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This is a "Post-Print" accepted manuscript, which has been published in "Journal of Dairy Science"

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Please cite this publication as follows:


You can download the published version at:

https://doi.org/10.3168/jds.2017-13441
INTERPRETIVE SUMMARY

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

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Methane is a greenhouse gas and is produced in the rumen of dairy cows during the digestion of feed. A study was carried out to investigate whether it would be possible to breed for lower methane emission by using methane measurements obtained from infrared sensors during automatic milking. Part of the variation in the sensor measured methane was due to the genetic background of the cow. This indicates that measurements obtained from infrared sensors during automatic milking can be used to select for lower methane emission by dairy cows during breeding.
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 methane ($\text{CH}_4$) and carbon dioxide ($\text{CO}_2$) on 1508 Dutch Holstein Friesian cows located on 11 commercial dairy farms were available. Phenotypes per AMS visit were the mean of $\text{CH}_4$ ($\text{CH}_4\text{mean}$), mean of $\text{CO}_2$ ($\text{CO}_2\text{mean}$), $\text{CH}_4\text{mean}$ divided by $\text{CO}_2\text{mean}$ ($\text{Ratiomean}$), and their log$_{10}$-transformations $\text{CH}_4\text{log}$, $\text{CO}_2\text{log}$, and $\text{Ratiolog}$. The repeatabilities of the log$_{10}$-transformated methane phenotypes were 0.27 for $\text{CH}_4\text{log}$, 0.31 for $\text{CO}_2\text{log}$, and 0.14 for $\text{Ratiolog}$. The heritabilities of these phenotypes were 0.11 for $\text{CH}_4\text{log}$, 0.12 for $\text{CO}_2\text{log}$, and 0.03 for $\text{Ratiolog}$. These results indicate that measurements taken using infrared sensors in AMS are repeatable and heritable and, thus, could be used for selection for lower $\text{CH}_4$ 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 $\text{CH}_4\text{log}$ instead of $\text{Ratiolog}$ would be expected to give a greater reduction of $\text{CH}_4$ emission by dairy cows.

**Keywords:** methane emission, dairy cow, AMS, non-dispersive 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 methane \((\text{CH}_4)\) (Gerber et al., 2013). Approximately 8% of the worldwide agricultural greenhouse gas emissions originate from enteric \(\text{CH}_4\) 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 \(\text{CH}_4\) emission by dairy cows.

Reduction of \(\text{CH}_4\) 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 \(\text{CH}_4\) emission. An example of breeding for reduced \(\text{CH}_4\) is the reduction of 13% of \(\text{CH}_4\) per 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 \(\text{CH}_4\) emission through selective breeding is wanted and requires quantification of possible genetic variation in \(\text{CH}_4\) emission.

To quantify possible genetic variation in \(\text{CH}_4\) emission, \(\text{CH}_4\) 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 \(\text{CH}_4\) and carbon dioxide \((\text{CO}_2)\) 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 \(\text{CH}_4\) emission on large numbers of cows.
Previous studies have shown that CH$_4$ measurements based on infrared sensors in AMS are repeatable. Lassen et al. (2012) summarized CH$_4$ measurements per AMS visit by taking the mean of CH$_4$, the mean of CO$_2$ and the mean of the ratio between CH$_4$ and CO$_2$.

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 CH$_4$ 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 CH$_4$ emission.

These CH$_4$ 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 impact CH$_4$ emission via the differences in feed regimes between farms (Bell et al., 2014a; Hammond et al., 2016). Hour of the day can influence CH$_4$ 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 CH$_4$ 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 CH$_4$ emission between cows. Lassen and Løvendahl (2016) found genetic variation in CH$_4$ emission that was summarized in several phenotypes. The heritabilities ranged between 0.16 and 0.21, providing support for the use of CH$_4$ concentrations measured using infrared sensors in AMS to decrease CH$_4$ emission through selective breeding.

The aim of this study was to quantify the genetic variation of CH$_4$ emission by Dutch dairy cows measured using infrared sensors installed in AMS. The dataset comprised of CH$_4$ and CO$_2$ 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 chambers (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 non-dispersive infrared (NDIR) technique, and logged twice per second. Phenotypes were defined as follows: CH₄ production (l/d) from CRC; CH₄ concentration (ppm) from sensor; and CH₄:CO₂ ratio from sensor.

**Data**

CH₄ and CO₂ concentrations (ppm) were measured on 1508 primiparous and multiparous dairy cows from 11 commercial farms in the Netherlands. On nine of these farms, cows were fed in the morning, whereas on one farm cows were fed in the evening. In addition, some farms had automatic feed pushers that compiled the feed continuously during the day, and one
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 automatic milking systems (AMS; Lely Astronaut A4, Lely Industries NV, Maassluis, the Netherlands) using NDIR sensors. A total of four sensors were used to collect all data by installing them consecutively in different AMS. Measurements were taken in a total of 23 AMS, one to four 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 two 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 and these days were, therefore, discarded. The number of days with measurements ranged from ten up to 81 per AMS. The second condition was that AMS visits should last at least 90 seconds and AMS visits shorter than 90 seconds were removed from the dataset. Removing AMS visits shorter than 90 seconds ensured that most AMS visits that did not result in a
milking, and would, thus, not provide a steady measurement, were removed from the dataset. 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 dataset, as these could not be linked to the pedigree. The fourth condition was that the week of lactation of the cow visiting the AMS should be known and cows should be between one and 60 weeks in lactation. The fifth condition was that AMS visits with missing CH\textsubscript{4} phenotypes (see phenotypes) or missing model effects (see model 1) were removed from the dataset. The sixth and last condition was that each cow should have at least four 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. AMS visits that had a standardized residual effect >3.5 based on model 1 for CH\textsubscript{4}mean, CH\textsubscript{4}log, CO\textsubscript{2}mean, CO\textsubscript{2}log, Ratiomean or Ratiolog were considered outliers and were removed. After removal of the outliers, the dataset consisted of 123,369 AMS visits from 1,508 dairy cows. This dataset was used to estimate the variance components and genetic parameters.

**Phenotypes**

The CH\textsubscript{4} and CO\textsubscript{2} measurements were summarized per AMS visit into six phenotypes. To correct for the background levels of CH\textsubscript{4} and CO\textsubscript{2} in the barn, offsets for CH\textsubscript{4} and CO\textsubscript{2} were calculated per AMS visit. The background levels of CH\textsubscript{4} were assumed to be 0 ppm and the offset for CH\textsubscript{4} was the mean of the 10 lowest values for CH\textsubscript{4} in a specific AMS visit. As the background levels of CO\textsubscript{2} were assumed to be 400 ppm, the offset for CO\textsubscript{2} was the mean of the lowest 10 values for CO\textsubscript{2} minus 400. The CH\textsubscript{4} offset was subtracted from the individual (twice per second) CH\textsubscript{4} measurements during a specific AMS visit and the CO\textsubscript{2} offset was subtracted from the individual CO\textsubscript{2} measurements. After adjustment for the offsets, the methane phenotypes were calculated. The first phenotype is the mean of CH\textsubscript{4} per AMS visit.
The second phenotype is the mean of CO\textsubscript{2} per AMS visit (CO\textsubscript{2}mean). The third phenotype is based on the ratio (CH\textsubscript{4}/CO\textsubscript{2}) per AMS visit (Ratiomean). The residuals of the traits based on model 1 were not normally distributed. For example, the residuals of CH\textsubscript{4} mean showed a thicker and longer right tail (Kurtosis=3.24). Therefore, phenotypes were log\textsubscript{10}-transformed and after transformation residuals became normally distributed (Kurtosis of log\textsubscript{10}-transformed CH\textsubscript{4}mean=0.55). These log\textsubscript{10}-transformations resulted in the fourth phenotype CH\textsubscript{4}log (log\textsubscript{10}(CH\textsubscript{4}mean)), fifth phenotype CO\textsubscript{2}log (log\textsubscript{10}(CO\textsubscript{2}mean)), and sixth phenotype Ratiolog (log\textsubscript{10}(Ratiomean)). Furthermore, milk yield in kg 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 following model:

\[
Y_{ijklm} = \mu + DayAMS_i + Lactationweek_j + Hour*Farm_k + Animal_l + Permanent_m + e_{ijklm}
\]

(model 1)

Where \(Y_{ijklm}\) is the dependent variable (CH\textsubscript{4}mean, CO\textsubscript{2}mean, Ratiomean, CH\textsubscript{4}log, CO\textsubscript{2}log, Ratiolog or milk yield); \(\mu\) is the mean; DayAMS\(_i\) is the combined effect of day of measurement, farm of measurement, AMS of measurement and sensor of measurement (991 levels); Lactationweek\(_j\) is the fixed effect of week of lactation (60 levels); Hour*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 (\(\sim N(0, A\sigma^2_{Animal})\)) with additive genetic relationship matrix \(A\) and additive genetic variance \(\sigma^2_{Animal}\); Permanent\(_m\) is the random permanent environmental effect (\(\sim N(0, I\sigma^2_{Permanent})\)) with identity matrix \(I\) and permanent environmental variance \(\sigma^2_{Permanent}\); and \(e_{ijklm}\) is the random error effect (\(\sim N(0, I\sigma^2_{Error})\)) with identity matrix \(I\) and residual variance \(\sigma^2_{Error}\).
Measurement Period

For analysis, all data available for each cow were used, ranging from 1 to 81 days per cow. To study the effect of length of measurement period a subset of the data was used consisting of data from one AMS on one 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, five individual datasets with that length in consecutive days were created by random sampling from the dataset of 50 days (e.g., five datasets containing 30 consecutive days). Datasets of the same measurement period length were sometimes partially overlapping. Repeatabilities were calculated for each measurement period dataset, and repeatabilities and their standard errors were averaged over the five datasets for each measurement period length.

Genetic Parameters

The repeatability was calculated as follows:

\[ \text{Repeatability} = \frac{\sigma_{\text{Animal}}^2 + \sigma_{\text{Permanent}}^2}{\sigma_{\text{Animal}}^2 + \sigma_{\text{Permanent}}^2 + \sigma_{\text{Error}}^2} \]

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

\[ h^2 = \frac{\sigma_{\text{Animal}}^2}{\sigma_{\text{Animal}}^2 + \sigma_{\text{Permanent}}^2 + \sigma_{\text{Error}}^2} \]

We presented the descriptive statistics on all six 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\textsubscript{4} emission for a cow was calculated as:

\[ \sqrt{\frac{mh^5}{(m-1)h^2+1}} \]
where \( m \) is the number of repeated sensor measurements in an AMS, \( h^2 \) is the heritability, and \( r \) is the repeatability. The accuracy of breeding value for \( \text{CH}_4 \) for a bull with half-sib daughters was calculated as:

\[
\sqrt{\frac{n x^2}{(n-1)x^2+4}} \]

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

**Descriptive Statistics**

Descriptive statistics on the six 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 \( \text{CH}_4\text{mean} \) and \( \text{CO}_2\text{mean} \) decreased, whereas the mean and standard deviation increased in an absolute sense for \( \text{Ratiomean} \). 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 dataset, despite removing AMS visits shorter than 90 seconds. We considered any AMS visit longer than 90 seconds suitable for \( \text{CH}_4 \) and \( \text{CO}_2 \) 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$_4$mean, 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 two distinct peaks in CH$_4$mean during the day: the first peak around 9 AM and the second peak around 8 PM. Farm B showed a strong increase in CH$_4$mean during the day compared with the night. Both types of patterns were present in the dataset, 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$_4$mean, as obtained from model 1, for the first 50 weeks of lactation is presented in Figure 2. The effect of week of lactation on CH$_4$mean increased rapidly during the first 12 weeks of lactation. After this strong increase, the effect of week of lactation on CH$_4$mean remained relatively constant until 35 weeks in lactation and decreased gradually thereafter. Between weeks 12 and 35, the effect of lactation week accounted for 3.7% of the variation in the mean of CH$_4$mean.

Genetic Parameters for Methane Emission

Repeatabilities and heritabilities of the log$_{10}$-transformated 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$_4$log and CO$_2$log, and were lower for Ratiolog. The heritabilities were lower than the repeatabilities and ranged between 0.03 and 0.12 for the methane phenotypes. Heritabilities were similar for CH$_4$log and CO$_2$log, and were lower for Ratiolog. 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 CH$_4$ emission expressed as CH$_4$log 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
$CH_4$ 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 three methane phenotypes showed higher repeatabilities in
measurement periods longer than 5 days compared with shorter measurement periods.

Measurement periods longer than 10 days 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 days.

Furthermore, repeatabilities of the measurement period of 10 days were not significantly
different from the repeatabilities of the dataset with all observations from that specific AMS.

**DISCUSSION**

The aim of this study was to quantify the genetic variation in methane phenotypes measured
with NDIR sensors in AMS. Methane phenotypes based on sensor measurements of $CH_4$ and
$CO_2$ 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 that there is genetic variation in these phenotypes.

**Methane Sensor Test**

High repeatability of $CH_4$ production obtained from CRC measurements and of $CH_4$
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 CH$_4$ 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 this study were measured in parts per million (ppm). In the literature concentration measurements (in ppm) have been transformed to CH$_4$ production (g/day) using a dilution factor or using CO$_2$ production (Madsen et al., 2010; Garnsworthy et al., 2012a; Lassen and Løvendahl, 2016). These transformations, however, are based on several assumptions, like a constant CO$_2$ production of a cow throughout the day, that may not always be met. The CH$_4$ production that is obtained after transformation is affected by the accuracy of these assumptions. For breeding, absolute values are not needed, as it focusses 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 Ratiomean, i.e., the mean of the ratio between CH$_4$ and CO$_2$. We included Ratiomean 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 Ratiomean 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 Ratiomean, i.e., CH$_4$mean and CO$_2$mean, 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 Ratiolog compared with the literature would not affect the direction of selection if Ratiolog 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 (s.e.=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 CH\textsubscript{4}log was 0.11 (s.e.=0.02), which is comparable with the heritability of milk yield per AMS visit. This indicates that there is 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 one farm at a time, with a single sensor installed per AMS. To study the impact of the DayAMS effect 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 CH\textsubscript{4}log, 27% for CO\textsubscript{2}log, and 82% for Ratiolog. 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 CH\textsubscript{4} 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 impact 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 material 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 the moment of feeding differed 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 CH$_4$ 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 weeks of lactation by Lassen and Løvendahl (2016), at 20 weeks of lactation by Garnsworthy et al. (2012b), and at 12 weeks 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 weeks 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 weeks of lactation (Garnsworthy et al., 2012b) and 80% of the peak methane emission at 44 weeks of lactation (Lassen and Løvendahl, 2016). In our study, methane emission decreased with about 33% of the peak methane emission at 50 weeks of lactation. The pattern found in our study is comparable with the patterns found in literature and the found differences in patterns might be explained by many different factors such as the used phenotypes. Our study used CH$_4$mean in ppm as phenotype whereas the other studies used methane in g/day that was either from the integral area under the peaks of methane emission (Garnsworthy et al., 2012b) or by the ratio between CH$_4$ and CO$_2$ 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 this study, repeatabilities of the log$_{10}$-transformed methane phenotypes ranged between 0.14 and 0.31. Other studies have reported repeatabilities of CH$_4$ measured in AMS that ranged between 0.34 for the mean of CH$_4$ and 0.86 for the mean of CO$_2$ (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 studies of Lassen and Bell used a model that corrects for diet effects and this 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 has influenced the repeatabilities found in our study, and other studies have chosen different measurement periods. Bell et al. (2014b) measured CH$_4$ and CO$_2$ for a 35-day period whereas Lassen et al. (2012) measured for a 3-day period. The results obtained from our study indicate that repeatabilities (and their standard errors) remained stable in measurement periods of at least 10 consecutive days. In other words, the value of additional repeated measurements beyond 10 days 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 (s.e.=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 log$_{10}$-transformed phenotypes in this study were 0.11 for CH$_4$ log, 0.12 for CO$_2$ log, and 0.03 for Ratiolog. 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 CH$_4$ and CO$_2$ (in ppm) gave a heritability of 0.16 and both CH$_4$ in g/day and CH$_4$ in g/kg fat and protein corrected milk gave a heritability of 0.21. These heritabilities were slightly higher compared with the heritabilities of CH$_4$ log and CO$_2$ log in our study. The heritability of Ratiolog of our study is considerably lower compared with the other heritabilities. Lassen et al. (2012) used the ratio between CH$_4$ and CO$_2$ 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 Ratiolog had relatively more total variation and less genetic variation than CH$_4$ log and CO$_2$ log. Therefore, based on the results of our study, the use of CH$_4$ log for selection instead of Ratiolog would be expected to give a greater reduction of methane emission by dairy cows.
The heritability of the phenotypes showed that there is genetic variation present 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

CH₄log, CO₂log, and Ratiolog were all repeatable and heritable, but Ratiolog had a lower repeatability and heritability than the other two 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 CH₄log for selection instead of Ratiolog would be expected to give a greater reduction of methane emission by dairy cows.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the 11 farmers for their input and collaboration with the project and Jan Kortmann for his technical assistance.
REFERENCES


emissions and mitigation opportunities. in Food and Agriculture Organization of the

user guide release 4.1 structural specification. VSN International Ltd, Hemel
Hempstead, UK.

Kebreab, M. A. Eugène, Z. Yu, K. J. Shingfield, A. Schwarm, A. N. Hristov, and C.
K. Reynolds. 2016. Review of current in vivo measurement techniques for quantifying

Gerrits. 2015. Design of climate respiration chambers, adjustable to the metabolic
mass of subjects. Pages 35-56 in Indirect calorimetry. Wageningen Academic
Publishers, Wageningen.

Montes, J. Oh, E. Kebreab, S. J. Oosting, P. J. Gerber, B. Henderson, H. P. S. Makkar,
and J. Firkins. 2013. Special topics —mitigation of methane and nitrous oxide
emissions from animal operations: Iii. A review of animal management mitigation

IPCC. 2014. Climate change 2014: Synthesis report. Contribution of working groups i, ii and
iii to the fifth assessment report of the intergovernmental panel on climate change, ed.

Lassen, J. and P. Løvendahl. 2016. Heritability estimates for enteric methane emissions from


Table 1. Descriptive statistics of phenotypes on methane (CH$_4$) and carbon dioxide (CO$_2$) measured with non-dispersive infrared (NDIR) sensors in automatic milking systems (AMS) on 1,508 Dutch dairy cows (123,369 AMS visits)

<table>
<thead>
<tr>
<th>Trait</th>
<th>Mean</th>
<th>SD$^1$</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH$_4$mean (ppm)</td>
<td>254</td>
<td>230</td>
<td>11</td>
<td>2073</td>
</tr>
<tr>
<td>CH$_4$log (ppm)$^2$</td>
<td>2.25</td>
<td>0.37</td>
<td>1.04</td>
<td>3.32</td>
</tr>
<tr>
<td>CO$_2$mean (ppm)</td>
<td>1443</td>
<td>681</td>
<td>408</td>
<td>9054</td>
</tr>
<tr>
<td>CO$_2$log (ppm)$^2$</td>
<td>3.11</td>
<td>0.20</td>
<td>2.61</td>
<td>3.96</td>
</tr>
<tr>
<td>Ratiomean</td>
<td>0.17</td>
<td>0.12</td>
<td>0.01</td>
<td>0.87</td>
</tr>
<tr>
<td>Ratiolog$^2$</td>
<td>-0.87</td>
<td>0.27</td>
<td>-1.92</td>
<td>-0.06</td>
</tr>
<tr>
<td>Milk (kg)$^3$</td>
<td>10.8</td>
<td>3.4</td>
<td>0.00</td>
<td>36.50</td>
</tr>
</tbody>
</table>

$^1$ Standard deviation.

$^2$ Log$_{10}$-transformed phenotypes.

$^3$ Milk production per AMS visit.
Table 2. Repeatabilities and heritabilities of phenotypes on methane (CH$_4$) and carbon dioxide (CO$_2$) measured with non-dispersive infrared (NDIR) sensors in automatic milking systems (AMS) on 1,508 Dutch dairy cows (123,369 AMS visits)$^1$

<table>
<thead>
<tr>
<th>Trait</th>
<th>repeatability</th>
<th>heritability</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH$_4$ log (ppm)$^2$</td>
<td>0.27 (0.008)</td>
<td>0.11 (0.02)</td>
</tr>
<tr>
<td>CO$_2$ log (ppm)$^2$</td>
<td>0.31 (0.009)</td>
<td>0.12 (0.02)</td>
</tr>
<tr>
<td>Ratiolog$^2$</td>
<td>0.14 (0.005)</td>
<td>0.03 (0.01)</td>
</tr>
<tr>
<td>Milk (kg)$^3$</td>
<td>0.45 (0.010)</td>
<td>0.17 (0.03)</td>
</tr>
</tbody>
</table>

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

$^2$ Log$_{10}$-transformed phenotypes.

$^3$ Milk production per AMS visit.
Table 3. The average repeatability of phenotypes on methane (CH$_4$) and carbon dioxide (CO$_2$) measured with non-dispersive infrared (NDIR) sensors in automatic milking systems (AMS) on Dutch dairy cows over different measurement period lengths$^{1,2}$

<table>
<thead>
<tr>
<th>Measurement period</th>
<th>N</th>
<th>CH$_4$log$^3$</th>
<th>CO$_2$log$^3$</th>
<th>Ratiolog$^3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total AMS period</td>
<td>8,851</td>
<td>0.19 (0.029)</td>
<td>0.16 (0.026)</td>
<td>0.19 (0.026)</td>
</tr>
<tr>
<td>3 days</td>
<td>376</td>
<td>0.12 (0.075)</td>
<td>0.12 (0.062)</td>
<td>0.14 (0.079)</td>
</tr>
<tr>
<td>5 days</td>
<td>650</td>
<td>0.15 (0.055)</td>
<td>0.12 (0.044)</td>
<td>0.15 (0.054)</td>
</tr>
<tr>
<td>10 days</td>
<td>1,295</td>
<td>0.22 (0.049)</td>
<td>0.17 (0.043)</td>
<td>0.22 (0.048)</td>
</tr>
<tr>
<td>20 days</td>
<td>2,567</td>
<td>0.23 (0.040)</td>
<td>0.18 (0.034)</td>
<td>0.23 (0.038)</td>
</tr>
<tr>
<td>30 days</td>
<td>3,827</td>
<td>0.23 (0.037)</td>
<td>0.18 (0.031)</td>
<td>0.22 (0.034)</td>
</tr>
<tr>
<td>50 days</td>
<td>6,296</td>
<td>0.22 (0.032)</td>
<td>0.16 (0.026)</td>
<td>0.21 (0.030)</td>
</tr>
</tbody>
</table>

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

$^2$The table contains the measurement period in days, the average number of AMS visit per measurement period (N), and the average repeatability per methane phenotype (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 standard error (s.e.) in parentheses.

$^3$Log$_{10}$-transformed phenotypes.
Figure 1. Effect of hour of the day on CH\textsubscript{4} mean (ppm) measured with non-dispersive infrared (NDIR) sensors in automatic milking systems (AMS) on 1,508 Dutch dairy cows. The figure shows two representative farms (A = 5,554 AMS visits; B = 20,458 AMS visits). The effect of hour 4 was set to zero for both farms to enable comparison and the other effects are expressed relative to hour 4.

Figure 2. Effect of week of lactation on CH\textsubscript{4} mean (ppm) measured with non-dispersive infrared (NDIR) sensors in automatic milking systems (AMS) on 1,508 Dutch dairy cows (123,369 AMS visits). The effect of week 4 of lactation was set to zero and the other effects are expressed relative to week 4.
Van Engelen et al., Figure 1

The figure illustrates the effect on CH₄ concentration (ppm) throughout the day, categorized into two groups labeled A and B. The x-axis represents the hour of the day (h), ranging from 0 to 23, while the y-axis denotes the effect on CH₄ concentration (ppm), ranging from -20 to 100.

Line A shows a peak around the 10th hour, reaching a high concentration, followed by a gradual decrease. Line B, on the other hand, exhibits a more fluctuating pattern with peaks around the 10th and 16th hours, maintaining a higher concentration compared to Line A.
Van Engelen et al., Figure 2

-20 -15 -10 -5 0 5 10 15 20

Week of lactation (weeks)

Effect on CH₄mean (ppm)