Heritability of methane emissions from dairy cows over a lactation measured on commercial farms¹

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ABSTRACT: Methane emission is currently an important trait in studies on ruminants due to its environmental and economic impact. Recent studies were based on short-time measurements on individual cows. As methane emission is a longitudinal trait, it is important to investigate its changes over a full lactation. In this study, we aimed to estimate the heritability of the estimated methane emissions from dairy cows using Fourier-transform infrared spectroscopy during milking in an automated milking system by implementing the random regression method. The methane measurements were taken on 485 Polish Holstein-Friesian cows at 2 commercial farms located in western Poland. The overall daily estimated methane emission was 279 g/d. Genetic variance fluctuated over the course of lactation around the average level of 1,509 (g/d)², with the high-

est level, 1,866 (g/d)², at the end of the lactation. The permanent environment variance values started at 2,865 (g/d)² and then dropped to around 846 (g/d)² at 100 d in milk (DIM) to reach the level of 2,444 (g/d)² at the end of lactation. The residual variance was estimated at 2,620 (g/d)². The average repeatability was 0.25. The heritability level fluctuated over the course of lactation, starting at 0.23 (SE 0.12) and then increasing to its maximum value of 0.3 (SE 0.08) at 212 DIM and ending at the level of 0.27 (SE 0.12). Average heritability was 0.27 (average SE 0.09). We have shown that estimated methane emission is a heritable trait and that the heritability level changes over the course of lactation. The observed changes and low genetic correlations between distant DIM suggest that it may be important to consider the period in which methane phenotypes are collected.

Key words: heritability, methane emission, random regression

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J. Anim. Sci. 2017.95:4813–4819
doi:10.2527/jas2017.1842

INTRODUCTION

Methane (CH₄) emission is an important trait in studies on ruminants due to its environmental impact. The estimated contribution of the livestock sector to the total anthropogenic greenhouse gas emissions is about 18% globally (Steinfeld et al., 2006) and about 13% in

the European Union (Leip et al., 2010). Methane emission is also related to the loss of GE (2 to 12%) provided with the feed (Murray et al., 1976; Johnson et al., 1993).

About 90% of the enteric CH₄ is released during eructation events and by breathing (Murray et al., 1976). This opens up a possibility for introducing breath analyzing techniques for quantifying CH₄ emissions from ruminants. Recently, a number of breath analyzing techniques using infrared (Garnsworthy et al., 2012a; Lassen et al., 2012; Negussie et al., 2016) or laser techniques (Chagunda et al., 2009) have been introduced. These techniques are noninvasive, fast, and cost effective and may be implemented in a production environment and, thus, meet standards for generating phenotypes for genetic analyses.

Recent studies, based on short-time measurements using noninvasive techniques, showed repeatability ranging from 0.33 to 0.46 (Garnsworthy et al., 2012b; Lassen et al., 2012; Lassen and Løvendahl, 2013; Negussie et al., 2016) and heritability ranging

¹This study was financed within NCN OPUS 2013/09/B/NZ9/03179. MP acknowledges the financial support of the Polish Ministry of Science and Higher Education. This study is partly based on the knowledge gained within the networks of COST Action FA1302 “Large-scale methane measurements on individual ruminants for genetic evaluations.” The authors would like to thank to Prof. Włodzimierz Nowak (Poznan University of Life Sciences, Poland) for his support and nutritional consultations and to Karol Polaszek, the Agricultural Company Długie Stare sp. z o.o. (Poland), the Agricultural Company Mariusz Duda (Poland), and farm staff for their care and technical support.

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Received June 19, 2017.

Accepted September 9, 2017.

from 0.16 to 0.21 (Lassen and Løvendahl, 2016), indicating a potential for genetic improvement. As CH₄ emission is a longitudinal trait, it is important to investigate its changes over a full lactation. This can be facilitated by using random regression models, which enable modelling changes in genetic and permanent environment variance across time (Jamrozik et al., 1997; Strabel and Jamrozik, 2006). In this study, we aim to estimate the heritability of CH₄ emission over a full lactation measured using the Fourier-transform infrared spectroscopy (FTIR) technique during milking in an automated milking system (AMS) by implementing the random regression method.

MATERIAL AND METHODS

All animal treatments were approved by the Local Ethical Committee for Experiments on Animals in Poznan, Poland (decision number 64/2012).

The CH₄ measurements were taken on Polish Holstein-Friesian cows at 2 commercial farms located in western Poland. Methane emission on Farm 1 was measured during 2 periods: one from December 2, 2014, to February 3, 2016, and the other from June 1, 2016, to September 17, 2016, starting at the ninth day in milk (DIM). On Farm 2, the measurements were taken from February 5, 2016, to March 14, 2016, starting at 5 DIM. The measurements were recorded during milking in an AMS (Lely Astronaut A4; Lely Holding, Maassluis, the Netherlands).

Nutrition

On both farms, the animals were fed ad libitum with a partial mixed ration (PMR) with nutrients covering the 25-kg level of milk yield, and additional production was covered by a concentrate distributed in AMS units depending on the animal's productivity. Farms differed in terms of the applied diet composition and feeding routine. The feeding routine on Farm 1 was the following: 0600 h, morning PMR feeding; 0730 h, feed pushing (pushing the feed from the previous feeding such that cows can reach it); 1130 h, feed pushing and PMR feeding; and 1800 h, feed pushing. On Farm 2, the feeding routine was as follows: 0700 h, morning PMR feeding, and 1700 h, afternoon PMR feeding. Feed pushing was performed several times a day at unspecified time points.

Methane Measurements

Methane concentration was measured using an FTIR analyzer (GASMET 4030; Gasmeter Technologies Oy, Helsinki, Finland). Air was sampled at the feed-

Table 1. Data description

Farm's characteristics	Farm 1		Farm 2	
No. of animals with records	356		127	
No. of measurements	437,888		5,645	
No. of daily observations	31,239		3,190	
Measuring period 1	Dec. 2, 2014	Feb. 3, 2016	Feb. 5, 2016	Mar. 14, 2016
Measuring period 2	June 1, 2016	Sept. 17, 2016	NA ¹	NA
	Lactation		Lactation	
	1	2+	1	2+
No. of records per lactation	288,272	149,616	2,060	3,585
No. of cows per lactation	250	165	45	84
Average measurement period, d	83	64	25	25
Minimum measurement period, d	7	5	5	5
Maximum measurement period, d	171	183	37	35

¹NA = not available.

ing bin of the AMS unit using a heated sampling line. Measurements were taken continuously and stored in the data set every 5 s as the 5-s averages. Every 24 h, gaseous nitrogen was used to automatically adjust the zero point of the analyzer. Farm 1 was equipped with 3 AMS units and Farm 2 was equipped with 2 AMS units. At each farm, the methane concentration in the AMS units was measured for 24 h. The change between AMS units was performed automatically.

Background concentration was the median value of the CH₄ concentration of the closest period preceding the analyzed milking when no cow was milked. Our analyses (results not shown) indicated that median value was a better estimate of the background concentration than the mean value. Observations up to 305 DIM were analyzed. In total, 443,533 milkings with CH₄ measurements recorded on 483 cows were available for analysis (for more details, see Table 1).

Daily estimated CH₄ emission was quantified in grams per day. First, CH₄ emission in liters per day was calculated according to Madsen et al. (2010) as follows: the ratios between CH₄ and CO₂ concentrations were averaged over each milking and multiplied by the expected CO₂ emission (L/d) calculated based on the heat production unit following Pedersen et al. (2008). Then, CH₄ emission was converted to grams per day. The conversion was made using the CH₄ density of 0.668 g/L (i.e., assuming normal temperature and pressure of 20°C and 101.325 kPa). In this way, multiple daily CH₄ outputs per cow were obtained, which were used in further analyses.

The fixed effects were selected based on the likelihood ratio test using a mixed model approach with the random cow effect using the lme4 package (Bates et al., 2015) implemented in the R software (R Development Core Team, 2013).

Figure 1. Diurnal variation in CH₄ emissions in 2 farms (Farm 1 = solid line and Farm 2 = dashed line).

Genetic components were estimated based on the predicted daily average values for methane per cow. The daily averages for the estimated CH₄ were obtained as follows: 1) Each data set was adjusted for the AMS unit and diurnal variation in the estimated CH₄ emissions and 2) corrected estimated CH₄ emissions were averaged within the same date for each cow. Separate correction for the AMS unit and diurnal variation was performed due to differences between farms (see Fig. 1). The corrections were made based on the following linear regression model:

$$CH_{4_{ijm}} = AMS_j + \sum_{n=1}^5 (f_{1n} \sin \varnothing + f_{2n} \cos \varnothing) + error_{ijm} \quad [1]$$

in which CH_{4_{ijm}} is the estimated CH₄ output for each milking of the *i*th cow milked at the *j*th AMS unit at time *m*. Diurnal variation was modeled using a Fourier series approach adopted after Løvendahl and Bjerring (2006) and Lassen et al. (2012): *f*_{1*m*} and *f*_{2*m*} are fixed regression coefficients of the Fourier series and $\varnothing$ is $2\pi s/86,400$, in which *s* is the second of the day in which the measurement was taken. The constant of 86,400 scales *s* to values ranging from 0 to 1. Initial analyses of the different regression order (not shown here) indicated that the fifth-order regression gave the best fit to the diurnal variation of CH₄. The adjustment of the CH₄ records was achieved by subtracting the effects of a particular AMS unit and the effect of the diurnal variation for a given milking estimated with Model [1].

The final model to estimate genetic components included random effects, which were selected based on a comparison of the Akaike information criteria and Bayesian information criteria obtained from ASReml 4.1 (Gilmour et al., 2014). The model included the

pedigree of the animals with methane records. The random animal and permanent environment effects were included in the model with up to the second level of Legendre polynomials (Kirkpatrick et al., 1990). The overall lactation curve was modeled with third-order polynomials. The final model used to estimate heritability was the following:

$$CH_{4_{ijkm}} = LAC_j \times \sum_{n=1}^4 \beta_n DIM_{kn} + YK_l + \sum_{n=1}^3 animal_{in} DIM_{kn} + \sum_{n=1}^3 pe_{in} DIM_{kn} + error_{ijkl} \quad [2]$$

in which CH_{4_{ijklm}} is the estimated and adjusted average daily CH₄ output of the *i*th cow in the *j*th lactation, the *k*th day in milk (DIM), and the *l*th year-week of the measurement, where year-week of the measurement (YK), where YK are farm specific; β_n are fixed regression coefficients. Lactation (LAC) had two levels: 1 and 2+, in which records from up to lactation 8 were included (for detailed distribution of cows with records over lactations, see Table 2). The general lactation curve was modeled using third-order Legendre polynomials. The structure of collected data made it possible to model random animal (*animal*) and permanent environment (*pe*) effects with second-order Legendre polynomials.

RESULTS AND DISCUSSION

Average CH₄ Emission

The overall daily estimated CH₄ emission was 279 g/d (SD 63.7). Farm 2 had higher CH₄ emissions by about 73 g/d, whereas animals kept on that farm were heavier than on Farm 1 by approximately 125 kg and produced approximately 3 kg more milk (see Table 3 for more details). This may indicate differences in feed intake or efficiency of the cows kept at different farms. To investigate this issue, the individual nutrition data would be necessary, which were, unfortunately, unavailable.

The average daily estimated CH₄ emission recorded in the present study is generally lower than in other reports. The findings in the literature are closer to results from Farm 2 than those from Farm 1. For example, Lassen and Løvendahl (2016), who used the same analyzer on Danish Holstein cows, showed the average emission of 315 g/d. Similar CH₄ emissions were found with different measuring techniques. For example, Chagunda et al. (2009) showed 357 g/d based on laser measurements on Holstein Friesian cows kept in Scotland. Negussie et al. (2016), who measured emissions using photoacoustic infrared spectroscopy based on 115 cows, reported an emission of 831 g/d (converted from 555 L/d). Grainger et al. (2007), who measured methane on a small sample

Table 2. Number of cows with CH₄ records per lactation per location

Lactation	Farm 1	Farm 2
1	250	44
2	138	42
3	23	19
4	7	11
5	1	4
6	2	3
7	0	1
8	1	2
9	0	1
2+	165	83

of Australian Holstein cows kept under experimental conditions, indicated an average daily CH₄ emission of 331 g when measured using the SF₆ tracer method and 322 g when using respiration chambers. Substantially higher values were reported by Bell et al. (2014; 422 g/d) and Garnsworthy et al. (2012a; 430 g/d).

The differences between the average emissions obtained in the present study and in the literature have to be considered with caution, because using a different density to convert from grams per day to liters per day leads to changes in the magnitude of the differences. For example, at an assumed temperature of 0°C, the methane density is higher (0.716 g/L) and, therefore, the differences between the presented values decrease. In the present study, we assumed normal temperature and pressure of 20°C and 101.325 kPa as the analyzer reports the results standardized to such conditions.

Variation in Cows' Heat Production

The results presented in this study are expressed in grams per day. To obtain this unit, the measured CH₄ concentrations were converted according to Madsen et al. (2010), who adopted the heat production unit from Pedersen et al. (2008). In this equation, the information on individual production, BW, and pregnancy length are incorporated to calculate the cow's daily CO₂ production. The calculations are made ignoring the existence of between-animal variation in the daily CO₂ production. Including such a variance would increase the accuracy of the methane emission phenotype. Yet at the moment, a different method allowing estimation of methane production based on the setup used in this study is not available.

Variation in Time

The diurnal variation pattern differed between farms (see Fig. 1). On Farm 1, the pattern was slightly smoother than on Farm 2 (i.e., estimated CH₄ emis-

Table 3. Average methane emissions, fat–protein corrected milk (FPCM) yields, and BW for analyzed farms

Trait	Farm 1		Farm 2		Farms jointly	
	Mean	SD	Mean	SD	Mean	SD
Methane, g/d	272	59.0	345	70.2	279	63.7
BW, kg	544	114.8	670	74.9	556	117.5
FPCM, kg	34	6.8	37	8.8	34	7.1

sion was less variable over the course of a day). The pattern of diurnal variation reported in this study was similar to the patterns shown by others (Garnsworthy et al., 2012a; Lassen et al., 2012; Bell et al., 2014). In general, the estimated CH₄ emission level increased after feeding events and dropped over the night period. The main driver for this pattern is connected with management routines, especially those related to feeding. As management routines differ between farms, diurnal patterns differed as well, as shown in Fig. 1. In the present study, the difference between SD of the hourly estimated CH₄ level on both farms was 4 g/d. Therefore, the adjustment for the diurnal variation had to be performed separately, at the farm level. The importance of adjusting the data for the time of day at which the measurement was taken is especially important when an AMS is used. It is possible that cows have individual preferences for the time of milking. Therefore, the evaluation of a cow that would be milked only at the time of the day with a high (or low) CH₄ level may be biased if the diurnal variation is not accounted for, and all the daily measurements are simply averaged.

Daily estimated CH₄ emission levels fluctuated over the measuring period, as shown in Fig. 2. Higher values were observed in warmer periods than in the colder ones. This seasonal variation, however, has to be interpreted with caution, because herd management changes over the year. In the summer months, cooling fans were used at high temperatures and wind blinds were open, causing an increased air flow in the barn. In contrast, in winter, the airflow in the barn was limited. This may have had some impact on the measurements of methane concentrations. Additionally, some seasonal changes in the diet may have an additional impact on CH₄ emission levels.

Lactation Pattern

The pattern of daily estimated CH₄ emission levels over the course of lactation differed between the first lactation and the other lactations grouped together (see Fig. 3). At the beginning of the first lactation, the CH₄ emission level was lower and then rapidly increased at around 20 DIM to rise further, this time steadily, for the remaining part of the lactation. In lactation 2+, the initial estimated CH₄ emission level was higher, with

Figure 2. Average estimated daily CH_4 emissions over the measuring period (based on Farm 1).

Figure 3. Estimated CH_4 emission over the course of lactation 1 (solid line) and lactation 2+ (dashed line) based on observations from Farm 1.

a steady decrease during the later stages of lactation. The phenotypic lactation pattern shown here somewhat differs from that presented by Lassen and Løvendahl (2016), which had a shape typical of a lactation curve. In the present study, the phenotypic lactation pattern was based on fewer animals and a smaller number of farms than in the study by Lassen and Løvendahl (2016), which may partly explain the differences. A high variation of the estimated CH_4 shown in the present study at the beginning of lactation may be partly attributed to the limited number of observations during that period, especially from the first days of the lactation.

Variance Components

Genetic, permanent environment, and residual variances over the course of lactation, estimated using model [2], are presented in Fig. 4. Genetic variance fluctuated over the course of lactation around the average level of $1,509 \text{ (g/d)}^2$, with the highest level, $1,866 \text{ (g/d)}^2$, at the end of the lactation. The permanent environment variance values started at $2,865 \text{ (g/d)}^2$ and then dropped to around 846 (g/d)^2 at 100 DIM to reach the level of $2,444 \text{ (g/d)}^2$ at the end of lactation. The residual variance was estimated at $2,620 \text{ (g/d)}^2$.

There is a limited number of studies reporting variance components and heritability estimates for direct measurements of CH_4 emissions, and to our knowledge, there is no study applying a random regression model to estimate heritability levels at particular DIM (except for studies based on fatty acid composition-based prediction [Vanrobays et al., 2016] or simulation studies [Yin et al., 2015]). A low number of such publications is mainly caused by the fact that it is expensive to obtain a high number of observations from long periods of time, whereas most studies focus on short periods to maximize the number of phenotyped animals. In our study, our aim

was to estimate variance components over the course of lactation and genetic relationships between particular DIM. This came at the cost of the number of phenotyped animals. Nonetheless, the present results show that the estimated CH_4 emissions may have a different genetic background at different lactation stages. At the beginning of lactation, genetic variance was much higher than at its end. Consecutive DIM that are close to each other in time were highly genetically correlated, whereas correlations between more distant DIM were low. Therefore, we hypothesize that it is important not only to focus on the number of phenotyped animals but also to ensure that individual farms are periodically revisited to collect phenotypes on the same cows at different time points of their lactations. A higher genetic variation at the beginning of lactation observed in this study may suggest that more emphasis should be put on the first period of lactation. Random regression models not only provide better modeling of changes in variance components over a full lactation but also produce means for better modeling of individual lactation curves for animals (Jamrozik et al., 1997). This is important, particularly in the light of low genetic correlations between estimated CH_4 at different stages of lactation. Ignoring this source of variation and estimation of breeding values based only on selected short time points may be a source of inaccuracy and reduce the value of this trait in selection programs.

The repeatability followed the trend of the permanent environment variance (see Fig. 5), starting at the level of 0.40 at the beginning of the lactation and then dropping to 0.17 in 100 DIM and steadily increasing afterward, reaching 0.35. The average repeatability was 0.25. The repeatability levels reported in the present study are in range of previously reported values (Garnsworthy et al., 2012b; Lassen et al., 2012; Lassen and Løvendahl, 2016; Negussie et al., 2016).

Figure 4. Genetic (solid line), permanent environment (dashed line), and residual (dotted line) variances [(g/d)²] over the course of lactation.

The heritability level fluctuated over the course of lactation (see Fig. 6), starting at 0.23 (SE 0.12), then increasing to its maximum value of 0.3 (SE 0.08) at 212 DIM, and ending at the level of 0.27 (SE 0.12). The observed fluctuations should be considered with caution, because the changes of the heritability are within the SE of the estimates. Therefore, more data should be collected to confirm these findings. Average heritability was 0.27 (average SE 0.09). The heritability obtained from a simplified model, which included only simple random effects of the animal and permanent environment (i.e., without modeling of the individual lactation curves for random effects), gave a similar estimate of the heritability (0.26 [SE 0.06]). The average heritability presented in this study is similar to the value of 0.21 presented by Lassen and Løvendahl (2016), who analyzed a similar phenotype (CH₄ in g/d). Heritability of other phenotypes reported by Lassen and Løvendahl (2013, 2016) ranged from 0.16 to 0.21. Heritability

Figure 6. Repeatability of estimated CH₄ emission over the course of lactation.

Figure 5. Heritability of estimated CH₄ emission over the course of lactation (solid line) with SE (dashed lines).

estimates for CH₄ emissions predicted from milk fat composition and based on feed intake and maintenance ranged from 0.12 to 0.44 (de Haas et al., 2011; van Engelen et al., 2015; Yin et al., 2015).

Genetic correlations between particular DIM, as shown in Fig. 7, indicated high similarity between the neighboring DIM (i.e., correlation of 1), which decreased when the distance between DIM increased, reaching 0 at the greatest distances. The average genetic correlation was 0.74. Phenotypic correlations (not shown) were much lower than the genetic ones and averaged 0.39

Conclusion

In this study, we analyzed methane measurements obtained during milking in an AMS with the use of an FTIR analyzer. We have shown that estimated daily CH₄ emissions are a heritable trait (0.27 [SE 0.09]) and that

Figure 7. Genetic correlations between CH₄ emissions on 5 (solid line), 200 (dashed line), and 305 d in milk (DIM; dotted line) and CH₄ emissions on the remaining DIM of lactation.

the heritability level changes over the course of lactation, ranging from 0.23 (SE 0.12) to 0.30 (SE 0.08). The observed changes and low genetic correlations between distant DIM suggest that it may be important to consider the period in which methane phenotypes are collected.

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