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Estimation of genetic parameters and single-step genome-wide association studies for eating time and rumination time in Holstein dairy cows

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ABSTRACT

The aims of this study were to estimate genetic parameters and to identify genomic regions associated with eating time (**EAT**) and rumination time (**RUT**) in Holstein dairy cows. Genetic correlations among EAT, RUT and milk yield traits were also estimated. The data were collected from 2019 to 2022 in 6 dairy herds located in the Walloon Region of Belgium. The data set consisted of daily EAT and RUT records on 284 Holstein cows, from which 41 cows had records only for the first parity, 101 cows had both first and second parities records, and 142 cows had records only for the second parity. The number of daily EAT and RUT records in the first-parity (**P1**) and second-parity (**P2**) cows were 18,569 (on 142 cows) and 34,464 (on 243 cows), respectively. Data on 28,994 single nucleotide polymorphisms (**SNP**) located on 29 Bos taurus autosomes (**BTA**) of 747 animals (435 males) were used. Random regression test-day models were used to estimate genetic parameters through the Bayesian Gibbs sampling method. The SNP solutions were estimated using a single-step genomic best linear unbiased prediction approach. The proportion of genetic variance explained by each 20-SNP sliding window (with an average size of 1.52 Mb) was calculated, and regions accounting for at least 1.0% of the total additive genetic variance were used to search for candidate genes. Mean (standard deviation (**SD**)) averaged daily EAT and RUT were 327.0 (85.66) and 559.4 (77.69) min/d for cows in P1 and 316.0 (82.24) and 574.2 (75.42) min/d for cows in P2, respectively. Means (standard deviation; SD) heritability (h2) estimates for daily EAT and RUT were 0.42 (0.09) and 0.45 (0.06) for cows in P1 and 0.45 (0.04) and 0.43 (0.02) for cows in P2, respectively. Mean (SD) daily genetic correlations between daily EAT and RUT were 0.27 (0.07) for P1 and 0.34 (0.08) for P2. Genome-wide association analyses identified 6 genomic regions distributed over 5 chromosomes (BTA1, BTA4, BTA11, BTA14 (2 regions), and BTA17) associated with EAT or RUT. The findings of this study increase our preliminary understanding of the genetic background of feeding behavior in dairy cows; however, larger data sets are needed to determine whether EAT and RUT might have the potential to be used in selection programs.
KEYWORDS: Rumination time, eating time, Holstein cows

INTRODUCTION

Milk yield per cow has more than doubled in the previous 40 years and many cows now produce more than 20,000 kg of milk per lactation. Increased milk yield is associated with changed in diets and increased energy requirements in dairy cows (Løvendahl and Munksgaard, 2016). The ability of an animal to increase daily energy intake (**EI**) depends on the net energy density of the diet and daily feed intake (**FI**) (Harvatine and Allen, 2005). While using a reliable method to measure FI or residual feed intake (**RFI;** an increasingly used trait to analyze feed efficiency in livestock) is too expensive for commercial farms, available sensor technologies provide information about the feeding behavior of the animals which are associated with RFI and FI (Byskov et al., 2015; De Mol et al., 2016; Byskov et al., 2017). Feeding behavior can be analyzed using traits such as eating time (**EAT**), meal frequency, duration of each meal, intake per meal, and rumination time (**RUT**) (Nkrumah et al., 2007; Kelly et al., 2010; Cavani et al., 2022). Several studies reported that variation in feeding behavior explained part of the variation observed in R(FI) in dairy cattle (Green et al., 2013; Lin et al., 2013; Cavani et al., 2022). De Mol et al. (2016) reported that the correlation between EAT and FI ranged from 0.53 to 0.56 in lactating dairy cows. Byskov et al. (2017) reported that RUT is genetically correlated with RFI and dry matter intake (**DMI**) in dairy cows. Byskov et al. (2015) reported that the daily intake of forage neutral detergent fiber (**NDF**) and starch are positively related

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to RUT, whereas intake of sugar is negatively related to RUT. Furthermore, rumination activity may effect CH_4 emissions (a major contributor to the global greenhouse gas emissions and also a loss of feed energy during production) through affecting feed particle size, microbial fermentation and rumen fluid pH (Zetouni et al., 2018; Mikuła et al., 2021). Moreover, FI, EAT and RUT can be used to monitor the health and reproduction of dairy cattle (DeVries et al., 2009; Calamari et al., 2014; Pahl et al., 2015). Therefore, feeding behavior like EAT and RUT can be considered useful indicators for R(FI) and CH_4 production in dairy cows (Schirmann et al., 2009; Byskov et al., 2017; Negussie et al., 2017; Mikuła et al., 2021; Cavani et al., 2022). The advantage of EAT and RUT over R(FI) or CH_4 is that EAT and RUT can be automatically recorded on a large-scale by sensor-based systems (Bikker et al., 2014). However, the use of EAT and RUT in dairy cattle breeding program requires a comprehensive understanding of genetic backgrounds for these traits. It has been documented that factors such as stage of lactation, season, parity, health disorders, housing conditions and composition of the feed ration may influence EAT and RUT (Nielsen et al., 2000; Kaufman et al., 2016; Løvendahl and Munksgaard, 2016; Schirmann et al., 2016); however, more studies are needed to have reliable and complete information about the genetic aspects of EAT and RUT in dairy cows. Thus, due to the lack of feeding behavior genetic studies in lactating dairy cattle, the aims of this study were to estimate genetic parameters and to identify genomic regions associated with EAT and RUT in Holstein dairy cows.

MATERIALS AND METHODS

Phenotypic Data

Six dairy herds located in the Walloon Region of Belgium participated in this study. Individual daily eating time (**EAT**) and rumination time (**RUT**) data were collected using SenseHub dairy sensors (Allflex Livestock Intelligence^{TM}). The animals included were equipped with SenseHub dairy sensors for the entirety of the study period. The data were collected from 2019 to 2022 and were edited to include only cows with known birth date, calving date, and parity number. Age at the first calving (**AFC**) was calculated as the difference between birth date and first calving date and restricted to the range of 540 to 1200 d. Only records from the first 2 parities were kept for the analyses. Records from days in milk (**DIM**) greater than 305 d were eliminated. Daily EAT and RUT were edited to remove records outside the range of mean ± 3 standard deviations (**SD**). The final data set consisted of daily EAT and RUT records on 284 Holstein cows, from which 41 cows had records only for the first parity, 101 cows had both first and second parities records, and 142 cows had records only for the second parity. The 142 s parity cows without first parity records were kept in the data set due to the limited number of records available. The number of daily EAT and RUT records in the first-parity (**P1**) and second-parity (**P2**) cows were 18,569 (on 142 cows) and 34,464 (on 243 cows), respectively. The recordings on EAT and RUT were summarized as weekly averages for each animal before being subjected to further analyses. Pedigree depth of the animals were traced back to 25 generations to include all ancestors of the animals. Full pedigree records included 7,312 animals (2,018 males).

Genotypic Data

Genotype data were available for 747 animals (435 males and 312 females) in the pedigree data set used. EAT and RUT records were available for 243 of the 312 genotyped females. The animals were genotyped using the BovineSNP50 Beadchip v1 to v3 (Illumina, San Diego, CA, USA). Single nucleotide polymorphisms (**SNP**) in common among the 3 chips were kept. Non-mapped SNPs, SNPs located on sex chromosomes, and triallelic SNPs were excluded. A minimum GenCall Score of 0.15 and minimum GenTrain Score of 0.55 were used to keep SNP. Minor allele frequency (**MAF**) less than 5% were excluded. The difference between the observed and expected heterozygosity was estimated, and if the difference was greater than 0.15, the SNP was excluded (Wiggans et al., 2009). Finally, 28,994 SNP located on 29 Bos taurus autosomes (**BTA**) remained for the genomic analyses.

Variance Component Estimation

The (co)variance components and breeding values for EAT and RUT were estimated based on the integration of the random regression test-day model (**RR-TDM**) into the single-step GBLUP procedure (**SS RR-TDM**) using the following 2-trait, 2-lactation (first 2 lactations) model (Paiva et al., 2022):

$$y_{ijklmno} = \mu + HTDp_i + HY_j + LS_k + \sum_{b=0}^{3} AS_l \Phi_b(t) + \sum_{b=0}^{3} pe_n \Phi_b(t) + \sum_{b=0}^{3} a_n \Phi_b(t) + e_{ijklmno},$$

where y_{ijklmn} is the weekly averaged daily records of EAT and RUT belonging to the DIM o of cow n in

parity m, belonging to ith class of HTDp, jth class of HY, kth stage of lactation, and lth class of AS. HTDp is the fixed effect of herd-testday-parity; HY is the fixed effect of herd-year of calving; LS is the fixed effect of lactation stage (11 classes were defined: DIM 1:15, 16: 35, and the rest of the lactation period was grouped into 9 30-d classes); AS is the fixed effect of age-season of calving defined as follows: age at calving class (3 and 2 classes of age at calving were defined for the first and second parity, respectively) × season of calving (4 seasons: winter from January to March, spring from April to June, summer from July to September and autumn from October to December); $\sum_{b=0}^{3} AS_j \Phi_b(t)$ is the fixed regression coefficients of the age-season of calving modeled using Legendre polynomials of order 3; $\sum_{b=0}^{3} pe_m \Phi_b(t)$ and $\sum_{b=0}^{3} a_m \Phi_b(t)$ are the random regression coefficients of permanent environmental, and additive effects modeled using Legendre polynomials of order 3, respectively; and e_{ijklmn} is the residual effect. The permanent environment, additive genetic and residual variances were assumed to be normally distributed with mean zero ($\mu = 0$) and variances as follows:

$$\text{Var}\begin{bmatrix} pe \\ a \\ e \end{bmatrix} = \begin{bmatrix} \mathbf{P} \otimes \mathbf{I} & 0 & 0 \\ 0 & \mathbf{Ga} \otimes \mathbf{H} & 0 \\ 0 & 0 & \mathbf{R} \end{bmatrix},$$

where $\mathbf{P}$ is the 16 × 16 covariance matrix of the permanent environmental regression coefficients; $\mathbf{Ga}$ is the 16 × 16 covariance matrix of the additive genetic regression coefficients, blocks within $\mathbf{R} = \sum^{+} \mathbf{r^p}$ contain residual variance ($\mathbf{r}$) that depends on parity ($\mathbf{p}$). Residual variance was assumed the same within each parity. The $\mathbf{H}$ is a matrix that combines pedigree and genomic relationships, where its inverse relies on the integration of additive and genomic relationship matrices, $\mathbf{A}$ and $\mathbf{G}$, 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 $\mathbf{A}$ is the numerator relationship matrix based on the pedigree for all animals; $\mathbf{A}_{22}$ is the numerator relationship matrix for genotyped animals; and $\mathbf{G}$ 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 $\mathbf{G^*}$ 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 $\mathbf{Z}$ is a matrix of gene content adjusted for allele frequencies (0, 1 or 2 for *aa*, *Aa* and *AA*, respectively); $\mathbf{D}$ is a diagonal matrix of weights for SNP variances ($\mathbf{D} = \mathbf{I}$); M is the number of SNPs, and p_i is the MAF of the ith SNP.

The (co)variance components were estimated by Bayesian inference using the GIBBS3F90 software (Aguilar et al., 2018). Gibbs sampling was used to obtain marginal posterior distributions for the various parameters using a single chain of 500,000 iterates with a sampling interval of 20 samples. The first 100,000 iterations of the chain were regarded as a burn-in period to allow sampling from the proper marginal distributions. Genetic (co)variances on each test-day were calculated using the equation described by Jamrozik and Schaeffer (1997). Daily heritability was defined as the ratio of genetic variance to the sum of the additive genetic, permanent environmental, and residual variances at a given DIM.

The vector of genomic estimated breeding values (**GEBV**) of the EAT and RUT for each animal i, which included daily GEBV from all DIM (1 to 305) 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, $\mathbf{GEBV_i} = \mathbf{T}\hat{\mathbf{g}}_\mathbf{i}$, where $\hat{\mathbf{g}}_\mathbf{i}$ is the vector of additive genetic predicted regression coefficients for animal i and $\mathbf{T}$ is a matrix of orthogonal covariates associated with the Legendre orthogonal polynomial functions.

Genome-Wide Association Study

Genome-wide association studies (**GWAS**) were performed for EAT and RUT in the first and second parities considering following 3 lactation stages: 1) from 1 to 60 DIM, representing the ascending production stage and lactation peak; 2) from 61 to 200 DIM, representing the middle lactation stage; and 3) from 201 to 365 DIM, representing the production decline up to the end of the lactation (Oliveira et al., 2019). Therefore, the GEBV for each lactation stage of each animal i were obtained by averaging the daily GEBV solutions of the specific DIM; that is,

$$\hat{GEBV1}_i = \left(\hat{GEBV}_{i1} + \hat{GEBV}_{i2} + \ldots + \hat{GEBV}_{i60}\right) / 60,$$

$$\hat{GEBV2}_i = \left(\hat{GEBV}_{i61} + \hat{GEBV}_{i62} + \ldots + \hat{GEBV}_{i200}\right) / 140,$$

and

$$\hat{GEBV3}_i = \left(\hat{GEBV}_{i201} + \hat{GEBV}_{i202} + \ldots + \hat{GEBV}_{i305}\right) / 105,$$

where $\hat{GEBV1}_i$, $\hat{GEBV2}_i$, and $\hat{GEBV3}_i$ are the GEBV for the first, second, and third lactation stages of animal i obtained by averaging the GEBV from 1 to 60, 61 to 200, and 201 to 305 DIM, respectively. Furthermore, the GEBV of animal i through the entire lactation were obtained by averaging the daily GEBV solutions of all DIM; that is,

$$\hat{GEBVe}_i = \left(\hat{GEBV}_{i1} + \hat{GEBV}_{i2} + \ldots + \hat{GEBV}_{i305}\right) / 305,$$

where $\hat{GEBVe}_i$ is the GEBV of animal i through the entire lactation, obtained by averaging the GEBV from 1 to 305.

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 relationship matrix ($\mathbf{G}$) was built with no weight. The SNP effects were obtained using the following equation:

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

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 following:

$$\frac{\mathrm{Var}\left(a_i\right)}{\sigma_a^2} \times 100\% = \frac{\mathrm{Var}\left(\sum_{j=1}^{20} Z_j \hat{u}_j\right)}{\sigma_a^2} \times 100,$$

where a_i is the genetic value of the ith region that consists of 20 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 20-SNP moving windows, with an average size of ~1.52 Mb, was calculated across the whole genome, and those 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 the genetic information such as the extent of linkage disequilibrium (**LD**) in neighboring SNPs (Habier et al., 2011).

Identification of positional candidate genes for the studied traits

Genes located inside the identified genomic regions (i.e., between the start and end of genomic coordinates of the identified regions) were defined as the positional candidate genes for EAT and RUT. We identified genes using the National Center for Biotechnology Information (NCBI) Map Viewer tool for the UMD3.1 assembly as the reference map.

RESULTS

The descriptive statistics of the analyzed traits are presented in Table 1. Daily milk yield (**MY**) averaged 25.6 kg (4.21% fat and 3.41% protein) and 32.4 kg (4.22% fat and 3.38% protein) for cows in P1 and P2, respectively. Mean (SD) weekly averaged daily EAT and RUT were 327.0 (85.7) and 559.4 (77.7) min/d for cows in P1 and 316.0 (82.24) and 574.2 (75.4) min/d for cows in P2, respectively. The coefficient of variations (CV%) of weekly averaged daily EAT and RUT was 26.2% and 13.9% in the first parity and 26.0% and 13.1% in the second parity, respectively.

The lactation curves for weekly averaged daily EAT and RUT are presented in Figure 1. The daily EAT was low at the day of calving, increased rapidly during the first month after calving, and reached the peak of 358 min/d in P1 and 325 min/d in P2 at 35 and 28 weeks in milk, respectively. The daily RUT was low at the day of calving, increased rapidly until 6 and 5 weeks after calving, when the peak of 591 min/d in P1 and 588 min/d in P2 was reached, respectively (Figure 1). Fac-

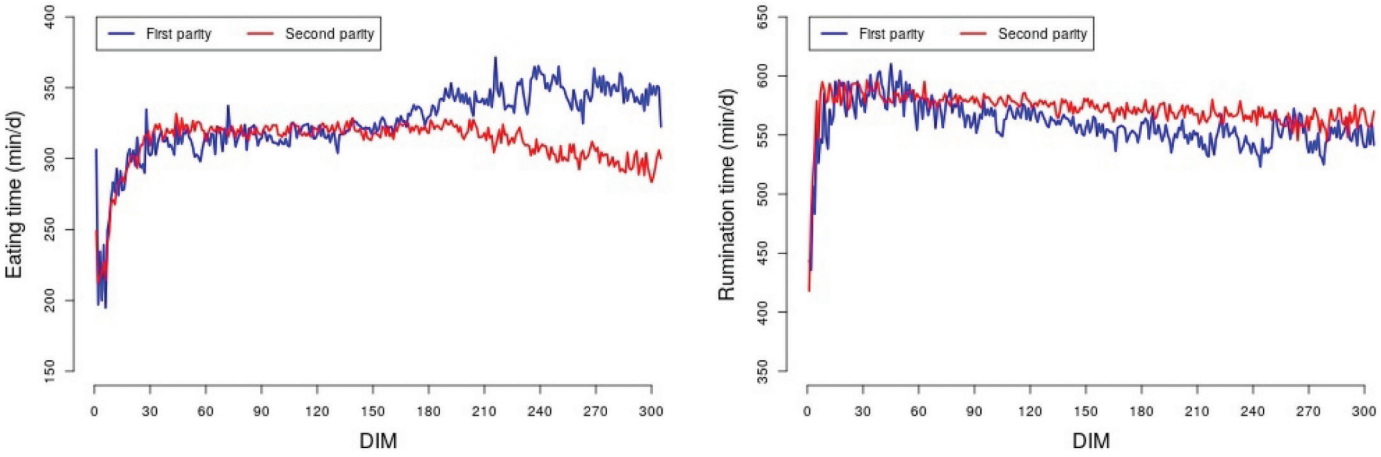

Figure 1. Lactation curves for weekly averaged daily eating time (EAT, min/d) and rumination time (RUT, min/d) for the first (blue) and second (red) parity in Walloon Holstein cows.

tors including herd, parity, sampling season, sampling year, and the weeks in milk showed significant effect on EAT and RUT ($P < 0.05$). Least squares means EAT was highest in sampling season of summer (357 min/d, SE = 1.0) followed by spring (338 min/d, SE = 1.0) and was lowest in winter (310 min/d, SE = 1.1) followed by autumn (320 min/d, SE = 1.0). Least squares means RUT was highest in sampling season of winter (576 min/d, SE = 1.1) followed by autumn (567 min/d, SE = 1.0) and was lowest in summer (551 min/d, SE = 1.0) followed by spring (558 min/d, SE = 1.0).

Heritability estimates and genetic correlations among the studied traits are presented in Table 2. Means (SD) daily heritability (h^2) estimates for weekly averaged daily EAT and RUT were 0.42 (0.09) and 0.45 (0.06) for cows in P1 and 0.45 (0.04) and 0.43 (0.02) for cows in P2, respectively. Mean genetic correlation estimates between weekly averaged EAT and RUT were 0.27 (ranged from 0.01 to 0.55) for P1 and 0.34 (ranged from 0.17 to 0.54) for P2. The mean genetic correlations between weekly averaged daily EAT and MY, fat percentage (**FP**), and protein percentage (**PP**) ranged from 0.15 to 0.17, 0.05 to 0.09, and 0.05 to 0.08, respectively. The corresponding values estimated for weekly averaged daily RUT ranged from 0.22 to 0.27 (MY), 0.12 to 0.17 (FP), and 0.11 to 0.16 (PP).

General information (start and end SNP numbers, window size, start and end genomic positions, and the variance explained by each windows) about the results of single-step GWAS (**ssGWAS**) for EAT and RUT are presented in Data S1-S16 [2 traits (EUT and RUT) × 2 parities × 4 stages per parity] (https://github.com/hadiatashi/eating-rumination-time-Holstein-cows). The Manhattan plots of the proportion of total additive genetic variance explained by 20-SNP windows for EAT and RUT are shown in Figures 2 and 3, respectively. The genomic regions associated with EAT and RUT along with corresponding genes are presented in Table 3. In total, 6 regions distributed over 5 chromosomes (BTA1, BTA4, BTA11, BTA14 (2 regions), and BTA17) were identified that are associated with EAT or RUT. The following are the

Table 1. Descriptive statistics for milk yield, fat percentage, protein percentage, weekly averaged daily eating time, and rumination time in Walloon Holstein cows[1]

	First lactation			Second lactation		
Traits[2]	Mean	SD	CV (%)	Mean	SD	CV (%)
MY (kg)	25.6	5.3	20.8	32.4	7.5	23.1
FP (%)	4.21	0.63	15.1	4.22	0.69	16.5
PP (%)	3.41	0.29	8.45	3.38	0.34	10.2
EAT (min/d)	327.0	85.7	26.2	316.0	82.2	26.0
RUT (min/d)	559.4	77.7	13.90	574.2	75.4	13.1

[1]The number of test-day records for EAT and RUT in first and second parity cows were 18,569 (on 142 cows) and 34,464 (on 243 cows), respectively.

[2]MY = milk yield (kg/d), FP = fat percentage (%), PP = protein percentage (%), EAT = eating time (min/d); RUT = rumination time (min/d).

results discussed by chromosome. The genomic regions are expressed based on the UMD3.1 assembly.

BTA1 The genomic region located from 103.0 to 105.2 Mb on BTA1 was associated with EAT. This region was 2.16 Mb in size and explained 1.37% of the total additive genetic variance for EAT during early lactation for P2. This region also explained 0.84% of the total additive genetic variance of RUT in the late lactation for P2.

BTA4 Genomic region located from 56.8 to 58.12 Mb on BTA4 explained 1.02% of the total additive genetic variance of RUT in late-lactation for P2. This region was 1.24 Mb in size and harbors genes including inner mitochondrial membrane peptidase subunit 2 (*IMMP2L*) and leucine rich repeat neuronal 3 (*LRRN3*).

BTA11 The genomic region located from 15.64 to 17.026 Mb on BTA11 was associated with RUT in the second lactation. This region was 1.38 Mb in size and explained 1.19% and 0.76% of the total additive genetic variance of RUT for the early and mid-lactation in P2, respectively. This region harbors genes including latent transforming growth factor β binding protein 1 (*LTBP1*), RAS guanyl releasing protein 3 (*RASGRP3*), and family with sequence similarity 98 member A (*FAM98A*).

BTA14 Two genomic regions located from 7.61 to 8.48 Mb and 26.3 to 27.4 Mb on BTA14 were associated with EAT and RUT. Hereafter, these regions are identified as BTA14-I, and BTA14-II.

The BTA14-I was 0.87 Mb in size and explained 1.09% of the total additive genetic variance of EAT during mid lactation for P1. This region harbors zinc finger and AT-hook domain containing (*ZFAT*) and microRNA mir-30d (*MIR30D*).

The BTA14-II explained 1.10% and 0.75% of the total additive genetic variance for EAT during mid-lactation for P1 and P2, respectively. This region explained 1.12% of the total additive genetic variance of RUT during mid lactation for P1. This region was 1.10 Mb in size and also explained 2.27% and 1.36% of the total additive genetic variance of RUT during mid and entire lactation for P2. Genes including UBX domain protein 2B (*UBXN2B*), thymocyte selection-associated high mobility group box (*TOX*), syndecan binding protein (*SDCBP*), cytochrome P450 family 7 subfamily A member 1 (*CYP7A1*), and neutral sphingomyelinase activation associated factor (*NSMAF*) were identified inside this region.

BTA17 The genomic region located from 57.8 to 59.0 Mb on BTA17 explained 1.37% and 0.64% of the total additive genetic variance of EAT during mid lactation and entire lactation for P1, respectively. In addition, more than 0.60% of the total additive genetic variance of EAT during mid lactation for P1 was explained by

Table 2. Mean (SD) daily heritability (diagonal) and mean (SD) genetic correlations (upper triangle) for milk yield, fat percentage, protein percentage, weekly averaged daily eating time, and rumination time estimated on a daily basis across the lactation in Walloon Holstein cows[1]

	First lactation					Second lactation				
Traits[2]	MY	FP	PP	EAT	RUT	MY	FP	PP	EAT	RUT
MY	0.39 (0.10)	−0.57 (0.12)	−0.55 (0.04)	0.17 (0.11)	0.27 (0.06)	0.42 (0.09)	−0.47 (0.07)	−0.44 (0.06)	0.15 (0.10)	0.22 (0.07)
FP		0.47 (0.10)	68 (0.12)	0.09 (0.12)	0.12 (0.09)		0.50 (0.09)	0.71 (0.08)	0.05 (0.11)	0.17 (0.08)
PP			0.45 (0.06)	0.08 (0.14)	0.11 (0.07)			0.52 (0.07)	0.05 (0.12)	0.16 (0.06)
EAT				0.42 (0.09)	0.27 (0.07)				0.45 (0.04)	0.34 (0.08)
RUT					0.45 (0.06)					0.43 (0.02)

[1]The number of test-day records for EAT and RUT in first and second parity cows were 18,569 (on 142 cows) and 34,464 (on 243 cows), respectively.
[2]MY = milk yield (kg/d), FP = fat percentage (%), PP = protein percentage (%), EAT = eating time (min/d); RUT = rumination time (min/d).

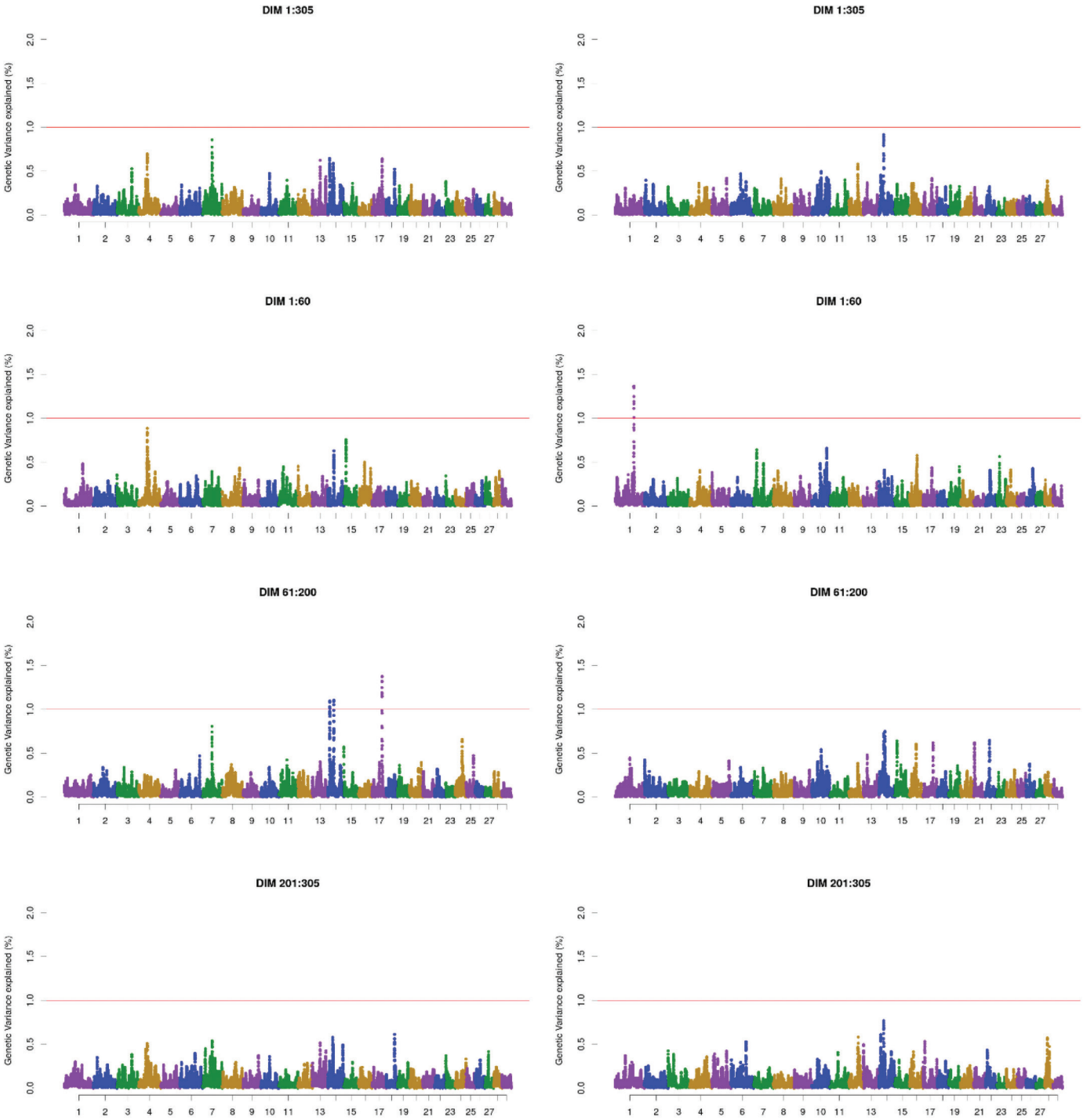

Figure 2. Additive genetic variance explained by windows of 20 adjacent SNPs across chromosomes for weekly averaged daily eating time (min/d) in different stages of lactation in the first (left) and second parity (right) Walloon Holstein cows.

this region. Genes including citron rho-interacting serine/threonine kinase and coiled-coil domain containing 60 (*CCDC60*) were inside this region.

DISCUSSION

The mean weekly averaged daily EAT ranged from 316 to 327 min. This result was in agreement with published mean daily EAT of 330 min (Senn et al., 1995)

and was in the range of 240 to 420 min reported by Beauchemin (1991). Dado and Allen (1994) reported that Holstein cows spent 301 min/d eating. However, some studies reported shorter daily EAT of 208 to 218 min/d (Meir et al., 2018), 185 to 214 min/d (Kononoff et al., 2003) and 248 to 264 min/d (Braun et al., 2015) in Holstein cows. Moreover, some studies reported longer daily EAT of 462 min/d (Schleisner et al., 1999)

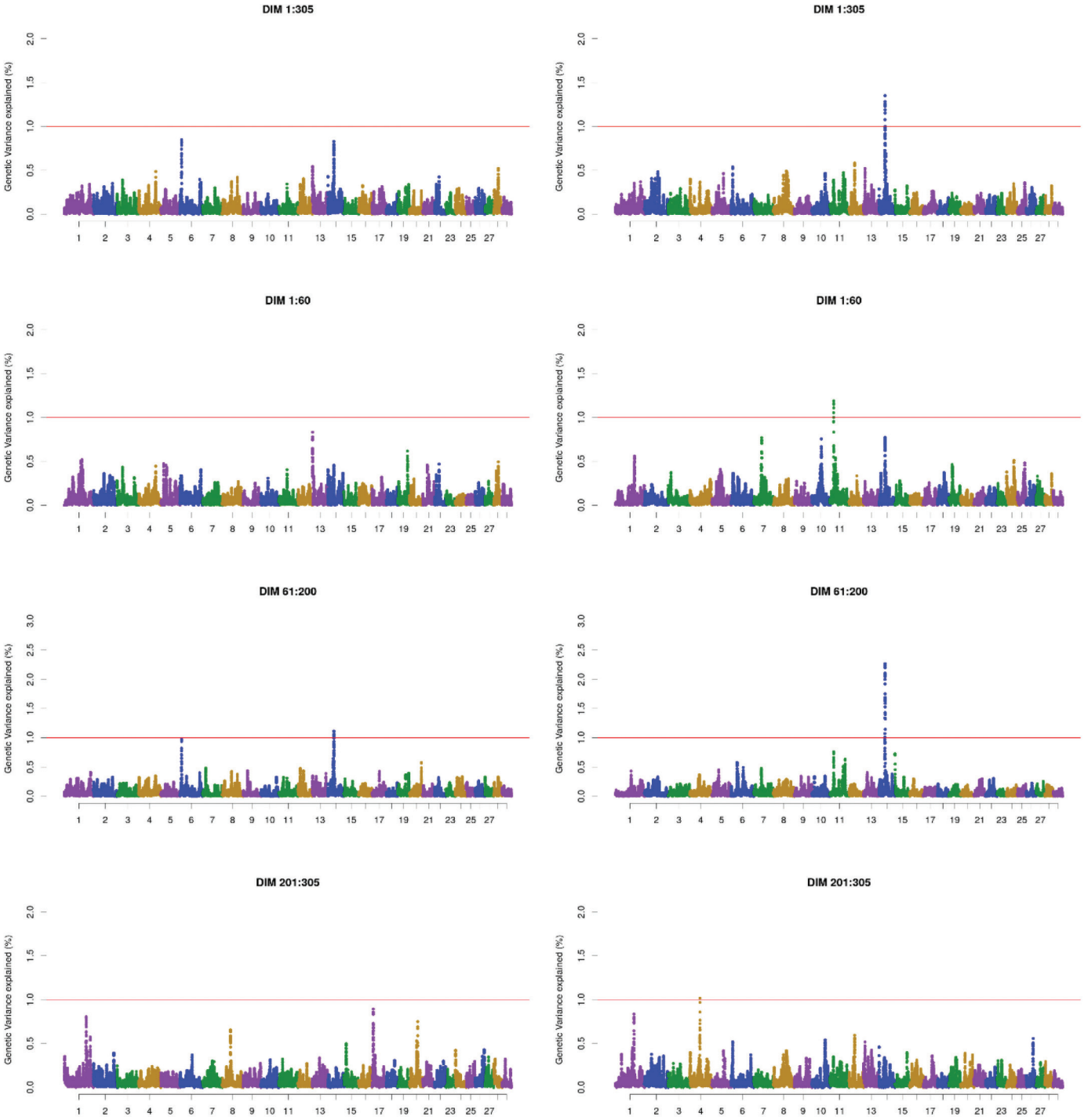

Figure 3. Additive genetic variance explained by windows of 20 adjacent SNPs across chromosomes for weekly averaged daily rumination time (min/d) in different stages of lactation in the first (left) and second parity (right) Walloon Holstein cows.

and 375 to 497 min/d (Braun et al., 2013). The mean weekly averaged daily RUT ranged from 559.4 to 574.2 min which is in agreement with published daily RUT of 464 to 579 min/d (Metz, 1975) and 498 to 584 min/d (Maekawa et al., 2002). Dado and Allen (1994) reported that Holstein cows spent 457 min/d ruminating. Soriani et al. (2012) reported that mean RUT during the early lactation (DIM 15 to 40) was 504 min/d in primiparous and 562 min/d in multiparous Holstein cows. However, mean RUT found in our study are higher than that reported by some previous studies. For instance, Byskov et al. (2017) reported that the mean RUT for Holstein cows is 413 min/d and varied from 413 to 448 min/d in different parts of lactation. Meir et al. (2018) reported values from 427 to 459 min/d for mean RUT. Lopes et al. (2022) reported values of 446 min/d for overall RUT in Canadian Holstein. López-Paredes et al. (2020) reported that mean of RUT is 473 min/d and Zetouni et al. (2018) reported that mean RUT varied from 415 to 443 min/d in Holstein cows.

Factors such as age, parity, lactation stage, milk yield, type of ration and feeding management affect FI and may explain the variation found for daily EAT and RUT in the literature. Daily EAT and RUT were low at calving and increased rapidly in the first month after calving, but showed little change in the rest of the lactation. Considerable divergence was found between EAT lines for first and second parity in late lactation, presumably due to higher lactation persistency and slower feeding rate in first parity animals. This study showed that first parity cows ate longer and had shorter RUT than second parity cows. The published studies in recent years provides new insights on eating and ruminating activity of dairy cows (Beauchemin, 2018). EAT is strongly influenced by eating rate and there are issues of social competition for eating, whereas RUT is largely a function of ration compositions; thus, RUT is considered as a physiological trait, whereas EAT has both physiological and behavioral origins. Braun et al. (2013) reported that the duration of eating and rumination are longer in older cows. Dado and Allen (1994) reported that older cows eat faster than younger cows (shorter EAT per kg DMI). Soriani et al. (2012) reported that daily means of RUT in primiparous cows are shorter than that in multiparous cow. The difference between the duration of rumination in primiparous and multiparous cows can be, at least in part, attributed to the differences in DMI as RUT adjusted for DMI of primiparous cows is usually less than or similar to that of multiparous cows (Dado and Allen, 1994). Kowsar et al. (2008) reported that means EAT and RUT per day and per kg DMI in primiparous are longer than those in multiparous cows. The lowest values for RUT and EAT were found on the day of calving which is in line with Soriani et al. (2012). The current study showed that the RUT in summer and spring is less than that in winter and autumn. Tapki and Şahin (2006) and Moallem et al. (2010) showed that RUT is reduced during heat stress in dairy cows. FI depends on EAT and intake per unit time. Mujibi et al. (2010) reported significant association between season and eating speed in beef cattle and showed that higher air temperature and higher solar radiation are associated with lower feeding rate; therefore, although mean EAT in summer and spring was higher than that in winter and autumn, kg FI may be less per minute eating. The CV for EAT was more than that for RUT. Dado and Allen (1994) reported that CV for EAT is about 17% and the reported CV for RUT ranged from 16% (Dado and Allen, 1994) to 48% (Byskov et al., 2015).

The mean daily h^2 estimates for EAT and RUT ranged from 0.42 to 0.45 (EAT) and from 0.43 to 0.45 (RUT),

Table 3. Genomic regions associated with weekly averaged daily eating time (EAT) and rumination time (RUT) in different stages of lactation in Walloon Holstein cows[1]

Chromosome	Position (bp)[2]	Gene[3]	Trait[4] (parity, stage of lactation, % variance explained)
BTA1	103,034,522 - 105,191,465	*SI*	EAT (2, 1, 1.37), RUT (2, 3, 0.84)
BTA4	56,875,012 - 58,115,832	*IMMP2L, LRRN3, IMMP2L*	RUT (2, 3, 1.01), EAT (1, 1, 0.51),
BTA11	15,644,634 –17,026,673	*LTBP1, RASGRP3, FAM98A*	RUT (2, 1, 1.19), RUT (2, 2, 0.76)
BTA14	7,612,098 - 8,479,608	*ZFAT, MIR30D, MIR30B*	EAT (1, 2, 1.09), EAT (2, 3, 0.61), EAT (2, e, 0.45)
BTA14	26,264,142 - 27,360,366	*NSMAF, CYP7A1, SDCBP, TOX, UBXN2B*	EAT (1, 2, 1.10); EAT (1, 1, 0.63); EAT (2, 2, 0.75); RUT (1, 2, 1.12); RUT (2, 2, 2.27); RUT (2, e, 1.36); RUT (1, 1, 0.46); RUT (1, e, 0.83); RUT (2, 1, 0.78)
BTA17	57,800,291 - 59,043,406	*TMEM233, PRKAB1, HSPB8, CCDC60, SRRM4, CIT*	EAT (1, 2, 1.37), EAT (1, e, 0.64), EAT (2, 2, 0.62)

[1].The GWAS analyses were performed for EAT and RUT considering 3 stages of lactation in each parity: (1) from 5 to 60 DIM, representing the ascending production stage and lactation peak; (2) from 61 to 200 DIM, representing the lactation persistency stage; (3) from 201 to 365 DIM, representing the production decline up to the end of the lactation, and (e) from 5 to 365 DIM representing the entire lactation.

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

[3].Genes inside the genomic region. Official gene symbol.

[4].EAT = eating time (min/d); RUT = rumination time (min/d).

indicating that a significant proportion of phenotypic variance of these traits are explained by additive genetic effects. Lopes et al. (2022) reported that h^2 of RUT in Canadian Holstein cows is 0.41 and Byskov et al. (2017) reported that h^2 of RUT ranged from 0.14 to 0.33 in Holstein cows. Low to moderate genetic correlations ranged from 0.27 to 0.34 were found between EAT and RUT indicating that cows that spend more time eating tend to ruminate longer. Beauchemin (2018) reported a direct relationship of 0.27 between EAT and RUT for dairy cows but Dado and Allen (1994) reported an inverse relationship between EAT and RUT. Moderate positive genetic correlations were found between EAT and RUT with MY which is in agreement with previous studies (Stone et al., 2017; Kaufman et al., 2018). Although the relationship between RUT and milk production has been investigated in few studies information is lacking on the relationship between EAT and milk yield and composition. Part of the variation found in the published studies may be due to the slightly different criteria used among studies to define RUT and in particular EAT, but RUT and EAT are also highly affected by feed management, physical and chemical composition of the diet, and inherent variability among animals. Soriani et al. (2013) reported a direct relationship between RUT and MY in Holstein cows. Lopes et al. (2022) reported that genetic correlation between RUT and MY in Canadian Holstein is 0.51. EAT and RUT were weakly correlated with FP and PP. López-Paredes et al. (2020) reported that RUT had positive correlations with MY and protein yield (**PY**) and negative correlations with fat yield (**FY**), PP, and FP. The mean genetic correlation found between FP and RUT was stronger than that found between FP and EAT. Milk fat is strongly influenced by changes in rumen fermentation; therefore, the high association between RUT and FP can be, at least in part, explained by the effect of rumination on rumen fermentation by breaking up large feed particles, increasing saliva and changing rumen pH (Bauman and Griinari, 2001; Zetouni et al., 2018; Mikuła et al., 2021).

Typically, GWAS methods are based on testing the significance of SNP effects on the traits of interest. However, SNPs within a genomic region can be highly correlated and jointly influence the phenotype. Furthermore, the genetic information in neighboring SNPs, such as the extent of LD, is not used in the GWAS because it depends on single SNP (Bao and Wang, 2017). Therefore, window-based GWAS procedure has been proposed as an effective method to estimate the combined effect of several consecutive SNPs in a specific region and to identify genomic regions that explain a given amount of genetic variance (Aguilar et al., 2019). The common form for declaring significance is to use a threshold on the additive genetic variance explained by individual window (Aguilar et al., 2019). However, it is unclear what window size is optimal, and no standard presently exists to define the threshold on explained genetic variance. Therefore, determining the proper window size is usually subjective and researchers often do not justify their choices or sometimes acknowledge that their choices are arbitrary. Fragomeni et al. (2014) examined different SNP window sizes and recommended windows of 20 adjacent SNP as a reasonable size. In this study, a window-based GWAS through the single-step genomic best linear unbiased predictor (**ssGBLUP**) was used. The results were presented by the proportion of total genetic variance explained by window of 20 adjacent SNP with an average size of ~1.52 Mb and windows explaining at least 1.0% of the total additive genetic variance were used to search for candidate genes. We used 1 SNP as the moving step of the window, which ensured that we do not miss genomic regions potentially associated with the traits due to the combination of SNPs. The results of the ssGWAS identified 6 genomic regions distributed over 5 chromosomes (BTA1, BTA4, BTA11, BTA14 (2 regions), and BTA17) associated with EAT and RUT. A genomic region located from 103.0 to 105.2 Mb on BTA1 was associated with EAT. This region has previously been reported to be associated with FI-related traits including FI, DMI, and RFI (Rolf et al., 2012; Seabury et al., 2017; Li et al., 2019). This region was also associated with milk yield traits and average daily gain (**ADG**) (Viitala et al., 2003; Meredith et al., 2012). Sucrase-isomaltase (*SI*) is the only functional gene identified inside this region. SI encodes a sucrase-isomaltase enzyme which is essential for the digestion of dietary carbohydrates.

The genomic region located from 56.8 to 58.12 Mb on BTA4 was associated with RUT. Previous studies reported that this region is associated with FP and PY in dairy cows (Lindersson et al., 1998; Schrooten et al., 2004). The genomic region located from 15.64 to 17.026 Mb on BTA11 was associated with RUT. This region has previously been reported to be associated with FI-related traits such as DMI, RFI and feed conversion ratio (**FCR**) (Marquez et al., 2009; Sherman et al., 2009). In addition, it has been reported that this region is associated with milk yield traits and milk fatty acid (**FA**) profile (Cole et al., 2011; Iung et al., 2019). *RASGRP3* gene, located inside this region, was reported to be associated with FP and PP in Holstein cows (Cole et al., 2011). The genomic region located from 7.61 to 8.48 Mb on BTA14 was associated with EAT. This region was reported to be associated with milk yield traits and milk FA profile in Holstein cows (Gebreyesus et al., 2019; Pedrosa et al., 2021). Buitenhuis et al. (2014)

reported that *ZFAT*, identified inside this region, is associated with FP in Holstein cows. The genomic region located from 26.3 to 27.4 Mb on BTA14 was associated with EAT and RUT and has been reported to be associated with milk yield traits (Boichard et al., 2003; Lund et al., 2008; Pedrosa et al., 2021). Among genes located inside this region, *CCDC60* was associated with PY in Holstein (Wang et al., 2022). The genomic region located from 57.8 to 59.0 Mb on BTA17 was associated with EAT and has been reported to be associated with milk yield traits, milk FA profile, and RFI in Holstein dairy cows (Bouwman et al., 2012; Seabury et al., 2017).

CONCLUSION

This study aimed to estimate genetic parameters and to identify genomic regions associated with EAT and RUT in Holstein cows. The results showed that EAT and RUT are moderately heritable and moderately correlated with MY. However, we strongly encourage future work to investigate genetic backgrounds of EAT and RUT using a bigger data set on dairy cattle to determine whether these traits might have the potential to be used in selection programs. Although several genomic regions were identified to be linked with EAT and RUT, combined explained lees than 2.5% of the total additive variances of the traits. This indicates that EAT and RUT are highly polygenic, in which many regions across the genome contribute to their genetic variations. Therefore, implementing marker based selection for these traits seems to be impractical at this stage.

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REFERENCES

Aguilar, I., A. Legarra, F. Cardoso, Y. Masuda, D. Lourenco, and I. Misztal. 2019. Frequentist p-values for large-scale-single step genome-wide association, with an application to birth weight in American Angus cattle. Genet. Sel. Evol. 51:1–8. https://doi.org/10.1186/s12711-019-0469-3.

Aguilar, I., I. Misztal, D. Johnson, A. Legarra, S. Tsuruta, and T. Lawlor. 2010. Hot topic: a unified approach to utilize phenotypic, full pedigree, and genomic information for genetic evaluation of Holstein final score. J. Dairy Sci. 93:743–752. https://doi.org/10.3168/jds.2009-2730.

Aguilar, I., I. Misztal, S. Tsuruta, A. Legarra, and H. Wang. 2014. PREGSF90–POSTGSF90: computational tools for the implementation of single-step genomic selection and genome-wide association with ungenotyped individuals in BLUPF90 programs. 10. World Congress on Genetics Applied to Livestock Production (WCGALP). American Society of Animal Science.

Aguilar, I., S. Tsuruta, Y. Masuda, D. Lourenco, A. Legarra, and I. Misztal. 2018. BLUPF90 suite of programs for animal breeding with focus on genomics. Proceedings of the world congress on genetics applied to livestock production, pp. 11–16.

Bao, M., and K. Wang. 2017. Genome-wide association studies using a penalized moving-window regression. Bioinformatics 33:3887–3894. https://doi.org/10.1093/bioinformatics/btx522.

Bauman, D., and J. M. Griinari. 2001. Regulation and nutritional manipulation of milk fat: low-fat milk syndrome. Livest. Prod. Sci. 70:15–29. https://doi.org/10.1016/S0301-6226(01)00195-6.

Beauchemin, K. 2018. Invited review: Current perspectives on eating and rumination activity in dairy cows. J. Dairy Sci. 101:4762–4784. https://doi.org/10.3168/jds.2017-13706.

Beauchemin, K. A. 1991. Ingestion and mastication of feed by dairy cattle. Vet. Clin. North Am. Food Anim. Pract. 7:439–463. https://doi.org/10.1016/S0749-0720(15)30794-5.

Bikker, J., H. Van Laar, P. Rump, J. Doorenbos, K. Van Meurs, G. Griffioen, and J. Dijkstra. 2014. Evaluation of an ear-attached movement sensor to record cow feeding behavior and activity. J. Dairy Sci. 97:2974–2979. https://doi.org/10.3168/jds.2013-7560.

Boichard, D., C. Grohs, F. Bourgeois, F. Cerqueira, R. Faugeras, A. Neau, R. Rupp, Y. Amigues, M. Y. Boscher, and H. Levéziel. 2003. Detection of genes influencing economic traits in three French dairy cattle breeds. Genet. Sel. Evol. 35:77–101. https://doi.org/10.1186/1297-9686-35-1-77.

Bouwman, A. C., M. H. Visker, J. A. van Arendonk, and H. Bovenhuis. 2012. Genomic regions associated with bovine milk fatty acids in both summer and winter milk samples. BMC Genet. 13:1–13. https://doi.org/10.1186/1471-2156-13-93.

Braun, U., L. Trösch, F. Nydegger, and M. Hässig. 2013. Evaluation of eating and rumination behaviour in cows using a noseband pressure sensor. BMC Vet. Res. 9:1–8. https://doi.org/10.1186/1746-6148-9-164.

Braun, U., S. Zürcher, and M. Hässig. 2015. Evaluation of eating and rumination behaviour in 300 cows of three different breeds using a noseband pressure sensor. BMC Vet. Res. 11:1–6. https://doi.org/10.1186/s12917-015-0549-8.

Buitenhuis, B., L. L. Janss, N. A. Poulsen, L. B. Larsen, M. K. Larsen, and P. Sørensen. 2014. Genome-wide association and biological pathway analysis for milk-fat composition in Danish Holstein and Danish Jersey cattle. BMC Genomics 15:1–11. https://doi.org/10.1186/1471-2164-15-1112.

Byskov, M., A. Fogh, and P. Løvendahl. 2017. Genetic parameters of rumination time and feed efficiency traits in primiparous Holstein cows under research and commercial conditions. J. Dairy Sci. 100:9635–9642. https://doi.org/10.3168/jds.2016-12511.

Byskov, M., E. Nadeau, B. Johansson, and P. Nørgaard. 2015. Variations in automatically recorded rumination time as explained by variations in intake of dietary fractions and milk production, and between-cow variation. J. Dairy Sci. 98:3926–3937. https://doi.org/10.3168/jds.2014-8012.

Calamari, L., N. Soriani, G. Panella, F. Petrera, A. Minuti, and E. Trevisi. 2014. Rumination time around calving: An early signal to detect cows at greater risk of disease. J. Dairy Sci. 97:3635–3647. https://doi.org/10.3168/jds.2013-7709.

Cavani, L., W. E. Brown, K. L. P. Gaddis, R. J. Tempelman, M. J. VandeHaar, H. M. White, F. Peñagaricano, and K. A. Weigel. 2022. Estimates of genetic parameters for feeding behavior traits and their associations with feed efficiency in Holstein cows. J. Dairy Sci. 105:7564–7574. https://doi.org/10.3168/jds.2022-22066.

Cole, J. B., G. R. Wiggans, L. Ma, T. S. Sonstegard, T. J. Lawlor Jr., B. A. Crooker, C. P. Van Tassell, J. Yang, S. Wang, L. K. Matukumalli, and Y. Da. 2011. Genome-wide association analysis of thirty one production, health, reproduction and body conformation traits in contemporary US Holstein cows. BMC Genomics 12:1–17. https://doi.org/10.1186/1471-2164-12-408.

Dado, R., and M. Allen. 1994. Variation in and relationships among feeding, chewing, and drinking variables for lactating dairy cows. J. Dairy Sci. 77:132–144. https://doi.org/10.3168/jds.S0022-0302(94)76936-8.

De Mol, R., R. Goselink, J. van Riel, H. Knijn, and A. Van Knegsel. 2016. The relation between eating time and feed intake of dairy cows. Precision Dairy Farming 2016:387–392.

DeVries, T., K. Beauchemin, F. Dohme, and K. Schwartzkopf-Genswein. 2009. Repeated ruminal acidosis challenges in lactating dairy cows at high and low risk for developing acidosis: Feeding, ruminating, and lying behavior. J. Dairy Sci. 92:5067–5078. https://doi.org/10.3168/jds.2009-2102.

Fragomeni, B. O., I. Misztal, D. L. Lourenco, I. Aguilar, R. Okimoto, and W. M. Muir. 2014. Changes in variance explained by top SNP windows over generations for three traits in broiler chicken. Front. Genet. 5:332. https://doi.org/10.3389/fgene.2014.00332.

Gebreyesus, G., A. Buitenhuis, N. Poulsen, M. Visker, Q. Zhang, H. van Valenberg, D. Sun, and H. Bovenhuis. 2019. Combining multipopulation 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.

Green, T., J. Jago, K. Macdonald, and G. Waghorn. 2013. Relationships between residual feed intake, average daily gain, and feeding behavior in growing dairy heifers. J. Dairy Sci. 96:3098–3107. https://doi.org/10.3168/jds.2012-6087.

Habier, D., R. L. Fernando, K. Kizilkaya, and D. J. Garrick. 2011. Extension of the Bayesian alphabet for genomic selection. BMC Bioinformatics 12:1–12. https://doi.org/10.1186/1471-2105-12-186.

Harvatine, K., and M. Allen. 2005. The effect of production level on feed intake, milk yield, and endocrine responses to two fatty acid supplements in lactating cows. J. Dairy Sci. 88:4018–4027. https://doi.org/10.3168/jds.S0022-0302(05)73088-5.

Iung, L., J. Petrini, J. Ramírez-Díaz, M. Salvian, G. Rovadoscki, F. Pilonetto, B. Dauria, P. Machado, L. Coutinho, G. Wiggans, and G. B. Mourão. 2019. Genome-wide association study for milk production traits in a Brazilian Holstein population. J. Dairy Sci. 102:5305–5314. https://doi.org/10.3168/jds.2018-14811.

Jamrozik, J., and L. Schaeffer. 1997. Estimates of genetic parameters for a test day model with random regressions for yield traits of first lactation Holsteins. J. Dairy Sci. 80:762–770. https://doi.org/10.3168/jds.S0022-0302(97)75996-4.

Kaufman, E., V. Asselstine, S. LeBlanc, T. Duffield, and T. DeVries. 2018. Association of rumination time and health status with milk yield and composition in early-lactation dairy cows. J. Dairy Sci. 101:462–471. https://doi.org/10.3168/jds.2017-12909.

Kaufman, E., S. LeBlanc, B. McBride, T. Duffield, and T. DeVries. 2016. Association of rumination time with subclinical ketosis in transition dairy cows. J. Dairy Sci. 99:5604–5618. https://doi.org/10.3168/jds.2015-10509.

Kelly, A., M. McGee, D. Crews Jr., A. Fahey, A. Wylie, and D. Kenny. 2010. Effect of divergence in residual feed intake on feeding behavior, blood metabolic variables, and body composition traits in growing beef heifers. J. Anim. Sci. 88:109–123. https://doi.org/10.2527/jas.2009-2196.

Kononoff, P., A. Heinrichs, and H. Lehman. 2003. The effect of corn silage particle size on eating behavior, chewing activities, and rumen fermentation in lactating dairy cows. J. Dairy Sci. 86:3343–3353. https://doi.org/10.3168/jds.S0022-0302(03)73937-X.

Kowsar, R., G. Ghorbani, M. Alikhani, M. Khorvash, and A. Nikkhah. 2008. Corn silage partially replacing short alfalfa hay to optimize forage use in total mixed rations for lactating cows. J. Dairy Sci. 91:4755–4764. https://doi.org/10.3168/jds.2008-1302.

Li, B., L. Fang, D. Null, J. Hutchison, E. Connor, P. VanRaden, M. VandeHaar, R. Tempelman, K. Weigel, and J. Cole. 2019. High-density genome-wide association study for residual feed intake in Holstein dairy cattle. J. Dairy Sci. 102:11067–11080. https://doi.org/10.3168/jds.2019-16645.

Lin, Z., I. Macleod, and J. Pryce. 2013. Estimation of genetic parameters for residual feed intake and feeding behavior traits in dairy heifers. J. Dairy Sci. 96:2654–2656. https://doi.org/10.3168/jds.2012-6134.

Lindersson, M., L. Andersson-Eklund, D.-J. De Koning, A. Lunden, A. Mäki-Tanila, and L. Andersson. 1998. Mapping of serum amylase-1 and quantitative trait loci for milk production traits to cattle chromosome 4. J. Dairy Sci. 81:1454–1461. https://doi.org/10.3168/jds.S0022-0302(98)75709-1.

Lopes, L., K. Houlahan, L. Alcantara, G. Oliveira Jr., D. Tulpan, F. Miglior, F. Schenkel, and C. Baes. 2022. Genetic parameters for rumination time and traits related to sustainable dairy production. Proceedings of 12th World Congress on Genetics Applied to Livestock Production (WCGALP) Technical and species orientated innovations in animal breeding, and contribution of genetics to solving societal challenges. Wageningen Academic Publishers, pp. 227–230.

López-Paredes, J., I. Goiri, R. Atxaerandio, A. García-Rodríguez, E. Ugarte, J. Jiménez-Montero, R. Alenda, and O. González-Recio. 2020. Mitigation of greenhouse gases in dairy cattle via genetic selection: 1. Genetic parameters of direct methane using noninvasive methods and proxies of methane. J. Dairy Sci. 103:7199–7209. https://doi.org/10.3168/jds.2019-17597.

Løvendahl, P., and L. Munksgaard. 2016. An investigation into genetic and phenotypic variation in time budgets and yield of dairy cows. J. Dairy Sci. 99:408–417. https://doi.org/10.3168/jds.2015-9838.

Lund, M. S., P. Sorensen, P. Madsen, and F. Jaffrézic. 2008. Detection and modelling of time-dependent QTL in animal populations. Genet. Sel. Evol. 40:177–194. https://doi.org/10.1186/1297-9686-40-2-177.

Maekawa, M., K. Beauchemin, and D. Christensen. 2002. Chewing activity, saliva production, and ruminal pH of primiparous and multiparous lactating dairy cows. J. Dairy Sci. 85:1176–1182. https://doi.org/10.3168/jds.S0022-0302(02)74180-5.

Marquez, G., R. Enns, M. Grosz, L. Alexander, and M. MacNeil. 2009. Quantitative trait loci with effects on feed efficiency traits in Hereford× composite double backcross populations. Anim. Genet. 40:986–988. https://doi.org/10.1111/j.1365-2052.2009.01946.x.

Meir, Y. B., M. Nikbachat, Y. Fortnik, S. Jacoby, H. Levit, G. Adin, M. C. Zinder, A. Shabtay, E. Gershon, and M. Zachut. 2018. Eating behavior, milk production, rumination, and digestibility characteristics of high-and low-efficiency lactating cows fed a low-roughage diet. J. Dairy Sci. 101:10973–10984. https://doi.org/10.3168/jds.2018-14684.

Meredith, B. K., F. J. Kearney, E. K. Finlay, D. G. Bradley, A. G. Fahey, D. P. Berry, and D. J. Lynn. 2012. Genome-wide associations for milk production and somatic cell score in Holstein-Friesian cattle in Ireland. BMC Genet. 13:1–11. https://doi.org/10.1186/1471-2156-13-21.

Metz, J. H. M. 1975. Time patterns of feeding and rumination in domestic cattle. Wageningen University and Research.

Mikuła, R., M. Pszczola, K. Rzewuska, S. Mucha, W. Nowak, and T. Strabel. 2021. The effect of rumination time on milk performance and methane emission of dairy cows fed partial mixed ration based on maize silage. Animals (Basel) 12:50. https://doi.org/10.3390/ani12010050.

Moallem, U., G. Altmark, H. Lehrer, and A. Arieli. 2010. Performance of high-yielding dairy cows supplemented with fat or concentrate under hot and humid climates. J. Dairy Sci. 93:3192–3202. https://doi.org/10.3168/jds.2009-2979.

Mujibi, F., S. Moore, D. Nkrumah, Z. Wang, and J. Basarab. 2010. Season of testing and its effect on feed intake and efficiency in growing beef cattle. J. Anim. Sci. 88:3789–3799. https://doi.org/10.2527/jas.2009-2407.

Negussie, E., Y. de Haas, F. Dehareng, R. Dewhurst, J. Dijkstra, N. Gengler, D. Morgavi, H. Soyeurt, S. van Gastelen, T. Yan, and F. Biscarini. 2017. Invited review: Large-scale indirect measurements for enteric methane emissions in dairy cattle: A review of proxies and their potential for use in management and breeding decisions. J. Dairy Sci. 100:2433–2453. https://doi.org/10.3168/jds.2016-12030.

Nielsen, B. L., R. F. Veerkamp, and A. B. Lawrence. 2000. Effects of genotype, feed type and lactational stage on the time budget of dairy cows. Acta Agric. Scand. A Anim. Sci. 50:272–278. https://doi.org/10.1080/090647000750069467.

Nkrumah, J., D. Crews Jr., J. Basarab, M. Price, E. Okine, Z. Wang, C. Li, and S. S. Moore. 2007. Genetic and phenotypic relationships of feeding behavior and temperament with performance, feed efficiency, ultrasound, and carcass merit of beef cattle. J. Anim. Sci. 85:2382–2390. https://doi.org/10.2527/jas.2006-657.

Pahl, C., E. Hartung, K. Mahlkow-Nerge, and A. Haeussermann. 2015. Feeding characteristics and rumination time of dairy cows around estrus. J. Dairy Sci. 98:148–154. https://doi.org/10.3168/jds.2014-8025.

Paiva, J. T., R. R. Mota, P. S. Lopes, H. Hammami, S. Vanderick, H. R. Oliveira, R. Veroneze, F. Fonseca e Silva, and N. Gengler. 2022. Random regression test-day models to describe milk production and fatty acid traits in first lactation Walloon Holstein cows. J. Anim. Breed. Genet. 139:398–413. https://doi.org/10.1111/jbg.12673.

Pedrosa, V. B., F. S. Schenkel, S.-Y. Chen, H. R. Oliveira, T. M. Casey, M. G. Melka, and L. F. Brito. 2021. Genomewide association analyses of lactation persistency and milk production traits in Holstein cattle based on imputed whole-genome sequence data. Genes (Basel) 12:1830. https://doi.org/10.3390/genes12111830.

Rolf, M., J. Taylor, R. Schnabel, S. McKay, M. McClure, S. Northcutt, M. Kerley, and R. Weaber. 2012. Genome-wide association analysis for feed efficiency in Angus cattle. Anim. Genet. 43:367–374. https://doi.org/10.1111/j.1365-2052.2011.02273.x.

Schirmann, K., M. A. von Keyserlingk, D. Weary, D. Veira, and W. Heuwieser. 2009. Validation of a system for monitoring rumination in dairy cows. J. Dairy Sci. 92:6052–6055. https://doi.org/10.3168/jds.2009-2361.

Schirmann, K., D. Weary, W. Heuwieser, N. Chapinal, R. Cerri, and M. Von Keyserlingk. 2016. Rumination and feeding behaviors differ between healthy and sick dairy cows during the transition period. J. Dairy Sci. 99:9917–9924. https://doi.org/10.3168/jds.2015-10548.

Schleisner, C., P. Nørgaard, and H. H. Hansen. 1999. Discriminant analysis of patterns of jaw movement during rumination and eating in a cow. Acta Agric. Scand. A Anim. Sci. 49:251–260. https://doi.org/10.1080/090647099424015.

Schrooten, C., M. Bink, and H. Bovenhuis. 2004. Whole genome scan to detect chromosomal regions affecting multiple traits in dairy cattle. J. Dairy Sci. 87:3550–3560. https://doi.org/10.3168/jds.S0022-0302(04)73492-X.

Seabury, C. M., D. L. Oldeschulte, M. Saatchi, J. E. Beever, J. E. Decker, Y. A. Halley, E. K. Bhattarai, M. Molaei, H. C. Freetly, S. L. Hansen, H. Yampara-Iquise, K. A. Johnson, M. S. Kerley, J. W. Kim, D. D. Loy, E. Marques, H. L. Neibergs, R. D. Schnabel, D. W. Shike, M. L. Spangler, R. L. Weaber, D. J. Garrick, and J. F. Taylor. 2017. Genome-wide association study for feed efficiency and growth traits in US beef cattle. BMC Genomics 18:1–25. https://doi.org/10.1186/s12864-017-3754-y.

Senn, M., B. Dürst, A. Kaufmann, and W. Langhans. 1995. Feeding patterns of lactating cows of three different breeds fed hay, corn silage, and grass silage. Physiol. Behav. 58:229–236. https://doi.org/10.1016/0031-9384(95)00044-J.

Sherman, E., J. Nkrumah, C. Li, R. Bartusiak, B. Murdoch, and S. Moore. 2009. Fine mapping quantitative trait loci for feed intake and feed efficiency in beef cattle. J. Anim. Sci. 87:37–45. https://doi.org/10.2527/jas.2008-0876.

Soriani, N., G. Panella, and L. Calamari. 2013. Rumination time during the summer season and its relationships with metabolic conditions and milk production. J. Dairy Sci. 96:5082–5094. https://doi.org/10.3168/jds.2013-6620.

Soriani, N., E. Trevisi, and L. Calamari. 2012. Relationships between rumination time, metabolic conditions, and health status in dairy cows during the transition period. J. Anim. Sci. 90:4544–4554. https://doi.org/10.2527/jas.2011-5064.

Stone, A., B. Jones, C. Becker, and J. Bewley. 2017. Influence of breed, milk yield, and temperature-humidity index on dairy cow lying time, neck activity, reticulorumen temperature, and rumination behavior. J. Dairy Sci. 100:2395–2403. https://doi.org/10.3168/jds.2016-11607.

Tapki, İ., and A. Şahin. 2006. Comparison of the thermoregulatory behaviours of low and high producing dairy cows in a hot environment. Appl. Anim. Behav. Sci. 99:1–11. https://doi.org/10.1016/j.applanim.2005.10.003.

VanRaden, P. M. 2008. Efficient methods to compute genomic predictions. J. Dairy Sci. 91:4414–4423. https://doi.org/10.3168/jds.2007-0980.

Viitala, S. M., N. F. Schulman, D.-J. de Koning, K. Elo, R. Kinos, A. Virta, J. Virta, A. Mäki-Tanila, and J. Vilkki. 2003. Quantitative trait loci affecting milk production traits in Finnish Ayrshire dairy cattle. J. Dairy Sci. 86:1828–1836. https://doi.org/10.3168/jds.S0022-0302(03)73769-2.

Wang, P., X. Li, Y. Zhu, J. Wei, C. Zhang, Q. Kong, X. Nie, Q. Zhang, and Z. Wang. 2022. Genome-wide association analysis of milk production, somatic cell score, and body conformation traits in Holstein cows. Front. Vet. Sci. 9:9. https://doi.org/10.3389/fvets.2022.932034.

Wiggans, G., T. Sonstegard, P. VanRaden, L. Matukumalli, R. Schnabel, J. Taylor, F. Schenkel, and C. Van Tassell. 2009. Selection of single-nucleotide polymorphisms and quality of genotypes used in genomic evaluation of dairy cattle in the United States and Canada. J. Dairy Sci. 92:3431–3436. https://doi.org/10.3168/jds.2008-1758.

Zetouni, L., G. F. Difford, J. Lassen, M. Byskov, E. Norberg, and P. Løvendahl. 2018. Is rumination time an indicator of methane production in dairy cows? J. Dairy Sci. 101:11074–11085. https://doi.org/10.3168/jds.2017-14280.