Weighted single-step genome-wide association studies for methane intensity in Chinese Holstein cattle

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Abstract

Reducing methane emissions from dairy cows has been a key area of research in recent decades. This study aimed to identify genomic regions associated with methane intensity (MeI) in Chinese Holstein cattle. MeI phenotype was either predicted by mid-infrared spectra (MIRS, R2cv= 0.66) or directly measured by sniffer. Data were collected from eight commercial farms in Beijing between 2017–2020 and 2024. A weighted single-step genome-wide association study (WssGWAS) was performed based on 1,120 genotypes, 4,995 phenotypic records, and pedigree of 10 911 individuals.

The mean MeI was 7.67 ± 1.52 (g/kg milk yield). The estimated heritability of MeI was 0.15±0.04, and the repeatability was 0.42±0.02. Eleven 10-SNP windows harboring 19 protein encoding genes explained 2.17% of the genomic variance, with genomic regions on BTA1, 5, 8, 15, 19, 20, 24, 26, and 27. Five of the windows were also associated with milk production or milk component traits, while one window contained the QTL linked to metabolic body weight. The region explaining the highest proportion of variance (0.34%) was located on BTA15, which included five protein encoding genes. Among them, *SCN4B* and *MPZL3* are proposed as candidate genes.

In total, the preliminary results show that MeI is a heritable, repeatable, and polygenic trait in Chinese Holstein population. The identified MeI-related genomic regions provide an insight for breeding dairy cows with lower methane emissions.

Key words: dairy cattle, genetic parameter, WssGWAS, methane intensity

Introduction

Methane emissions from ruminants are a significant contributor to greenhouse gas emissions in agriculture. In China, approximately 24% of total methane emissions come from the production of livestock (Wang et al., 2024). In the past 30 years, the contribution of dairy cattle has notably increased, rising from 1.9% to 7% of the total emissions (Wang et al., 2024). Reducing methane emissions from cows is an issue that requires worldwide attention.

As we all know, animal breeding is a helpful method to reach this goal. To apply breeding techniques, large-scale recording of individual enteric methane emissions is essential (de Haas et al., 2017). However, methane emission is difficult to measure, and only few methods can costly generate large amount of data, such as sniffer and milk mid-infrared spectra (MIRS). With sniffer, individual cows can be recorded on a wide scale and at a reasonable cost (Garnsworthy et al., 2019). Using sniffers placed in the feed bin of automatic milking systems (AMS), this method measures the concentrations of gases. The present study also employed MIRS to predict the methane intensity of dairy cows. It is simple, high-throughput, and shows a great deal of potential for predicting methane emissions from dairy animals. The ability of MIRS to predict methane emissions has been widely reported (Coppa et al., 2022, Dehareng et al., 2012).

Among the various methane emission traits, the definition if methane intensity (MeI) is methane output relative to output such as milk production (de Haas et al., 2017). Specifically, MeI measures the amount of methane (CH_4) emitted per kilogram of output product, such as milk (g/kg), and is strongly influenced by both the milk production levels and the energy required for this process.

The main objective of this study is to 1) measure sniffer-based methane intensity and predict methane intensity based on MIRS in Chinese Holstein population; 2) estimate genetic parameters for methane intensity and, 3) identify candidate genomic regions for methane intensity.

Materials and Methods

Data and Sampling

Animals

Data were collected from July 2024 to November 2024 at two commercial farms in Beijing. A total of 208 cows were recorded during experiment.

Breath Sampling

All cows had access to an AMS (DeLaval International AB, Tumba, Sweden) for milking. Each barn was equipped with two AMS, but only one of them was installed with a sniffer. Cows were free to enter either AMS, with or without the sniffer (Guardian NG/Gascard, Edinburgh Instruments Ltd, Livingston, UK).

Data segments with no record of a cow entering the AMS within 5 minutes before or after were classified as ambient values. The ambient values recorded on a given day were averaged and used as the daily ambient mean.

While cows were inside the AMS, their heads could approach the gas collector positioned in the feed bin, as shown in Figure 1. Records of cows spending less than 2 minutes inside the AMS were excluded from the analysis. The raw data were preprocessed in four steps: (1) matching data from the AMS and sniffer to match a sniffer measurement with an identification number; (2) removing the first minute of each record; (3) using the 'findpeaks' function in R v4.3.2 to identify belching peaks. At least one peak must be found (exceed the mean ambient CH_4 concentration for the day by 200 ppm); (4) deleting consecutive when CO_2 concentration dropped below the lower 25%

Figure 1. Gas collector in the feed bin

quartile of the mean CO_2 concentration for more than 10 seconds, indicating that the cow's head had left.

After processing, ambient-corrected gas concentrations for CH_4 and CO_2 were obtained by subtracting the ambient mean from the measured concentrations. The mean values of the gas concentrations and their ratio were calculated for each measurement. Subsequently, a three-step data quality control process was employed: (1) daily averages for the ambient-corrected gas concentrations were calculated after collecting all records for a single day. A twofold standard deviation quality control was used to eliminate records with excessively high or low gas contents; (2) records for measurement days with fewer than 10 cows were removed to avoid potential machine errors; (3) records with concentration ratios greater than the mean ± standard deviation of the concentration ratios for the same cow were removed.

Milk Yield, Body Weight, and Feed

For milk yield, the 3-day average was used as the daily milk yield (DMY). Milk composition data, including milk fat percentage, lactose percentage, and protein percentage, were collected from DHI. The closest DHI record to the methane measurement date (within 15 days) was selected for subsequent calculation.

Records with milk fat >7% or <2%, milk protein >5% or <2%, and daily milk yield <5 kg or >100 kg were excluded. Additionally, records with days in milk (DIM) <15 or >300 were removed. Energy-corrected milk was calculated using the formula from Sjaunja et al. (1990).

Body weight was expressed as weekly averages after a two-step quality control process: (1) cows whose body weight exceeded the upper or lower limits were removed (first parity: 450–750 kg; 2+ parities: 500–900 kg); (2) measurements within a single parity that differed by >50 kg from the mean were removed. After this, weekly averages of body weight were calculated. Since first-parity cows have greater weight variability, their weekly body weight average only represented the current week's weight. In contrast, body weight data from cows of later parities can represent the averages of the current, previous, and next week's body weight.

Feed data was provided by farm. Descriptive statistics of individual information, daily milk yield, body weight and diet crude fat for dairy cows is shown in Table 1.

Table 1: Descriptive statistics of individual information, daily milk yield, body weight and diet crude fat in Chinese Holstein cattle.

Trait	mean	SD	min	max
parity	2.46	1.33	1	7
days in milk	132.81	77.81	15	299
daily milk yield (kg)	42.85	9.03	17.14	66.48
body weight (kg)	690.53	87.81	481	888
diet crude fat (%DM)	5.58	0.42	4.91	6.42

Methane Intensity

Following the 'Model 2' developed by Kjeldsen et al. (2024), CO_2 production (CO_2P) was calculated. Subsequently, the methane and CO_2 concentrations from each measurement were averaged. Since the gases originated from the same breath, their concentrations were multiplied by their molecular weights before calculating the ratio to obtain the mass ratio (CH_4:CO_2). Methane intensity (MeI) was calculated as:

$$MeI = \frac{CH_4{:}\,CO_2 \times CO_2P}{DMY}$$

Given the variability in methane emissions at different times of day, a single measurement cannot accurately reflect an animal's true methane emission level. Therefore, weekly averages were used as the methane emission traits in this study. Weekly averages were calculated by retaining records from weeks with more than 4 measurements. Finally, 758 weekly averages were retained for subsequent analyses.

MIRS Prediction

Most of the milk spectral data were collected by the farm for DHI testing. In addition to the DHI sample collections, we also collected milk samples between two DHI samplings. All milk samples were analyzed using the same spectrometer (Banteley), which generates a spectrum of 899 wavelength transmittance values in the mid-infrared (MIR) region. The following spectral regions were retained for analysis, including 968.1–1 577.5 cm^{-1}, 1 731.8–1 762.6 cm^{-1}, 1 781.9–1 808.9 cm^{-1}, and 2 831.0–2 966.0 cm^{-1} followed Grelet et al. (2021), leaving a total of 215 wavenumbers. The spectra were preprocessed using Savitzky-Golay second-order derivatives, with spectral quality control conducted using pcout (Filzmoser et al., 2008). In addition to the MIRS data, individual information (parity, DIM, and DMY) were also included in the dataset for prediction. The data were processed to match a total of 227 records from 120 cows, which formed the training set (Dataset A).

Prediction Equation Development

Partial least squares regression (PLSR) was used to develop the prediction equation. Under 10-fold cross-validation, the model achieved an R^2 (coefficient of determination) of 0.66 and a Root mean square error of prediction (RMSE) of 1.25.

Then the prediction formula was employed in the dataset B (21 772 records with MIRS and individual information) to obtain phenotypes for a larger population. Dataset A was contained by dataset B. To ensure the usability of the prediction equations, the Mahalanobis distance (Mahalanobis, 1936) was calculated for MIRS in dataset B. Only data with Mahalanobis distance within that of dataset A were retained. Predictive equations for methane emission traits were built based on the training set and applied to dataset B for quality control of predicted methane emission phenotypes. When the records within the same individual parity was less than 3, all values for that parity were deleted. Subsequently, the coefficient of variation (CV) was calculated for each cow in single parity. Records with a CV greater than 25% were removed, leaving a total of 4 995 records from 1 187 cows.

Pedigree and Genotype

The pedigree of the cows with phenotypic records were traced back as many generations as possible. The final pedigree included 10 911 cows.

A total of 1,120 cows were genotyped using the Illumina 150K Bovine Bead Chip (Illumina Inc.). Genomic quality control was performed using PLINK v1.90 software (Purcell et al., 2007). Single nucleotide polymorphisms (SNPs) with minor allele frequencies lower than 0.1 or those with extreme deviations from Hardy–Weinberg equilibrium (P-value < 10^{-6}) were excluded. After quality control, a total of 109 619 SNPs were used in the study.

WssGWAS

The (co)variance components were estimated using AI-REML and EM-REML procedure implemented in the AIREMLF90 package from BLUPF90 (Misztal et al., 2014).

The variance components and genetic parameters was estimated based on the model:

$$y = X_1\beta + X_2\phi + Z\alpha + Wpe + e$$

y was the vectors of methane intensity. β was the vector of fixed effects for colostrum quality traits, including farm-season-year of calving (45 levels), parity (3 levels); ϕ was the regression coefficient of days in milk. α was the vector of random additive genetic effects, following $\alpha \sim N(0, H\sigma_a^2)$; pe was the permanent environment effect following $pe \sim N(0, I\sigma_{pe}^2)$; e was the vectors of random residual effects following $e \sim N(0, I\sigma_e^2)$; X_1, X_2, Z, and W, were the corresponding incidence matrices; H was the matrix of additive genetic relationships constructed from the pedigree and genotype; σ_a^2 was the additive genetic variance; I was an identity matrix, σ_{pe}^2 was the permanent environment variance, and σ_e^2 was the residual variance. The inverse of the H matrix (H^{-1}) was calculated as follows:

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

where A^{-1} is the inverse of the pedigree-based relationship matrix; A_{22}^{-1} is the A^{-1} for the genotyped animals; and $G^{-1}$is the inverse of the genomic relationship matrix. The G matrix was calculated according to(VanRaden, 2008):

$$G = \frac{ZDZ'}{2\sum_{i=1}^{M} P_i(1-P_i)}$$

where Z is the matrix of genotypes adjusted for allele frequencies (0, 1, or 2 for aa, Aa, and AA, respectively); D is a diagonal matrix of weights for SNP variances (initially $D = I$); M is the number of SNPs, and P_i is the minor allele frequency of the i^{th} SNP.

The estimates of SNP effects and weights for the WssGWAS analyses (four iterations) for colostrum quality traits were obtained according to (Wang et al., 2014). The weight for each SNP was calculated as: $d_i = 1.125^{\frac{|\hat{a}_i|}{sd(\hat{a}_i)}-2}$ (VanRaden, 2008), where i is the i^{th} SNP. The percentage of the total addictive genetic variance explained by the i^{th} region was calculated as:

$$\frac{Var(a_i)}{\sigma_a^2} \times 100\% = \frac{Var\left(\sum_{j=1}^{10} Z_j \hat{u}_j\right)}{\sigma_a^2} \times 100\%$$

where a_i is genetic value of the i^{th} region that consists of contiguous 10 SNPs, σ_a^2 is the total additive genetic variance, Z_j is a vector of gene content of the j^{th} SNP for all individuals, and $\hat{u}_j$ is the marker effect of the jth SNP within the i^{th} region.

Non-overlapping contiguous genomic windows that explained 0.15% or more of the total additive genetic variance were considered to be associated with the trait. Candidate genes were identified by examining genomic windows based on the ARS-UCD1.2. The biological functions of these genes, Gene Ontology (GO) terms (Ashburner et al., 2000) enrichment were identified using the R package "BiomaRt" (Durinck et al., 2009) and "clusterProfiler" (Wu et al., 2021). The genomic regions were compared to cattle QTL database (Hu et al., 2022).

Results & Discussion

As presented in Table 2, the average MeI was 7.22 ± 1.99 g/kg in the current population. The predicted methane intensity closely followed the observed values, with a predicted MeI of 7.67 ± 1.52 g/kg.

Table 2: Descriptive statistics of methane intensity (MeI) and predicted methane intensity (PMeI) in Chinese Holstein cattle.

Trait	mean	SD	min	max
MeI (g/kg)	7.22	1.99	3.11	15.04
PMeI (g/kg)	7.67	1.52	3.14	13.62

Different MeI values have been recorded in earlier studies. In a mixed cow herd, MeI ranged from 3.0 to 36.0 g/kg, with an average of 13.5 ± 3.92 g/kg reported by Niu et al. (2018). Similarly, in a population of French Holstein cattle, Fresco et al. (2023) reported a MeI of 11.7 ± 2.6 g/kg. In this study, MeI was lower than those found in these studies, but it was closer to 8.61 ± 1.15 g/kg in dairy cattle reported by Lassen and Løvendahl's (2016).

PMeI showed moderate heritability according to our research. The results indicate that the heritability estimate for PMeI was 0.15±0.04 and the repeatability was 0.42±0.02. In previous study, MeI or PMeI heritability ranged from 0.04 to 0.35. In a population of 1 091 Swiss Brown cows, Bittante and Cecchinato et al. (2020) observed a heritability of 0.12 ± 0.06, which is similar to our study. While Fresco et al. (2024) reported a heritability of 0.35 ± 0.04 using a very large dataset (n = 167 514), Lassen and Løvendahl (2016) estimated a heritability of 0.21 ± 0.06 using a population of 3 121 cows. Higher heritability values than those obtained in our study were found in both of these studies. However, our result was lower such as the heritability of 0.04 ± 0.03 estimated by Manzanilla-Pech et al. (2022) using of 1 962 Danish Holstein cows. The breed, gas measurement techniques and equipment, and raising conditions of dairy cows are some of the variables that affect the heritability estimate of MeI or PMeI in various populations. The heritability estimates in the current population are in the medium range when compared to the findings of other studies.

In this study, we identified eleven genomic regions on *Bos taurus autosome* (BTA) 1, 5, 8, 15, 19, 20, 24, 26, and 27 that explained more than 0.15% of the genetic variance as Figure 2. These regions, which harbor a total of 19 protein-coding genes, accounted for 2.17% of the genomic variance. The window that explained the highest genetic variance was located on BTA15, which explained 0.34% of additive genetic variance and contained five genes, including *JAML*, *SCN2B*, *TMPRSS4*, *SCN4B*, and *MPZL3*. Two of these genes were enriched by the significant GO terms, which were *SCN2B* and *MPZL3*.

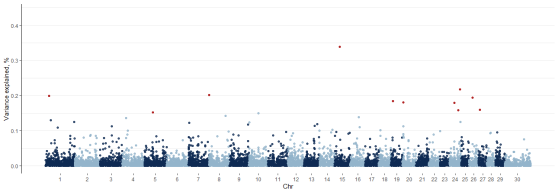

Figure 2. Proportion of the total additive genetic variance of 10-SNP genomic windows based on the weighted single-step genome association study for predicted methane intensity. Red points represent the windows exceed the 0.15% threshold of the total additive genetic variance.

Table 3: Quantitative trait loci reported for *Bos taurus* associated with genomic regions that explained more than 0.15% of the additive genetic variance for predicted Methane intensity

Chr	Regions (Mb)	Explained genetic variance, %	Associated trait
1	20.03-20.29	0.20	MP
5	44.96-45.20	0.15	MF, MY
24	47.10-47.32	0.16	MF
24	56.77-56.93	0.22	MP, BW
26	19.74-20.23	0.19	MF, MP

MP: milk protein, MF: milk fat, MY: milk yield, BW: body weight

Additionally, we referred to the Cattle QTL database to examine potential QTL overlaps with genomic regions that explained more than 0.15% of the additive genetic variance. Table 3 shows five genomic regions containing QTLs associated with milk protein, milk fat, milk yield, and body weight. However, the relationships of MeI with these traits still needs to be further explored.

Conclusions

Methane intensity can be measured and predicted by milk mid-infrared spectra. It is a moderate heritable, polygenic trait in Chinese Holstein population. However, these are relatively preliminary findings, and further research is still necessary.

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