least 30% of the day were kept. Days with less than 30% data were mostly without data or the data present were fragmentary; therefore, these days were discarded. The number of days with measurements ranged from 10 up to 81 per AMS. The second condition was that AMS visits should last at least 90 s; AMS visits shorter than 90 s were removed from the data set. Removing AMS visits shorter than 90 s ensured that most AMS visits that did not result in a milking, and would, thus, not provide a steady measurement, were removed from the data set. The third condition was that the ID of the cow visiting the AMS should be known; AMS visits without cow ID were removed from the data set, as these could not be linked to the pedigree. The fourth condition was that week of lactation of the cow visiting the AMS should be known and cows should be between 1 and 60 wk in lactation. The fifth condition was that AMS visits with missing CH₄ phenotypes (see phenotypes) or missing model effects (see model 1) were removed from the data set. The sixth and last condition was that each cow should have at least 4 AMS visits. After editing, a total of 129,900 AMS visits on 1,508 dairy cows that had on average 86 AMS visits (range = 4–295 AMS visits) were available for analysis. The number of cows ranged between 62 and 224 per farm.

The AMS visits that had a standardized residual effect >3.5 based on model 1 for mean and log-transformed CH₄, mean and log-transformed CO₂, and mean and log-transformed ratio of CH₄ to CO₂ were considered outliers and were removed. After removal of the outliers, the data set consisted of 123,369 AMS visits from 1,508 dairy cows. This data set was used to estimate the variance components and genetic parameters.

Phenotypes

The CH₄ and CO₂ measurements were summarized per AMS visit into 6 phenotypes. To correct for the background levels of CH₄ and CO₂ in the barn, offsets for CH₄ and CO₂ were calculated per AMS visit. The background levels of CH₄ were assumed to be 0 ppm and the offset for CH₄ was the mean of the 10 lowest values for CH₄ in a specific AMS visit. As the background levels of CO₂ were assumed to be 400 ppm, the offset for CO₂ was the mean of the lowest 10 values for CO₂ minus 400. The CH₄ offset was subtracted from the individual (twice per second) CH₄ measurements during a specific AMS visit and the CO₂ offset was

subtracted from the individual CO₂ measurements. After adjustment for the offsets, the methane phenotypes were calculated. The first phenotype was the mean of CH₄ per AMS visit (**CH₄m**); the second phenotype was the mean of CO₂ per AMS visit (**CO₂m**). The third phenotype was based on the ratio (CH₄/CO₂) per AMS visit and is calculated as the mean of CH₄ divided by the mean CO₂ per AMS visit (**RATm**). The residuals of the traits based on model 1 were not normally distributed. For example, the residuals of CH₄m showed a thicker and longer right tail (kurtosis = 3.24). Therefore, phenotypes were log₁₀-transformed, and after transformation residuals became normally distributed (kurtosis of log₁₀-transformed CH₄m = 0.55). These log₁₀-transformations resulted in the fourth phenotype [log₁₀(CH₄m) (**CH₄l**)], fifth phenotype [log₁₀(CO₂m) (**CO₂l**)], and sixth phenotype [log₁₀(RATm) (**RATl**)]. Furthermore, milk yield in kilograms per AMS visit was included as a general trait.

Data Analysis

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

$$Y_{ijklm} = \mu + \text{DayAMS}_i + \text{Lactationweek}_j + \text{Hour} \\ \times \text{Farm}_k + \text{Animal}_l + \text{Permanent}_m + e_{ijklm}, \quad [1]$$

where Y_{ijklm} is the dependent variable (CH₄m, CO₂m, RATm, CH₄l, CO₂l, RATl, or milk yield); μ is the mean; DayAMS_i is the combined effect of day of measurement-farm of measurement, AMS of measurement, and sensor of measurement (DayAMS; 991 levels); Lactationweek_j is the fixed effect of week of lactation (60 levels); $\text{Hour} \times \text{Farm}_k$ is the fixed interaction of hour of the day (24 levels) and farm of measurement (11 levels); Animal_l is the random additive genetic effect of animal $\left[\sim N(0, \mathbf{A}\sigma_{\text{animal}}^2) \right]$, with additive genetic relationship matrix $\mathbf{A}$ and additive genetic variance σ_{animal}^2 ; Permanent_m is the random permanent environmental effect $\left[\sim N(0, \mathbf{I}\sigma_{\text{permanent}}^2) \right]$, with identity matrix $\mathbf{I}$ and permanent environmental variance $\sigma_{\text{permanent}}^2$; and e_{ijklm} is the random error effect $\left[\sim N(0, \mathbf{I}\sigma_{\text{error}}^2) \right]$, with identity matrix $\mathbf{I}$ and residual variance σ_{error}^2 .

Measurement Period

For analysis, all data available for each cow were used, ranging from 1 to 81 d/cow. To study the effect of

length of measurement period a subset of the data were used consisting of data from 1 AMS on 1 farm during 50 consecutive days from December 2015 to February 2016. The measurement period lengths that were tested were 3, 5, 10, 20, and 30 consecutive days. For each of these lengths, 5 individual data sets with that length in consecutive days were created by random sampling from the data set of 50 d (e.g., 5 data sets containing 30 consecutive days). Data sets of the same measurement period length were sometimes partially overlapping. Repeatabilities were calculated for each measurement period data set, and repeatabilities and their standard errors were averaged over the 5 data sets for each measurement period length.

Genetic Parameters

The repeatability was calculated as

$$\text{Repeatability} = \frac{\sigma_{\text{animal}}^2 + \sigma_{\text{permanent}}^2}{\sigma_{\text{animal}}^2 + \sigma_{\text{permanent}}^2 + \sigma_{\text{error}}^2},$$

with additive genetic variance σ_{animal}^2 , permanent environmental variance $\sigma_{\text{permanent}}^2$, and residual variance σ_{error}^2 . The heritability (h^2) was calculated as

$$h^2 = \frac{\sigma_{\text{animal}}^2}{\sigma_{\text{animal}}^2 + \sigma_{\text{permanent}}^2 + \sigma_{\text{error}}^2}.$$

We presented the descriptive statistics on all 6 phenotypes, but the genetic parameters of only the $\log_{10}$ -transformed phenotypes were shown. As the residuals of the untransformed phenotypes were not normally distributed, this could affect the results found for these genetic parameters. Effects of the model parameters were presented on the untransformed phenotypes as these effects are then easier to interpret.

The accuracy of the breeding value for CH_4 emission for a cow was calculated as

$$\sqrt{\frac{mh^2}{(m-1)t+1}},$$

where m is the number of repeated sensor measurements in an AMS, h^2 is the heritability, and t is the repeatability. The accuracy of breeding value for CH_4 for a bull with half-sib daughters 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 accuracy of the breeding value of the daughters with 25 repeated sensor measurements each.

RESULTS

Methane Sensor Test

During the test, the repeatabilities of CH_4 production (L/d), CH_4 concentration (ppm) and $\text{CH}_4:\text{CO}_2$ ratio were calculated using data averaged per cow per day. Repeatability of CH_4 production obtained from CRC measurements was 0.87 (SE = 0.04), repeatability of CH_4 concentration obtained from sensor measurements was 0.90 (SE = 0.04), and repeatability of $\text{CH}_4:\text{CO}_2$ ratio obtained from sensor measurements was 0.94 (SE = 0.02). The correlation between CH_4 production obtained from CRC and CH_4 concentration obtained from sensor was 0.71 (SE = 0.10). The correlation between CH_4 production obtained from CRC and $\text{CH}_4:\text{CO}_2$ ratio obtained from sensor was 0.49 (SE = 0.18).

Descriptive Statistics

Descriptive statistics on the 6 methane phenotypes and on milk production per AMS visit are represented in Table 1. After the $\log_{10}$ -transformation, the means and standard deviations of CH_4m and CO_2m decreased,

Table 1. Descriptive statistics of phenotypes on CH_4 and CO_2 measured with nondispersive infrared (NDIR) sensors in automatic milking systems (AMS) on 1,508 Dutch dairy cows (123,369 AMS visits)

Trait	Mean	SD	Minimum	Maximum
CH_4 mean (ppm)	254	230	11	2,073
$\text{CH}_4 \log^1$ (ppm)	2.25	0.37	1.04	3.32
CO_2 mean (ppm)	1,443	681	408	9,054
$\text{CO}_2 \log^1$ (ppm)	3.11	0.20	2.61	3.96
$\text{CH}_4:\text{CO}_2$ ratio mean	0.17	0.12	0.01	0.87
$\text{CH}_4:\text{CO}_2$ ratio $\log^1$	-0.87	0.27	-1.92	-0.06
Milk ² (kg)	10.8	3.4	0.00	36.50

¹ $\log_{10}$ -transformed phenotypes.

²Milk production per AMS visit.

whereas the mean and standard deviation increased in an absolute sense for RAT_m. A milk yield of 0 kg for 619 AMS visits illustrates that during these AMS visits the cows were not milked by the AMS. These AMS visits were still present in the data set, despite removing AMS visits shorter than 90 s. We considered any AMS visit longer than 90 s suitable for CH₄ and CO₂ measurements, irrespective if the cow was being milked during that visit.

Effect of Hour of the Day on Methane Emission

In general, the effect of hour of the day on CH_{4m}, as obtained from model 1, was lower during the night and higher during the day. This general pattern showed variation between farms, as demonstrated by farms A and B in Figure 1. Farm A showed 2 distinct peaks in CH_{4m} during the day: the first peak around 0900 h and the second peak around 2000 h. Farm B showed a strong increase in CH_{4m} during the day compared with the night. Both types of patterns were present in the data set, but most farms had a pattern similar to that of farm A.

Effect of Week of Lactation on Methane Emission

The effect of week of lactation on CH_{4m}, as obtained from model 1, for the first 50 wk of lactation is presented in Figure 2. The effect of week of lactation on CH_{4m} increased rapidly during the first 12 wk of lactation. After this strong increase, the effect of week of lactation on CH_{4m} remained relatively constant until

35 wk in lactation and decreased gradually thereafter. Between wk 12 and 35, the effect of lactation week accounted for 3 to 7% of the variation in the mean of CH_{4m}.

Genetic Parameters for Methane Emission

Repeatabilities and heritabilities of the log₁₀-transformed methane phenotypes and milk production are presented in Table 2. The repeatabilities ranged between 0.14 and 0.31 for the methane phenotypes, were similar for CH_{4l} and CO_{2l}, and were lower for RAT_l. The heritabilities were lower than the repeatabilities and ranged between 0.03 and 0.12 for the methane phenotypes. Heritabilities were similar for CH_{4l} and CO_{2l} and were lower for RAT_l. Milk yield per AMS visit had a higher repeatability (0.45) and heritability (0.17) than the methane phenotypes; standard errors of the repeatabilities and heritabilities were between 0.005 and 0.03.

The accuracy of the breeding value for CH₄ emission expressed as CH_{4l} for a cow based on 25 repeated sensor measurements in an AMS was 0.61. For a bull with 25 daughters, where each daughter has 25 repeated sensor measurements, the accuracy of the breeding value for CH₄ emission was 0.85.

Effect of Measurement Period

The average repeatabilities and standard error of each measurement period length are presented in Table 3. All 3 methane phenotypes showed higher repeatabilities

Figure 1. Effect of hour of the day on CH₄ mean (ppm) measured with nondispersive infrared (NDIR) sensors in automatic milking systems (AMS) on 1,508 Dutch dairy cows. The figure shows 2 representative farms (A = 5,554 AMS visits; B = 20,458 AMS visits). The effect of h 4 was set to zero for both farms to enable comparison and the other effects are expressed relative to h 4.

Figure 2. Effect of week of lactation on CH₄ mean (ppm) measured with nondispersive infrared (NDIR) sensors in automatic milking systems (AMS) on 1,508 Dutch dairy cows (123,369 AMS visits). The effect of wk 4 of lactation was set to zero and the other effects are expressed relative to wk 4.

Table 2. Repeatabilities and heritabilities of phenotypes on CH₄ and CO₂ measured with nondispersive infrared (NDIR) sensors in automatic milking systems (AMS) on 1,508 Dutch dairy cows (123,369 AMS visits)¹

Trait	Repeatability	Heritability
CH ₄ log ² (ppm)	0.27 (0.008)	0.11 (0.02)
CO ₂ log ² (ppm)	0.31 (0.009)	0.12 (0.02)
CH ₄ :CO ₂ ratio log ²	0.14 (0.005)	0.03 (0.01)
Milk ³ (kg)	0.45 (0.010)	0.17 (0.03)

¹This table contains the repeatability ($\text{repeatability} = \sigma^2_{\text{animal}} + \sigma^2_{\text{permanent}} / \sigma^2_{\text{animal}} + \sigma^2_{\text{permanent}} + \sigma^2_{\text{error}}$) and the heritability ($\text{heritability} = \sigma^2_{\text{animal}} / \sigma^2_{\text{animal}} + \sigma^2_{\text{permanent}} + \sigma^2_{\text{error}}$) with their respective SE in parentheses, where σ^2_{animal} , $\sigma^2_{\text{permanent}}$, and σ^2_{error} are additive genetic, permanent environmental, and residual variance, respectively.

²Log₁₀-transformed phenotypes.

³Milk production per AMS visit.

in measurement periods longer than 5 d compared with shorter measurement periods. Measurement periods longer than 10 d did not lead to further improvements of the repeatabilities. Standard errors decreased with increasing measurement period length, but the largest decrease occurred with the increase in measurement period from 3 to 5 d. Furthermore, repeatabilities of the measurement period of 10 d were not significantly different from the repeatabilities of the data set with all observations from that specific AMS.

DISCUSSION

The aim of our study was to quantify the genetic variation in methane phenotypes measured with NDIR sensors in AMS. Methane phenotypes based on sensor measurements of CH₄ and CO₂ on Dutch dairy cows were both repeatable and heritable. The repeatabilities of these phenotypes ranged between 0.14 and 0.31. The heritabilities of these phenotypes ranged between 0.03

and 0.12, indicating genetic variation in these phenotypes.

Methane Sensor Test

High repeatability of CH₄ production obtained from CRC measurements and of CH₄ concentration obtained from sensors agree with the literature (Bell et al., 2014b; Donoghue et al., 2016). The high repeatabilities found in the present study demonstrate high consistency between subsequent measurements on the same animal, implying high precision of measurement and suggesting consistent differences between animals. High repeatabilities and moderate correlations demonstrate the potential of the sensor method for the collection of phenotypes on CH₄ emission for large numbers of individual animals. Repeatabilities are expected to be lower when installed in AMS because of more variable conditions on the farm. This limitation is most likely compensated by the ability to perform large-scale collection of data on commercial dairy farms. This facilitates repeated measures on a single animal and recording large numbers of animals, both contributing to the accuracy of estimated breeding values.

Methane Phenotypes

Phenotypes used in our study were measured in parts per million. In the literature, concentration measurements (in ppm) have been transformed to CH₄ production (g/d) using a dilution factor or using CO₂ production (Madsen et al., 2010; Garnsworthy et al., 2012a; Lassen and Løvendahl, 2016). These transformations, however, are based on several assumptions, such as a constant CO₂ production of a cow throughout the day

Table 3. The average repeatability of phenotypes on CH₄ and CO₂ measured with nondispersive infrared (NDIR) sensors in automatic milking systems (AMS) on Dutch dairy cows over different measurement period lengths^{1,2}

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

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

²The table contains the measurement period in days, the average number of AMS visit per measurement period (N), and the average repeatability per methane phenotype ($\text{repeatability} = \sigma^2_{\text{animal}} + \sigma^2_{\text{permanent}} / \sigma^2_{\text{animal}} + \sigma^2_{\text{permanent}} + \sigma^2_{\text{error}}$) with their respective average SE in parentheses, where σ^2_{animal} , $\sigma^2_{\text{permanent}}$, and σ^2_{error} are additive genetic, permanent environmental, and residual variance, respectively.

³Log₁₀-transformed phenotypes.

that may not always be met. The CH₄ production obtained after transformation is affected by the accuracy of these assumptions. For breeding, absolute values are not needed, as it focuses on the relative differences between animals to select the best animals.

Phenotypes similar to those used in our study were also used in other studies (e.g., Madsen et al., 2010; Lassen et al., 2012; Bell et al., 2014b). The absolute values of such similar phenotypes, however, have not been published except for RATm (i.e., the mean of the ratio between CH₄ and CO₂). We included RATm in our study because it was reported in other studies and can be used to quantify methane production (Madsen et al., 2010). The absolute value of RATm in our study was considerably higher than the one reported by Lassen et al. (2012; i.e., 0.17 vs. 0.065). The reason for this difference is unclear, as the absolute values of the underlying traits to RATm (i.e., CH₄m and CO₂m) were not reported by Lassen et al. (2012). Breeding, however, does not depend on absolute values, and, therefore, it is expected that the difference in absolute value of RATl compared with the literature would not affect the direction of selection if RATl would be used for selection.

Milk Yield

Selective breeding for milk yield has led to substantial genetic progress over time. Heritability for milk yield per AMS visit in our study was 0.17 (SE = 0.03). This heritability is slightly lower than the heritability of 0.24 reported (Mulder et al., 2004) for milk yield per day recorded in AMS. The heritability of CH₄l was 0.11 (SE = 0.02), which is comparable with the heritability of milk yield per AMS visit. This indicates potential for a reduction in methane emission through selective breeding when using sensor measurements in AMS.

Effect of DayAMS

The DayAMS effect that was used in the model includes the effects of the day, farm, AMS, and sensor of measurement. These effects could not be disentangled in our study, because most measurements took place on 1 farm at a time, with a single sensor installed per AMS. To study the effect of DayAMS on the methane phenotypes, an additional analysis was performed in which DayAMS was included in model 1 as a random effect instead of as a fixed effect. This analysis showed that the percentage of total variation that was explained by the DayAMS effect was 56% for CH₄l, 27% for CO₂l, and 82% for RATl. These results indicate that the phenotypes were largely influenced by the DayAMS effect, and illustrates that accounting for the effects of day

of measurement, farm, AMS, and sensor is important when analyzing methane phenotypes. Farm conditions that are known to influence CH₄ measurements are season, air flow, and barn management (Wu et al., 2016). The large effect of DayAMS agrees with the other studies that acknowledge the effect of farm of measurement and farm conditions on methane measurements (Bell et al., 2014a; Hammond et al., 2016).

Effect of Hour of the Day on Methane Emission

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

Effect of Week of Lactation on Methane Emission

Week of lactation had a significant effect in the model, with a *P*-value below 0.001 when DayAMS was included in model 1 as random. The size of the effect is relatively small compared with the effect of DayAMS. As feed composition and intake usually changes throughout lactation, week of lactation can affect CH₄ emission (Garnsworthy et al., 2012b; Bell et al., 2014a; Lassen and Løvendahl, 2016). Previous studies also reported effects of lactation stage on methane emission measured in AMS (Garnsworthy et al., 2012b; Bell et al., 2014a; Lassen and Løvendahl, 2016). Similar to our study, these studies found an increase in methane emissions during the first weeks of lactation. The highest level of methane emission was found at around 10 wk of lactation by Lassen and Løvendahl (2016), at 20 wk of lactation by Garnsworthy et al. (2012b), and at 12 wk of lactation in our study. After the initial increase in methane emission per week of lactation, either a stable level of methane emission until 50 wk in lactation was reported (Bell et al., 2014a) or a decrease in methane

emission per week of lactation was reported (Garnsworthy et al., 2012b; Lassen and Løvendahl, 2016). The extent of this decrease varied between 20% of the peak methane emission at 50 wk of lactation (Garnsworthy et al., 2012b) and 80% of the peak methane emission at 44 wk of lactation (Lassen and Løvendahl, 2016). In our study, methane emission decreased with about 33% of the peak methane emission at 50 wk of lactation. The pattern found in our study is comparable with the patterns found in literature, and the differences in patterns might be explained by many different factors such as the used phenotypes. Our study used CH₄m in parts per million as the phenotype whereas other studies used methane in grams per day that was either from the integral area under the peaks of methane emission (Garnsworthy et al., 2012b) or by the ratio between CH₄ and CO₂ in relation to heat-producing units (Lassen and Løvendahl, 2016). Based on the results of our study and of the literature, inclusion of lactation stage into the model to analyze methane emission is recommended.

Repeatabilities of Methane Phenotypes

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

Effect of Measurement Period

Measurement period influenced the repeatabilities found in our study, and other studies have chosen different measurement periods. Bell et al. (2014b) measured CH₄ and CO₂ for a 35-d period, whereas Lassen et al. (2012) measured for a 3-d period. The results obtained from our study indicate that repeatabilities (and their SE) remained stable in measurement periods of at least 10 consecutive days. In other words, the value of additional repeated measurements beyond 10 d of measurements on the same individual was close to zero.

Although Lassen et al. (2012) used a shorter measurement period than our study, their reported standard errors are small (SE = 0.003–0.006). This indicates that the repeatability reported will likely not be affected by increasing the measurement period.

Genetic Parameters for Methane Emission

The heritabilities of the log₁₀-transformed phenotypes in this study were 0.11 for CH₄l, 0.12 for CO₂l, and 0.03 for RATl. Lassen and Løvendahl (2016) measured methane using infrared sensors in AMS on 3,121 Holstein cows and calculated heritabilities of methane emission. Methane emission calculated using the ratio between CH₄ and CO₂ (in ppm) gave a heritability of 0.16, and both CH₄ in grams per day and CH₄ in grams per kilogram of fat- and protein-corrected milk gave a heritability of 0.21. These heritabilities were slightly higher compared with the heritabilities of CH₄l and CO₂l in our study. The heritability of RATl of our study is considerably lower compared with the other heritabilities. Lassen et al. (2012) used the ratio between CH₄ and CO₂ to create a more stable phenotype that was less influenced by the position of the head of the cow to the sensor. In our study, however, we found that RATl had relatively more total variation and less genetic variation than CH₄l and CO₂l. Therefore, based on the results of our study, the use of CH₄l for selection instead of RATl would be expected to give a greater reduction of methane emission by dairy cows.

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

CONCLUSIONS

The CH₄l, CO₂l, and RATl were all repeatable and heritable, but RATl had a lower repeatability and heritability than the other 2 traits. It is recommended to measure CH₄ and CO₂ on at least 10 consecutive days to maximize repeatabilities of the methane phenotypes. It is important to account for farm, AMS, day

of measurement, time of day, and lactation stage when estimating genetic parameters for methane phenotypes. The use of CH₄ for selection instead of RATI would be expected to give a greater reduction of methane emission by dairy cows.

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