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Genetic parameters of mid-infrared-predicted methane production and its relationship with production traits in Walloon Holstein dairy cows

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ABSTRACT

Genetic selection to reduce methane production from dairy cows may be an efficient way for reducing the impact of dairy production on climate change. In this study genetic parameters and genomic regions associated with 2 commonly used daily methane features (predicted daily methane emission [PME; g/d]), and log-transformed predicted methane intensity (LMI = log [PME/milk yield (kg)]) were investigated. The PME (g/d) data, predicted using routinely recorded milk mid-infrared spectra, collected between 2007 and 2023 on 285,530 first-parity (1,920,130 test-day records), 224,643 second-parity (1,516,843 test-day records), and 160,226 third-parity (1,072,725 test-day records) Holstein cows distributed in 1,520 herds in the Walloon region of Belgium were used. Data of 565,049 SNPs, located on 29 *Bos taurus* autosomes (BTA), on 7,375 animals (1,798 bulls) were used. Random regression test-day models were used to estimate genetic parameters through the Bayesian Gibbs sampling method. The SNP solutions were estimated through a single-step genomic BLUP approach. The proportion of genetic variance explained by windows of 50 consecutive SNPs (with an average size of ~212 kb) was calculated, and regions accounting for at least 1.0% of the total additive genetic variance were used to search for positional candidate genes. Mean (SD) daily PME per cow was 324.3 (66.88) g/d, 355.0 (68.75) g/d, and 367.1 (71.42) g/d, while the mean daily LMI was 2.64 (0.36), 2.61 (0.39), and 2.58 (0.40) for the first, second, and third lactation, respectively. Mean (SD) h^2 estimates for PME were 0.22 (0.05), 0.20 (0.05), and 0.21 (0.05) and for LMI were 0.25 (0.05), 0.23 (0.05), and 0.22 (0.05) in the first, second and third lactation, respectively. Average genetic correlations (SD) estimated between PME and LMI were 0.53 (0.04), 0.46 (0.12), and 0.43 (0.16) in the first, second, and third lactation, respectively. The genetic correlations estimated between PME and production traits, including milk yield (MY), fat percentage (FP), protein percentage (PP), milk urea concentration (MU), and SCS, ranged from −0.12 (MY) to 0.42 (FP), −0.09 (MY) to 0.47 (FP), and −0.07 (MY) to 0.43 (FP) for the first, second, and third lactations, respectively. For LMI, the estimated genetic correlations ranged from −0.89 (MY) to 0.56 (FP), −0.91 (MY) to 0.55 (FP), and −0.90 (MY) to 0.50 (FP) for the first, second, and third lactations, respectively. Genome-wide association analyses identified 4 genomic regions (BAT1 144.38–144.47 Mb and BAT14 1.52–2.15 Mb, BAT14 2.19–2.57 Mb and BAT14 2.67 – 2.98 Mb) harboring genes including the *SLC37A1* (BTA1), *AHARPIN*, *MROH1*, *DGAT1*, *FAM83H*, *TIGD5*, *MROH6*, *NAPRT*, *GML*, *LYPD2*, and *JPK* (BTA14) that were associated with the studied methane features. The findings of this study help to unravel the genomic background of methane emissions and can be used for the future implementation of genomic evaluation of methane emissions in Walloon Holstein cows.

Key words: methane emissions, genomic association, Holstein

INTRODUCTION

Emissions of GHG such as carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O), and halocarbons have a considerable impact on climate change and global warming (Knapp et al., 2014). Agrifood systems account for one-third of the total anthropogenic GHG emissions (Crippa et al., 2021; Tubiello et al., 2021; Tubiello et al., 2022) mainly in the form of CO_2 followed by CH_4 and N_2O (FAO, 2022). The main agricultural sources of GHG emissions are soil nitrification and denitrification, enteric fermentation by ruminants, manure management, and rice production (FAO, 2022). Dairy cattle production is a major contributor to the global human-induced GHG emissions mainly in the form of methane (de Haas et al., 2021). Each dairy cow emits between 60 and 160 kg of methane per year (Hristov et al., 2013). Methane is produced during the microbial fermentation of feed in

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the rumen. The produced methane represents a portion of the feed energy that is not used by the animal for productive purposes, with the majority being released into the atmosphere through eructation and respiration. It has been reported that between 2% and 12% of the total gross energy intake in dairy cows is lost in the form of methane (Johnson and Johnson, 1995; Boadi and Wittenberg, 2002; Benchaar and Greathead, 2011). Therefore, in addition to its environmental impact, methane production negatively affects energy utilization efficiency and may have direct economic implications, potentially offering financial incentives for dairy farmers.

Opportunities for nutritional and microbial manipulation to reduce enteric methane emissions in dairy cows have been extensively studied (Benchaar and Greathead, 2011; Tseten et al., 2022). However, genetic selection for low-methane-emitting cows could be added as an effective tool to any combination of strategies, offering a permanent, cumulative, and long-term contribution to reduce methane production in dairy cattle across generations (González-Recio et al., 2020; Manzanilla-Pech et al., 2022c). The first requirement for successful genetic selection is to establish a method for measuring the trait or phenotype of interest in a large number of animals at a low cost. Additionally, it is essential to estimate the genetic variation of the trait within the population to determine whether the trait is heritable. Moreover, it is necessary to estimate genetic correlations between the trait of interest and those already included in the current breeding objectives. This allows the conduct of cost-benefit analyses to determine the economic value of the new trait. Accurate direct measurement of methane production in a large number of animals requires complex and expensive techniques. Therefore, the use of milk composition (e.g., milk fatty acid [**FA**] contents) or milk mid-infrared (**MIR**) spectra have been proposed as effective tools to indirectly predict methane emissions in dairy cows (Dehareng et al., 2012; van Engelen et al., 2014; van Gastelen and Dijkstra, 2016).

Milk MIR spectra are currently used to predict various milk compositions including fat, protein, lactose, groups and individual FA, milk mineral contents, as well as cheese-making properties in the Walloon dairy cows (Soyeurt et al., 2006; Soyeurt et al., 2011; Bastin et al., 2016). This procedure has also been proven as a fast and inexpensive method for predicting the amount of daily methane produced by individual dairy cows (Kandel et al., 2017; Vanlierde et al., 2021). Despite the incorporation of MIR-based predicted methane production data into the routine milk recording of dairy cows in the Walloon region of Belgium since 2007, the potential contribution of this trait to the breeding program of Walloon dairy cows is still being investigated (Atashi et al., 2022b,c).

The desired reduction in methane emissions must be achieved while ensuring that there are no negative effects on cow health and performance. Therefore, it is imperative to gain a comprehensive understanding of the genetic background of methane-related traits and their relationships with other traits of interest. Although several studies have investigated the genetic correlations between MIR-based methane traits and production traits, there remains a lack of consensus regarding the magnitude and direction of these correlations. Moreover, limited research has investigated the relationships between MIR-based methane traits and critical traits, such as SCS and milk urea concentration (**MU**), in Holstein dairy cows. The latter is particularly pertinent given the growing global concerns about nitrogen-related environmental pollution within the agricultural sector. Furthermore, exploring the genomic architecture of methane production traits could yield valuable insights into the genomic regions and specific genes that contribute to their genetic variation, thereby enhancing our understanding of the biological pathways involved. Therefore, the objectives of the present study were to estimate genetic parameters, identify genomic regions associated with 2 MIR-based methane traits, and evaluate their genetic correlations with production traits in Walloon Holstein cows.

MATERIALS AND METHODS

No human or animal subjects were used, so this analysis did not require approval by an Institutional Animal Care and Use Committee or Institutional Review Board.

Phenotypic Data

The dataset used in this study consisted of test-day records of milk yield (**MY**), SCC, MIR-based predicted fat percentage (**FP**), protein percentage (**PP**), milk urea (**MU**), and MIR-predicted methane emissions (**PME**, g/d). These data were collected between 2007 and 2023 during the official milk recording conducted in the Walloon region of Belgium by the Walloon Breeding Association (Ciney, Belgium). To generate the spectral (MIR) data, the milk samples were analyzed using Foss Milkoscan FT6000 spectrometers (Hillerød, Denmark) at the milk laboratory Comité du Lait (Battice, Belgium). Methane emissions in dairy cows can be defined in terms of production (PME, g/d), methane intensity (g/kg of MY), and methane yield (g/kg of DMI).

In this study, PME were predicted from the recorded milk MIR spectra using the lactation-stage-dependent equation (Vanlierde et al., 2021). A total of 1,089 measurements of CH_4 collected on 299 cows in the whole lactation were used to develop the predicted equation based on a modified partial least squares regression model. The

CH_4 measurements (n = 1,089; g/d) were collected using the SF6 tracer technique (n = 513) and using respiration chambers (n = 576). The R^2 and SE of calibration and cross-validation for the prediction equation based on the 4 factors (MIR data, MY, parity, and breed) were 0.73 and 0.68, 55 g/d and 59 g/d, respectively. The PME records were limited to 100 to 800 g/d. Methane intensity (**PMI**) data were calculated as the PME divided by the daily MY (kg/d) on the same day. The PMI was then log-transformed to achieve a normal distribution (**LMI**). Test-day SCC records were transformed into SCS using the following formula: SCS = $\log_2$(SCC/100,000) + 3. Daily MY, FP, and PP were restricted within the ranges of 3 to 70 kg, 1% to 9%, and 1% to 7%, respectively (ICAR, 2022). The MU was restricted between 2 and 70 mg/dL milk. The data were edited to ensure that only cows with a known birth date, calving date, and parity number were included. Only records from the first 3 parities that had complete data for all included traits on a given test day were retained. The records with DIM less than 5 d or exceeding 365 d were excluded. The age at the first calving (**AFC**) was calculated by subtracting the first calving date from the birth date and was limited to a range of 540 to 1,200 d. Within cows, if parity 3 was present, then parity 1 and 2 were also present, and if parity 2 was present, then parity 1 was also present. In the end, there were a total of 1,920,130 test-day records from 285,530 Holstein cows during their first lactation; 1,516,843 records from 224,643 cows in the second lactation; and 1,072,725 records from 160,226 cows in the third lactation. On average, 6.72, 6.75, and 6.70 test-day records were available per cow per parity. The pedigree was traced back 5 generations and encompassed 439,214 Holsteins in total, including 13,834 bulls.

Genotypic Data

Genotypic data were available for 7,375 phenotyped Holsteins, including 1,798 bulls, either directly phenotyped or represented in the analyzed pedigree. Individuals were genotyped using the BovineSNP50 Beadchip versions 1 to 3 and EuroG MD (SI) version 9 from Illumina (San Diego, CA). Only SNPs that were common across all 4 chips were kept, and nonmapped SNP, SNP on sex chromosomes, and triallelic SNP were removed. Additionally, SNPs with Mendelian conflicts and those with a minor allele frequency (**MAF**) below 5% were also excluded. Subsequently, genotypes were imputed to high density (BovineHD Beadchip) using a reference panel consisting of 4,352 HD individuals (1,046 bulls) using FImpute V2.2 software (Sargolzaei et al., 2014). Also, any SNPs with more than 15% difference between observed and expected heterozygosity were eliminated (Wiggans et al., 2009). Finally, a total of 565,049 SNPs located on 29 *Bos taurus* autosomes (**BTA**) were used in the genomic analyses.

Variance Component Estimation

The (co)variance components and GEBV for the methane traits studied (PME and LMI) were estimated by integration of the random regression test-day model (**RR-TDM**) into the single-step GBLUP procedure (**SS RR-TDM**) using a 3-trait (PME_1, PME_2, and PME_3 for PME; and LMI_1, LMI_2, and LMI_3 for LMI) model. The effects in the model were the same as the current genetic evaluation model used for production traits in the Walloon region of Belgium (Paiva et al., 2022):

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Q}(\mathbf{Wh} + \mathbf{Zp} + \mathbf{Za}) + \mathbf{e},$$

where **y** is a vector of PME (LMI) in the first 3 parities (PME_1, PME_2, and PME_3 for PME; and LMI_1, LMI_2, and LMI_3 for LMI), **b** is a vector of fixed effects (herd × test-day, stage of lactation [72 classes, DIM was divided by 5 d, except for DIM 360 to 365, which was considered as one class], stage of lactation × age at calving × season of calving, gestation stage], **h** is a vector of herd × year of calving common environmental random regression coefficients; **a** is a vector of additive genetic random regression coefficients; **p** is a vector of permanent environmental random regression coefficients; **e** is a vector of random residuals; **X**, **W**, and **Z** are incidence matrixes; and **Q** is the covariate matrix for second-order Legendre polynomials associated with DIM d as the following:

$$q_{0(d)} = 1.0,$$

$$q_{1(d)} = 3.0^{0.5}x,$$

$$q_{2(d)} = \left(\frac{5}{4}\right)^{0.5}\left(3.0x^2 - 1\right),$$

where $x = -1 + 2\left(\frac{d-1}{365-1}\right)$.

Residual covariances among traits were considered to be zero. Homogeneity of residual variance was checked visually by computing and plotting the SD of observed residuals (difference between observed and predicted values) for each class of DIM in the first 3 parities. The following (co)variance structures were assumed:

$$\text{Var}\begin{bmatrix}\mathbf{h}\\ \mathbf{p}\\ \mathbf{a}\\ \mathbf{e}\end{bmatrix} = \begin{bmatrix}\mathbf{HY}\otimes\mathbf{I}_c & 0 & 0 & 0\\ 0 & \mathbf{P}\otimes\mathbf{I}_p & 0 & 0\\ 0 & 0 & \mathbf{G}\otimes\mathbf{H} & 0\\ 0 & 0 & 0 & \mathbf{R}\end{bmatrix},$$

where **HY** is the coefficient of the herd × year of calving (co)variance (9 × 9 matrix); **P** is the coefficient of the permanent environment (co)variance (9 × 9 matrix; 3 traits × 3 regression coefficients, as the second-order Legendre polynomials were used); **G** is the coefficients of the additive genetic (co)variance (9 × 9 matrix); $\mathbf{I}_c$ is an identity matrix of dimension c (number of herd × year of calving classes); $\mathbf{I}_p$ is an identity matrix of dimension p (number of cows with records); **R** is a diagonal matrix of dimension n (total number of PME [LMI] records across the 3 parities) with diagonal elements equal to $\sigma^2_{e(t)}$, which is the residual variance for trait t (parity) in which PME (LMI) was recorded; ⊗ represents the Kronecker product function; and **H** is the combined pedigree and genomic relationships among animals, and its inverse involved the combination of additive (**A**) and genomic (**G**) relationship matrixes, respectively (Aguilar et al., 2010).

$$\mathbf{H}^{-1} = \mathbf{A}^{-1} + \begin{bmatrix}0 & 0\\ 0 & \mathbf{G}^{-1}-\mathbf{A}_{22}^{-1}\end{bmatrix},$$

where **A** is the numerator relationship matrix based on the pedigree for all animals; $\mathbf{A}_{22}$ is the numerator relationship matrix for genotyped animals; and **G** is the weighted genomic relationship matrix obtained using the following function:

$$\mathbf{G} = \mathbf{G}^* \times 0.95 + \mathbf{A}_{22} \times 0.05.$$

The **G*** is the genomic relationship matrix obtained using the following function described by VanRaden (2008):

$$\mathbf{G}^* = \frac{\mathbf{ZDZ}'}{\sum_{i=1}^{M} 2p_i(1-p_i)},$$

where **Z** is a matrix of gene content adjusted for allele frequencies (0, 1 or 2 for *aa*, *Aa*, and *AA*, respectively); **D** is a diagonal matrix of weights for SNP variances (**D** = **I**); M is the number of SNPs, and p_i is the MAF of the ith SNP. The **H** matrix was built scaling **G** based on $\mathbf{A_{22}}$, considering that the average of the diagonal of **G** is equal to the average of the diagonal of $\mathbf{A_{22}}$, and the average of the off-diagonal **G** is equal to the average of the off-diagonal $\mathbf{A_{22}}$.

The (co)variance components were estimated by Bayesian inference using the GIBBS3F90 software (Aguilar et al., 2018). A single chain of 500,000 iterates with a sampling interval of 20 samples was employed for Gibbs sampling to derive marginal posterior distributions for the parameters. The initial 100,000 iterates were considered as a burn-in period to ensure proper sampling from the marginal distributions. Genetic (co)variances for each test day were computed using the formula following (Jamrozik and Schaeffer, 1997).

$$\sigma^2_g(d) = \mathbf{q}'_d\ \mathbf{G}\mathbf{q}_d,$$

where **q** is the covariate matrix for second-order Legendre polynomials associated with DIM, d, and **G** is the additive genetic (co)variance (9 × 9 matrix). Daily h^2 was determined as the genetic variance divided by the sum of the additive genetic, permanent environmental, herd-year calving, and residual variances at a specific DIM.

The vector of **GEBV** of the studied methane features (PME, and LMI) for each animal i, which included daily GEBV from all DIM (5 to 365) in each parity, was estimated by multiplying the vector of additive genetic predicted regression coefficients by the matrix of Legendre orthogonal polynomial covariates; that is, $\mathbf{GEBV_i} = \mathbf{T}\hat{\mathbf{g}}_i$,, where $\hat{\mathbf{g}}_i$ is the vector of additive genetic predicted regression coefficients for animal i; and **T** is a matrix of orthogonal covariates associated with the Legendre orthogonal polynomial functions. Furthermore, the same RR-TDM through multiple-trait (6 traits), was used to estimate correlation between the methane traits (PME, LMI) and MY and selected milk composition traits (fat yield [**FY**], protein yield [**PY**], FP, PP, MU, and SCS).

GWAS

The GWAS analyses were performed for PME and LMI in the first 3 lactations focusing on different lactation stages: (1) from DIM 5 to 60, which includes the ascending production stage and lactation peak, (2) from DIM 61 to 200, which represents the lactation persistency stage, and (3) from DIM 201 to 365, which signifies the production decline until the end of the lactation period (Oliveira et al., 2019). The GEBV for each lactation stage of animal i (for each trait) was calculated by averaging the daily GEBV solutions of the specific DIM:

$$\text{GE}\hat{\text{B}}\text{V1}_i = (\text{GE}\hat{\text{B}}\text{V}_{i5} + \text{GE}\hat{\text{B}}\text{V}_{i6} + \ldots + \text{GE}\hat{\text{B}}\text{V}_{i60}) / 56,$$

$$\text{GE}\hat{\text{B}}\text{V2}_i = (\text{GE}\hat{\text{B}}\text{V}_{i61} + \text{GE}\hat{\text{B}}\text{V}_{i62} + \ldots + \text{GE}\hat{\text{B}}\text{V}_{i200}) / 140,$$

and

$$\widehat{GEBV3}_i = (\widehat{GEBV}_{i201} + \widehat{GEBV}_{i202} + \ldots + \widehat{GEBV}_{i365}) / 165,$$

where $\widehat{GEBV1}_i$, $\widehat{GEBV2}_i$, and $\widehat{GEBV3}_i$ are the GEBV for the first, second, and third lactation stages of animal i obtained by averaging the GEBV from DIM 5 to 60, 61 to 200, and 201 to 365, respectively. Furthermore, the GEBV of animal i through the entire lactation were obtained by averaging the daily GEBV from DIM 5 to 365; that is,

$$\widehat{GEBV}_i = \left(\widehat{GEBV}_{i5} + \widehat{GEBV}_{i6} + \ldots + \widehat{GEBV}_{i365}\right) / 361,$$

where $\widehat{GEBV}_i$ is the GEBV of animal i through the entire lactation. The SNP effects were estimated using the postGSf90 software (Aguilar et al., 2014). The animal effects were decomposed into those for genotyped ($\mathbf{a_g}$) and ungenotyped animals ($\mathbf{a_n}$). The animal effects of genotyped animals are a function of the SNP effects, $\mathbf{a_g} = \mathbf{Zu}$, where $\mathbf{Z}$ is a matrix relating genotypes of each locus and $\mathbf{u}$ is a vector of the SNP marker effect. The variance of animal effects was assumed as:

$$\mathrm{Var}\left(\mathbf{a_g}\right) = \mathrm{Var}\left(\mathbf{Zu}\right) = \mathbf{ZDZ}'\sigma_u^2 = \mathbf{G}\sigma_a^2,$$

where $\mathbf{D}$ is a diagonal matrix of weights for variances of markers ($\mathbf{D} = \mathbf{I}$) and σ_u^2 is the additive genetic variance captured by each SNP marker when the weighted relationship matrix ($\mathbf{G}$) was built with no weight. The SNP effects were obtained using the following equation:

$$\hat{u} = »\mathbf{DZ}'\ \mathbf{G}^{-1}\ \hat{a}_g = \mathbf{DZ}'\left[\mathrm{ZDZ}'\right]^{-1}\hat{a}_g,$$

where λ was defined by VanRaden (2008) as a normalizing constant, as described below:

$$\lambda = \frac{\sigma_u^2}{\sigma_a^2} = \frac{1}{\sum_{i=1}^{M} 2p_i(1-p_i)}.$$

The percentage of the total additive genetic variance explained by the ith genomic region was estimated as follows:

$$\frac{\mathrm{Var}\left(a_i\right)}{\sigma_a^2} \times 100\% = \frac{\mathrm{Var}\left(\sum_{j=1}^{50} \mathbf{Z}_j \hat{u}_j\right)}{\sigma_a^2} \times 100,$$

where a_i is the genetic value of the ith region that consists of 50 adjacent SNPs; σ_a^2 is the total additive genetic variance; $\mathbf{Z_j}$ is the vector of the SNP content of the jth SNP for all individuals; and $\hat{u}_j$ is the marker effect of the jth SNP within the ith region. The additive genetic variance explained by 50-SNP moving windows, with an average size of ~216 kb, was calculated across the whole genome, and windows explaining at least 1.0% of the total additive genetic variance were considered promising regions and used to identify positional candidate genes. The concept of grouping SNP into windows was adopted as a way to better capture genetic information such as the extent of linkage disequilibrium in neighboring SNPs (Habier et al., 2011).

Identification of Positional Candidate Genes for the Traits Studied

The animals were genotyped using Illumina's BovineSNP50 BeadChip v1 to v3 and the EuroG MD (SI) v9 chip (Illumina, San Diego, CA). The genotypes were imputed to the BovineHD Beadchip, which is based on the bovine reference genome assembly UMD3.1. However, there is a new bovine reference genome assembly, ARS-UCD1.2, which provides long sequencing reads and addresses gaps and repetitive regions present in the UMD3.1 assembly and provides more reliable annotation information (Rosen et al., 2020). To convert the coordinate ranges of the identified genomic regions from UMD3.1 to ARS-UCD1.2 assembly, we used the Lift Genome Annotations tool (https://genome.ucsc.edu/cgi-bin/hgLiftOver). Then, genes located inside the identified genomic regions were identified as potential positional candidate genes associated with the methane features under study. For this purpose, we used the National Center for Biotechnology Information Map Viewer tool, with the ARS-UCD1.2 assembly serving as the reference map (https://www.ncbi.nlm.nih.gov/datasets/genome/GCF_002263795.1).

RESULTS

Descriptive Statistics

The descriptive statistics for the studied MY traits and methane features are presented in Table 1. The average daily MY was 23.56 kg (3.99% fat and 3.36% protein) in the first lactation, 26.91 kg (4.07% fat and 3.45% protein) in the second lactation, and 28.58 kg (4.07% fat and 3.41% protein) in the third lactation. Environmental factors including herd, calving year, calving season, AFC, parity, and lactation stage affected the studied methane features ($P < 0.05$). The PME exhibited higher values in multiparous cows, with mean (SD) values of 324.3 (66.88), 355.0 (68.75), and 367.1 (71.42) g/d for the first, second, and third lactation, respectively. The LMI showed higher values in primiparous cows, with mean

Table 1. Descriptive statistics for the defined methane traits[1] and MY traits[2] in the first three parities in Walloon Holstein cows[3]

Parity	PME	LMI	MY	FY	PY	FP	PP	MU	SCS
First	324.3 (66.88)	2.64 (0.36)	23.56 (6.13)	0.93 (0.24)	0.79 (0.20)	3.99 (0.67)	3.36 (0.35)	25.23 (8.28)	2.50 (1.60)
Second	355.0 (68.75)	2.61 (0.39)	26.91 (8.30)	1.07 (0.32)	0.91 (0.25)	4.07 (0.71)	3.45 (0.38)	24.53 (8.30)	2.81 (1.77)
Third	367.1 (71.42)	2.58 (0.40)	28.58 (9.09)	1.15 (0.36)	0.96 (0.27)	4.08 (0.72)	3.41 (0.39)	24.20 (8.16)	3.17 (1.87)

[1]PME stands for methane emission predicted from the recorded milk MIR spectra (g/d); LMI stands for log-transformed methane intensity based on the ratio of PME divided by the daily MY (kg/d).

[2]MY = milk yield (kg/d), FY = fat yield (kg/d), PY = protein yield (kg/d), FP = fat percentage, PP = protein percentage, MU = milk urea concentration (mg/dL milk); SCS = $\log_2$(SCC/100,000) + 3.

[3]In the first, second, and third lactations, there were 1,920,130 test-day records (on 285,530 animals), 1,516,843 test-day records (on 224,643 animals), and 1,072,725 test-day records (on 160,226 animals), respectively.

(SD) values of 2.64 (0.36), 2.61 (0.39), and 2.58 (0.40) for the first, second, and third lactation, respectively. The CV for PME were 21%, 20%, and 20%, and for LMI, they were 14%, 15%, and 15% for the first, second, and third lactations, respectively.

The trend in concentrations of PME over the course of lactation is given in Figure 1A. The PME for multiparous cows exhibited a more abrupt increase and an earlier plateau than that for primiparous cows. The lowest value for PME was found at the beginning of the lactation, increased rapidly with DIM, reached its peak at the middle of lactation, then slightly decreased with DIM to the end of the lactation. The middle of lactation was identified as the most consistent and indicative phase of PME levels during the lactation period. The trend of LMI across lactation is given in Figure 1B. The lowest value for LMI was found at the beginning of lactation, increased rapidly at the beginning of lactation, and increased slightly for the rest of lactation period.

Heritability and Genetic Correlation

Heritability estimates for PME and LMI changed over the course of lactation. For PME, the h^2 level started at ~0.16 (DIM = 5), increasing to a maximum value of ~0.32 (DIM ~200), and ended at the level of ~0.09 (DIM = 365; Figure 1C). Mean h^2 estimates for PME were 0.22 (0.05), 0.20 (0.05), and 0.21 (0.05) for the first, second and third lactation, respectively. For LMI h^2 level started at ~0.13 at DIM 5, increased to its maximum value of ~30 at DIM ~210, and ended at the level of ~0.18 (DIM = 365; Figure 1D). Mean h^2 estimates for LMI were 0.25 (0.05), 0.23 (0.05), and 0.22 (0.05) for the first, second and third lactation, respectively.

The trajectories of the genetic correlations between PME and LMI are shown in Figure 2. Through the course of lactation, correlations between PME and LMI were always positive (ranging from 0.43 to 0.53). The genetic correlation between PME and LMI was high in the early stage of lactation; then, this correlation experienced a slight decline before stabilizing during mid lactation for primiparous cows. However, in multiparous cows, the correlation between PME and LMI decreased from the beginning to the end of lactation.

The genetic correlations between the methane traits and the production traits studied are presented in Table 2. In the first, second, and third lactations, the genetic correlation between PME and production traits ranged from −0.12 (MY) to 0.42 (FP), −0.09 (MY) to 0.47 (FP), and −0.07 (MY) to 0.43 (FP), respectively. For LMI, the corresponding values ranged from −0.93 (MY) to 0.58 (FP), −0.96 (MY) to 0.57 (FP), and −0.96 (MY) to 0.57 (FP) across the 3 lactations. The mean genetic correlation between methane traits and MU was approximately zero, with values ranging from −0.03 to −0.02 for PME and −0.03 to −0.01 for LMI, indicating no significant relationship between methane and MU.

QWAS

General information (start and end SNP numbers, windows size, start and end genomic positions, and the variance explained by each window) for the results of single-step GWAS for the studied methane traits are presented in Supplemental Data S1–S24 (see Notes; 6 traits [PME_1, PME_2, PME_3, and LMI_1, LMI_2, LMI_3] × 4 stages per parity; https://github.com/hadiatashi/Holstein-CH4). The Manhattan plots displaying the percentage of total additive genetic variance explained by 50-SNP windows for PME and LMI can be found in Figures 3 and 4, respectively. Table 3 shows the 50-SNP windows linked to the methane traits and their respective genes. A total of 4 genomic regions spanning across 2 chromosomes (BAT1 144.38–144.47 Mb and BAT14 1.52–2.15 Mb, 2.19–2.57 Mb, 2.67–2.98 Mb) have been identified to linked with PME, LMI, or both. The results are detailed by chromosome in the following sections.

BTA1. The genomic segment spanning from 144.38 to 144.47 Mb (UMD3.1 assembly) on BTA1 was associated with PME, explaining 1.99% to 2.11%, and 1.37% to 1.42% of the total additive genetic variance of PME in DIM 61 to 200 and DIM 5 to 365, respectively. This

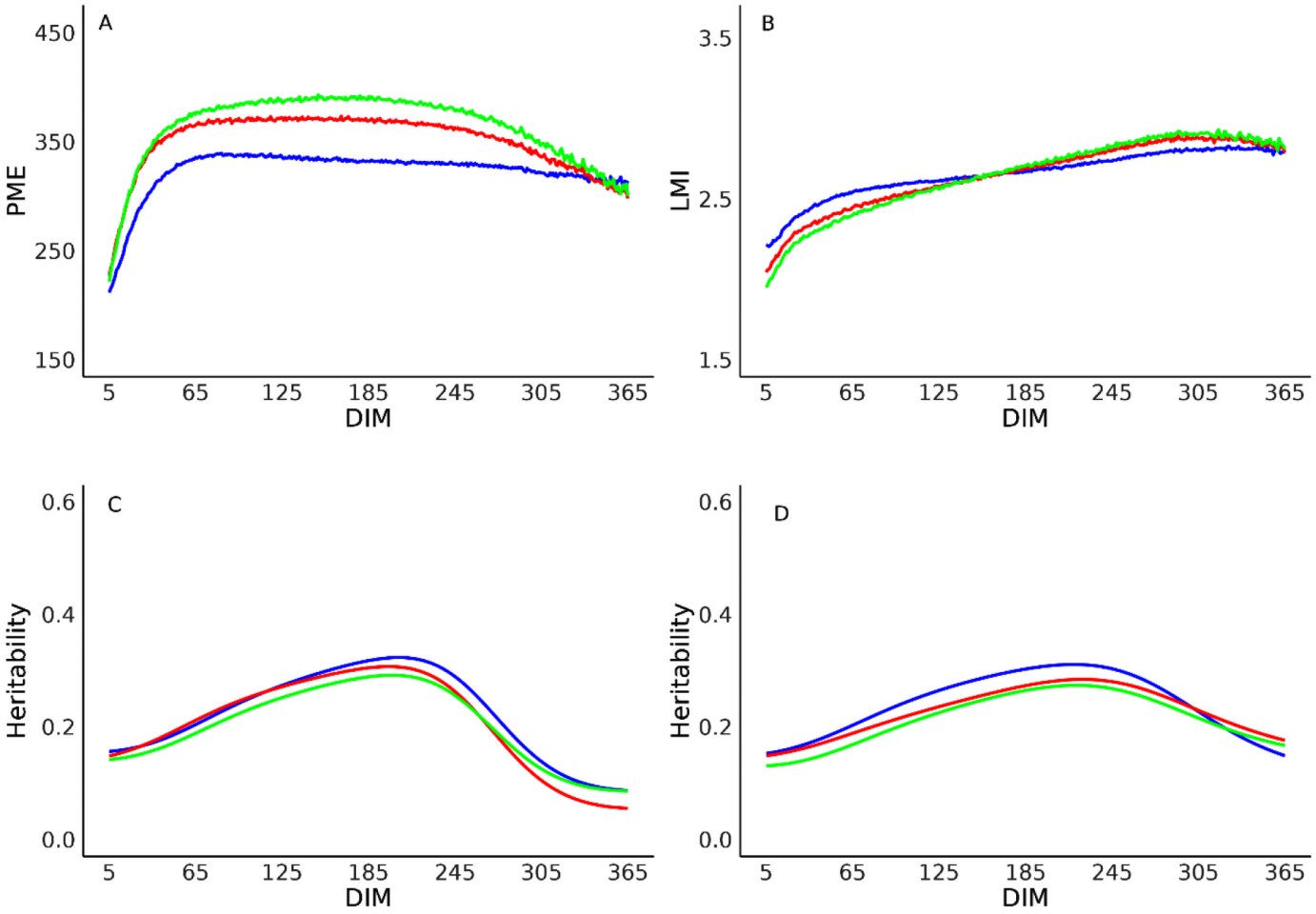

Figure 1. The lactation curves for PME (A) and LMI (B) for the first (blue), second (red) and third (green) parities in Walloon Holstein cows. Heritability of PME (C) and LMI (D) over the course of lactation for the first (blue), second (red) and third (green) parities in Walloon Holstein cows. PME stands for methane emission predicted from the recorded milk MIR spectra (g/d); LMI stands for log-transformed methane intensity based on the ratio of PME divided by the daily MY (kg/d).

region is 84.82 kb in size and harbors the solute carrier family 37 member 1 (*SLC37A1*) gene.

BTA14. Three distinct genomic regions on BTA14 were identified to be associated with PME, LMI, or both. These regions are located from 1.52 to 2.15 Mb, 2.19 to 2.57 Mb, and 2.67 to 2.98 Mb (UMD3.1 assembly) on BTA14, identified as BTA14-I, BTA14-II, and BTA14-III. The subsequent results are based on the regions identified on BTA14.

BTA14-I. The genomic region located from 1.52 to 2.15 Mb on BTA14 was associated with PME and LMI. This region explained 0.38% to 3.84% and 2.42% to 7.22% of the total additive genetic variance of PME and LMI, respectively. This region was 633.27 kb in size and harbors more than 30 genes, including scratch family transcriptional repressor 1 (*SCRT1*), diacylglycerol O-acyltransferase 1 (*DGAT1*), cleavage and polyadenylation specific factor 1 (*CPSF1*), tonsoku like, DNA repair protein (*TONSL*), and spermatogenesis and centriole associated 1 (*SPATC1*).

BTA14-II. The region located from 2.19 to 2,57 Mb on BTA14 was associated with LMI. The region explained 0.38% to 1.22%, 0.65% to 1.33%, and 0.59% to 1.36% of the total additive genetic variance of LMI in the first, second, and third lactation, respectively. This region was 374.22 kb in size and harbors genes including nicotinate phosphoribosyltransferase (*NAPRT*), family with sequence similarity 83 member H (*FAM83H*), eukaryotic translation elongation factor 1 delta (*EEF1D*), pyrroline-5-carboxylate reductase 3 (*PYCR3*), scribble planar cell polarity protein (*SCRIB*), lymphocyte antigen-6 family member H (*LY6H*), and mitogen-activated protein kinase 15 (*MAPK15*).

BTA14-III. An additional region explaining large proportions of the genetic variance of PME and LMI were found between 2.67 and 2.98 Mb on BTA14, where 15

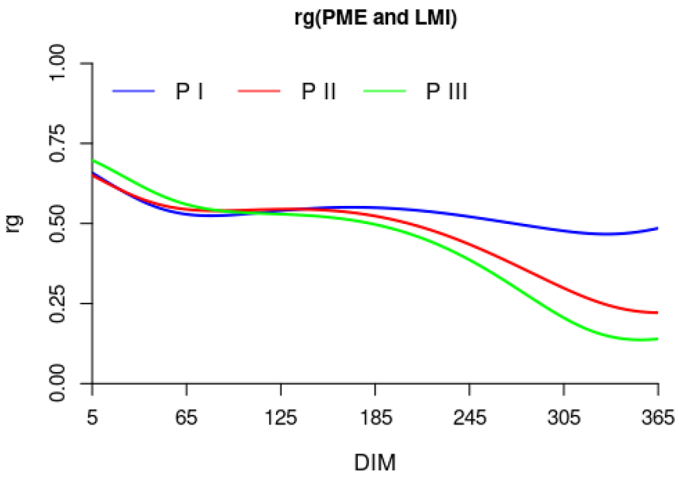

Figure 2. The genetic correlation (rg) between PME and LMI over the course of lactation for the first (blue), second (red) and third (green) parities in Walloon Holstein cows. PME stands for methane emission predicted from the recorded milk MIR spectra (g/d); LMI stands for log-transformed methane intensity based on the ratio of PME divided by the daily MY (kg/d).

genes, including adhesion G protein-coupled receptor B1 (*ADGRB1*), glycosylphosphatidylinositol anchored molecule like (*GML*), and lymphocyte antigen-6 family member D and K (*LY6D* and *LY6K*), are located. This region explained 0.14% to 1.01% and 0.63% to 1.93% of the total additive genetic variance of PME and LMI, respectively.

DISCUSSION

The mean PME (324.3–367.1 g/d) is in close agreement with Manzanilla-Pech et al. (2022b), 354.83 g/d, and Lassen and Løvendahl (2016), 315 g/d; however, lower averages for PME were reported by Pickering et al. (2015), 30.59 g/d, and Pszczola et al. (2017), 279 g/d, and higher averages were reported by Richardson et al. (2021), 469 g/d; Kamalanathan et al. (2023), 463.5 g/d; and Niu et al. (2018), 392 g/d. It is not clear how much the method of measurement for CH_4 or the diet or the combination of both could affect the average.

Moreover, PME was significantly higher in multiparous cows compared with primiparous cows, and LMI was lower in multiparous animals, reflecting their higher MY. This pattern has been observed by Kandel et al. (2017), 433 versus 453 g/d for PME and 2.93 versus 2.86 for LMI, for the first- and second-parity Holstein cows. Fresco et al. (2023), using methane emission data predicted using a GreenFeed device, reported that primiparous cows produce less methane than multiparous cows while LMI was lower in multiparous cows. Both PME and LMI showed significant variation through the course of lactation. The lowest value for PME was found at the beginning of the lactation period, rapidly increased with DIM in the first month of milking, and stayed at its high level for around 8 mo, with a steady decrease during the last stage of the lactation period. The variation found at the end of lactation for PME may be partly attributed to the limited number of observations during this period. The lactation pattern found for PME is the same as that presented by Pszczola et al. (2017) and Fresco et al. (2023); however, Lassen and Løvendahl (2016) presented a shape typical of a lactation curve for methane production. The LMI increased during the lactation period. The same findings were reported by Fresco et al. (2023), de Haas et al. (2021), and Kandel et al. (2017) based on the methane production predicted using GreenFeed device, feed intake, and milk near-infrared spectra, respectively.

Mean h^2 estimates for PME ranged from 0.20 to 0.22, and they ranged from 0.22 to 0.25 for LMI, which are in the range reported by some previous studies (Manza-

Table 2. Mean (range) genetic correlation between the studied methane traits (PME, LMI)[1] and MY traits[2] estimated across the lactation in the first three parities in Walloon Holstein cows[3]

	PME			LMI		
Item	First parity	Second parity	Third parity	First parity	Second parity	Third parity
LMI	0.53 (0.47 to 0.66)	0.46 (0.22 to 0.65)	0.43 (0.14 to 0.70)			
MY	−0.12 (−0.18 to 0.00)	−0.09 (−0.14 to 0.08)	−0.07 (−0.14 to 0.15)	−0.89 (−0.93 to −0.73)	−0.91 (−0.96 to −0.76)	−0.90 (−0.95 to −0.73)
FY	0.27 (−0.05 to 0.37)	0.34 (0.15 to 0.41)	0.33 (0.04 to 0.40)	−0.37 (−0.60 to −0.24)	−0.36 (−0.82 to −0.13)	−0.38 (−0.82 to −0.14)
PY	0.01 (−0.10 to 0.03)	0.05 (−0.11 to −0.09)	0.09 (−0.15 to 0.22)	−0.74 (−0.80 to −0.67)	−0.73 (−0.91 to −0.64)	−0.72 (−0.90 to −0.64)
FP	0.42 (−0.10 to 0.59)	0.47 (0.17 to 0.58)	0.43 (0.05 to 0.55)	0.56 (0.01 to 0.69)	0.55 (0.29 to 0.68)	0.50 (0.18 to 0.67)
PP	0.28 (−0.19 to 0.48)	0.29 (−0.11 to 0.47)	0.28 (−0.19 to 0.47)	0.57 (0.22 to 0.68)	0.56 (0.27 to 0.65)	0.51 (0.14 to 0.63)
MU	−0.02 (−0.13 to 0.23)	−0.02 (−0.12 to 0.11)	−0.03 (−0.11 to 0.10)	−0.03 (−0.10 to 0.16)	−0.01 (−0.06 to 0.05)	−0.02 (−0.04 to 0.11)
SCS	−0.02 (−0.06 to 0.05)	−0.05 (−0.07 to 0.02)	−0.05 (−0.07 to 0.03)	0.10 (−0.15 to 0.27)	0.14 (−0.04 to 0.54)	0.15 (−0.02 to 0.49)

[1]PME stands for methane emission predicted from the recorded milk MIR spectra (g/d); LMI stands for log-transformed methane intensity based on the ratio of PME divided by the daily MY (kg/d).

[2]MY = milk yield (kg/d), FY = fat yield (kg/d), PY = protein yield (kg/d), FP = fat percentage, PP = protein percentage, MU = milk urea concentration (mg/dL milk); SCS = $\log_2$(SCC/100,000) + 3.

[3]In the first, second, and third lactations, there were 1,920,130 test-day records (on 285,530 animals), 1,516,843 test-day records (on 224,643 animals), and 1,072,725 test-day records (on 160,226 animals), respectively.

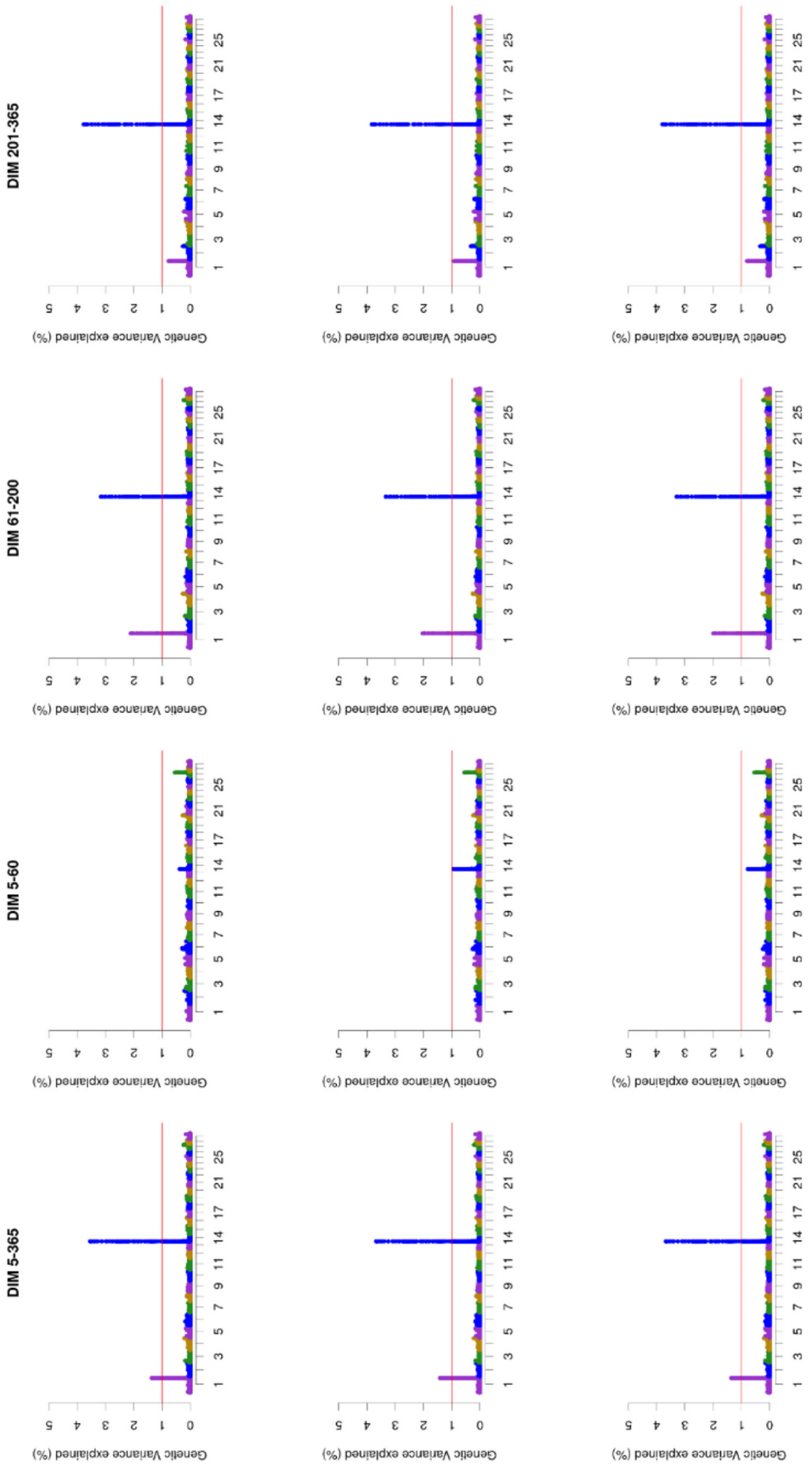

Figure 3. Additive genetic variance explained by windows of 50 adjacent SNPs across chromosomes for the predicted methane emission (PME g/d) in the different lactation stages in the first (first row), second (second row), and third parity (third row) in Walloon Holstein cows.

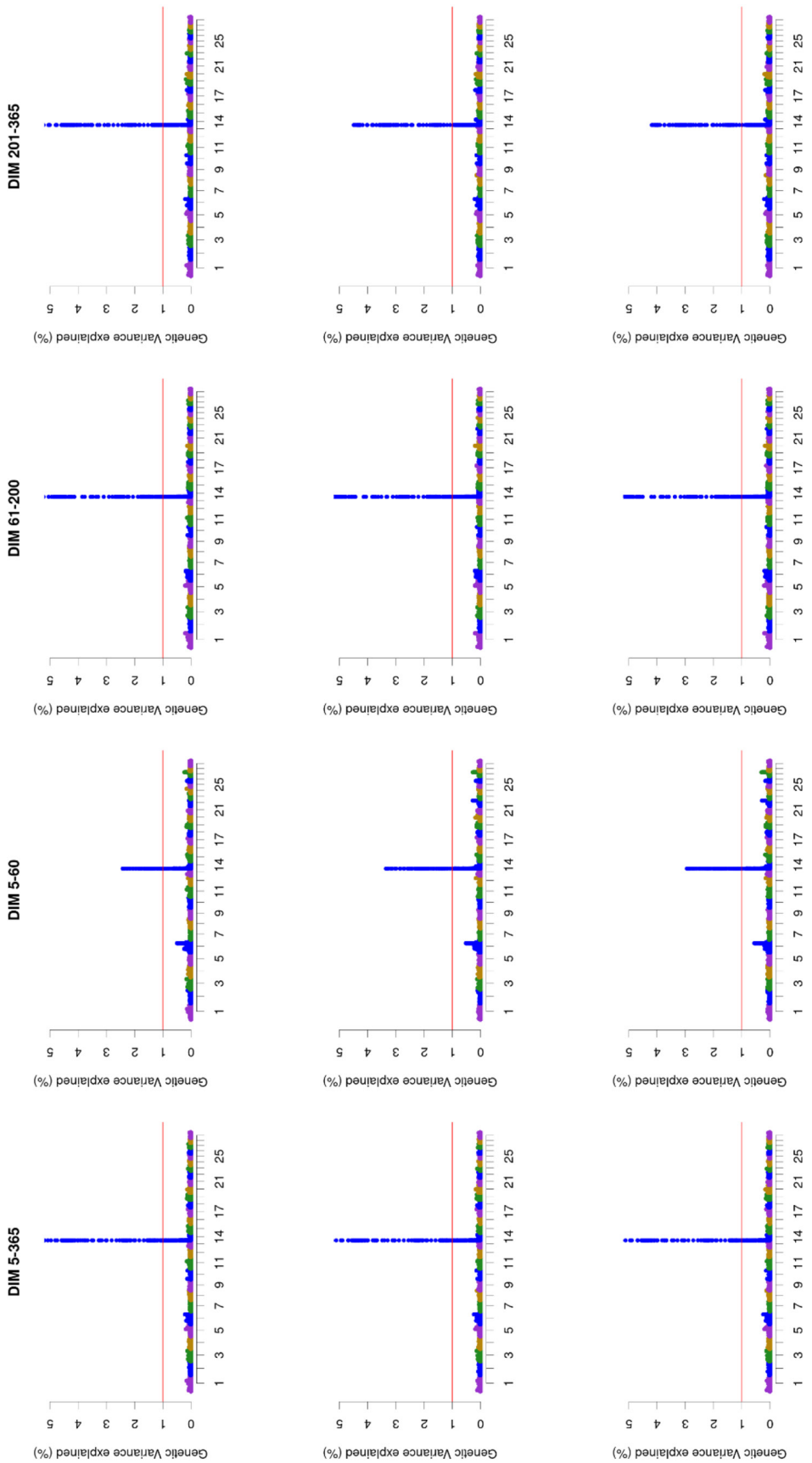

Figure 4. Additive genetic variance explained by windows of 50 adjacent SNP across chromosomes for the log-transformed methane intensity (LMI) in the different lactation stages in the first (first row), second (second row), and third parity (third row) in Walloon Holstein cows.

Table 3. Genomic regions associated with the studied methane traits (PME and LMI) in the first three parities in Walloon Holstein cows[1]

Chromosome	Position (bp)[2]	Position (bp)[3]	Gene[4]	Trait[5] (parity, stage of lactation,[6] % variance explained)
BTA1	144380987–144465804	142817724–142902514	*SLC37A1*	PME (1, 1, 0.11), PME (1, 2, 2.11), PME (1, 3, 0.77), PME (1, e, 1.37), PME (2, 1, 0.12), PME (2, 2, 2.04), PME (2, 3, 0.92), PME (2, e, 1.42), PME (3, 1, 0.11), PME (3, 2, 1.99), PME (3, 3, 0.81), PME (3, e, 1.37)
BTA14	1517553–2150825	481648–870500	–*SHARPIN*, *MROH1*, *TSSK5*, *GPAA1*, *TMEM276-ZFTRAF1*, *PLEC*, *VPS28*, *CPSF1*, *HSF1*, *SLC39A4*, *OPLAH*, *GRINA*, *SMPD5*, *WDR97*, *FBXL6*, *TONSL*, *SLC52A2*, *ADCK5*, *SCRT1*, *TMEM249*, *CYC1*, *HGH1*, *SPATC1*, *EXOSC4*, *BOP1*, *PARP10*, *DGAT1*, *SCX*, *MAF1*, *MIR1839*	PME (1, 1, 0.38), PME (1, 2, 3.17), PME (1, 3, 3.78), PME (1, e, 3.55), PME (2, 1, 0.94), PME (2, 2, 3.34), PME (2, 3, 3.84), PME (2, e, 3.67), PME (3, 1, 0.78), PME (3, 2, 3.31), PME (3, 3, 3.79), PME (3, e, 3.68), LMI (1, 1, 2.42), LMI (1, 2, 6.61), LMI (1, 3, 5.89), LMI (1, e,6.17), LMI (2, 1, 3.35), LMI (2, 2, 7.08), LMI (2, 3, 4.49), LMI (2, e, 6.05), LMI (3, 1, 2.95), LMI (3, 2, 7.22), LMI (3, 3, 4.19), LMI (3, e, 6.06)
BTA14	2194228–2568452	999900–1366351	*EEF1D*, *PYCR3*, *MIR193A-2*, *GFUS*, *MAFA*, *SCRIB*, *LY6H*, *GSDMD*, *GLI4*, *ZC3H3*, *ZNF623*, *IQANK1*, *RHPN1*, *TIGD5*, *ZNF696*, *GPIHBP1*, *CCDC166*, *MAPK15*, *FAM83H*, *MROH6*, *NAPRT*, *TOP1MT*	LMI (1, 1, 0.38), LMI (1, 2, 1.22), LMI (1, 3, 0.96), LMI (1, e, 1.06), LMI (2, 1, 0.65), LMI (2, 2, 1.33), LMI (2, 3, 0.65), LMI (2, e, 1.01), LMI (3, 1, 0.59), LMI (3, 2, 1.36), LMI (3, 3, 0.59), LMI (3, e, 1.01)
BTA14	2673388–2978629	1435245–1818117	*THEM6*, *LOC112441461*, *ARC*, *LY6K*, *SLURP1*, *LYNX1*, *LOC101905222*, *ADGRB1*, *GML*, *PSCA*, *LYPD2*, *LOC112449566*, *CYP11B1*, *LY6D*, *JRK*	PME (1, 1, 0.14), PME (1, 2, 0.93), PME (1, 3, 0.95), PME (1, e, 1.00), PME (2, 1, 0.32), PME (2, 2, 0.96), PME (2, 3, 0.95), PME (2, e, 1.01), PME (3, 1, 0.27), PME (3, 2, 0.95), PME (3, 3, 0.92), PME (3, e, 1.00), LMI (1, 1, 0.63), LMI (1, 2, 1.79), LMI (1, 3, 1.38), LMI (1, e, 1.55), LMI (2, 1, 0.94), LMI (2, 2, 1.89), LMI (2, 3, 0.92), LMI (2, e, 1.43), LMI (3, 1, 0.86), LMI (3, 2, 1.93), LMI (3, 3, 0.83), LMI (3, e, 1.42)

[1]In the first, second, and third lactations, there were 1,920,130 test-day records (on 285,530 animals), 1,516,843 test-day records (on 224,643 animals), and 1,072,725 test-day records (on 160,226 animals), respectively.

[2]The positions of the identified genomic regions based on the UMD3.1 assembly.

[3]The positions of the identified genomic regions, based on the UMD3.1 assembly, transformed to those in the ARS-UCD1.2 assembly.

[4]Genes inside the genomic region. Official gene symbol (Assembly ARS-UCD1.2, annotation release 105).

[5]PME stands for methane emission predicted from the recorded milk MIR spectra (g/d); LMI stands for log-transformed methane intensity based on the ratio of PME divided by the daily MY (kg/d).

[6]The genome-wide association studies analyses were performed for PME and LMI in the first three lactations focusing on different lactation stages: (1) from DIM 5 to 60, which includes the ascending production stage and lactation peak; (2) from DIM 61 to 200, which represents the lactation persistency stage; (3) from DIM 201 to 365, which signifies the production decline until the end of the lactation period; and (e) from DIM 5 to 365 representing the entire lactation.

nilla-Pech et al. 2022b; Pszczola et al., 2017; Fresco et al., 2024). However, de Haas et al. (2021) reported that h^2 estimates for CH_4 emigrants predicted based on feed intake is 0.35. In contrast, lower h^2 has been reported by Kamalanathan et al. (2023): 0.16 for methane production and 0.21 for methane intensity. The variation found for the h^2 of methane features studied in the literature can be explained by the differences in the studied breeds, number of records, the method of measurement for CH_4, statistical models, and the length of the period of data collection. For example, van Engelen et al. (2015) reported that h^2 of methane predicted based on the different sets of milk FA ranged from 0.12 to 0.44 in Dutch dairy cows.

The results showed that the h^2 of PME and LMI changes over the course of lactation, and its maximum value was found at DIM ~200 for PME and DIM ~210 for LMI. Manzanilla-Pech et al. (2022a) reported that estimated h^2 for PME vary between 0.14 and 0.25 at the beginning of lactation, between 0.28 and 0.47 in the middle, and between 0.11 and 0.29 at the end of lactation in Danish Holstein cows.

The genetic correlation between PME and LMI was high in the early stage of lactation, decreased slightly, and then stabilized in mid lactation for primiparous cows. In multiparous cows the correlation between PME and LMI decreased from the beginning to the end of lactation. The genetic correlation pattern found between PME and LMI is the same as that presented by Fresco et al. (2024). Moderate negative genetic correlations were estimated between PME and MY and a weak positive genetic correlation was found between PY and PME; however, FY, FP, and PP were positively correlated with PME. The same findings were reported by Kandel et al. (2017). Pszczola et al. (2019), using 34,429 daily methane production records collected from 483 cows, reported low positive correlation between methane production and production traits, ranging from 0.21 (FY) to 0.04 (FP) for Polish-Holstein cows. A British study found moderate positive genetic correlations between methane production and MY throughout lactation (Breider et al., 2019). Lassen and Løvendahl (2016) reported a correlation of 0.43 between fat- and protein-corrected milk and methane production. The estimated genetic correlations between PME and SCS and MU were close to zero. Pszczola et al. (2017) reported that methane production is positively correlated with FY (0.21), MY (0.15), and SCS (0.11).

Four genomic regions distributed over 2 chromosomes (BTA1 [1 region] and BTA14 [3 regions]) were associated with PME, LMI, or both. The following are the results discussed by regions and chromosomes.

The genomic region located from 144.38 to 144.47 Mb on BTA1 (UMD3.1 assembly) was associated with PME. This region is located within QTL previously reported to be linked to various milk-related traits such as MY traits, FA profile, and cheese-making properties in Dual-Purpose Blue and Walloon Holstein cows (Atashi et al., 2022a,d; Atashi et al., 2023a,b). Furthermore, this region has also been associated with CN percentage, milk FA, FY, MY, and SCS in Holstein cows (Harder et al., 2006; Schopen et al., 2009; Bouwman et al., 2011). The *SLC37A1* gene, located within this region, has been shown to exhibit high expression in the mammary glands and encodes a glucose-6-phosphate transporter involved in blood glucose homeostasis and sugar transport. Furthermore, Sanchez et al. (2021) reported that *SLC37A1* is associated with the milk mineral content of magnesium, potassium, sodium, and phosphorus in Montbéliarde dairy cows.

BTA14. Three genomic regions located from 1.52 to 2.15 Mb, 2.19 to 2.57 Mb, and 2.67 to 2.98 Mb (UMD3.1 assembly) on BTA14 were associated with PME, LMI, or both. The following are the results discussed by regions identified on BTA14.

BTA14-I. The genomic region located from 1.52 to 2.15 on BTA14 was associated with PME and LMI. This region has been reported to be associated with MY, FP, PP, CN percentage, calcium content, cheese-making properties (Atashi et al., 2023a), nitrogen efficiency index, and milk true protein nitrogen (Chen et al., 2023) in Walloon Holstein cows. It has been reported that PME is positively correlated with predicted nitrogen lose and negatively correlated with nitrogen use efficiency in Walloon Holstein dairy cows (Atashi et al., 2022b). Clancey et al. (2019) reported an association between SNPs within this region and MY in Holstein cows. This region has been reported to be associated with body energy content, body temperature, cumulative effective energy balance, blood glucose level, ketosis, and lifetime in Holstein cows (Oikonomou et al., 2009; Nayeri et al., 2016; Nayeri et al., 2019; Luo et al., 2021). Ornelas et al. (2019) reported that lower methane intensity is associated with higher digestible energy, energy balance, and the ratio between metabolizable and digestible energy. This BTA14-I region in this study is 633.27 kb in size and contains many genes such as *SCRT1*, *DGAT1*, *CPSF1*, *TONSL*, and *SPATC1*. *DGAT1*, involved in the last step of the synthesis of triacylglycerol, has a major effect on milk production traits (Clancey et al., 2019; Nayeri et al., 2019). The *SHANK* gene product is involved in regulating immune and inflammatory responses (Wang et al., 2012) and has been associated with colostrum and serum albumin concentrations in Holstein cows (Lin et al., 2020).

BTA14-II. The region located from 2.19 to 2.57 Mb on BTA14 was associated with LMI. This region has been reported to be associated with 305-d MY, lactation curve parameters, FP, PP, and cheese-making proprietaries in Holstein cows (Atashi et al., 2020, 2023a). This region

is 374.22 kb in size and contains 22 genes. Those genes located within this region, including *NAPRT*, *FAM83H*, *EEF1D*, *PYCR3*, *SCRIB*, *LY6H*, and *MAPK15*, have been previously suggested as potential candidate genes for milk production traits in Holstein cows (Li et al., 2010; Buitenhuis et al., 2014; Ning et al., 2017; Wang et al., 2019).

BTA14-III. An additional region explaining a large proportion of the genetic variance of PME and LMI was found between 2.67 to 2.98 Mb on PTA14. This region has been reported to be associated with lactation curve parameters, MY, FP, PP, and cheese-making traits (Atashi et al., 2020, 2023a) nitrogen efficiency index and milk true protein nitrogen (Chen et al., 2023) in Holstein cows. Among genes identified inside this region, *ADGRB1*, *GML*, and *LY6D* have been previously reported as candidate genes for MY, FP, PP, FY, PY and milk FA profiles in Holstein cows (Cole et al., 2011; Buitenhuis et al., 2014; Ning et al., 2017; Jiang et al., 2019). The cytochrome P405 family 11 subfamily B member 1 (*CYP11B1*) gene is involved in glucose and lipid metabolism and has been reported as a functional candidate gene for milk production in dairy cows (Bülow and Bernhardt, 2002; Kaupe et al., 2007).

This study has 2 limitations that should be addressed in future research. First, MIR-based predicted methane, as used in this study, is a proxy for CH_4 emissions and may not accurately reflect actual CH_4 production. Future research should verify whether genomic selection for MIR-predicted methane aligns with selection based on sniffer-measured methane breeding values, to assess the suitability of MIR-predicted methane as a proxy for genetic improvement of actual methane emissions. Second, CH_4 production (direct CH_4 or its proxies) is negatively correlated with key production traits, indicating that genetic selection for reduced CH_4 emissions could inadvertently decrease production performance. Therefore, future research should focus on developing breeding strategies that reduce CH_4 emissions without diminishing overall production efficiency or herd profitability.

CONCLUSIONS

Genetic parameters of PME and LMI and their genetic correlations with production traits were estimated. The results showed that large variations exist in MIR-predicted methane emissions expressed as PME or LMI, encouraging the implementation of genetic selection for environmentally friendly cows. The results of GWAS showed that the methane emissions traits studied are highly polygenic; many regions across genome contribute to their genetic variation.

NOTES

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Nonstandard abbreviations used: AFC = age at first calving; BTA = *Bos taurus* autosomes; FA = fatty acids; FP = fat percentage; FY = fat yield; LMI = log-transformed PMI; MAF = minor allele frequency; MIR

= mid-infrared; MU = milk urea; MY = milk yield; PME = predicted methane emissions; PMI = (predicted?) methane intensity; PP = protein percentage; PY = protein yield; rg = genetic correlation; RR-TDM = random regression test-day model; SS RR-TDM = single-step GBLUP procedure integrated with RR-TDM.

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