Abstract

Methane emissions in ruminant livestock has become a hot topic, given the pressure to reduce greenhouse gas emissions drastically in the European Union over the next 10 to 30 yr. During the 2021 United Nations Climate Change conference, countries also made collective commitments to curb methane emissions by 2050. Genetic selection for low-methane-emitting animals, particularly dairy cows, is one possible strategy for mitigation. However, it is essential to understand how methane emissions in lactating animals vary along lactation and across lactations. This understanding is useful when making decisions for future phenotyping strategies, such as the frequency and duration of phenotyping within and across lactations. Therefore, the objectives of this study were to estimate (1) genetic parameters for 2 methane traits: methane concentration (MeC) and methane production (MeP) at 2 parity levels in Danish Holstein cows across the entire lactation using random regression models; (2) genetic correlations within and between methane traits across the entire lactation; and (3) genetic correlations between the methane traits and economically important traits throughout first lactation. Methane concentration (n = 19,639) records of 575 Danish Holstein cows from a research farm measured between 2013 and 2020 were available. Subsequently, CH4 production in grams/day (MeP; n = 13,866) was calculated; MeP and MeC for first and second lactation (L1 and L2) were analyzed as separate traits: MeC_L1, MeP_L1, MeC_L2, and MeP_L2. Heritabilities, variance components, and genetic correlations within and between the 4 CH4 traits were estimated using random regression models with Legendre polynomials. The additive genetic and permanent environmental effects were modeled using second-order Legendre polynomial for lactation weeks. Estimated heritabilities for MeP_L1 ranged between 0.11 and 0.49, for MeC_L1 between 0.10 and 0.28, for MeP_L2 between 0.14 and 0.36, and for MeC_L2 between 0.13 and 0.29. In general, heritability estimates of MeC traits were lower and more stable throughout lactation and were similar between lactations compared with MeP. Genetic correlations (within trait) at different lactation weeks were generally highly positive (0.7) for most of the first lactation, except for the correlation of early lactation (<10 wk) with late lactation (>40 wk) where the correlation was the lowest (<0.5). Genetic correlations between methane traits were moderate to highly correlated during early and mid lactation. Finally, MeP_L1 has stronger genetic correlations with energy-corrected milk and dry matter intake compared with MeC_L1. In conclusion, both traits are different along (and across) lactation(s) and they correlated differently with production, maintenance, and intake traits, which is important to consider when including one of them in a future breeding objective.

Key words: methane production, methane concentration, efficiency, genetic correlation, random regression

Introduction

Enteric fermentation by ruminants contributes to 44.3% of global livestock emissions (FAO, 2018). Methane gas has been classified as a short-lived air pollutant (Tong et al., 2015), making its reduction a possible solution for global warming in the short term. Reducing enteric methane (CH4) emissions in ruminants is imperative, given the commitment by the European Commission (2019) to reduce greenhouse gas emissions by 55% in 2030 and become neutral by 2050. During the last decade, several approaches have been proposed to reduce methane in livestock (Knapp et al., 2014), including nutritional approaches such as feed additives (Roque et al., 2019), manure management (Hristov et al., 2013), genetic selection (Heagarty et al., 2007; González-Recio et al., 2020), reductions in the num-
number of animals, and reductions in meat consumption (Stoll-Kleemann and Schmidt, 2017). Genetic selection for low-CH₄-emitting cows can be an effective and sustainable approach to reduce greenhouse gas production from dairy cattle (Lassen and Difford, 2020), given that genetic progress is cumulative over generations. Thus, selecting low-CH₄-emitting animals could yield significant reductions in emissions within a few generations. However, this methane reduction should be achieved without affecting the health status of the cow or its production. For that reason, it is important to understand the genetic architecture behind methane traits and their relationship with other traits (i.e., health and production traits).

Despite the above-mentioned extensive research over the last decade, little is known about the variation of CH₄ emissions during lactation and across lactations. This can be partly attributed to the difficulty in recording CH₄ emissions and to the fact that considerable time and resources are needed to longitudinally record CH₄ emission over the typical 305-d lactation and over multiple lactations in cohorts of animals large enough to estimate genetic parameters. Thus, given the scarcity of longitudinal CH₄ data in adequately large populations, studies that have explored genetic variation along lactation and across lactations are limited to predicted CH₄ emissions from milk infrared spectra (Kandel et al., 2017) or short spot measurements of CH₄ emissions in a few herds (Pszczola et al., 2017; Sypniewski et al., 2021). From these studies (Breider et al., 2019; Sypniewski et al., 2021), it is clear that additive genetic variation changes within and across lactations for CH₄ production and CH₄ breath concentration in dairy cows.

The use of sniffers, which only measure the methane concentration in cow's breath (Garnsworthy et al., 2019), require more validation as a methane emission trait. This is a simpler alternative to calculation of production of CH₄ in grams per day, using the ratio CH₄: CO₂ of gas concentrations and ECM together with BW (Madsen et al., 2010). Evidence suggests that estimation of CH₄ production leads to an artificially induced covariance structure between CH₄ with ECM and BW, with high genetic correlations (Manzanilla-Pech et al., 2020, 2021). Previous studies have presented methane concentration as a trait that could be used as proxy for methane emissions (Difford et al., 2020; González-Recio et al., 2020; Manzanilla-Pech et al., 2021).

Knowledge about the genetic variation in measured CH₄ emission traits during and across lactations from long-term experiments would be useful to better elucidate temporal genetic variation in CH₄ emission and its possible interaction with other traits. Additionally, this knowledge would help to form the basis for designing efficient testing and selection schemes. Currently, in this study, we have gathered the largest amount of longitudinal data for CH₄ emission in Holstein cows, based on continuously recording over a period of 7 yr in a research farm with around 600 cows over first and second lactations.

Therefore, the objectives of this study were to estimate (1) genetic parameters for 2 methane traits (methane concentration, MeC; and methane production, MeP) at 2 parity levels in Danish Holstein dairy cows across the entire lactation using random regression models; (2) genetic correlations within and between the methane traits across the entire lactation; and (3) genetic correlations between the methane traits and other traits along first lactation.

**MATERIALS AND METHODS**

The phenotypic records in the present study were collected on Danish Holstein cows from the Danish Cattle Research Center (DCRC, Tjele, Denmark); DCRC has collected DMI, ECM, and BW data since its inception in 2001, and “sniffer” equipment was installed at DCRC in 2013 for the recording of MeC. The number of cows, phenotype recording, and data editing steps are described sequentially below for the methane database and for the intake and production database. Ethical approval was not needed for this study because no animal procedures were performed, as only existing data were used.

**Methane Data Collection and Editing**

Methane concentration records from 649 Holstein cows between 2013 and 2020 from DCRC were available. Data are described in part by Zetouni et al. (2018), Difford et al. (2020), and Manzanilla-Pech et al. (2020). Methane concentration was measured using a nondispersive infrared CH₄ sensor (Guardian NG, Edinburgh Instruments Ltd.) and, in parallel, CO₂ was measured using same technique (Gascard, Edinburgh Instruments Ltd.). More than 200,000 daily MeC records on 649 cows were available; however, weekly (average) MeC records were calculated to match the weekly records of ECM and BW available, which were used to calculate MeP (described below). Data included weekly averages in which up to 3 d of data were allowed to be missing within a week, and individual cows required a minimum of 3 weekly measurements to be retained for further analysis. After editing, only first and second lactations were used, resulting in 19,639 weekly MeC records from 575 Danish Holstein cows and 13,866 MeP records from 504 cows being analyzed. This reduction from MeC records to MeP records is due to the lack of...
of ECM or BW, traits that are needed in the calculation of MeP. Methane concentration distribution per lactation is shown in Table 1. Methane concentration (CH₄ in parts per million, ppm; MeC) was transformed using natural logarithm (ln) to achieve normality and then multiplied by 100 to avoid problems with the scale of the other traits. Methane production (CH₄ in g/d; MeP) was calculated as follows. First, CH₄ in liters/day was computed using the formula of Madsen et al. (2010) based on heat-producing units (HPU):

\[
CH₄ (L/d) = (CH₄/CO₂) \times 180 \times 24 \times HPU,
\]

where

\[
HPU = 5.6 \text{ metabolic BW} + 22 \text{ ECM} + 1.6 \times 10^{-5} \times (\text{days carried calf})^3.
\]

Second, CH₄ in liters per day was converted to CH₄ in grams per day using the formula

\[
MeP (CH₄ g/d) = \text{density} \times CH₄ (L/d),
\]

where the density of CH₄ at 20°C = 0.668 g/L.

Given that the traits would be analyzed by random regression, the analyses were performed per trait per lactation (L1 and L2), resulting in the following traits: MeC_L1, MeP_L1, MeC_L2, and MeP_L2.

Production, Maintenance, and Intake Data Collection and Editing

Records on ECM, BW, and DMI from 960 primiparous cows belonging to the DCRC research farm (Table 2) from 2003 to 2019 were available. Cows were fed with automated feeders (Insentec, RIC system). Cows were part of numerous nutritional experiments, and diets included primarily rolled barley, corn silage, grass clover silage, rapeseed meal, and soybean meal. Body weight was measured automatically at each milking, and averaged per week (Li et al., 2017). The barn was a loose housing system with access to automatic milking stations (DeLaval International AB). Each milking system was fitted with a weighing platform (Danvaegt) that recorded BW at each milking. Energy-corrected milk was calculated using the following formula (Sjaunja et al., 1991):

\[
\text{ECM (kg) = 0.25 milk (kg) + 12.2 fat content (kg) + 7.7 protein content (kg).}
\]

Variance Components Estimation

Variance components for the 4 methane traits (MeC_L1, MeC_L2, MeP_L1, MeP_L2) were estimated using a single-trait model with the average information (AI)-REML algorithm in DMU software (Version 6, Release 5.4; Madsen and Jensen, 2014) using a pedigree relationship matrix. A pedigree containing the identification of the cow, sire, and dam with 11,778 animals (after pruning) in the relationship matrix was used, with an average of 10 generations deep. The pedigree included 152 sires with an average of 4.3 daughters per sire. The relationship matrix for animals with methane phenotypes is shown in Supplemental Figure S1 (https://dataverse.harvard.edu/dataverse/supplemental1; Manzanilla-Pech, 2022).

The model used to estimate the variance components was

\[
y_{ijklm} = TYS_j + ACC_l + LACW_t + \sum_{k=0}^{n=2} a_{ik} \Phi_{itk} + \sum_{k=0}^{p=2} p_{ek} \Phi_{itk} + e_{ijklm},
\]

where \( y_{ijklm} \) is the phenotype for MeC_L1, MeC_L2, MeP_L1, and MeP_L2; \( TYS \) is the fixed effect for trial-year-season (98 classes); \( ACC \) is the \( l \) effect of age of cow at calving in months as a covariate (10 classes for L1 and 17 for L2); \( LACW \) is the fixed effect of time \( t \) for lactation week (1 to 45 weeks). Random effects are as follows: \( a_{ik} \) is the \( k \)th regression coefficient of Legendre polynomial for the additive genetic effect of the \( i \)th cow; \( \Phi_{itk} \) is the term of the second-order Legendre polynomial for cow \( i \) measured at \( LACW_t \); \( p_{ek} \) is the \( k \)th regression coefficient of Legendre polynomial for the permanent environmental effect of the \( i \)th cow.

Table 1. Descriptive statistics for methane concentration (MeC) and methane production (MeP) at first (MeC_L1, MeP_L1) and second lactation (MeC_L2, MeP_L2)

| Trait   | Unit                  | No. of cows | No. of records | Mean  | SD   | Minimum | Maximum | CV (%) |
|---------|-----------------------|-------------|----------------|-------|------|----------|---------|--------|
| MeC_L1  | Log(ppm) × 100        | 489         | 11,243         | 575.7 | 53.9 | 414.2    | 731.5   | 9.4    |
| MeC_L2  |                       | 368         | 8,405          | 573.4 | 50.5 | 414.3    | 714.2   | 8.8    |
| MeP_L1  | g/d                   | 425         | 8,065          | 306.6 | 77.6 | 77.3     | 588.0   | 25.3   |
| MeP_L2  |                       | 318         | 5,801          | 354.5 | 81.5 | 88.9     | 598.2   | 22.9   |
and $e$ is the residual error of $y_{ijklm}$. Residual errors were assumed to have heterogeneous variances and were divided into 11 classes according to lactation month (1 to 11). Residual covariances within the same trait at different lactation month were assumed to be zero. Genetic correlations were estimated through pairwise bivariate analyses between the traits without heterogeneous residual variances to avoid over-parametrization of the model. The (co)variance structure for a is $\text{Var}(a) = A \otimes G$, where $A$ = additive genetic relationship matrix, $\otimes = $ Kronecker product function, and $G = $ additive genetic covariance matrix of the genetic random regression coefficients. Similarly, $\text{Var}(pe) = I \otimes \text{PE}$, where $I = $ identity matrix and $\text{PE} = $ permanent environmental covariance matrix of the random regression coefficients (description below) and describes the permanent environmental component of a lactation for a cow. The residual variance structure $[\text{Var}(e)]$ was assumed diagonal and constant over lactation month.

Estimated (co)variance components of the random regression were used together with Legendre polynomial coefficients to calculate genetic and permanent environmental variances and covariances for each lactation month using the methodology of Fischer et al. (2004):

$$G = \Phi \, K \, \Phi'$$ and

$$\text{PE} = \Phi \, \text{KPE} \, \Phi'$$ [6]

where $G$ is the genetic variance-covariance matrix within trait per lactation week (matrix $n \times n$), $\Phi$ is a matrix of order $t \times n$ ($t = 3$ and $n = 45$), which contains 3 orthogonal polynomial coefficients for each of the traits through 45 lactation weeks; $K$ is a matrix of order $t \times t$, which contains the estimated covariance function describing the genetic variance components for the random regression coefficients. Likewise, $\text{PE}$ is the permanent environmental variance-covariance matrix and $\text{KPE}$ is a matrix of order $t \times t$, which contains the covariance function for the permanent environmental variance components. For bivariate analyses, $G$ and $\text{PE}$ were across traits and lactation week, being of size $2n (= 90)$, where $K$ and $\text{KPE}$ were size $2t (= 6)$.

Finally, to disentangle the relationship (genetic correlation) of both methane traits with production, maintenance, and intake traits, bivariate analyses were performed between $\text{MeC}_L1$ and $\text{MeP}_L1$ with ECM, DMI, and BW for first lactation. The model for ECM, DMI, and BW used in the bivariate analyses with methane traits was the same model as in Eq. [5]. Second-lactation data were excluded from this analysis due to an insufficient number of cows and records available across these traits, particularly for methane production ($\text{MeP}_L2$). Heritabilitys, permanent environmental ratio, repeatabilities, and genetic correlations between traits were calculated for each week of lactation. Standard errors (SE) were calculated per lactation week based on the methodology of Fischer et al. (2004) and reported by quartile (Q2, Q3), where Q2 is the median and Q3 (upper quartile) is the value under which 75% of data points are found when arranged in increasing order.

### RESULTS AND DISCUSSION

#### Descriptive Statistics

Descriptive statistics of the 4 methane traits ($\text{MeP}_{L1}$, $\text{MeP}_{L2}$, $\text{MeC}_{L1}$, $\text{MeC}_{L2}$) are shown in Table 1. The average for $\text{MeP}_{L1}$ (first lactation) was 307 g/d and for $\text{MeP}_{L2}$ (second lactation) was 355 g/d. Averages for $\text{MeP}$ were lower than reported (396–503 g/d) by Sypniewski et al. (2021) in multiparous Polish Holsteins (in 2 farms) and Richardson et al. (2021; 469 g/d) in multiparous Australian Holsteins. However, our averages for $\text{MeP}$ in both lactations were higher than those presented by López-Paredes et al. (2020; 183 g/d) in Spanish Holstein cows. As expected, methane production showed a lower average for first-lactation cows than for second-lactation cows (307 g/d), as they are lighter animals with lower intake and production (Table 2). The average for $\text{MeC}_{L1}$ was 576 and that for $\text{MeP}_{L2}$ was 574, which were higher than reported (505–517) by Sypniewski et al. (2021). Descriptive statistics for ECM, BW, and DMI for first lactation are presented in Table 2. The average for ECM was 30.2 kg/d, whereas that for BW was 617 kg, and for DMI was 19.4 kg/d. Averages for ECM, BW, and DMI were

| Trait    | Unit | No. of cows | No. of records | Mean  | SD   | Minimum | Maximum | CV (%) |
|----------|------|-------------|----------------|-------|------|---------|---------|--------|
| ECM_L1   | kg/d | 921         | 30,498         | 30.2  | 5.7  | 8.7     | 57.9    | 18.9   |
| BW_L1    | kg   | 963         | 32,038         | 617.0 | 66.5 | 421.7   | 893.0   | 10.7   |
| DMI_L1   | kg/d | 958         | 32,275         | 19.4  | 2.9  | 9.8     | 33.0    | 14.9   |

Table 2. Descriptive statistics for ECM, BW, and DMI for first-lactation cows (ECM_L1, BW_L1, and DMI_L1)
within the range of values previously reported for primiparous Holstein cows (Manzanilla-Pech et al., 2014; Li et al., 2017; Difford et al., 2020).

**Heritabilities, Permanent Environmental Ratio, and Repeatabilities Across Lactation(s)**

Estimated heritabilities ($h^2$) per lactation week for MeP_L1, MeP_L2, MeC_L1, and MeC_L2 are reported in Figure 1. Heritabilities for MeP were higher than those for MeC for both lactations. Methane concentration (MeC_L1, and MeC_L2) heritabilities were more similar to each other along lactation compared with MeP heritabilities, which showed a higher heritability for first lactation than for second lactation. Estimated heritabilities for MeC traits varied from 0.10 to 0.20 at the beginning of the lactation, from 0.25 to 0.53 in mid lactation, and from 0.11 to 0.24 at the end of lactation (Q2–Q3 SE = 0.05–0.06); for MeP, estimated heritabilities varied from 0.14 to 0.25 at the beginning of the lactation, from 0.28 to 0.47 in mid lactation, and from 0.11 to 0.29 at the end of lactation (Q2–Q3 SE = 0.07–0.13). Sypniewski et al. (2021) reported a similar pattern of heritability along lactation and similar values (from 0.13 to 0.26) for MeP in Holstein cows, including different lactations. Breider et al. (2019) presented heritabilities ranging from 0.12 to 0.45 along the lactation (until wk 52) in multiparous Holstein cows; however, their heritability values showed a very different pattern across lactation, with the lowest point occurring at wk 20. Furthermore, Sypniewski et al. (2021) presented different patterns of heritability for MeC across lactation, with low heritabilities close to zero at the beginning of lactation and a constant increase toward end of the lactation (up to 0.14). Even though Breider et al. (2019) and Sypniewski et al. (2021) used the same method (sniffer) to measure methane, there are differences in their results compared with the current study. These differences could be due to the inclusion of several lactations (4 and 8, respectively) in the analyses, whereas, in the current study, we analyzed traits separately per lactation and only for the first and second lactations. Furthermore, it is interesting to observe that the distribution of heritability estimates across lactation for MeP is similar to the ECM and DMI curves previously reported by Manzanilla-Pech et al. (2014) and Li et al. (2017) in primiparous Holstein cows. This further supports the notion that heritability estimates for MeP are influenced by the genetic architecture of ECM heritabilities along lactation. This makes it complicated to disentangle which part of the genetic variation of MeP is due to MeC and which is due to addition of ECM in the calculation of MeP. To summarize, estimates of heritability for MeP vary along the lactation from low to moderate; however, there is no clear consensus on the shape of changes during lactation across the few studies that have reported heritabilities along the lactation.

Estimated permanent environmental ratio ($pe^2$) per lactation week for MeP_L1, MeP_L2, MeC_L1, and MeC_L2 were lower for MeP than for MeC. Estimated $pe^2$ for MeC traits varied from 0.49 to 0.62 at the beginning of the lactation, from 0.50 to 0.58 in mid lactation and from 0.45 to 0.63 at the end of lactation (Q2–Q3 SE = 0.02–0.05); for MeP, estimated $pe^2$ varied from 0.26 to 0.44 at the beginning of the lactation, from 0.23 to 0.28 in mid lactation, and from 0.26 to 0.68 at the end of lactation (Q2–Q3 SE = 0.11–0.18). Additionally, repeatability estimates ($t$) per lactation week for MeP_L1, MeP_L2, MeC_L1, and MeC_L2 are reported in Supplemental Figure S2 (https://dataverse.harvard.edu/dataverse/supplementals1; Manzanilla-Pech, 2022). Estimated repeatabilities ranged from moderate to high for all traits: 0.63 to 0.86 for MeC_L1, 0.64 to 0.79 for MeC_L2, 0.62 to 0.82 for MeC_L2, and 0.53 to 0.82 for MeC_L2, with Q2–Q3 SE between 0.03 and 0.09.

**Genetic Correlations Within Trait and Lactation**

Estimated genetic correlations ($r_g$) per lactation week for MeC_L1, MeC_L2, MeP_L1, and MeP_L2 are shown in Figure 2. Genetic correlations within lactation were, as expected, highest between weeks close to each other in time and decreased as the time between measurements increased. First-lactation traits (MeC_L1 and MeP_L1) showed similar patterns of correlations, with predominantly high correlations within trait and the weakest correlations between early and late lactation (as low as 0.4). However, MeP_L1 was marginally more strongly correlated between early and mid lactation. In contrast, the second-lactation traits (MeC_L2 and MeP_L2) showed differential correlation patterns along lactation weeks, with $r_g$ for MeC_L2 being mostly high and positive (>0.8) during the entire lactation, and MeP_L2 having lower $r_g$ between early and mid lactation for MeP_L2 (as low as 0.3). Similar genetic correlations at different lactation weeks have been reported by Manzanilla-Pech et al. (2014) and Li et al. (2018) for ECM, DMI, and BW in primiparous Holstein cows. For MeP_L2, it is not clear whether these lower correlations between mid and late lactation are related to the use of ECM in the calculation of MeP.
Genetic Correlation Between Traits in the Same Lactation Week

Estimated \( r_g \) at the same lactation week for the 4 traits are shown in Figure 3. All 4 pairwise \( r_g \) followed the same pattern, with moderate to high correlations (up to 0.9) during early and mid lactation, with a peak around wk 32, falling after this point until the end of the lactation (as low as 0.45). The highest \( r_g \) was for the MeP_L1–MeP_L2, which was close to 0.8 during most of the lactation, followed closely by MeC_L1–MeP_L1 and MeC_L2–MeP_L2, meaning that the highest \( r_g \) was for the same trait (MeP) across lactations (L1–L2) followed by \( r_g \) at the same lactation between traits. Finally, the lowest \( r_g \) was for MeC_L1–MeC_L2, with \( r_g \) from 0.45 at the beginning of lactation increasing steeply toward a peak at wk 32 and decreasing after this point. Standard errors (Q2–Q3) between traits at the same parity ranged between 0.11 and 0.15, whereas those for same trait at different parity were considerably larger (Q2–Q3 = 0.20–0.30). Based on these results, MeC could be genetically more stable across lactations than MeP; however, due to the large SE, we cannot draw firm conclusions. Analysis including more data and (possibly) further lactations is recommended to verify the trends reported in this study. These results are important because it could be assumed that methane is the same trait across lactations; however, this might not be always the case, as genetic correlation varies across lactations (as we can see in MeP and MeC, where the genetic correlation between lactations is not unity). It is important to mention that we did not find additional studies that analyze methane traits across and along lactation, given the global scarcity of recording schemes for CH\(_4\) emissions. This study is the first of its kind to estimate genetic correlations for methane traits across and between lactations. Another key finding of this study was the high genetic correlations between MeC and MeP (>0.7 for most of the lactation), showing that MeC could be used as a proxy for methane production, especially when the measuring method is a sniffer, avoiding the need to calculate MeP using ECM.

Genetic Correlations Between Methane Traits and Production, Maintenance, and Intake Traits Along First Lactation

Estimated \( r_g \) at the same lactation week between methane concentration and methane production during
first lactation (MeC_L1, MeP_L1) with ECM, BW, and DMI at first lactation (ECM_L1, BW_L1, DMI_L1) are presented in Figure 4. Due to the restrictive size of the databases, SE of the \( r_g \) between MeC and MeP with ECM, BW, and DMI were large (Q2–Q3 = 0.30–0.42). Genetic correlations between MeC and ECM, BW, and DMI at first lactation were mostly positive during early and mid lactation and became negative for late lactation (after wk 30). All 3 genetic correlations following the same sigmoidal pattern throughout first lactation, with MeC_L1–DMI_L1 having the highest positive correlation (up to 0.55) and MeC_L1–BW_L1 having the highest negative correlation (as low as −0.4). The estimated genetic correlations between MeC_L1 and ECM_L1 ranged from low–moderate positive (up to 0.44) during early and mid lactation to low–moderate negative in late lactation (as low as −0.25). Difford et al. (2020) reported moderate positive genetic correlations between MeC and ECM (0.37) and between MeC and DMI (0.60) in Danish Holstein cows using a repeatability model. Genetic correlations between MeP_L1 and ECM_L1 and DMI_L1 were moderately positive (>0.5) for most of the lactation, whereas those between MeP_L1 and BW_L1 were close to zero for

Figure 2. Estimated genetic correlations (\( r_g \)) per lactation week (LACT WEEK) for (a) methane concentration (MeC) for first lactation (MeC_L1), (b) methane production (MeP) for first lactation (MeP_L1), (c) MeC for second lactation (MeC_L2), and (d) MeP for second lactation (MeP_L2).
most of the lactation, with a slight increase toward wk 30 followed by a decrease, becoming negative at the end of the lactation. Only a single study has estimated genetic correlations between methane and production and maintenance traits in Holstein cows along lactation using random regression models (Breider et al., 2019). Breider et al. (2019) presented moderate (0.38–0.57) genetic correlations between MeP and milk yield during the entire lactation, with a maximum at mid lactation (20–30 wk) in multiparous UK Holstein cows. Likewise, they reported genetic correlations between MeP and BW close to zero along the lactation. As methane is derived from fermentation of the feed, it is not surprising that the genetic correlations across lactation between MeP and DMI follow a similar pattern as the feed intake curve across lactation, with sharp increases from early to mid lactation and a steady decline toward the end of lactation (Harder et al., 2019).

Previous studies using single CH4 phenotypes per lactation in multiparous cows have reported (using linear animal models) a wide range of genetic correlations between MeP and ECM, ranging from −0.08 to 0.74 (Breider et al., 2018; Richardson et al., 2021) in Holstein cows from the UK and Australia. However, Richardson et al. (2021) used a different method to measure CH4, the SF6 tracer method previously described by Deighton et al. (2013), which could explain some of the differences in results. Furthermore, previous studies in Danish cows have reported genetic correlations between MeP and ECM from 0.45 to 0.6 (Lassen and Løvendahl, 2016; Manzanilla-Pech et al., 2020) with a population that includes part of the population used in this study. To summarize, MeP has shown higher genetic correlations with ECM and DMI compared with MeC. These analyses were performed in an attempt to better elucidate the similarities between the studied methane traits, genetic correlations between the methane traits and with production, maintenance, and intake traits in the first lactation. As we have explained before, we incorporate ECM and BW when we transform MeC into MeP, with the aim of accounting for the size and production of the animal. However, this incorporation could lead to induced higher correlations between MeP and ECM and BW. This is particularly true for the higher correlation between MeP and ECM compared with that between MeC and ECM. However, more important is the genetic correlation of MeC with ECM and DMI, as MeC is the raw unadulterated trait and that correlation is the true genetic correlation between methane and production and intake.

Implications

One of the methods used to measure methane, given its large scale and low cost, are the sniffers installed in automated milking systems (Garnsworthy et al., 2012; Pszczola et al., 2017; Lassen and Difford, 2020; López-Paredes et al., 2020). However, the output of this method is methane concentration and, to calculate methane production (in g/d), the ratio of recorded CH4:CO2 and ECM and BW are required (Madsen et al., 2010), which evidently cause high correlations with ECM and
BW. For this reason, in the last couple of years, the use of methane concentration as a proxy trait for methane emissions has become more popular (Calderón-Chagoya et al., 2019; Difford et al., 2020; Manzanilla-Pech et al., 2021; Sypniewski et al., 2021). The results of this study bring to light the genetic architecture behind methane production and methane concentration and their correlations with production traits. This analysis shows the necessity of creating a phenotype independent of milk production to be able to select for methane without compromising production. One of the suggested traits is residual methane production, which could be different for first-lactation and later-lactation cows, as it is for residual feed intake. However, given the small data sets currently available, the results are limited and should be extended for further lactations to validate this hypothesis.

**CONCLUSIONS**

Methane concentration traits had lower heritabilities that were more stable across lactation and between lactations than MeP traits. However, the higher heritabilities for MeP traits could be due to induced covariance from component traits such as ECM and BW, with well-documented higher heritabilities and more drastic
changes within and across lactations. Estimated genetic correlations (within trait) at different lactation weeks were similar in MeC and MeP in first lactation but different in second lactation. Most of the genetic correlations between traits (MeP and MeC) in the same lactation week were moderate to highly correlated during early and mid lactation, except between first and second parity for MeC, which were weaker than for MeP in early lactation. This suggests the possible use of MeC as a proxy for MeP without the use of ECM. Furthermore, compared with MeC, MeP (at first lactation) had stronger genetic correlations with ECM and DMI. In conclusion, both traits are different along lactation and correlate differently with production, maintenance, and intake traits, which is important to consider when including one of them in future breeding objectives. These results are useful for understanding the genetic relationship between MeC and MeP along and across lactation(s), as well as between methane traits with ECM, BW, and DMI in first lactation.

ACKNOWLEDGMENTS

This work was funded by a grant from the Danish Milk Levy Fund (Aarhus, Denmark) for the project “Reduceret klimaaftryk på KO-niveau og BEDRIFTS-niveau.” Per Madsen (Aarhus University, Denmark) is kindly acknowledged for his help in the realization of this study. The authors have not stated any conflicts of interest.

REFERENCES

Breider, I. S., E. Wall, and P. C. Garnsworthy. 2019. Short communication: Heritability of methane production and genetic correlations with milk yield and body weight in Holstein–Friesian dairy cows. J. Dairy Sci. 102:7277–7281. https://doi.org/10.3168/jds.2018-15099.

Breider, I. S., E. Wall, P. C. Garnsworthy, and J. E. Pryce. 2018. Genetic relationships between methane emission and milk yield, live weight and dry matter intake. Proceedings of the 11th World Congress on Genetics Applied to Livestock Production (WCGALP), Volume Challenges - Environmental, page 134, 2018. WCGALP, New Zealand.

Calderón-Chagoya, R., J. H. Hernandez-Medrano, F. J. Ruiz-López, A. García-Ruiz, V. E. Vega-Murillo, M. Montano-Bermúdez, M. E. Arechavala-Velasco, E. Gonzalez-Padilla, E. I. Mejía-Melchor, N. Saunders, J. A. Bonilla-Cardenas, P. C. Garnsworthy, and S. I. Román-Ponce. 2019. Genome-wide association studies for methane production in dairy cattle. Genes (Basel) 10:905. https://doi.org/10.3390/genes10120995.

Deighton, M. H., B. M. O’Loughlin, S. R. O. Williams, P. J. Moate, E. Kennedy, T. M. Boland, and R. J. Eckard. 2013. Declining sulphur hexafluoride permeability of polytetrafluoroethylene membranes causes overestimation of calculated ruminant methane emissions using the tracer technique. Anim. Feed Sci. Technol. 180:86–95.

Difford, G. F., P. Levandahl, R. F. Veenkamp, H. Bouwman, M. H. P. W. Visker, J. Lassen, and Y. de Haas. 2020. Can greenhouse gases in breath be used to genetically improve feed efficiency of dairy cows? J. Dairy Sci. 103:2442–2459. https://doi.org/10.3168/jds.2019-16906.

European Commission. 2019. A European Green Deal: Striving to be the first climate-neutral continent. Accessed Jan. 15, 2022. https://ec.europa.eu/info/strategy/priorities-2019-2024/european-green-deal_en.

FAO. 2018. The State of Food Security and Nutrition in the World 2018. Building Climate Resilience for Food Security and Nutrition. FAO (Food and Agriculture Organization of the United Nations), IFAD (International Fund for Agricultural Development), UNICEF, WFP (World Food Program), and WHO (World Health Organization).

Fischer, T. M., A. Glimour, and J. H. J. van der Werf. 2004. Computing approximate standard errors for genetic parameters derived from random regression models fitted by average information REML. Genet. Sel. Evol. 36:363. https://doi.org/10.1186/1297-9686-36-363.

Garnsworthy, P. C., J. Craigion, J. H. Hernandez-Medrano, and N. Saunders. 2012. Variation among individual dairy cows in methane measurements made on farm during milking. J. Dairy Sci. 95:3181–3189. https://doi.org/10.3168/jds.2011-4606.

Garnsworthy, P. C., G. F. Difford, M. J. Bell, A. R. Bayat, P. Huhtanen, B. Kuhl, J. Lassen, N. Peiren, M. Pszczola, D. Sorg, M. H. P. W. Visker, and T. Yan. 2019. Comparison of methods to measure methane for use in genetic evaluation of dairy cattle. J. Dairy Sci. 102:9377–9387. https://doi.org/10.3168/jds.2019-15087.

González-Recio, O., J. López-Paredes, L. Ouatahar, N. Charfeddine, E. Ugarte, R. Alenda, and J. A. Jiménez-Montero. 2020. Mitigation of greenhouse gases in dairy cattle via genetic selection: 2. Incorporating methane emissions into the breeding goal. J. Dairy Sci. 103:7210–7221. https://doi.org/10.3168/jds.2019-17598.

Harder, I., E. Stamer, W. Jung, and G. Thaller. 2019. Lactation curves and model evaluation for feed intake and energy balance in dairy cows. J. Dairy Sci. 102:7204–7216. https://doi.org/10.3168/jds.2018-15300.

Hegarty, R. S., J. P. Goopy, R. M. Herd, and B. McCorkell. 2007. Cattle selected for lower residual feed intake have reduced daily methane production. J. Anim. Sci. 85:1479–1486.

Hristov, A. N., J. Oh, C. Lee, R. Meinen, P. Montes, T. Ott, J. Firkins, A. Rotz, C. Dell, A. Adesogan, W. Yang, J. Tricarico, E. Kebrab, G. Waghorn, J. Dijkstra, and S. Oosting. 2013. Mitigation of greenhouse gas emissions in livestock production: A review of technical options for non-CO2 emissions. Animal Production and Health Paper No. 177. Food and Agriculture Organization of the United Nations (FAO).

Kandel, P. B., M. L. Varnrobays, A. Vanlierde, F. Dehareneg, E. Froidmont, N. Gengler, and H. Soyeurt. 2017. Genetic parameters of mid-infrared methane predictions and their relationships with milk production traits in Holstein cattle. J. Dairy Sci. 100:5578–5591. https://doi.org/10.3168/jds.2016-11954.

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

Lassen, J., and G. F. Difford. 2020. Genetic and genomic selection as a methane mitigation strategy in dairy cattle. Animal 14:473–483. https://doi.org/10.1017/S175173120001561.

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

Li, B., B. Berglund, W. F. Fikse, J. Lassen, M. H. Lidauer, P. Mäntysaari, and P. Løvendahl. 2020. Can greenhouse gases in livestock production be used to quantitatively measure methane for use in genetic evaluation of dairy cattle. J. Dairy Sci. 103:9076–9084. https://doi.org/10.3168/jds.2019-12775.

Li, B., W. F. Fikse, P. Levandahl, J. Lassen, M. H. Lidauer, P. Mäntysaari, and B. Berglund. 2018. Genetic heterogeneity of feed intake, energy-corrected milk, and body weight across lactation in primiparous Holstein, Nordic Red, and Jersey cows.J. Dairy Sci. 101:1001–10021. https://doi.org/10.3168/jds.2018-14611.

López-Paredes, J., I. Goiri, R. Atxaerandio, A. García-Rodriguez, E. Ugarte, J. A. Jiménez-Montero, R. Alenda, and O. Gonzalez-Re.
Manzanilla-Pech, C. I. V., D. Gordo, G. F. Difford, P. Løvendahl, D. Mansan Gordo, G. F. Madsen, P., and J. Jensen. 2010. Methane and carbon dioxide ratio in excreted air for quantification of the methane production from ruminants. Livest. Sci. 129:223–227. https://doi.org/10.1016/j.livsci.2010.01.001.

Manzanilla-Pech, C. I. V. 2022. Supplemental Figures JDS. Harvard Dataverse, V1. https://doi.org/10.7910/DVN/D7WRLT.

Manzanilla-Pech, C. I. V., D. Gordo, G. F. Difford, P. Lovendahl, and J. Lassen. 2020. Multitrait genomic prediction of methane emissions in Danish Holstein cattle. J. Dairy Sci. 103:9195–9206. https://doi.org/10.3168/jds.2019-17857.

Manzanilla-Pech, C. I. V., P. Lovendahl, D. Mansan Gordo, G. F. Difford, J. E. Pryce, F. Schenkel, S. Wegmann, F. Miglior, T. C. Chud, P. J. Moate, S. R. O. Williams, C. M. Richardson, P. Stothard, and J. Lassen. 2021. Breeding for reduced methane emission and feed-efficient Holstein cows: An international response. J. Dairy Sci. 104:8983–9001. https://doi.org/10.3168/jds.2020-19889.

Manzanilla Pech, C. I. V., R. F. Veerkamp, M. P. L. Calus, R. Zetouni, L., M. Kargo, E. Norb erg, and J. Lassen. 2018. Genetic correlation of methane production and concentration measured in the breath of Polish Holstein-Friesian cattle. Animals (Basel) 11:3175. https://doi.org/10.3390/ani11113175.

Roque, B. M., C. G. Brooke, J. Ladhau, T. Polley, L. J. Marsh, N. Najafi, P. Pandey, L. Singh, R. Kinley, J. K. Salwen, E. Eloefradosh, E. Kebreab, and M. Hess. 2019. Effect of the macroalgae *Asparagopsis taxiformis* on methane production and rumen microbiome assemblage. Anim. Microbiome 1.3. https://doi.org/10.1186/s42523-019-0004-4.

Sjauja, L. O., L. Bævre, L. Junkkarinen, J. Pedersen, and J. Setala. 1991. A Nordic Proposal for and Energy Corrected Milk (ECM) Formula. EAAP Publication 50: Performance Recording of Animals. State of the Art 1990, Centre for Agricultural Publishing and Documentation (PUDOC), Wageningen, the Netherlands.

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

Tong, F., P. Jaramillo, and I. M. L. Azevedo. 2015. Comparison of life cycle greenhouse gases from natural gas pathways for light-duty vehicles. Energy Fuels 29:6008–6018. https://doi.org/10.1021/acs.energyfuels.5b01063.

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

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Journal of Dairy Science Vol. 105 No. 12, 2022