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Genome-wide association study for methane emission traits in Danish Holstein cattle

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ABSTRACT

Selecting for lower methane emitting cows requires insight into the most biologically relevant phenotypes for methane emission, which are close to the breeding goal. Several methane phenotypes have been suggested over the last decade. However, the (dis)similarity of their underlying genetic architecture and correlation structures are poorly understood. Therefore, the objective of this study was to test association of SNP and genomic regions through GWAS on 8 CH_4 emission traits in Danish Holstein cattle. The traits studied were methane concentration (MeC; ppm), methane production (MeP ; g/d), 2 definitions of residual methane (RMETc and RMETp: MeC and MeP regressed on metabolic body weight and energy-corrected milk, respectively), 2 definitions of methane intensity (MeI; MeIc = MeC/ECM and MeIp = MeP/ECM); 2 definitions of methane yield per kilogram of dry matter intake (MeY; MeYc = MeC/dry matter intake and MeYp = MeP/dry matter intake). A total of 1,962 cows with genotypes (Illumina BovineSNP50 Chip or Eurogenomic custom SNP chip) and repeated records of the above-mentioned 8 methane traits were analyzed. Strong associations were found with 3 traits (MeC, MeP, and MeYc) on chromosome 13 and with 5 traits (MeC, MeP, MeIp, MeYp, and MeYc) on chromosome 26. For MeIc, MeIp, RMETc, MeYc, and MeYp, some suggestive association signals were identified on chromosome 1. Genomic segments of 1 Mbp (n = 2,525) were tested for their association with these traits, which identified between 33 to 54 significantly associated regions. In a pairwise comparison, MeC and MeP were the traits that shared the highest number of significant segments (17). The same trend was observed when comparing SNP significantly associated with the traits MeC and MeP shared from 23 to 25 SNP (most of which were located in chromosomes 11, 13, and 26). Based on our results on GWAS and genetic correlations, we conclude that MeC is (genetically) more closely linked to MeP than any of the other methane traits analyzed.

Key words: genome-wide association study, methane yield, methane intensity, residual methane

INTRODUCTION

Enteric fermentation by ruminants contributes to 44.3% of the global livestock emissions (FAO, 2018). Western European livestock produces 15% of the world GHG emissions while concurrently contributing to 25% of the world's milk production (FAO, 2018). Genetic selection of low methane (CH_4) emitting cows can be an effective and sustainable approach to reduce GHG production from dairy cattle (Garnsworthy et al., 2012; Lassen and Difford, 2020). Given that genetic progress is cumulative over generations, selecting low CH_4 emitting animals could yield significant reductions in emissions within a few generations. However, multiple CH_4 phenotypes have been proposed and there is currently a lack of consensus on the optimal CH_4 trait for the breeding goal (de Haas et al., 2017; Løvendahl et al., 2018).

The lack of consensus stems from the different combinations of the directly measured methane production in g/d (**MeP**) and other traits such as DMI (which is a substantial driver in variation in MeP) as well as BW and ECM, which are energy sinks for DMI (Tempelman et al., 2015). The most prominent combination traits include ratio traits such as methane intensity (**MeI**; CH_4 per kilogram of milk, milk yield, or ECM) and methane yield (**MeY**; CH_4 per kg of DMI), as well as residual methane emission traits, which are estimated using multiple linear regression on various combinations of metabolic body weight (**MBW**), ECM, and DMI (Donoghue et al., 2016; Hayes et al., 2016; Richardson et al., 2021). The residual CH_4 traits can be further divided into genetic residual methane and phenotypic residual methane (Manzanilla-Pech et al., 2016; Rich-

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ardson et al., 2021). The purpose of computing different linear combinations is to remove the covariance between MeP and other traits driving variation in CH_4 (DMI, ECM, BW), making CH_4 emission statistically independent of these traits.

The need for cost-effective, noninvasive, and large-scale phenotyping under commercial conditions has given rise to an additional class of CH_4 traits, namely CH_4 breath concentration (referred to as CH_4 concentration, **MeC**; Madsen et al., 2010; van Engelen et al., 2015; Difford et al., 2020). The majority of genetic research has been conducted using sniffers installed in automated milking stations (Lassen and Difford, 2020), but also a highly portable handheld laser CH_4 detector (Mühlbach et al., 2018). However, to calculate CH_4 g/d (referred to as MeP) and subsequently the other CH_4 traits from MeC, the ratio of recorded CH_4:CO_2 gas concentrations is multiplied by predicted CO_2 tracer gas using ECM and BW (Madsen et al., 2010). This could lead to an artificially induced covariance structure between traits resulting in high correlations with ECM and BW, and potentially with DMI as well. Somewhat counter-intuitively, researchers are then required to remove the covariance with ECM and BW through calculating the ratio or residual traits discussed above. For this reason, some authors have used MeC directly in genetic parameter estimations (Difford et al., 2020; Manzanilla-Pech et al., 2020).

Investigations into the underlying genomic architecture of different CH_4 traits can generate knowledge of the origin of genetic variation in different CH_4 phenotypes. This will indicate the genetic closeness of different CH_4 phenotypes. Only few authors (Manzanilla-Pech et al., 2016; Pszczola et al., 2018; Calderon-Chagoya et al., 2019) have investigated the genomic architecture behind CH_4 traits. However, due to the inherently small data sets and populations with CH_4 records available in the world, only a few studies have performed GWAS with a limited number of animals ($n < 300$; Manzanilla-Pech et al., 2016; Pszczola et al., 2018; Calderon-Chagoya et al., 2019). Therefore, the objective of this study was to test association of SNP and genomic regions through GWAS on 8 CH_4 emission traits in a larger data set with Danish Holstein cattle. The traits studied were MeC (CH_4 concentration), MeP (CH_4 production; g/d), 2 definitions of residual CH_4 (RMETc and RMETp: MeC and MeP regressed on MBW and ECM, respectively), 2 definitions of CH_4 intensity (MeIc = MeC/ECM and MeIp = MeP/ECM); 2 definitions of CH_4 yield per kg DMI (MeYc = MeC/DMI and MeYp = MeP/DMI). In this study, we used both MeC and MeP to calculate different definitions of MeI, MeY, and residual CH_4, respectively.

MATERIALS AND METHODS

Data Collection and Traits

A total of 1,962 Danish Holstein cows with weekly records on CH_4 breath concentration (7,227 records), BW (7,295 records), milk yield (7,311 records), and DMI (4,785 records) were available from the Danish Cattle Research Center (**DCRC**, Tjele, Denmark) and 10 commercial farms in Denmark. Data were collected between 2011 and 2016, and were previously described by Zetouni et al. (2018), Difford et al. (2020), and Manzanilla-Pech et al. (2020). Methane concentration was measured by 2 sniffer methods (Garnsworthy et al., 2012; Lassen et al., 2012): the nondispersive infrared CH_4 sensor (Guardian NG, Edinburgh Instruments Ltd.) in the research farms and some commercial farms and the portable Fourier transform infrared Gasmet DX-4000 (Gasmet; Gasmet Technologies Oy) in the commercial farms. Both methodologies were described and compared previously (Difford et al., 2016). Methane concentration (CH_4 in ppm, referred to as **MeC**) was not normally distributed, thus, a natural logarithm transformation was applied, and finally it was multiplied by 100 to avoid problems with the scale of the other traits. Data from research and commercial farms were filtered to only include weekly averages where a maximum of 3 d was allowed to be missing within a week, and a cow had a minimum of 3 weekly measurements. Methane production (CH_4 in g/d, referred to as **MeP**) was calculated as follows: first, CH_4 was computed in liters using the formula of Madsen et al. (2010) based on heat-producing units (**HPU**):

$$CH_4\ (L/d) = (CH_4/CO_2) \times 180 \times 24 \times HPU, \quad [1]$$

where

$$HPU = 5.6\ MBW + 22\ ECM + 1.6 \times 10^{-5} \times \text{days carried calf}. \quad [2]$$

Second, CH_4 was converted in L/d to g/d using the formula:

$$MeP = CH_4\ g/d = \text{density} \times CH_4\ (L/d), \quad [3]$$

where the density of CH_4 at 20°C = 0.668 g/L.

Cows were located at the DCRC (Foulum, Denmark) and were fed automatically with feeders (Insentec, RIC system) as reported in Li et al. (2017). Cows at DCRC were part of several nutritional experiments and diets included primarily rolled barley, corn silage, grass clover silage, rapeseed meal, and soybean meal.

The DCRC barn is a loose housing system with access to automatic milking systems (**AMS**; DeLaval International AB). Milk composition was determined using infrared technology at Eurofins using CombiFoss equipment (Foss). The AMS was fitted with a weighing platform (Danvaegt) that recorded BW at each milking from which weekly averages were calculated (full description can be found in Li et al., 2017). For the 10 commercial farms, weekly average milk yield and milk components were available from the national recording scheme (RYK, Skejby, Denmark). Body weight in commercial farms was measured with weighing scales in the AMS as well. Metabolic BW was defined as $BW^{0.75}$.

Energy-corrected milk was calculated using the following formula (Sjaunja et al., 1990):

$$\text{ECM (kg)} = 0.25 \text{ milk (kg)} + 12.2 \text{ fat (kg)} + 7.7 \text{ protein (kg)}. \quad [4]$$

After calculating MeP, residual methane (**RMETp**) was the residual of the partial regression of MeP on MBW and ECM along with fixed effects described later in model [5]. Methane intensity [**MeIp**; MeP (g/d)/ECM (kg/d)] was calculated using MeP divided by ECM, CH_4 yield [**MeYp**; MeP (g/d)/DMI (kg/d)] was calculated using MeP divided by DMI. With the purpose of testing the use of MeC instead MeP, **RMETc** (residual of the partial regression of MeC on MBW and ECM), **MeIc** (MeC divided by ECM), **MeYc** (MeC divided by DMI) were calculated as well. The reasoning behind this is that for MeIp, ECM, and BW are needed to calculate MeP, whereas only ECM is used to calculate CH_4 intensity when using MeC (**MeIc**), leaving a portion of BW in the trait. Unlike other CH_4 traits, residual methane emission traits are independent of the traits used as regressors (in this case both ECM and BW). Both CH_4 yield traits (MeYc and MeYp) resulted in a reduced number of animals and observations (Table 1) as commercial herds do not have DMI records available.

Genotypes

Two sets of genotypes were available; 1,747 cows were genotyped with BovineSNP50 BeadChip (Illumina), 466 cows were genotyped with Eurogenomics custom SNP chip (LD chip; Boichard et al., 2018), and 74 cows were genotyped with both SNP chips. The genotypes were edited for quality control with the PLINK software (Purcell et al., 2007). Quality control included a minimum of 0.02 for minor allele frequency, a maximum of 10% genotypes missing per SNP, a maximum of 15% genotypes missing per animal, and Hardy-Weinberg disequilibrium ($P = 0.001$). In addition, 50K SNP genotypes were kept when an animal was genotyped with both LD and 50K SNP chips. Furthermore, SNP located on sex chromosomes, unmapped SNP, and SNP with duplicate or uncertain positions were deleted. Posteriorly, the LD chip genotypes were imputed to 50K with the software FImpute (Sargolzaei et al., 2014). After editing and removing duplicates, 1,962 cows (663 cows from DCRC and 1,299 cows on 10 commercial farms) with 38,253 SNP remained.

Variance Component Estimation (Genomic Heritabilities and Genomic Correlations)

Variance components for the 8 traits were estimated using the single trait model with AI-REML algorithm in DMU software (Version 6, Release 5.4; Madsen and Jensen, 2014) using a genomic relationship matrix (GRM) with all genotypes. Genetic correlations for the 4 pair traits were estimated through bivariate analyses. The model for the univariate and bivariate analyses was defined as follows:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Z_1a} + \mathbf{Z_2c} + \mathbf{e}, \quad [5]$$

Table 1. Descriptive statistics for 8 methane traits[1]

Trait	Number of animals	Number of records	Unit	Mean	SD	Minimum	Maximum	CV (%)
MeC	1,962	7,227	log (ppm × 100)	576.77	45.85	450.00	699.00	8
MeP	1,844	5,554	g/d	354.83	73.17	101.80	671.49	21
MeIc	1,956	7,227	MeC/kg of ECM	16.37	5.67	1.00	61.65	35
MeIp	1,839	5,554	g of CH_4/kg of ECM	9.30	3.50	1.00	36.37	38
RMETc	1,548	7,227	log (ppm × 100)	0.00	34.31	−120.00	120.00	—
RMETp	1,537	5,554	g/d	0.00	45.19	−190.00	280.00	—
MeYc	379	4,758	MeC/kg of DMI	25.33	5.72	1.00	68.91	22
MeYp	353	2,762	g of CH_4/kg of DMI	16.38	3.02	1.00	31.08	18

[1]MeC = methane concentration; MeP = methane production; MeIc = methane intensity calculated using MeC; MeIp = methane intensity calculated using MeP; RMETc = residual methane on metabolic BW and ECM using MeC; RMETp = residual methane on metabolic BW and ECM using MeP; MeYc = methane yield calculated using MeC; MeYp = methane yield using MeC.

where $\mathbf{y}$ is the vector of phenotypes; $\mathbf{b}$ represents the vector of fixed effects (herd-trial-year-season, lactation week modeled with the Wilmink function, type of sniffer, and parity number as 1, 2, and ≥3), for all traits except the residual traits (RMETc and RMETp only have SNP as a fixed effect as they have been corrected before for the other effects), $\mathbf{X}$ is the incidence matrix relating observations with fixed effects; $\mathbf{a}$ is the vector of direct additive genetic effects; $\mathbf{Z_1}$ is the incidence matrix relating observations with random genetic effects; $\mathbf{c}$ is the vector of permanent environmental effects; $\mathbf{Z_2}$ is the incidence matrix relating observations with random permanent environmental effect; and $\mathbf{e}$ is the vector of residual effects. Distributions of the random effects were $\text{var}(\mathbf{a}) = \mathbf{G}\sigma_a^2$, where $\mathbf{G}$ is the genomic relationship matrix and σ_a^2 is the additive genomic variance, $\text{var}(\mathbf{c}) = \mathbf{I}\sigma_c^2$, where $\mathbf{I}$ is identity matrix of order equal to the number of individuals with records and σ_c^2 is the permanent environmental variance, and $\text{var}(\mathbf{e}) = \mathbf{I}\sigma_e^2$, where $\mathbf{I}$ is an identity matrix of an order equal to the number of observations and σ_e^2 is the residual variance.

GWAS

Genome-wide association studies were performed using a single SNP regression analyses with DMU software (Version 6, Release 5.4; Madsen and Jensen, 2014) to determine the association of each SNP with the 8 analyzed traits (MeC, MeP, RMETc, RMETp, MeIc, MeIp, MeYc, MeYp,) using a linear mixed model and repeated records. The following univariate model was used per SNP for each of the 8 traits:

$$\mathbf{y} = \mathbf{Xb} + \beta_1\mathbf{SNP_i} + \mathbf{Z_1a}_{(\mathbf{without_j})} + \mathbf{Z_2c} + \mathbf{e}, \qquad [6]$$

where $\mathbf{y}$ is the vector of phenotypes; $\mathbf{b}$ represents the vector of fixed effects (same as in [5]), $\mathbf{X}$ is the incidence matrix relating observations with fixed effects; β_1 is the effect of SNP_i of the individual examined, $\mathbf{SNP_i}$ is the vector of SNP for the ith SNP genotype indicator variable coded as 0, 1, or 2 for an individual; $\mathbf{a}_{(\mathbf{without_j})}$ is the vector of direct additive genetic effects due to all the SNP except those on chromosome j where SNP_i is located; $\mathbf{Z_1}$ is the incidence matrix relating observations with random genetic effects; $\mathbf{c}$ is the vector of permanent environmental effects; $\mathbf{Z_2}$ is the incidence matrix relating observations with random permanent environmental effect; and $\mathbf{e}$ is the vector of residual effects. Distributions of the random effects were $\text{var}(\mathbf{a}) = \mathbf{G}\sigma_a^2$, where $\mathbf{G}$ is the genomic relationship matrix constructed leaving out chromosome j and σ_a^2 is the additive genomic variance, $\text{var}(\mathbf{c}) = \mathbf{I}\sigma_c^2$, where $\mathbf{I}$ is identity matrix of order equal to the number of individuals with records and σ_c^2 is the permanent environmental variance, and $\text{var}(\mathbf{e}) = \mathbf{I}\sigma_e^2$, where $\mathbf{I}$ is an identity matrix of an order equal to the number of observations and σ_e^2 is the residual variance. After SNP effects were estimated with DMU, P-values were calculated from a t-value ($\boldsymbol{t}$, from t distribution), which is the coefficient β_1 divided by its standard error $\left(t = \beta_1/\text{SE}\right)$ with n − 2 degrees of freedom, where n is the sample size.

Segments (Base Pair Windows) and SNP Comparison Between Traits

Using the GWAS results, a total of 2,525 nonoverlapping windows of 1 Mbp (1 million bp) across the 29 chromosomes were created, these segments contained on average 15 SNP. The aim of these windows was to identify regions significantly associated with each trait and posteriorly to determine regions in common across traits using a χ^2 pairwise test. Given that there could be some significant SNP in the same region without being exactly in the same bp position (same SNP; Khansefid et al., 2014). Second, these windows were created to determine significant SNP associated with more than one trait, pairwise comparisons of the data sets of significant SNP associated with the 8 different traits were used with the same procedure. Statistical significance of each SNP was determined using a χ^2 pairwise test to evaluate if the same SNP were significant in both traits rather than the expected by chance (Khansefid et al., 2014). Finally, the segments were compared with the cattle QTL database (Hu et al., 2013). The current release of the Cattle QTL database contains 160,659 QTL/associations from 1,030 publications, with 675 different traits, related to feed, production, maintenance and health (CH_4 traits are not included yet).

RESULTS AND DISCUSSION

Descriptive statistics for CH_4 traits are presented in Table 1. The estimates of the averages for MeP and MeIp are in agreement with Lassen and Løvendahl (2016) of 315 g/d and 8.61 g/L ECM using a subset of the data set in this study. However, Pszczola et al. (2017) reported much lower average (279 g/d) for MeP (calculated similarly as this study) in Polish Holstein cows. Oppositely, Richardson et al. (2021) reported greater averages, 469 g/d for MeP, 18.15 g/kg ECM for MeI, and 19 g/kg DMI for MeY, in grazing Australian Holstein cows with the SF_6 method. It is unclear how much the method of measurement for CH_4 or the diet or the combination of both could affect the average

MeP. For example, Garnsworthy et al. (2019) observed that MeP from the SF_6 method was consistently greater than all other methods (sniffers, laser, chambers, GreenFeed), but this method is also primarily used on grazing animals, which are expected to have greater roughage to concentrate intake ratio and thus greater CH_4. Finally, the average for MeC (5.6; without multiplication of 100) was similar to values by Difford et al. (2020) with a subset of the data set used in this study.

Estimated Genetic Parameters

Estimated heritabilities (Table 2) for all traits ranged between 0.09 (MeYc) and 0.21 (RMETc), being 0.14 for MeC and 0.15 for MeP. Lassen and Løvendahl (2016) have reported a heritability of 0.21 for both MeP and MeI in Danish Holstein cows from multiple commercial herds, using a single average weekly record per cow (average MeP and MeI distributed over the lactation). Difford et al. (2020) reported heritabilities of 0.16 for MeC in Dutch Holstein cows and 0.26 for MeC in Danish Holstein cows mainly using mostly longitudinal data from a single research farm in each country. It appears that heritability estimates tend to be greater in research herds with continuous recording than in commercial herds with recording from short intervals. The heritability in the current study is based on data from both commercial and research herds and thus slightly lower heritability may reflect the greater number of records from commercial herds. Similar heritability for MeP (0.16) was reported by Richardson et al. (2021) in Australian Holstein cows, and was greater for MeY (0.23) and MeI (0.33) using SF_6. Furthermore, Pszczola et al. (2017) reported heritabilities for daily MeP, obtained via sniffers, ranging from 0.23 to 0.30 across lactation in 2 herds. Heritability estimates for residual traits are not directly comparable as trait definition varied widely across studies. In Australian Holstein cattle, Richardson et al. (2021) reported heritabilities between 0.11 to 0.21 for 9 trait definitions of residual methane involving genetic and phenotypic regression of MeP on a combination of DMI and ECM corrected for DIM, parity, and experimental batch using phenotypes or direct genomic values. Furthermore, most of the above-mentioned studies, except Richardson et al. (2021), used pedigree relationships matrices instead genomic relationship matrices to estimate the heritabilities, which could affect the size of the heritability estimates presented here.

Genetic correlations between traits are presented in Table 2. Genetic correlations between all traits were estimated via bivariate analyses, but did not converge for some of the combinations (partially due to the few animals and records available for MeY traits). For this reason, only the genetic correlations between similar pair traits were reported, all of which did converge. The genetic correlation between MeC and MeP was 0.91 (SE = 0.07) in this study. Genetic correlations between other trait pairs were 0.46 (SE = 0.19) for MeIc and MeIp, 0.78 (SE = 0.14) for MeYc and MeYp, and 0.65 (SE = 0.23) for RMETc and RMETp. The genetic correlation between MeC and MeP showed a closer similarity between these 2 traits than between the other trait pairs. This does not come as a surprise; a positive correlation between these traits was expected, as MeC is an important part for the calculation of MeP (as the ratio between CH_4 and CO_2 concentrations is used in the calculation of formula [1]). Correlations between other trait pairs were also expected to be lower as they involve traits such as ECM, BW, and DMI, all of them with larger genetic variance than CH_4 itself. Genetic correlations between these trait pairs have not been reported before, as MeC is not a trait widely studied.

GWAS Results

Genome-wide association plots [$-\log_{10}$ (P)] of MeP, MeC, MeIc, MeIp, RMETc, RMETp, MeYc, and MeYp are presented in Figure 1. Quantile-quantile plots for MeP, MeC, MeIc, MeIp, RMETc, RMETp, MeYc, and MeYp are presented as Supplemental Figure S1 (https://dataverse.harvard.edu/dataverse/suplementalS1). There was no common pattern between the Manhattan plots of the 8 CH_4 traits, meaning that there are no chromosomes with significant SNP presented for all traits. Strong associations were established on chromosome 13 in 3 traits (MeC, MeP, and MeYc) and on chromosome 26 in 5 traits (MeC, MeP, MeIp, MeYp, and MeYc). However, for MeIc, MeIp, RMETc, MeYc, and MeYp, some suggestive association signals were identified on chromosome 1. Additionally, RMETc and MeIc

Table 2. Estimated heritabilities (h^2) of 8 methane traits and genetic correlations (r_g; SE in parentheses) between similar pair traits[1]

Trait	h^2	r_g
MeP	0.12 (0.03)	0.91 (0.07)
MeC	0.15 (0.03)	
MeIc	0.04 (0.03)	0.46 (0.19)
MeIp	0.04 (0.03)	
RMETc	0.11 (0.03)	0.65 (0.23)
RMETp	0.21 (0.03)	
MeYc	0.09 (0.04)	0.78 (0.14)
MeYp	0.14 (0.04)	

[1]MeC = methane concentration; MeP = methane production; MeIc = methane intensity calculated using MeC; MeIp = methane intensity calculated using MeP; RMETc = residual methane on metabolic BW and ECM using MeC; RMETp = residual methane on metabolic BW and ECM using MeP; MeYc = methane yield calculated using MeC; MeYp = methane yield using MeC.

showed associations on chromosomes 2 and 4. Similarly, Pszczola et al. (2018) reported strong associations between MeP (calculated from MeC) and SNP located on chromosomes 1, 4, 9, 13, and 25 using a Bayesian approach in 287 Holstein cows in Poland. Likewise, Calderon-Chagoya et al. (2019) reported associations between MeP (calculated from MeC) and chromosomes 1, 3, 13, and 20 in 280 dairy crossbred-dual purpose Mexican cows. Moreover, Manzanilla-Pech et al. (2016) reported strong associations on chromosomes 3, 6, and 13 for MeP, MeY, and MeI in Holstein cows in Australia recorded using SF_6. Nevertheless, all those studies had relatively small populations (<300 cows). Furthermore, van Engelen (2018) studied approximately 1,700 Dutch Holstein cows and reported significant associations on chromosome 14 for MeP and MeY predicted from milk mid-infrared spectra (**MIR**), and on chromosomes 14 and 19 for 2 definitions of MeP predicted from milk fatty acids. However, they did not find any significant association for MeI (predicted from MIR). In summary, there appears to be a reliable association on chromosome 13 for MeP based on the results of previous studies in several populations of Holstein in different countries (Manzanilla-Pech et al., 2016; Pszczola et al., 2018; Calderon-Chagoya et al., 2019). In the present study, this association was also present for MeC, as expected because MeC is used in the calculation of MeP (as showed in [1]). The GWAS for MeC or any other trait calculated with MeC have not been reported before, as MeC is a relatively understudied trait. However, one of the aims of this paper was to provide some insight about the genetic architecture of MeC and other CH_4 traits calculated using MeC, in comparison to the traditional CH_4 traits (MeP, MeI, and MeY). Adjusted CH_4 traits had weaker associations across all the chromosomes compared with MeC and MeP, except for MeYc and MeYp. Finally, adjusted traits using MeP (MeIp, RMETp, and MeYp,) had fewer SNP associated across chromosomes compared with adjusted traits using MeC (MeIc, RMETc, and MeYc). Our hypothesis is that adding ECM and BW to calculate MeP and subsequently correcting for ECM and BW could be causing the loss of some association signals, meaning that these traits have been statistically overcorrected. This is especially clear in RMETp, which shows practically no significant associations between the SNP and the trait. In Figure 1, MeY traits showed very strong association signals for chromosome 1 ($P = 8.22 \times 10^{-12}$; MeYp) and 24 ($P = 1.59 \times 10^{-14}$; MeYc); however, these results should be taken carefully, as the number of animals/genotypes for these traits is much lower than for the other traits (Table 1). According to Gebreyesus et al. (2019) who performed a power detection test on a several Holstein populations, as a function of sample size and proportion of explained variance by a QTL, a population of 2,880 animals could have a detection power of 0.97, whereas a population of 1,566 animals could have a detection power of 0.57 and a population of 614 animals only 0.05 to detect QTL explaining 5% of the genetic variance. Based on these results (Gebreyesus et al., 2019), we can infer that we have much more power of QTL detection for MeC (n = 1962) and MeP (n = 1,844) than for the other traits, especially for MeYc (n = 379) and MeYp (n = 353). Therefore, ideally, we would need around 3,000 animals per trait to have an optimal power of detection for QTL explaining at least 5% of the genetic variance.

Significant Segments and SNP Within and Between Traits

In Table 3, significant ($P < 0.001$) segments of 1 Mbp associated with and between all traits (MeC, MeP, MeIc, MeIp, RMETc, RMETp, MeYc, and MeYp) are presented. The total number of segments across the chromosomes was 2,525, and significant segments across traits ranged from 33 (MeYp) to 54 (MeP, MeIc). Less significant segments were found for traits that used MeP instead MeC, except for the residual traits. The highest number of significant segments in common between traits was 17 between MeC and MeP. The number of significant segments in common between pair traits with similar definitions varied; for instance, the intensity ratio traits (MeIc and MeIp) shared 4 segments, whereas residual traits (RMETc and RMETp) and the yield ratio traits (MeYc and MeYp) shared one segment per pair. Furthermore, significant SNP ($P < 0.001$) associated with each of the 8 traits and between traits (using a chi-squared test) are presented in Table 4. The number of significant SNP per trait varied from 36 (RMETc) to 71 (MeYc). Significant SNP in common from a chi-squared pairwise comparison showed that 25 (out of the 66) significant SNP for MeP were also significant for MeC, and 23 (out of the 42) significant SNP for MeC were also significant for MeP. However, MeIc and MeIp shared many fewer SNP (5 from 65 SNP and 4 from 45 SNP). In Table 5, significant SNP associated with 2 traits, with their chromosomal locations, are presented. As we have seen in previous tables, the pair of traits sharing most of the significant SNP were MeC and MeP, especially on chromosomes 11, 13, and 26. Another pair of traits that shared significant SNP was MeIc and MeIp on chromosomes 4 and 6. van Engelen (2018) reported strong associations for MeY predicted both from milk MIR and milk fatty acids on chromosome 14, and the SNP were identified as the ones coding for diacylglycerol O-acyltransferase 1 (DGAT1). However, it is hard to disentangle if this

strong signal could be due to use of milk mid-infrared spectra to predict MeI or if the addition of fat- and protein-corrected milk (**FPCM**) as the denominator (MeI = MeP/FPCM) could have influenced this result. In more detail, Pszczola et al. (2018) reported 16 significant SNP detected for MeP on 6 different chromosomes (1, 4, 9, 13, 20, and 25) and their respective candidate QTL regions. In this study, we identified 2 significant SNP in common with that study: one on chromosome 1 significantly associated ($P = 0.004$) with MeIp (BTA-89820-nors, 46,321,775 bp), and the other on chromosome 4 associated ($P = 0.003$) with MeYc (Hapmap39581-BTA-70101, 9203380 bp). According to Pszczola et al. (2018), the SNP on chromosome 1 is associated with 12 candidate genes, and the SNP on chromosome 4 is associated with 14 candidate genes.

Overlap with Cattle QTL Database

Although QTL for CH_4 traits are not in the cattle QTL database, other economically important and CH_4-related traits, including production, health, and feed-maintenance traits, are in this database. Some QTL associated with milk production, feed efficiency, weight, and conformation traits in the cattle QTL database overlap with the significant association segments for CH_4 traits identified in this study. In Table 6, we present a summary of QTL per chromosome reported for

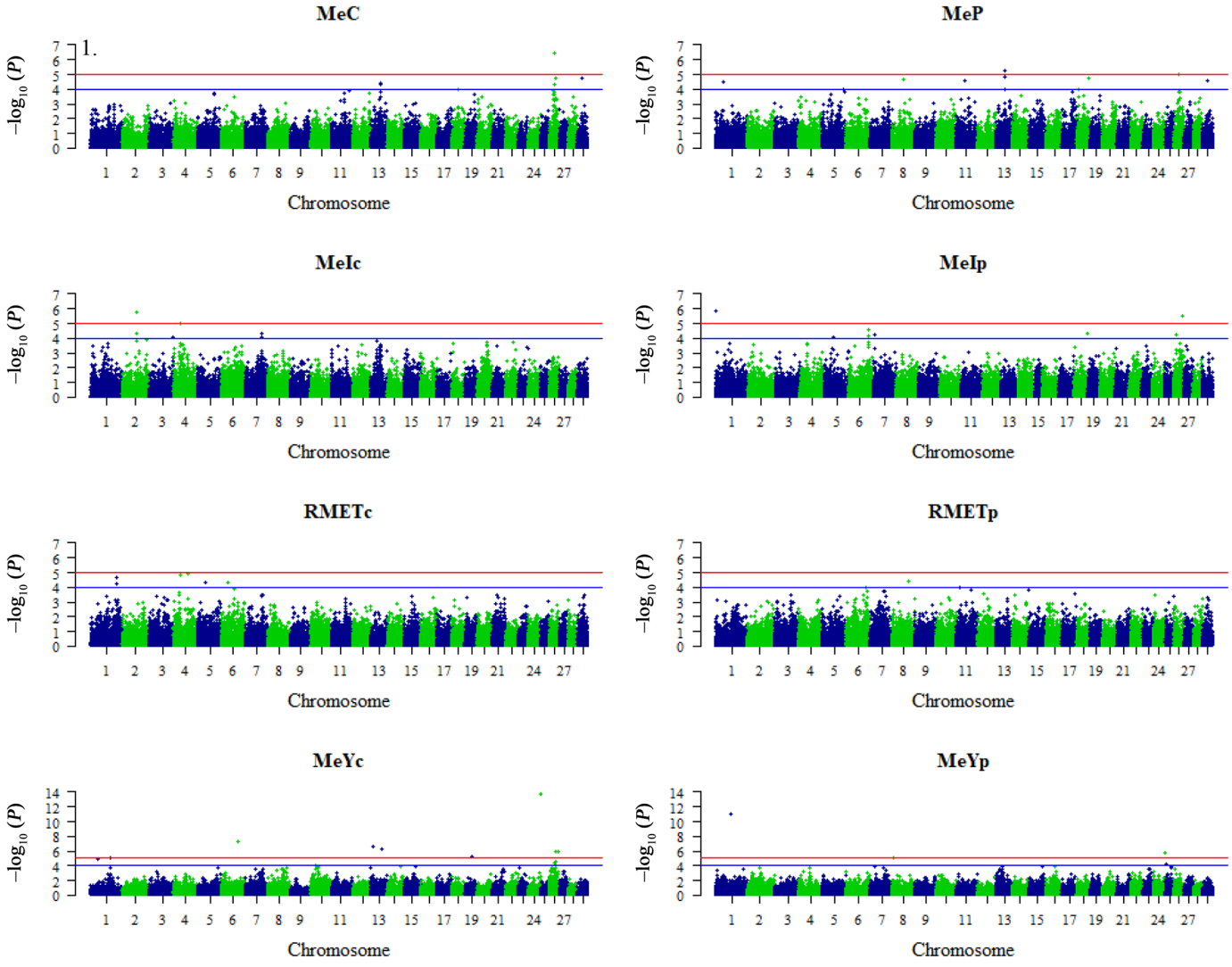

Figure 1. Genome-wide association plots [$-\log_{10}(P)$] of methane concentration (MeC), methane production (MeP), methane intensity based on MeC (MeIc), methane intensity based on MeP (MeIp), residual methane based on MeC (RMETc), residual methane based on MeP (RMETp), methane yield based on MeP (MeYc), and methane yield based on MeP (MeYp). The genome-wide significance level (lower line) is set at 10×10^{-5} and is plotted as the blue line. The red line represents the threshold for GWAS significance (upper line) after a Bonferroni correction (10×10^{-6}).

Table 3. Segments significantly ($P < 0.001$) associated with each trait (diagonal, bold) and each pair of traits (below diagonal)

Trait[1]	MeC	MeP	MeIc	MeIp	RMETc	RMETp	MeYc	MeYp
MeC	**34**							
MeP	17	**54**						
MeIc	1	1	**54**					
MeIp	1	2	4	**37**				
RMETc	0	0	4	0	**35**			
RMETp	0	0	1	0	1	**37**		
MeYc	5	3	2	5	3	0	**52**	
MeYp	1	0	0	1	1	1	1	**33**

[1]MeC = methane concentration; MeP = methane production; MeIc = methane intensity calculated using MeC; MeIp = methane intensity calculated using MeP; RMETc = residual methane on metabolic BW and ECM using MeC; RMETp = residual methane on metabolic BW and ECM using MeP; MeYc = methane yield calculated using MeC; MeYp = methane yield using MeC. Total number of segments = 2,525.

Bos taurus associated with significant ($P < 0.001$) segments for MeC, alone or in combination with other CH_4 traits. MeC was chosen because it does not have any artificially induced dependency with ECM and BW, as opposed to MeP that requires ECM and BW for its calculation. Chromosomes 11, 13, and 26 had the most overlapping associations with QTL reported in the database. Among the representative traits associated with these reported QTL (https://www.animalgenome.org/cgi-bin/QTLdb/index) are fat, protein, and lactose (in percentage and content), milk production, lifetime milk production, DMI, residual feed intake, BW, and several conformation traits highly correlated with weight (stature, chest width, body depth, dairy form, and strength; Manzanilla-Pech et al., 2016). Thirty-one QTL (from 15 chromosomes) were reported to have overlapping regions with the significant regions detected for MeC; 30 of those are associated with milk production traits, 18 with weight, 11 with conformation, and 7 with feed efficiency. Despite the scarcely available literature comparing genomic regions and QTL for important traits in cows, the genetic correlations between CH_4 traits and production (Breider et al., 2018; Difford et al., 2020), feed efficiency (Difford et al., 2020; Richardson et al., 2021), weight, and conformation traits (Breider et al., 2018; Zetouni et al., 2018) are well documented. Thus, associations between CH_4 traits and QTL reported for those traits are expected. Moreover, Pszczola et al. (2018) reported genomic regions controlling CH_4 associated with 3 QTL for feed efficiency traits in chromosome 4, 3 QTL for maintenance traits in chromosomes 4 and 9, and several QTL for milk production in 5 different chromosomes.

Implications

We have stated previously that ECM and BW are needed in the calculation of MeP (when using the sniffer method), resulting in artificially induced high correlations between MeP with ECM and BW. Subsequently, for the calculation of MeYp, MeIp, and RMETp under the conventional definition (using MeP), it is required to remove the covariance with ECM and BW, to have an independent CH_4 trait. One of the main problems in MeY and MeI as ratio traits is the strong negative correlation with the denominator trait (DMI and ECM) and an antagonism between the response in the numerator and the denominator. This problem could be solved by

Table 4. SNP significantly associated ($P < 0.001$) with each trait (in parentheses) and number of these SNP validated on the other traits[1]

Trait	SNP	MeC	MeP	MeIc	MeIp	RMETc	RMETp	MeYc	MeYp
MeC	(42)	—	23	1	0	0	0	9	0
MeP	(66)	25	—	2	2	0	1	8	0
MeIc	(65)	1	2	—	5	0	1	3	2
MeIp	(45)	0	3	4	—	0	0	5	1
RMETc	(36)	0	0	0	0	—	1	2	2
RMETp	(42)	0	1	1	0	1	—	0	1
MeYc	(71)	7	5	1	5	5	0	—	1
MeYp	(39)	0	0	2	1	2	1	1	—

[1]MeC = methane concentration; MeP = methane production; MeIc = methane intensity calculated using MeC; MeIp = methane intensity calculated using MeP; RMETc = residual methane on metabolic BW and ECM using MeC; RMETp = residual methane on metabolic BW and ECM using MeP; MeYc = methane yield calculated using MeC; MeYp = methane yield using MeC. Total number of SNP = 38,253. The table should be read per line.

Table 5. SNP significantly ($P < 0.001$) associated with more than one trait,[1] chromosome, and base pair position

SNP	Chromosome	Position (bp)	Traits associated
ARS-BFGL-NGS-93180	1	138,832,098	RMETc, MeYp
ARS-BFGL-NGS-24888	4	3,583,133	MeC, MeP
Hapmap59221-rs29014908	4	35,939,871	MeIc, MeIp
Hapmap44201-BTA-114510	4	36,842,170	MeIc, MeIp
Hapmap51046-BTA-75812	6	61,984,747	MeC, MeP
Hapmap52436-rs29009653	6	99,732,094	MeIc, MeIp
ARS-BFGL-NGS-47330	11	44,562,022	MeC, MeP
ARS-BFGL-NGS-12929	11	64,313,748	MeC, MeP
Hapmap26463-BTA-159947	11	92,086,008	MeC, MeP
Hapmap49571-BTA-32781	13	47,583,553	MeC, MeP
BTB-00525367	13	47,915,618	MeC, MeP
ARS-BFGL-NGS-70206	13	48,622,655	MeC, MeP
BTA-115847-no-rs	13	48,826,815	MeC, MeP
BTA-37116-no-rs	15	57,228,610	MeC, MeP
ARS-BFGL-NGS-32691	18	34,159,637	MeC, MeP
ARS-BFGL-NGS-54767	18	7,605,307	MeIc, MeIp
UA-IFASA-7562	19	49,438,164	MeC, MeP
ARS-BFGL-NGS-103202	24	61,455,723	MeYc, MeYp
Hapmap33073-BTA-162864	26	21,180,893	MeC, MeP
ARS-BFGL-NGS-2180	26	24,477,962	MeC, MeP
ARS-BFGL-NGS-1092	26	24,531,763	MeC, MeP
ARS-BFGL-NGS-18194	26	24,575,207	MeC, MeP
ARS-BFGL-NGS-81009	26	26,491,674	MeC, MeP
Hapmap38478-BTA-20824	26	28,723,721	MeC, MeP
Hapmap40449-BTA-61103	26	31,213,256	MeC, MeP
Hapmap19519-rs29022379	27	19,017,466	MeIp, MeYc
ARS-BFGL-NGS-60192	28	25,609,489	MeC, MeP
ARS-BFGL-NGS-24205	29	25,325,889	MeC, MeP

[1]MeP = methane production; MeC = methane concentration; MeIc = methane intensity based on MeC; RMETc = residual methane based on MeC; MeIp = methane intensity based on MeP; RMETp = residual methane based on MeP.

the use of a residual methane trait that is adjusted for ECM, BW, and DMI. However, an underlying problem would be to have ECM or ECM and BW "in" and "out" of these traits (MeIp, RMETp), and how this could affect the genetic variances and therefore their heritabilities. In this study, we explored through GWAS, and genomic correlations, the differences and similarities of MeC and MeP, and the other trait definitions (MeYc, MeIc, RMETc) using MeC instead MeP. Although our results showed clear similarities between MeC and MeP given the genetic correlation (0.91 ± 0.07) and the number of significant SNP and segments in common between these 2 traits, we cannot conclude based only on the results that those 2 traits are interchangeable. For that, we would need further investigation, taking into account the genetic correlations with other traits such as ECM, BW, and DMI and if possible to study further the correlated responses to selection of these and other traits when including MeP or MeC in the breeding goal. Moreover, the other pairs of ratio and residual traits using either MeP or MeC consistently showed weaker genetic correlations and practically no similarities in their GWAS results, having fewer SNP and segments in common between them.

Further Research Needs

Although this is the largest sample size for a GWAS on CH_4 traits in dairy cattle (near to 2,000 animals) compared with previous GWAS studies for CH_4 traits, this sample size is still considered small compared with GWAS conducted for milk production and other economic traits in Holstein cattle (Jiang et al., 2019; Liu et al., 2020). More CH_4 data are necessary to achieve higher power in the GWAS study to detect QTL associated with CH_4 traits and be able to calculate the percentage of genetic variance associated with it. According to Gebreyesus et al. (2019), QTL explaining 5% of genetic variance can be detected with a power of 0.97 with records from 3,000 cows. Ideally, those animals should also have milk production, weight, and feed intake data, not only to calculate some of the composite CH_4 traits (as MeY, MeI, and RMET) but also to estimate the genetic correlations between those traits. Though phenotyping of CH_4 and DMI are challenging, they are critical to have reliable phenotypes and accurate estimates of parameters that will help us to select for lower CH_4 emitting animals in the future. Multitrait GWAS approaches involving CH_4, production, and feed

Table 6. Quantitative trait loci reported for *Bos taurus* associated with significant ($P < 0.001$) segments for MeC as primary trait[1]

Traits	Chromosome	Starting (Mbp)	Ending (Mbp)	QTL ID	QTL associated with		
					Production	Feed efficiency	Maintenance
MeC	3	102	103	25,800	FY, PY, MY	RFI	
MeC, MeYp	4	59	60	26,166	PY		
MeC	5	84	85	20,529	FA, FP, FY, PP, PY, NM, PL		ADG, WWT, BD, RUMWD, STA, STR
MeC, MeP	6	61	62	42,034	FY, PY, FP, PP, NM, MY, PL		BW, W365, WWT, STR
MeC	8	90	91	14,659	PP, FY, PY, NM, MY, PL	RFI, DMI, FCR	BWG
MeC, MeP	11	44	45	45,389	FP, FY, PP, PY, NM, LACTPER		W365, BWG
MeC	11	63	64	45,488	PP, NM, PL, FP, AMY, LACTYD		W365, BWG, BD, RUMWD, STA, STR
MeC, MeP	11	64	65	34,920	FP		BWG
MeC	11	66	67	64,596	FP, FY, PY		W365, BWG, BD, RUMWD, STA, STR
MeC	12	85	860	23,828	MY, LMY	RFI	W365
MeC	12	86	870	37,104	LMY		
MeC, MeIc	13	46	47	26,522	FP, PP, NM, MY		BD, RUMWD, STA, STR
MeC, MeP	13	47	48	34,379	FP, PP		BWG
MeC, RMETp	13	48	49	26,335	PY, FP, FY, NM, MY, PP		BWG, BD, STA, STR
MeC	13	76	77	47,433	NM, PL		BW, RUMWD, STA
MeC, MeP	15	57	58	36,484	MY	DMI	
MeC, MeP	18	34	35	35,370	FY		
MeC	19	47	48	15,515	FA	ADFI	ADG, DYF, RUMWD, STA
MeC, MeP	19	49	50	19,040	PY, FA		
MeC	20	1	2	69,074	FA		BW
MeC	20	20	21	26,859	PP		
MeC, RMETc	26	18	19	52,715	FY, PY, NM, PL, FA		DYF, STR, BD, RUMWD, STA,
MeC, MeP	26	21	22	20,244	FY, FP, MY, FA, PP, PY, NM, PL		STR
MeC, MeP, MeYc	26	24	25	34,161	FA		
MeC, MeP, MeYc	26	26	27	26,104	FY, FA		WWT, BWG
MeC, MeP	26	28	29	29,971	FA		BW
MeC, MeYc	26	29	30	26,721	FP, FY, PP, PY, NM, MY, PL, FA		DYF
MeC, MeYc, MeIp	26	30	31	34,852	FA, FY		BWG, W365
MeC, MeP, MeYc	26	31	32	26,945	PP, FA		
MeC, MeP	28	25	26	26,112	FY		W365
MeC, MeP	29	25	26	56,562		DMI	BWG

[1]MeC = methane concentration; MeP = methane production; MeIc = methane intensity calculated using MeC; MeIp = methane intensity calculated using MeP; RMETc = residual methane on metabolic BW and ECM using MeC; RMETp = residual methane on metabolic BW and ECM using MeP; MeYc = methane yield calculated using MeC; MeYp = methane yield using MeC; FY = fat yield; PY = protein yield; MY = milk yield; AMY = average milk yield; LMY = lifetime milk yield; NM = net merit; FA = fatty acids; FP = fat percentage; PP = protein percentage; LACTPER = lactose percentage; LACTYD = lactose yield; PL = length of productive life; RFI = residual feed intake; ADFI = average daily feed intake; FCR = feed conversion ratio; W365 = weight at 365 d; BWG = body weight gain; WWT = weaning weight; CWT = carcass weight; BD = body depth; STA = stature; STR = structure; RUMWD = rump width; DYF = dairy form.

efficiency traits could also be beneficial when investigating the similarities on genetic architecture of the CH_4 traits and its correlations with other important traits. We demonstrate unequivocally that the genetic architecture shared by CH_4 traits is limited and the trait definition has profound implications for genomic regions detected. Other avenues of future research include Bayesian analyses, which offer more flexibility on the assumed distribution of SNP effects and will be of value as a basis for comparison with the current study. Selecting one CH_4 trait for the breeding goal should be accompanied with the proper foundational research about the implications (consequences) of selecting for that trait, such as the correlated response to other economically important traits.

CONCLUSIONS

Using the sniffer method to measure CH_4 has multiple benefits such as the ability to measure many animals with the same equipment during a short period when used in association with AMS at a reduced cost compared with other methods. However, the output trait of the sniffers is CH_4 concentration (MeC). To calculate MeP, ECM and BW need to be included in the equation, which creates collinearity with milk production and weight that could affect the correlated response of these traits when selecting for lower CH_4 emitting animals. The results of this paper show that MeC and MeP are genetically more similar than any other pair of traits analyzed, based on their genetic correlation and number of significant SNP and associated genome segments in common. Furthermore, MeC seems to have associations with QTL regions previously reported for milk production, maintenance, and feed efficiency traits.

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REFERENCES

Boichard, D., M. Boussaha, A. Capitan, D. Rocha, C. Hoze, M.P. Sanchez, T. Tribout, R. Letaief, P. Croiseau, C. Grohs, W. Li, C. Harland, C. Charlier, M. S. Lund, G. Sahana, M. Georges, S. Barbier, W. Coppieters, S. Fritz, and B. Guldbrandtsen. 2018. Experience from large scale use of the EuroGenomics custom SNP chip in cattle. Page 675 in Proceedings of the World Congress on Genetics Applied to Livestock Production, Vol. Molecular Genetics 4. Accessed Oct. 29, 2021. http://www.wcgalp.org/system/files/proceedings/2018/experience-large-scale-use-eurogenomics-custom-snp-chip-cattle.pdf.

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 World Congress on Genetics Applied to Livestock Production, Volume Challenges - Environmental, 134, 2018. WCGALP New Zealand. Accessed Oct. 29, 2021. http://www.wcgalp.org/proceedings/2018/genetic-relationships-between-methane-emission-and-milk-yield-live-weight-and-dry.

Calderon-Chagoya, R., J. H. Hernandez-Medrano, F. J. Ruiz-Lopez, A. Garcia-Ruiz, V. E. Vega-Murillo, M. Montano-Bermudez, M. E. Arechavaleta-Velasco, E. Gonzalez-Padilla, E. I. Mejia-Melchor, N. Saunders, J. A. Bonilla-Cardenas, P. C. Garnsworthy, and S. I. Roman-Ponce. 2019. Genome-wide association studies for methane production in dairy cattle. Genes (Basel) 10:995. https://doi.org/10.3390/genes10120995.

de Haas, Y., M. Pszczola, H. Soyeurt, E. Wall, and J. Lassen. 2017. Invited review: Phenotypes to genetically reduce greenhouse gas emissions in dairying. J. Dairy Sci. 100:855–870. https://doi.org/10.3168/jds.2016-11246.

Difford, G. F., J. Lassen, and P. Løvendahl. 2016. Interchangeability between methane measurements in dairy cows assessed by comparing precision and agreement of two non-invasive infrared methods. Comput. Electron. Agric. 124:220–226. https://doi.org/10.1016/j.compag.2016.04.010.

Difford, G. F., P. Løvendahl, R. F. Veerkamp, H. Bovenhuis, 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.2019-16966.

Donoghue, K. A., T. Bird-Gardiner, P. F. Arthur, R. M. Herd, and R. F. Hegarty. 2016. Genetic and phenotypic variance and covariance components for methane emission and postweaning traits in Angus cattle. J. Anim. Sci. 94:1438–1445. https://doi.org/10.2527/jas.2015-0065.

FAO, IFAD, UNICEF, WFP, and WHO. 2018. The state of food security and nutrition in the world 2018. Building climate resilience for food security and nutrition. FAO.

Garnsworthy, P. C., J. Craigon, 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. Kuhla, 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. Animals (Basel) 9:837.

Gebreyesus, G., A. J. Buitenhuis, N. A. Poulsen, M. H. P. W. Visker, Q. Zhang, H. J. F. van Valenberg, D. Sun, and H. Bovenhuis. 2019. Combining multi-population datasets for joint genome-wide association and meta-analyses: The case of bovine milk fat composition traits. J. Dairy Sci. 102:11124–11141. https://doi.org/10.3168/jds.2019-16676.

Hayes, B. J., K. A. Donoghue, C. M. Reich, B. A. Mason, T. Bird-Gardiner, R. M. Herd, and P. F. Arthur. 2016. Genomic heritabilities and genomic estimated breeding values for methane traits in Angus cattle. J. Anim. Sci. 94:902–908. https://doi.org/10.2527/jas.2015-0078.

Hu, Z. L., C. A. Park, X. Wu, and J. M. Reecy. 2013. Animal QTLdb: An improved database tool for livestock animal QTL/association data dissemination in the post-genome era. Nucleic Acids Res. 41:D871–D879. https://doi.org/10.1093/nar/gks1150.

Jiang, J., L. Ma, D. Prakapenka, P. M. VanRaden, J. B. Cole, and Y. Da. 2019. A large-scale genome-wide association study in U.S. Holstein cattle. Front. Genet. 10:412. https://doi.org/10.3389/fgene.2019.00412.

Khansefid, M., J. E. Pryce, S. Bolormaa, S. P. Miller, Z. Wang, C. Li, and M. E. Goddard. 2014. Estimation of genomic breeding values

for residual feed intake in a multibreed cattle population. J. Anim. Sci. 92:3270–3283. https://doi.org/10.2527/jas.2014-7375.
Lassen, J., and G. F. Difford. 2020. Genetic and genomic selection as a methane mitigation strategy in dairy cattle. Animal 14:s473–s483. https://doi.org/10.1017/S1751731120001561.
Lassen, J., and P. Løvendahl. 2016. Heritability estimates for enteric methane emissions from Holstein cattle measured using noninvasive methods. J. Dairy Sci. 99:1959–1967. https://doi.org/10.3168/jds.2015-10012.
Lassen, J., P. Løvendahl, and J. Madsen. 2012. Accuracy of noninvasive breath methane measurements using Fourier transform infrared methods on individual cows. J. Dairy Sci. 95:890–898. https://doi.org/10.3168/jds.2011-4544.
Li, B., B. Berglund, W. F. Fikse, J. Lassen, M. H. Lidauer, P. Mäntysaari, and P. Løvendahl. 2017. Neglect of lactation stage leads to naïve assessment of residual feed intake in dairy cattle. J. Dairy Sci. 100:9076–9084. https://doi.org/10.3168/jds.2017-12775.
Liu, L., J. Zhou, C. J. Chen, J. Zhang, W. Wen, J. Tian, Z. Zhang, and Y. Gu. 2020. GWAS-based identification of new loci for milk yield, fat, and protein in Holstein cattle. Animals (Basel) 10:2048. https://doi.org/10.3390/ani10112048.
Løvendahl, P., G. F. Difford, B. Li, M. G. G. Chagunda, P. Huhtanen, M. H. Lidauer, J. Lassen, and P. Lund. 2018. Selecting for improved feed efficiency and reduced methane emissions in dairy cattle. Animal 12(s2):s336–s349. https://doi.org/10.1017/S1751731118002276.
Madsen, J., B. S. Bjerg, T. Hvelplund, M. R. Weisbjerg, and P. Lund. 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.
Madsen, P., and J. Jensen. 2014. A user's guide to DMU, version 6, release 5.0. Cent. Quant. Genet. Genomics Dept. Mol. Biol. Genet. Univ. Aarhus Res Cent., Tjele, Denmark.
Manzanilla-Pech, C. I. V., Y. de Haas, B. J. Hayes, R. F. Veerkamp, M. Khansefid, K. A. Donoghue, P. F. Arthur, and J. E. Pryce. 2016. Genome-side association study of methane emissions in Angus beef cattle with validation in dairy cattle. J. Anim. Sci. 94:4151–4166. https://doi.org/10.2527/jas.2016-0431.
Manzanilla-Pech, C. I. V., D. Gordo, G. F. Difford, P. Løvendahl, 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.
Mühlbach, S., D. Sorg, F. Rosner, J. Kecman, and H. H. Swalve. 2018. Genetic analyses for CH_4 concentrations in the breath of dairy cows measured on-farm with the Laser Methane Detector. Proceedings of the 11th World Congress on Genetics Applied to Livestock Production. WCGALP New Zealand. Accessed Oct. 29, 2021. http://www.wcgalp.org/system/files/proceedings/2018/genetic-analyses-ch4-concentrations-breath-dairy-cows-measured-farm-laser-methane-detector.pdf.
Pszczola, M., K. Rzewuska, S. Mucha, and T. Strabel. 2017. Heritability of methane emissions from dairy cows over a lactation measured on commercial farms. J. Anim. Sci. 95:4813–4819. https://doi.org/10.2527/jas2017.1842.
Pszczola, M., T. Strabel, S. Mucha, and E. Sell-Kubiak. 2018. Genome-wide association identifies methane production level relation to genetic control of digestive tract development in dairy cows. Sci. Rep. 8:15164. https://doi.org/10.1038/s41598-018-33327-9.
Purcell, S., B. Neale, K. Todd-Brown, L. Thomas, M. A. R. Ferreira, D. Bender, J. Maller, P. Sklar, P. I. W. De Bakker, M. J. Daly, and P. C. Sham. 2007. PLINK: A tool set for whole-genome association and population-based linkage analyses. Am. J. Hum. Genet. 81:559–575. https://doi.org/10.1086/519795.
Richardson, C. M., T. T. T. Nguyen, M. Abdelsayed, P. J. Moate, S. R. O. Williams, T. C. S. Chud, F. S. Schenkel, M. E. Goddard, I. van den Berg, B. G. Cocks, L. C. Marett, W. J. Wales, and J. E. Pryce. 2021. Genetic parameters for methane emissions traits in Australian dairy cows. J. Dairy Sci. 104:539–549. https://doi.org/10.3168/jds.2020-18565.
Sargolzaei, M., J. P. Chesnais, and F. S. Schenkel. 2014. A new approach for efficient genotype imputation using information from relatives. BMC Genomics 15:478. https://doi.org/10.1186/1471-2164-15-478.
Sjaunja, L. O., L. Baevre, L. Junkkarinene, J. Pedersen, and J. Setala. 1990. A Nordic proposal for an energy corrected milk formula. 27th Session of the International Commission for Breeding and Productivity of Milk Animals. Paris, France.
Tempelman, R. J., D. Spurlock, M. P. Coffey, R. F. Veerkamp, L. Armentano, K. Weigel, Y. de Haas, C. Staples, E. E. Connor, Y. Lu, and M. VandeHaar. 2015. Heterogeneity in genetic and non-genetic variation and energy sink relationships for residual feed intake across research stations and countries. J. Dairy Sci. 98:2013–2026. https://doi.org/10.3168/jds.2014.8510.
van Engelen, S. 2018. The genetic background of methane emission by dairy cows. PhD thesis. Animal Breeding and Genomics, Wageningen University, Wageningen, the Netherlands. https://doi.org/10.18174/430631.
van Engelen, S., H. Bovenhuis, J. Dijkstra, J. A. van Arendonk, and M. H. Visker. 2015. Short communication: Genetic study of methane production predicted from milk fat composition in dairy cows. J. Dairy Sci. 98:8223–8226. https://doi.org/10.3168/jds.2014-8989.
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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