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Single-step genome-wide association for selected milk fatty acids in Dual-Purpose Belgian Blue cows

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ABSTRACT

The aim of this study was to estimate genetic parameters and identify genomic regions associated with selected individual and groups of milk fatty acids (FA) predicted by milk mid-infrared spectrometry in Dual-Purpose Belgian Blue cows. The used data were 69,349 test-day records of milk yield, fat percentage, and protein percentage along with selected individual and groups FA of milk (g/dL milk) collected from 2007 to 2020 on 7,392 first-parity (40,903 test-day records), and 5,185 second-parity (28,446 test-day records) cows distributed in 104 herds in the Walloon Region of Belgium. Data of 28,466 SNPs, located on 29 *Bos taurus* autosomes (BTA), of 1,699 animals (639 males and 1,060 females) 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 25-SNP sliding window (with an average size of ~2 Mb) was calculated, and regions accounting for at least 1.0% of the total additive genetic variance were used to search for candidate genes. Average daily heritability estimated for the included milk FA traits ranged from 0.01 (C4:0) to 0.48 (C12:0) and 0.01 (C4:0) to 0.42 (C12:0) in the first and second parities, respectively. Genetic correlations found between milk yield and the studied individual milk FA, except for C18:0, C18:1 *trans*, C18:1 *cis*-9, were positive. The results showed that fat percentage and protein percentage were positively genetically correlated with all studied individual milk FA. Genome-wide association analyses identified 11 genomic regions distributed over 8 chromosomes [BTA1, BTA4, BTA10, BTA14 (4 regions), BTA19, BTA22, BTA24, and BTA26] associated with the studied FA traits, though those found on BTA14 partly overlapped. The genomic regions identified differed between parities and lactation stages. Although these differences in genomic regions detected may be due to the power of quantitative trait locus detection, it also suggests that candidate genes underlie the phenotypic expression of the studied traits may vary between parities and lactation stages. These findings increase our understanding about the genetic background of milk FA and can be used for the future implementation of genomic evaluation to improve milk FA profile in Dual-Purpose Belgian Blue cows.

Key words: genetic parameter, milk composition, milk fatty acid, dual-purpose cattle

INTRODUCTION

The fat content of milk is of economic importance due to its effect on milk price in most countries of the world. The raw material for the production of butter is milk; thus, the quality of produced butter is highly correlated with contents and composition of milk fat (Gonzalez et al., 2003; Chen et al., 2004; Staniewski et al., 2021). For example, Gonzalez et al. (2003) reported that degree of unsaturation in milk fat affected melting characteristics of butter. Bobe et al. (2003) suggested that selection of individual cows with a more UFA composition can be used to produce butter with a softer texture and better spreadability. Furthermore, in recent decades, human consumption patterns of dairy products have changed, and consumers pay more attention to the health aspects of the food (Thorning et al., 2016; Sajdakowska et al., 2021). Milk fat contains approximately 400 different fatty acids (**FA**) that, depending on the degree of saturation/unsaturation in the carbon chain, can be divided into SFA, containing no double bond; MUFA, containing one double bond; and PUFA, containing 2 or more double bonds. In addition to the positive effect of milk UFA on the human health (Langley et al., 2020), increasing the proportion of UFA in bovine milk fat has positive effects on the technological properties of butter (Couvreur et al., 2006; Bobe et al., 2007; Staniewski

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et al., 2021). Interest in increasing the ratio of UFA to SFA in bovine milk is growing leading the researchers to evaluate the feasibility of changing milk FA profile in dairy cattle (Lock and Bauman, 2004; Couvreur et al., 2006; Lanier and Corl, 2015). The variation in the amount of FA and their composition in bovine milk fat is highly affected by environmental factors such as feeding strategies (i.e., related to fiber and energy intake, dietary fats), management of the cow, udder health, season, and physiological stage (e.g., parity, lactation stage) of the animal (Vanhatalo et al., 2007; Soyeurt et al., 2008; Lanier and Corl, 2015; Toledo-Alvarado et al., 2018). The FA profile of milk can be improved in several ways, including dietary modification and genetic selection. Although diet-induced changes are observed earlier, the latter leads to permanent and cumulative changes at the population level (Shingfield et al., 2006; Vanhatalo et al., 2007; Shingfield et al., 2013; Narayana et al., 2017). Genetic analyses, using chromatographic data and milk mid-infrared (**MIR**) based predictions, have revealed genetic variations in the FA profile of bovine milk, suggesting that the genetic improvement of the quality of milk based on its FA profile may be possible (Soyeurt et al., 2008; Bastin et al., 2011; Pegolo et al., 2016; Knutsen et al., 2022). However, to apply selection programs based on the performance recording and prediction of breeding values, genetic parameters for milk FA profile have to be estimated. Genetic variation associated with bovine milk fat composition has been investigated in various breeds of dairy cattle, with estimated heritabilities for individual FA being low to moderate (Mele et al., 2009; Bastin et al., 2013; Hein et al., 2018; Freitas et al., 2020).

The Belgian Blue breed, originating in central and upper Belgium in the 19th century, is composed of 2 strains including Beef Belgian Blue and Dual-Purpose Belgian Blue (**DPBB**) (Gengler et al., 2007; Mota et al., 2017; Wilmot et al., 2022). The DPBB is the second important cattle breed reared by dairy farmers in Walloon Region of Belgium. This breed is also reared by a few farmers in the northeast of France and recognized as the "Bleue du Nord." In 2007, the conservation status of the Bleue du Nord was listed by the FAO as endangered-maintained (Rischkowsky and Pilling, 2007). Atashi et al. (2022b) showed a continuous decrease in the effective population size of DPBB, indicating the need for effective conservation programs in this local breed. The improvement of economically important traits is a critical step to convince more farmers to use DPBB cows in their farms and, consequently, to preserve the breed. In this regard, making targeted modifications to the milk FA profile in DPBB cows may have the potential to contribute to the production of dairy products with higher added value. However, there is no information about the genetic parameters of milk FA profile in DPBB cattle. Furthermore, identification of genomic regions and individual genes responsible for genetic variation in milk fat composition improves our understanding of biological pathways involved in FA synthesis and can be used for changing milk fat composition via genetic selection. Although GWAS using typical models with milk production traits defined as 305 d need lower computational costs, this approach has a non-negligible limitation: there is substantial evidence that the genetic background of milk production traits changes during lactation and the genetic effects of QTL related to milk production traits are not constant during the lactation period (Lu and Bovenhuis, 2019; Atashi et al., 2020; Lu et al., 2020). Thus, many QTL whose genetic effects change during lactation might not be detected in this approach (Lund et al., 2008; Ning et al., 2018). In this regard, random regression (**RR**) models which directly model changes in random effects during lactation can be used to scan the whole genome for genomic regions whose effects on milk production traits change during lactation (Lu and Bovenhuis, 2019; Lu et al., 2020). Therefore, the aim of this study was to use RR test-day models (**RR-TDM**) to estimate genetic parameters and identify genomic regions associated with selected individual and groups of milk FA in DPBB cows.

MATERIALS AND METHODS

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

Phenotypic Data

The data used consisted of test-day records of milk yield (**MY**), fat percentage (**FP**), protein percentage (**PP**), and selected predicted individual and groups of milk FA (g/dL milk) on DPBB cows. Cows with missing birth date, calving date, or parity number were excluded. Only records from the first and second parities that had data for all traits on a given test-day were kept. Records from DIM lower than 5 d and greater than 365 d were eliminated. Age at the first calving was calculated as the difference between birth date and first calving date and restricted to the range of 640 to 1,500 d. Daily MY, FP, and PP were restricted to range from 1 to 70 kg, 1 to 9% and 1 to 7%, respectively. Test-day records of the studied milk FA were edited to remove records outside the range of mean ± 5 standard deviations (**SD**). Within cow, if parity 2 was present, parity 1 had to be present. Herds were required to have a

minimum of 10 cows in the data set to be included. The edited data consisted of 69,349 test-day records of MY, FP, PP along with predicted individual and groups of milk FA collected from 2007 to 2020 on 12,577 lactations of 7,392 animals distributed in 104 herds in the Walloon Region of Belgium. The number of test-day records on the first and second-parity cows were 40,903 (on 7,392 cows) and 28,446 (on 5,185 cows), respectively. On average across the data set, 5.51 test-day records were available per cow per lactation. Pedigree data were extracted from the database used for the official Walloon genetic evaluation. Pedigree depth of the animals was traced back to 20 generations to include all ancestors from genotyped and phenotyped animals. Full pedigree records included 44,428 females and 7,076 males.

Predicted Concentrations of FA in Milk

Milk samples were collected during the routine milk recording on the individual cows. The milk sample was composed of 50% of morning milk and 50% of evening milk. All milk samples were analyzed by MIR spectrometers (Foss MilkoScan FT6000; Foss), an instrument that also provided the standard milk recording analyses, to generate the milk MIR spectrum on which FA predicting equations were applied. The methodology used to predict the milk FA contents was fully described by Soyeurt et al. (2011). Soyeurt et al. (2011) used 6 methods to develop the most accurate prediction equations for predicting individual and groups of milk FA (g/dL milk) and suggested the method developed based on partial least squares regression (**PLS**) + the first-derivative pretreatment on the spectral data as the best predictor for most individual and groups of milk FA (g/dL milk). Therefore, this method was used to predict individual and groups of milk FA included in this study. Only equations predicting FA with a reasonably reliability [i.e., with cross-validation coefficients of determination (**R^2cv**) above or equal to 0.80 in the study of Soyeurt et al. (2011)] were considered in this study (Supplemental Table S1, https://github.com/hadiatashi/Supporting-information-to-SS-GWAS-for-selected-milk-FA-in-DPBB-cows). Soyeurt et al. (2011) used multiple breeds, countries, and production approaches to develop the calibration equations allowed to cover the natural variability of milk FA. A part of the data used for developing the equations for predicting included FA data belonged to DPBB; therefore, it can be expected that the variation found in the current data set (DPBB cows) is included in the variation found in the data set used by Soyeurt et al. (2011) with 6 cattle breeds.

The following individual and groups of milk FA were included in this study as described by Bastin et al. (2013): Individual FA are C4:0, C6:0, C8:0, C10:0, C12:0, C14:0, C16:0, C18:0, and C18:1 *cis*-9. The FA groups are SFA, MUFA, PUFA, short-chain fatty acids (**SCFA**), medium-chain fatty acids (**MCFA**), and long-chain fatty acids (**LCFA**). The group of SFA includes C4:0, C6:0, C8:0, C10:0, C12:0, C12:0 *iso*, C12:0 *anteiso*, C13:0 *iso*, C14:0, C14:0 *iso*, C14:0 *anteiso*, C15:0, C15:0 *iso*, C16:0, C16:0 *iso*, C16:0 *anteiso*, C17:0, C17:0 *iso*, C17:0 *anteiso*, C18:0, C19:0, C20:0 and C22:0. The group of MUFA includes C10:1, C12:1 *cis*, C14:1 *cis*, C16:1 *cis*, C16:1 *trans*, C18:1 *cis*-9, C18:1 *cis*-11, C18:1 *cis*-12, C18:1 *trans*-6–11, C18:1 *trans*-12–14, C18:1 *cis*-13 + *cis*-14 + *trans*-16, C20:1 *cis*-9 and C20:1 *cis*-11. The group of PUFA includes C18:2 *cis*-9 *trans*-13, C18:2 *trans*-8 *cis*-12, C18:2 *cis*-9 *trans*-12, C18:2 *trans*-8 *cis*-13, C18:2 *trans*-11 *cis*-15, C18:2 *trans*-9 *cis*-12, C18:2 *cis*-9 *cis*-12, C18:2 *cis*-9 *trans*-11 (CLA), C18:3 *cis*-9 *cis*-12 *cis*-15, C20:3 (n-6), C20:4 (n-6), C20:5 EPA (n-3), and C22:5 DPA. The group of SCFA includes FA with 4 to 10 carbons, the group of MCFA includes FA with 12 to 16 carbons and LCFA includes FA with 17 to 22 carbons. The infrared predictions of C6:0, C8:0, C10:0, C12:0, C14:0, C16:0, C18:1 *cis*-9, SFA, MUFA, SCFA, MCFA, and LCFA are more accurate than the predictions of C4:0, C18:0, C18:1 *trans*, and PUFA ($0.90 < R^2\text{cv} > 1.00$ vs. $0.80 < R^2\text{cv} > 0.90$).

Genotypic Data

Genotypic data were available for 1,699 animals (639 males and 1,060 females). The animals were genotyped using the BovineSNP50 Beadchip v1 to v3 and EuroG MD (SI) v9 (Illumina). The SNPs in common among the 4 chips were kept. Nonmapped SNPs, SNPs located on sexual 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 frequencies (**MAF**) less than 5% were excluded. The difference between observed and expected heterozygosity was estimated, and if the difference was greater than 0.15, the SNP was excluded (Wiggans et al., 2009). In total, 28,466 SNP located on 29 BTA were used in the genomic analysis.

Variance Component Estimation

The (co)variance components and genomic breeding value for selected milk FA were estimated based on the integration of the RR-TDM into single-step genomic BLUP procedure (**SS RR-TDM**) using a model

adapted for DPBB data structure (Atashi et al., 2021). The following SS RR-TDM through multiple-trait (4 traits), multiple-lactation (first 2 lactations) was used:

$$y_{ijklmn} = \mu + HTDp_i + \sum_{b=0}^{4} AS_j \phi_b(t) + \sum_{b=0}^{2} HY_k \phi_b(t) + \sum_{b=0}^{2} pe_m \phi_b(t) + \sum_{b=0}^{2} a_m \phi_b(t) + e_{ijklmn},$$

where y_{ijklmn} is the test-day record (MY, FP, PP, and the included individual and groups of milk FA) belonging to the DIM n of cow m in parity l, belonging to ith class of HTDp, jth class of AS, and kth class of herd-year of calving (HY); HTDp is the fixed effect of herd-test day-parity; AS is the fixed effect of age-season of calving defined as follows: age at calving class (6 and 4 classes of age at calving were created 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); $\sum_{b=0}^{4} AS_j \phi_b(t)$ is the fixed regression coefficient of the age-season of calving modeled using Legendre polynomials of order 4; $\sum_{b=0}^{2} HY_k \phi_b(t)$, $\sum_{b=0}^{2} pe_m \phi_b(t)$, and $\sum_{b=0}^{2} a_m \phi_b(t)$ are, respectively, the random regression coefficients of herd-year of calving (HY), permanent environmental (pe), and additive effects (a) modeled using Legendre polynomials of order 2; and e_{ijklmn} is the residual effect. The herd-year of calving, permanent environment, additive genetic, and residual variances were obtained as follows:

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

where **HY** is the 24 × 24 covariance matrix of the herd-year of calving regression coefficients; **I** is an identity matrix, ⊗ represents the Kronecker product function, **P** is the 24 × 24 covariance matrix of the permanent environmental regression coefficients; **Ga** is the 24 × 24 covariance matrix of the additive genetic regression coefficients; and blocks within $\mathbf{R} = \sum^{+} \mathbf{R}^{p}$ contain diagonal matrices (4 × 4) of residual covariances between traits with elements that depend on parity (p). Residual covariances between traits on the same test day were therefore allowed to be different from zero, and residual covariances were the same within each parity. The **H** is a matrix that combines pedigree and genomic relationships, whose inverse consists of the integration of additive and genomic relationship matrices, **A** and **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 **A** is the numerator relationship matrix based on pedigree for all animals; $\mathbf{A}_{22}$ is the numerator relationship matrix for genotyped animals; and **G** is the weighted genomic relationship matrix obtained using the following function:

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

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

The (co)variance components were estimated by Bayesian inference using the GIBBS3F90 software (Aguilar et al., 2018). Gibbs sampling was used to obtain marginal posterior distributions for the various parameters using a single chain of 200,000 iterates. The first 50,000 iterates 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, herd-year calving, and residual variances at a given DIM.

The vector of genomic EBV (**GEBV**) of the included individual and groups of milk FA for each animal i, which included daily GEBV from all DIM (5–365) in each parity, was estimated by multiplying the vector of additive genetic predicted regression coefficients by the

matrix of Legendre orthogonal polynomial covariates; that is, $\mathbf{GEBV_i} = \mathbf{T}\hat{\mathbf{g}}_\mathbf{i}$, 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

The GWAS analyses were performed for all included traits in the first and second lactations considering following 3 lactation stages 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 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 (for each trait in each parity) were obtained by summing the daily GEBV solutions of the specific DIM; that is,

$$\hat{\text{GEBV}}1_\text{i} = \hat{\text{GEBV}}_{\text{i}5} + \hat{\text{GEBV}}_{\text{i}6} + \ldots + \hat{\text{GEBV}}_{\text{i}60},$$

$$\hat{\text{GEBV}}2_\text{i} = \hat{\text{GEBV}}_{\text{i}61} + \hat{\text{GEBV}}_{\text{i}62} + \ldots + \hat{\text{GEBV}}_{\text{i}200}, \text{ and}$$

$$\hat{\text{GEBV}}3_\text{i} = \hat{\text{GEBV}}_{\text{i}201} + \hat{\text{GEBV}}_{\text{i}202} + \ldots + \hat{\text{GEBV}}_{\text{i}365},$$

where $\hat{\text{GEBV}}1_\text{i}$, $\hat{\text{GEBV}}2_\text{i}$, and $\hat{\text{GEBV}}3_\text{i}$ are the GEBV for the first, second, and third lactation stages of animal i obtained by summing the GEBV from 5 to 60, 61 to 200, and 201 to 365 DIM, respectively. The SNP effects for each lactation stage were estimated individually for each trait in each parity 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

$$\text{Var}\left(\mathbf{a_g}\right) = \text{Var}\left(\mathbf{Zu}\right) = \mathbf{ZDZ}'\sigma^2_\text{u} = \mathbf{G}\sigma^2_\text{a},$$

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

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

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

$$\lambda = \frac{\sigma^2_\text{u}}{\sigma^2_\text{a}} = \frac{1}{\sum_{\text{i}=1}^{\text{M}} 2\text{p}_\text{i}(1-\text{p}_\text{i})}.$$

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

$$\frac{\text{Var}\left(\text{a}_\text{i}\right)}{\sigma^2_\text{a}} \times 100\% = \frac{\text{Var}\left(\sum_{\text{j}=1}^{25}\text{Z}_\text{j}\hat{\text{u}}_\text{j}\right)}{\sigma^2_\text{a}} \times 100,$$

where a_i is the genetic value of the ith region that consists of 25 adjacent SNPs, σ^2_a is the total additive genetic variance, $\mathbf{Z_j}$ is the vector of the SNP content of the jth SNP for all individuals, and $\hat{\text{u}}_\text{j}$ is the marker effect of the jth SNP within the ith region. The additive genetic variance explained by 25-SNP moving windows, with an average size of ~2 Mb, was calculated across the whole genome and those windows that explained at least 1% of the total additive genetic variance were considered promising regions and used to identify potential candidate genes. The concept of grouping SNPs into windows was adopted as a way to better capture the effect of a QTL instead of a single SNP (Habier et al., 2011).

Identification of Candidate Genes for the Studied Milk FA Profile

The animals included in this study were genotyped using the BovineSNP50 Beadchip v1 to v3 and EuroG MD (SI) v9 (Illumina), which are based on the bovine reference genomes assembly UMD3.1. However, new bovine reference genome assembly ARS-UCD1.2, assembled using long sequencing reads, filled gaps and resolved repetitive regions of the UMD3.1 assembly, and has more credible annotation information. The Lift Genome Annotations tool, available through a simple web interface (https://genome.ucsc.edu/cgi-bin/hgLiftOver), was used to convert coordinate ranges of the identified genomic regions from the UMD3.1 to the ARS-UCD1.2 assembly. Then, to identify possible candidate genes associated with the considered milk FA traits, genes located within the identified genomic regions (i.e., between the start and end of genomic coordinates of the identified regions based on the ARS-UCD1.2 assembly) were further investigated. We identified genes using the National Center for Biotechnology Information Map Viewer tool for the ARS-UCD1.2 assembly (https://

Table 1. Descriptive statistics for milk yield, fat percentage, protein percentage, and selected milk fatty acid contents in Dual-Purpose Belgian Blue[1]

	First lactation			Second lactation		
Trait	Mean	SD	CV (%)	Mean	SD	CV (%)
Milk yield (kg)	12.56	4.08	32.52	14.69	5.49	37.39
Fat percentage (%)	3.63	0.59	15.99	3.66	0.63	17.07
Protein percentage (%)	3.34	0.37	10.95	3.34	0.38	11.31
C4:0 (g/dL)	0.095	0.019	19.63	0.096	0.020	20.75
C6:0 (g/dL)	0.062	0.013	21.57	0.064	0.014	22.64
C8:0 (g/dL)	0.038	0.010	24.82	0.040	0.010	25.16
C10:0 (g/dL)	0.089	0.027	30.41	0.094	0.028	29.68
C12:0 (g/dL)	0.108	0.035	32.18	0.115	0.035	30.82
C14:0 (g/dL)	0.401	0.083	20.63	0.412	0.086	20.91
C16:0 (g/dL)	1.046	0.280	26.73	1.061	0.289	27.22
C18:0 (g/dL)	0.383	0.105	27.37	0.362	0.102	28.20
C18:1 *trans* (g/dL)	0.129	0.065	49.88	0.123	0.062	50.39
C18:1 *cis*-9 (g/dL)	0.816	0.200	24.47	0.788	0.199	25.28
SFA (g/dL)	2.400	0.455	18.96	2.420	0.483	19.96
MUFA (g/dL)	1.137	0.272	23.97	1.102	0.271	24.58
PUFA (g/dL)	0.162	0.044	27.07	0.162	0.043	26.52
SCFA[2] (g/dL)	0.299	0.063	21.06	0.310	0.067	21.80
MCFA[3] (g/dL)	1.837	0.411	22.38	1.871	0.427	22.83
LCFA[4] (g/dL)	1.577	0.394	24.96	1.522	0.386	25.35

[1]The numbers of test-day records in first- and second-parity cows were 40,903 (on 7,392 lactations) and 28,446 (on 5,185 lactations), respectively.

[2]Short-chain fatty acids = C4 to C10.

[3]Medium-chain fatty acids = C12 to C16.

[4]Long-chain fatty acids = C17 to C22.

www.ncbi.nlm.nih.gov/genome/annotation_euk/Bos_taurus/106/) as the reference map.

RESULTS

Descriptive Statistics

The descriptive statistics for MY, FP, PP, and individual and groups of milk FA in the first and second lactations are presented in Table 1. The mean (SD) of daily MY was 12.56 (4.08) kg with 3.63% (0.59) of fat and 3.34% (0.37) of protein in the first parity, whereas the mean (SD) of MY was 14.69 (5.49) kg with 3.66% (0.63) of fat and 3.34% (0.38) of protein in the second parity. The highest coefficient of variation (CV) was found for C18:1 *trans*, whereas the lowest CV was found for SFA. The mean (SD) relative proportion of SFA (SFA to total fat) was 66.06% (7.42) and 66.29% (7.91) in the first and second lactations, respectively. The mean (SD) relative proportion of MUFA (MUFA to total fat) was 31.28% (5.47), and 30.21% (5.47) in the first and second lactations, respectively. The mean (SD) relative proportion of PUFA (PUFA to total fat) was 4.47% (1.05) and 4.45% (1.02) in the first and second lactations, respectively. In regard to saturation of fat, SFA was the most abundant group of FA in milk, followed by MUFA and PUFA. In regard to chain length, MCFA was the most abundant group of FA, followed by LCFA and SCFA. Concerning individual FA, C16:0 was the most abundant FA in milk, followed by C18:1 *cis*-9, and then C14:0.

Heritabilities and Genetic Correlations

The estimated genetic parameters for the included traits in the first and second lactations are presented in Tables 2 and 3, respectively. The mean daily h^2 estimates (SD of daily h^2 estimates across lactation) for MY, FP, and PP was 0.35 (0.04), 0.30 (0.05), and 0.37 (0.09) in the first lactation, respectively. The corresponding estimated values for the second lactation were 0.27 (0.02), 0.28 (0.04), and 0.37 (0.04), respectively. Average h^2 estimated for the studied individual and groups of milk FA ranged from 0.01 (C4:0) to 0.48 (C12:0) and 0.01 (C4:0) to 0.42 (C12:0) in the first and second lactations, respectively. Among the even-chain SFA (C4.0 to C18.0), C4:0 had the lowest and C12:0 had the highest h^2 in both lactations. In regard to chain length, SCFA, and MCFA showed moderate to high h^2 and LCFA showed low to moderate h^2. In regard to saturation of fat, the mean h^2 estimated for milk SFA was higher than that found for UFA and mean h^2 found for milk MUFA was lower than that estimated for PUFA.

Table 2. Genetic parameter estimates (mean and range across the lactation) for milk fatty acids and their genetic correlations with milk yield, fat percentage, and protein percentage in first-parity Dual-Purpose Belgian Blue cows[1]

	First lactation							
	h^2		Milk yield		Fat percentage		Protein percentage	
Trait	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Milk yield (kg)	0.35	0.24 to 0.40			−0.15	−0.24 to 0.25	−0.48	−0.61 to −0.35
Fat percentage (%)	0.30	0.17 to 0.34					0.55	0.28 to 0.61
Protein percentage (%)	0.37	0.24 to 0.50						
C4:0 (g/dL)	0.01	0.00 to 0.01	0.17	0.08 to 0.38	0.70	0.65 to 0.73	0.21	0.17 to 0.26
C6:0 (g/dL)	0.14	0.08 to 0.26	0.03	−0.01 to 0.10	0.07	0.00 to 0.12	0.04	0.00 to 0.06
C8:0 (g/dL)	0.25	0.20 to 0.35	0.04	−0.02 to 0.16	0.05	−0.07 to 0.11	0.03	−0.06 to 0.12
C10:0 (g/dL)	0.46	0.35 to 0.53	0.16	−0.17 to 0.35	0.67	0.30 to 0.78	0.35	0.14 to 0.50
C12:0 (g/dL)	0.48	0.37 to 0.55	0.08	−0.22 to 0.26	0.71	0.36 to 0.82	0.43	0.19 to 0.54
C14:0 (g/dL)	0.45	0.30 to 0.52	0.05	−0.09 to 0.17	0.80	0.63 to 0.88	0.47	0.41 to 0.54
C16:0 (g/dL)	0.32	0.20 to 0.41	0.01	−0.29 to 0.12	0.85	0.73 to 0.90	0.41	0.34 to 0.53
C18:0 (g/dL)	0.23	0.18 to 0.26	−0.30	−0.40 to 0.08	0.72	0.66 to 0.83	0.31	0.20 to 0.42
C18:1 *trans* (g/dL)	0.20	0.13 to 0.27	−0.38	−0.47 to −0.06	0.61	0.35 to 0.77	0.44	0.31 to 0.50
C18:1 *cis*-9 (g/dL)	0.19	0.14 to 0.24	−0.38	0.65 to 0.38	0.59	0.51 to 0.82	0.43	0.01 to 0.57
SFA (g/dL)	0.35	0.22 to 0.45	0.02	−0.14 to 0.06	0.93	0.88 to 0.95	0.44	0.36 to 0.51
MUFA (g/dL)	0.19	0.14 to 0.22	−0.40	−0.66 to 0.36	0.69	0.64 to 0.84	0.55	0.16 to 0.65
PUFA (g/dL)	0.26	0.24 to 0.28	−0.38	−0.48 to −0.03	0.72	0.68 to 0.84	0.63	0.35 to 0.68
SCFA[2] (g/dL)	0.43	0.27 to 0.52	0.18	−0.01 to 0.33	0.75	0.56 to 0.81	0.33	0.21 to 0.48
MCFA[3] (g/dL)	0.35	0.21 to 0.45	0.02	−0.10 to 0.09	0.86	0.80 to 0.93	0.47	0.40 to 0.53
LCFA[4] (g/dL)	0.20	0.15 to 0.22	−0.39	−0.61 to 0.26	0.73	0.69 to 0.87	0.50	0.18 to 0.58

[1]The number of test-day records in first-parity cows was 40,903 on 7,392 lactations.

[2]Short-chain fatty acids = C4 to C10.

[3]Medium-chain fatty acids = C12 to C16.

[4]Long-chain fatty acids = C17 to C22.

Table 3. Genetic parameter estimates (mean and range across the lactation) for milk fatty acids and their genetic correlations with milk yield, fat percentage, and protein percentage in second-parity Dual-Purpose Belgian Blue cows[1]

	Second lactation							
	h^2		Milk yield		Fat percentage		Protein percentage	
Trait	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Milk yield (kg)	0.27	0.24 to 0.30			0.01	−0.07 to 0.08	−0.29	−0.35 to −0.24
Fat percentage (%)	0.27	0.19 to 0.33					0.60	0.42 – 0.65
Protein percentage (%)	0.37	0.29 to 0.41						
C4:0 (g/dL)	0.01	0.00 to 0.02	0.15	0.08 to 0.30	0.47	0.32 to 0.67	0.22	0.09 to 0.33
C6:0 (g/dL)	0.17	0.14 to 0.29	0.02	−0.01 to 0.07	0.06	0.03 to 0.11	0.11	0.07 to 0.14
C8:0 (g/dL)	0.27	0.22 to 0.35	0.00	−0.11 to 0.12	0.03	−0.08 to 0.11	0.13	0.06 to 0.17
C10:0 (g/dL)	0.22	0.17 to 0.27	0.25	−0.03 to 0.57	0.69	0.35 to 0.83	0.45	0.16 to 0.59
C12:0 (g/dL)	0.42	0.35 to 0.48	0.18	−0.02 to 0.44	0.68	0.29 to 0.82	0.47	0.13 to 0.59
C14:0 (g/dL)	0.39	0.30 to 0.45	0.20	0.07 to 0.43	0.83	0.67 to 0.90	0.51	0.39 to 0.58
C16:0 (g/dL)	0.32	0.23 to 0.40	0.13	−0.02 to 0.19	0.88	0.78 to 0.92	0.46	0.30 to 0.61
C18:0 (g/dL)	0.24	0.19 to 0.28	−0.10	−0.19 to 0.14	0.71	0.66 to 0.82	0.27	0.10 to 0.40
C18:1 *trans* (g/dL)	0.18	0.11 to 0.23	−0.06	−0.24 to 0.16	0.59	0.38 to 0.67	0.31	0.17 to 0.40
C18:1 *cis*-9 (g/dL)	0.20	0.13 to 0.23	−0.25	−0.44 to 0.23	0.58	0.43 to 0.83	0.44	0.03 to 0.67
SFA (g/dL)	0.32	0.23 to 0.41	0.14	0.08 to 0.22	0.93	0.88 to 0.96	0.51	0.39 to 0.60
MUFA (g/dL)	0.20	0.14 to 0.22	−0.24	−0.39 to 0.20	0.70	0.21 to 0.87	0.54	0.09 to 0.71
PUFA (g/dL)	0.21	0.19 to 0.24	−0.16	−0.31 to 0.10	0.74	0.70 to 0.78	0.59	0.36 to 0.66
SCFA[2] (g/dL)	0.40	0.32 to 0.45	0.29	0.09 to 0.57	0.78	0.55 to 0.86	0.43	0.25 to 0.52
MCFA[3] (g/dL)	0.34	0.22 to 0.42	0.19	0.10 to 0.29	0.90	0.83 to 0.94	0.55	0.39 to 0.64
LCFA[4] (g/dL)	0.20	0.15 to 0.22	−0.23	−0.38 to 0.21	0.73	0.67 to 0.87	0.49	0.10 to 0.60

[1]The number of test-day records in second-parity cows was 28,446 on 5,185 lactations.

[2]Short-chain fatty acids = C4 to C10.

[3]Medium-chain fatty acids = C12 to C16.

[4]Long-chain fatty acids = C17 to C22.

Table 4. Phenotypic and genetic variances for milk fatty acids in Dual-Purpose Belgian Blue cows and their phenotypic and genetic correlations between the first and second parities[1]

	Phenotypic variance		Genetic variance (SE)		Correlation	
Trait	First parity	Second parity	First parity	Second parity	Phenotypic	Genetic (SE)
Milk yield (kg)	9.51E1	15.97E1	3.29E1 (2.81E-1)	4.31E1 (5.07E-1)	0.48	0.88 (0.02)
Fat percentage (%)	3.01E-1	4.06E-1	8.88E-2 (6.63E-3)	1.08E-1 (9.89E-3)	0.33	0.83 (0.02)
Protein percentage (%)	1.05E-1	1.43E-1	3.84E-2 (3.17E-3)	5.33E-2 (4.73E-3)	0.45	0.86 (0.03)
C4:0 (g/dL)	5.47E-3	2.13E-2	7.96E-5 (6.15E-5)	3.04E-4 (1.30E-4)	0.29	0.53 (0.12)
C6:0 (g/dL)	8.12E-2	1.28E-1	1.24E-2 (2.38E-2)	2.41E-2 (4.86E-2)	0.14	0.34 (0.10)
C8:0 (g/dL)	1.78E-1	2.81E-1	4.41E-2 (6.74E-2)	7.59E-2 (9.23E-2)	0.21	0.38 (0.16)
C10:0 (g/dL)	4.13E-4	1.04E-3	1.88E-4 (1.26E-5)	2.32E-4 (1.61E-5)	0.37	0.89 (0.08)
C12:0 (g/dL)	6.27E-4	8.26E-4	3.01E-4 (1.61E-5)	3.51E-4 (2.37E-5)	0.49	0.89 (0.07)
C14:0 (g/dL)	4.00E-3	5.64E-3	1.78E-3 (9.79E-5)	2.18E-3 (1.43E-4)	0.47	0.79 (0.08)
C16:0 (g/dL)	4.00E-2	5.32E-2	1.28E-2 (9.44E-4)	1.69E-2 (1.38E-3)	0.39	0.84 (0.07)
C18:0 (g/dL)	7.45E-3	8.62E-3	1.74E-3 (1.78E-4)	2.03E-3 (2.21E-4)	0.26	0.79 (0.09)
C18:1 *trans* (g/dL)	1.44E-3	1.60E-3	2.90E-4 (3.07E-5)	2.80E-4 (3.96E-5)	0.21	0.77 (0.08)
C18:1 *cis*-9 (g/dL)	2.73E-2	3.38E-2	5.32E-3 (5.96E-4)	6.72E-3 (8.19E-4)	0.24	0.79 (0.08)
SFA (g/dL)	1.46E-1	2.02E-1	5.15E-2 (3.67E-3)	6.53E-2 (5.34E-3)	0.40	0.87 (0.06)
MUFA (g/dL)	4.71E-2	5.83E-2	9.11E-3 (9.43E-4)	1.16E-2 (1.41E-3)	0.24	0.78 (0.06)
PUFA (g/dL)	8.33E-4	9.80E-4	2.18E-4 (2.09E-5)	2.06E-4 (2.19E-5)	0.28	0.71 (0.07)
SCFA[2] (g/dL)	2.76E-3	3.82E-3	1.19E-3 (7.52E-5)	1.53E-3 (1.04E-4)	0.47	0.89 (0.06)
MCFA[3] (g/dL)	9.43E-2	1.30E-1	3.34E-2 (2.22E-3)	4.37E-2 (3.34E-3)	0.41	0.87 (0.07)
LCFA[4] (g/dL)	9.41E-2	1.15E-1	1.88E-2 (2.07E-3)	2.25E-2 (2.94E-3)	0.23	0.77 (0.08)

[1]The numbers of test-day records in first- and second-parity cows were 40,903 (on 7,392 lactations) and 28,446 (on 5,185 lactations), respectively.
[2]Short-chain fatty acids = C4 to C10.
[3]Medium-chain fatty acids = C12 to C16.
[4]Long-chain fatty acids = C17 to C22.

The results showed that FP and PP were positively genetically correlated with all studied individual and groups of milk FA. Genetic correlations found between MY and all studied individual and groups of milk FA, except for C18:0, C18:1 *trans*, C18:1 *cis*-9, LCFA, MUFA, and PUFA, were positive. Genetic correlations estimated between individual even-chain SFA (C4.0 to C18.0) with MY, FP, and PP ranged from −0.30 (C18:0) to 0.17 (C4:0), 0.05 (C8:0) to 0.85 (C16:0), and 0.03 (C8:0) to 0.47 (C14:0) in the first lactation. The corresponding values found in the second lactation ranged from −0.10 (C18:0) to 0.25 (C10:0), 0.03 (C8:0) to 0.88 (C16:0), and 0.11 (C6:0) to 0.51 (C14:0), respectively. Mean daily genetic correlation estimated between MY and SFA, MUFA, and PUFA in the first lactation were, respectively, 0.02, −0.40, and −0.38. The corresponding values found in the second lactation were 0.14, −0.24, and −0.16. The estimated phenotypic and additive genetic variances for the studied traits and their phenotypic and genetic correlations in the first and second lactations are presented in Table 4. Genetic correlations among selected DIM for the studied traits are presented in Supplemental Figures S1–S32 (https://github.com/hadiatashi/Supporting-information-to-SS-GWAS-for-selected-milk-FA-in-DPBB-cows). As expected, the magnitudes of genetic correlations among adjacent DIM were high and the correlations decayed as the interval between DIM increased.

Genome-Wide Association Study

General information about the results of single-step GWAS for the studied traits in 3 defined lactation stages of each parity is described in Supplemental Data S1–S96 (16 traits × 2 parities × 3 lactation stages per each parity; https://github.com/hadiatashi/milk-fatty-acids-DBBB). The windows associated with the studied traits along with corresponding genes are presented in Table 5. In total, 11 genomic regions [BTA1, BTA4, BTA10, BTA14 (4 regions), BTA19, BTA22, BTA24, and BTA26] were identified to be associated with the studied individual and groups of milk FA (Supplemental Figures S33–S48, https://github.com/hadiatashi/Supporting-information-to-SS-GWAS-for-selected-milk-FA-in-DPBB-cows).

BTA1. The genomic region located between 143.6 to 145.2 Mb on BTA1 was associated with MUFA in the first and second lactations. This region was 1.6 Mb in size and harbors 19 genes including the phosphodiesterase 9A (*PDE9A*) and ATP binding cassette subfamily G member 1 (*ABCG1*).

BTA4. The genomic region located between 41.6 to 44.2 Mb on BTA4 was associated with C18:0 in the second lactation. The size of this region was 2.6 Mb and contains 10 genes.

BTA10. A genomic region located between 44.4 to 46.5 Mb on BTA10 was linked with C18:0, C18:1 *trans*,

Table 5. Genomic regions associated with selected milk fatty acids in different stages[1] of lactation in first- (left) and second-lactation (right) Dual-Purpose Belgian Blue cows

Chromosome	Position[2] (bp)	Position[3] (bp)	Gene[4]	Trait[5] (parity, stage of lactation, % variance explained)
BTA1	143,615,536–145,222,792	142,114,021–143,445,242	*U2AF1*, *PDE9A*, *C2CD2*, *CBS*, *TFF3*, *CRYAA*, *TFF2*, *UBASH3A*, *ZBTB21*, *PRDM15*, *SLC37A1*, *ABCG1*, *RSPH1*, *WDR4*, *UMODL1*, *NDUFV3*, *TFF1*, *TMPRSS3*, *PKNOX1*	MUFA (1, 2, 1.28), MUFA (2, 2, 1.01)
BTA4	41,610,894–44,177,870	41,234,638–43,942,271	*GSAP*, *LOC107132395*, *PHTF2*, *LOC112446509*, *LOC112446492*, *LOC100296900*, *PTPN12*, *TMEM60*, *RSBN1L*, *CCDC146*	C18:0 (2, 3, 1.02)
BTA10	44,417,569–46,450,370	44,368,506–46,392,179	*DAPK2*, *RTRAF*, *NID2*, *ZNF609*, *GNG2*, *OAZ2*, *LOC112448615*, *SNX1*, *CIAO2A*, *PLEKHO2*, *TRIP4*, *CSNK1G1*, *PPIB*, *RBPMS2*, *SNX22*, *LOC112448649*, *FRMD6*, *PTGDR*, *PIF1*, *PCLAF*, *HERC1*	C18:0 (1, 2, 1.06), C18:0 (1, 3, 1.04), C18:1 *trans* (1, 2, 1.08), PUFA (1, 2, 1.04)
BTA14	22,392,760–24,326,513	20,742,454–22,671,158	*RP1*, *LOC112449652*, *ATP6V1H*, *MRPL15*, *LOC112449633*, *TCEA1*, *ST18*, *RGS20*, *LYPLA1*, *SOX17*, *XKR4*, *RB1CC1*, *PCMTD1*, *OPRK1*, *NPBWR1*, *LOC112449643*	C6:0 (1, 2, 1.03)
BTA14	23,853,811–25,857,110	22,199,112–24,172,762	*RP1*, *LOC112449660*, *BPNT2*, *PLAG1*, *SDR16C6*, *CHCHD7*, *TMEM68*, *LYN*, *SOX17*, *SDR16C5*, *PENK*, *LOC112449630*, *TGS1*, *XKR4*, *LOC112449628*, *MOS*, *RPS20*	C16:0 (2, 1, 1.40), MCFA (2, 1, 1.51), MCFA (2, 2, 1.05), SFA (2, 1, 1.05)
BTA14	24,524,205–26,800,529	22,867,321–25,117,380	*LOC112449660*, *BPNT2*, *UBXN2B*, *PLAG1*, *CYP7A1*, *SDR16C6*, *LYN*, *CHCHD7*, *TMEM68*, *SDR16C5*, *PENK*, *LOC101902490*, *LOC112449630*, *TGS1*, *XKR4*, *TOX*, *LOC112449628*, *NSMAF*, *LOC112449629*, *SDCBP*, *FAM110B*, *MOS*, *RPS20*	C16:0 (1, 1, 1.44), MCFA (1, 1, 1.31)
BTA14	25,401,722–27,155,254	23,725,488–25,472,332	*LOC101902490*, *LOC112449660*, *BPNT2*, *TOX*, *NSMAF*, *LOC112449629*, *SDCBP*, *FAM110B*, *UBXN2B*, *CYP7A1*	C4:0 (1, 2, 1.34), C4:0 (2, 2, 1.08), C4:0 (2, 3, 1.01)
BTA19	34,319,757–36,437,188	33,716,989–35,808,547	*FLCN*, *SLC47A2*, *MAP2K3*, *PRPSAP2*, *MPRIP*, *NME1*, *SMCR8*, *SREBF1*, *MIEF2*, *TOP3A*, *USP22*, *DRG2*, *COPS3*, *NT5M*, *PEMT*, *UTP18*, *ALDH3A1*, *RNF112*, *LOC112442830*, *SHMT1*, *KCNJ12*, *FLII*, *ULK2*, *EPN2*, *MIR33B*, *MED9*, *DHRS7B*, *DRC3*, *TOM1L2*, *SLC5A10*, *FAM83G*, *LLGL1*, *MBTD1*, *SPAG9*, *GRAP*, *TMEM11*, *PLD6*, *ATPAF2*, *MFAP4*, *RASD1*, *ALKBH5*, *MAPK7*, *ALDH3A2*, *GID4*, *B9D1*, *AKAP10*, *NME2*, *RAI1*, *TNFRSF13B*	C16:0 (2, 3, 1.10), MCFA (1, 3, 1.10), MCFA (2, 3, 1.02), SFA (1, 3, 1.05), SFA (2, 3, 1.04), LCFA (2, 3, 1.00), MUFA (2, 3, 1.03), PUFA (1, 3, 1.01), PUFA (2, 3, 1.08)
BTA22	16,696,579–18,391,209	16,651,416–18,340,348	*CAMK1*, *JAGN1*, *IRAK2*, *IL17RE*, *CRELD1*, *PRRT3*, *CAV3*, *ARPC4*, *MTMR14*, *LHFPL4*, *EMC3*, *TADA3*, *TTLL3*, *CIDEC*, *RPUSD3*, *LOC112443539*, *FANCD2OS*, *BRK1*, *FANCD2*, *PRRT3*, *BRPF1*, *SSUH2*, *SRGAP3*, *OGG1*, *THUMPD3*, *LMCD1*, *SETD5*, *VHL*, *IL17RC*, *OXTR*, *RAD18*, *CPNE9*	C10:0 (2, 1, 1.01), C12:0 (2, 1, 1.13), SFA (1, 1, 1.09), SFA (2, 1, 1.00)
BTA24	33,581,573–35,667,926	33,201,418–35,286,213	*GREB1L*, *TMEM241*, *MIR544B-2*, *SNRPD1*, *LOC107131776*, *ROCK1*, *MIB1*, *MIR2285O-3*, *RBBP8*, *LOC112444225*, *MGC133647*, *MIR133A-2*, *GATA6*, *COLEC12*, *MIR1–2*, *LOC112444245*, *CABLES1*, *THOC1*, *ESCO1*, *LOC112444227*, *ABHD3*, *USP14*	C18:0 (2, 1, 1.01)
BTA26	20,444,634–22,913,016	20,575,233–23,039,524	*PITX3*, *ELOVL3*, *NDUFB8*, *HIF1AN*, *SEC31B*, *HPS6*, *WNT8B*, *LDB1*, *BLOC1S2*, *DNMBP*, *ABCC2*, *ARMH3*, *SLF2*, *SCD (SCD1)*, *LOC511498*, *PKD2L1*, *TWNK*, *MRPL43*, *COX15*, *PDZD7*, *POLL*, *LZTS2*, *FBXW4*, *DPCD*, *KAZALD1*, *LOC112444557*, *ERLIN1*, *NFKB2*, *PAX2*, *PSD*, *CUTC*, *CPN1*, *GBF1*, *LOC112444554*, *LOC101906134*, *PPRC1*, *ENTPD7*, *TLX1*, *LOC112444556*, *LOC112444545*, *OGA*, *NPM3*, *CHUK*, *CWF19L1*, *SLC25A28*, *SEMA4G*, *NOLC1*, *SFXN3*, *LOC112444563*, *LBX1*, *FGF8*, *BTRC*, *KCNIP2*, *LOC112444561*	C18:1 *cis*-9 (1, 3, 1.89), C18:1 *cis*-9 (2, 3, 1.58), C18:1 *trans* (1, 3, 1.78), C18:1 *trans* (2, 2, 1.04), C18:1 *trans* (2, 3, 1.12), LCFA (1, 3, 1.81), LCFA (2, 3, 1.75), MUFA (1, 3, 2.12), MUFA (2, 3, 1.62), PUFA (1, 3, 2.35), PUFA (2, 3, 2.48), PUFA (1, 2, 1.55), PUFA (2, 2, 1.59)

[1]The GWAS analyses were performed for all traits included for each parity, considering 3 stages of lactation: (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; and (3) from 201 to 365 DIM, representing the production decline up to the end of the lactation.

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

[3]The positions of the identified genomic regions based on the ARS-UCD1.2 assembly.

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

[5]SCFA = short-chain fatty acids (C4 to C10); MCFA = medium-chain fatty acids (C12 to C16); LCFA = long-chain fatty acids (C17 to C22).

and PUFA in the first lactation. This region was 2.1 Mb in size and harbors 21 genes.

BTA14. A genomic region located between 22.4 to 24.3 Mb on BTA14, which harbors 16 genes, was associated with C6:0 in the first lactation. The genomic region located between 23.9 to 25.9 Mb on BTA14 was associated with C16:0, MCFA, and SFA in the second lactation. The size of this region was 2.0 Mb and harbors 17 genes. The genomic region located between 24.5 to 26.8 Mb on BTA14 was associated with C16:0 and MCFA in the first lactation. This size of this region was 2.3 Mb and contains 23 genes. The region located between 25.4 to 27.2 Mb on BTA14 was associated with C4:0 in the first and second lactations. This region was 1.8 Mb in size and harbors 10 genes.

BTA19. The region located between 34.3 to 36.4 Mb on BTA19 was associated with C16:0, MCFA, SFA, PUFA, and LCFA in the first lactation. This region was also associated with C16:0 and LCFA in the second lactation. This region was 2.1 Mb in size and contains 49 genes including sterol regulatory element binding transcription factor 1 (*SREBF1*), retinoic acid induced 1 (*RAI1*), and phosphatidylethanolamine *N*-methyltransferase (*PEMT*).

BTA22. A genomic region located between 16.7 to 18.4 Mb on BAT22 was associated with SFA, C10:0, and C12:0. This region was 1.7 Mb in size and contains 32 genes.

BTA24. The genomic region located between 33.6 to 35.7 Mb on BTA24 was associated with C18:0 in the second lactation. The size of this region was 2.1 Mb and contains 22 genes.

BTA26. A region located between 20.4 to 22.9 Mb on BTA26 was associated with traits including C18:1 *cis*-9, C18:1 *trans*, LCFA, MUFA, and PUFA. This region was 2.5 Mb in size and harbors 54 genes including stearoyl-CoA desaturase (*SCD*), polycystin 2 like 1, transient receptor potential cation channel (*PKD2L1*), and β-transducin repeat containing E3 ubiquitin protein ligase (*BTRC*).

DISCUSSION

Mean relative proportion of SFA in DPBB was lower than those reported for Holstein and Jersey (Soyeurt et al., 2006, 2008; Bastin et al., 2013). Bastin et al. (2020) reported that milk fat produced by DPBB cows has a higher ratio of UFA to SFA than that produced by dairy breeds such as Holstein, Jersey, or Brown Swiss.

The mean h^2 estimates for the analyzed milk even-chain SFA ranged from 0.01 (C4:0) to 0.48 (C12:0) and 0.01 (C4:0) to 0.42 (C12:0) in the first and second lactations, respectively. The additive genetic variances estimated for C4:0, C6:0, and C8:0 showed high standard error (**SE**); therefore, their h^2 need to be interpreted with more caution. Moderate h^2 were estimated for C18:1 *trans* and C18:1 *cis*-9. In this study, milk FA were measured in g/dL milk. However, milk FA can also be expressed in g/100 g fat (Soyeurt et al., 2008; Stoop et al., 2008; Mele et al., 2009) or in g/d (Bobe et al., 2008). Milk FA expressed in g/dL milk and g/100 g fat are different traits, which should be taken into account when comparing different studies. Bobe et al. (2008) reported that h^2 estimated for individual even-chain SFA (C4.0 to C16.0; expressed in g/L milk) ranged from 0.01 (C4:0) to 0.40 (C10:0) in US Holstein cows. Stoop et al. (2008) reported that h^2 for individual even-chain SFA (C4.0 to C18.0; expressed in g/100 g fat) ranged from 0.19 (C18:0) to 0.54 (C10:0) in Dutch Holstein. Bastin et al. (2011) reported that h^2 for even-chain SFA (C4.0 to C18.0; expressed in g/dL milk) ranged from 0.23 (C18:0) to 0.44 (C8:0) in Walloon Holstein cows. In regard to chain length, our results showed moderate to high h^2 for SCFA and MCFA, whereas low to moderate h^2 was found for LCFA. Previous studied have already shown that SCFA and MCFA, which are synthesized de novo in the mammary gland, have moderate to high h^2; while LCFA, derived from blood lipids that originate mainly from the diet and endogenous lipids, have low to moderate h^2 (Bastin et al., 2011, 2013; Fleming et al., 2018). In regard to saturation of fat, h^2 estimates for SFA were higher than those estimated for MUFA and PUFA, in a close agreement with previous studies that used the same measuring unit (g/dL milk; Bobe et al., 2008; Soyeurt et al., 2008; Bastin et al., 2013; Fleming et al., 2018).

The results showed a weak positive genetic correlation between MY and SFA, and moderate negative genetic correlations between MY and UFA (MUFA and PUFA). Therefore, it can be concluded that genetic selection to increase MY in DPBB cows would result in a slight concomitant increase in the proportion of SFA and a more noticeable decrease in the proportion of UFA. Bastin et al. (2013) reported medium negative genetic correlation between MY and SFA, MUFA, and PUFA (expressed in g/dL milk) in Walloon Holstein cows. Petrini et al. (2016) reported moderate negative genetic correlations between MY and SFA and UFA (expressed in g/dL milk) in Holstein cows reared under tropical conditions. Moderate to high positive genetic correlations were found between FP and SFA, MUFA and PUFA, and low to moderate genetic correlations were found between PP and SFA, MUFA, and PUFA, which agree with previous studies (Bastin et al., 2013; Petrini et al., 2016; Fleming et al., 2018). Fleming et al. (2018) reported that SFA, UFA, SCFA, MCFA, and LCFA (expressed in g/dL milk) were positively genetically correlated with FP and negatively genetically cor-

related with MY and PP in Canadian Holstein cows. The genetic correlations found between MY and the analyzed individual FA, except for C18:0, C18:1 *trans*, and C18:1 *cis*, were positive. However, all analyzed individual FA showed positive genetic correlations with FP and PP. The heritability estimated for C4:0 was close to zero; hence, the estimated correlation between C4:0 and milk traits (MY, FP, and PP) should be interpreted with more caution. Bastin et al. (2013) reported that milk FA including C4:0 to C18:0 as well as C18:1 *cis*-9 were negatively genetically correlated with MY, while positive genetic correlations were found between these FA and FP and PP in Walloon Holstein cows. Petrini et al. (2016) reported negative genetic correlations between FP and C16:0, C18:0, and C18:1 (expressed in g/dL milk) in Holstein cows.

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 linkage disequilibrium, is not used in the GWAS depends on single SNP (Bao and Wang, 2017). Therefore, window-based GWAS procedure have been proposed as an effective procedure 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). However, the absence of a universal approach for hypothesis testing is an important challenge of window-based GWAS, even though it is quite a common procedure in genetic studies. Window-based GWAS may use different window types (distinct or sliding windows) and variable window sizes (defined as the number of SNP or the number of base pairs). 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 have not justified their choices or sometimes have acknowledged that their choices are arbitrary. For example, Han and Peñagaricano (2016) considered 1.5-Mb windows that explained more than 0.50% of the total genetic variance as the threshold to declare significance. Suwannasing et al. (2018) considered windows that explained more than 1% of the total genetic variance as the threshold to declare significance. Tiezzi et al. (2015) calculated the variance absorbed by 10-SNP moving windows and reported the 10 windows explaining the largest amount of genomic variance as most important windows. Fragomeni et al. (2014), examining different SNP window sizes, reported large noise with small window sizes and absence of peaks with large window sizes, and recommended windows of 20 SNPs as a reasonable size. In this study, a window-based GWAS through the single-step genomic BLUP was used. The results were presented by the proportion of total genetic variance explained by window of 25 adjacent SNPs with an average size of ~2 Mb and windows explaining for 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.

In total, 11 genomic regions distributed over 8 chromosomes [BTA1, BTA4, BTA10, BTA14 (4 regions), BTA19, BTA22, BTA24, and BTA26] were found to be linked with milk FA profile. The genomic regions identified differed between parities and lactation stages. The number of records was not equal between the first and second parities, nor between different DIM for each parity. Therefore, the SE of the estimated variance components and the power to detect QTL is expected to differ between parities and between lactation stages which can explain a part of the variation found in the identified genomic associations. However, it is known that the genetic variances for several milk production traits change during lactation and genetic correlations between these traits at different lactation stages differ from unity. Therefore, significant genotype by lactation stage interaction effects could be expected (Lu and Bovenhuis, 2019; Lu et al., 2020). In addition, MY and milk composition (e.g., milk FA content) are influenced by management practices, environmental conditions, animal physiological stage (e.g., parity, stage of lactation), and genetic merit of the animal, which may explain, at least in part, the variation found in the identified associations. For example, changes in genetic effects in early lactation might be related to negative energy balance (**NEB**) and those in late lactation might be caused by pregnancy (Lu and Bovenhuis, 2020).

The genomic region located between 143.6 to 145.2 Mb on BTA1 was associated with MUFA. The association between SNPs inside this region with traits including MY (Iung et al., 2019; Silva et al., 2020), fat yield (**FY**), protein yield (**PY**; Yang et al., 2015), FP, PP (Pausch et al., 2017; Atashi et al., 2022c), and SFA, UFA, and PUFA (expressed in g/100 g fat; Olsen et al., 2017; Shi et al., 2021) has been previously reported in various breeds of cattle. Olsen et al. (2017) reported significant association between SNPs inside this region and C6:0 to C12:0, C15:0, C18:1 *trans*-11, and PUFA (expressed in g/100 g fat). Li et al. (2014) detected significant associations between SNPs inside this region and C18:0 (expressed in g/100 g fat) at 146 Mb in

Chinese Holstein. Atashi et al. (2022a) reported that this region is associated with cheese-making properties including titratable acidity, casein percentage, calcium content, curd firmness, and coagulation time in DPBB cows. Wang and Bovenhuis (2018) reported that the genomic region located between 145.4 to 147.3 Mb on BTA1 is linked with selected wavenumbers on milk MIR in Dutch Holstein. This region harbors genes including *PDE9A*, *ABCG1*, and *SLC37A1.* The *PDE9A* is highly expressed in mammary glands and is associated with milk production traits (Yang et al., 2015). The protein encoded by *PDE9A* catalyzes the hydrolysis of cAMP and cGMP to their corresponding monophosphates and plays a role in signal transduction by regulating the intracellular concentration of these cyclic nucleotides. The *ABCG1* involves in macrophage cholesterol and phospholipid transport and may regulate cellular lipid homeostasis in other cell types (Klucken et al., 2000). This region also contains the *SLC37A1* gene, which encodes a membrane bound protein involved in the translocation of glycerol-3-phosphate to the endoplasmic reticulum (Bartoloni et al., 2000). Buitenhuis et al. (2015) and Kemper et al. (2016) suggested *SLC37A1* as the most likely candidate gene for milk phosphorus concentration. The *SLC37A1* has been reported to be associated with FP, PP, titratable acidity, casein percentage, calcium content, milk, curd firmness, and casein percentage in DPBB cows (Atashi et al., 2022a,c).

The genomic region located between 41.6 to 44.2 Mb on BTA4 was associated with C18:0. da Cruz et al. (2021) reported that this region is associated with 305-d MY in Girolando crossbreed cows. The gamma-secretase activating protein (*GSAP*) gene, located in this region, has been reported to be associated with clinical mastitis and SCS in Holstein cows (Asselstine et al., 2019). The genomic region located between 44.4 to 46.5 Mb on BTA10 was associated with C18:0, C18:1 *trans*, and PUFA. Bastin et al. (2013) reported a high positive genetic correlation between PUFA and C18:0 in Holstein cows. Previous studies showed that this region is associated with MY traits (Georges et al., 1995; Jiang et al., 2019; Pedrosa et al., 2021) and milk FA profile (Knutsen et al., 2018). Atashi et al. (2022c) reported that this region is associated with MY, PY, and SCS in DPBB cows. Among functional genes identified inside this region, the death associated protein kinase 2 (*DAPK2*) has been found to be linked with PP and FP in Holstein cows (Kolbehdari et al., 2009; Jiang et al., 2019), and FP in Buffalo (Vohra et al., 2021).

Four genomic regions, with a total size of 4.8 Mb and more or less overlapping, on BTA14 were associated with milk FA profile. These regions are located between 22.4 to 24.3 Mb, 23.9 to 25.9 Mb, 24.5 to 26.8 Mb, and 25.4 to 27.2 Mb on BTA14. The overlaps found among the identified regions may be partly explained by the difference in power of QTL detection caused by unequal number of records in the first and second parities or in lactation stages of each parity. The genomic region located between 22.4 to 24.3 Mb on BTA14 was associated with C6:0 in the first lactation. This region has been previously reported to be associated with MY, PY, FP, and PP (Lund et al., 2008; Cole et al., 2011; Marques et al., 2011) in dairy cows. The ATPase H^+ transporting V1 subunit H (*ATP6V1H*) is located in this region and has been reported to be linked with residual feed intake in Australian Angus cattle (de las Heras-Saldana et al., 2019). The additive genetic variance estimated for C6:0 showed high SE; therefore, the association found between this genomic region and C6:0 should be interpreted more conservatively. The genomic region located between 23.9 to 25.9 Mb on BTA14 was linked with C16:0, MCFA and SFA in the second lactation. The C16:0 is grouped into MCFA and SFA categories; therefore, these traits are correlated and can be affected be the same genes. Bastin et al. (2013) reported high positive genetic correlations (0.97) among C16:0, SFA, and MCFA (expressed in g/dL milk) in Walloon Holstein cows. This region has been previously reported to be associated with MY, FY, PY, FP, and PP in various cattle breeds (Marques et al., 2011; Fink et al., 2017); however, its association with milk FA profile has not been previously reported. Among functional genes identified inside this region, *SDR16C5* has been reported to be associated with FP in Holstein dairy cows (Marques et al., 2011), and feed efficiency and growth traits in beef cattle (Seabury et al., 2017). The genomic region located between 24.5 to 26.8 Mb on BTA14 was associated with C16:0 and MCFA. Previous studies showed that this region is associated with MY, FY, PY, FP, PP, C13:0, and CLA (expressed in g/100 g fat; Marques et al., 2011; Strillacci et al., 2014; Ibeagha-Awemu et al., 2016; Fink et al., 2017). The region located between 25.4 to 27.2 Mb on BTA14 was associated with C4:0. This region has been previously found to be associated with traits including MY, FY, PY, PP, and CLA (expressed in g/100 g fat) (Boichard et al., 2003; Marques et al., 2011; Ibeagha-Awemu et al., 2016; Jiang et al., 2019). The additive genetic variance estimated for C4:0 showed high SE and its estimated h^2 was close to zero; therefore, the association found between this region and C4:0 should be interpreted with caution.

A base substitution in exon 8 (*K232A*) of the *DGAT1* gene has been proposed as an important gene affecting milk production traits (Grisart et al., 2002). In the used SNP data, rs109421300 [located on position

1,801,116 bp of BTA14 (UMD3.1 assembly)] was the only SNP located in *DGAT1*. The SNP rs109421300 is 1,153 bp upstream of the *K232A* causal mutation and has been reported to be associated with MY, fat yield, and protein yield in Holstein cows (Jiang et al., 2019). However, windows harboring rs109421300 did not explain a considerable part of the total additive genetic variance of the analyzed traits in our study. It can be concluded that the SNP rs109421300 is not in linkage disequilibrium with *K232A* in the DPBB population. Furthermore, the association between *DGAT1* and milk production traits varied among breeds and populations. For example, Oliveira et al. (2019) reported that genomic regions associated with MY were located on BTA11, BTA16, and BTA28 for the Ayrshire; BTA6, BTA13, and BTA14 (multiple genes including *DGAT1*) for the Holstein; and BTA2, BTA5, BTA11, BTA20, BTA26, and BTA27 for the Jersey breed. Lu and Bovenhuis (2020) reported that the effect of pregnancy on milk production traits differed between cows with different *DGAT1* genotypes.

The region located between 34.3 to 36.4 Mb on BTA19 was associated with C16:0, SFA, MCFA, LCFA, MUFA, and PUFA. Bastin et al. (2013) reported high positive genetic correlations among C16:0, SFA, MCFA, LCFA, MUFA, and PUFA in Walloon Holstein cows which may explain why these traits are affected by the same genomic region. This region has been previously found to be associated with FY, FP, and milk FA profile (expressed in g/100 g fat) in dairy cows (Bouwman et al., 2011; Sasago et al., 2017; Pedrosa et al., 2021). The region located between 33 to 62 Mb on BTA19 has been reported to be linked with multiple milk FA, in particular, de novo synthesized FA C8:0, C10:0, C12:0 and C14:0 (expressed in g/100 g fat; Bouwman et al., 2012; Li et al., 2014; Gebreyesus et al., 2019). Among functional genes identified inside this region, *SREBF1*, *RAI1*, and *PEMT* have been reported to be associated with FP and milk FA profile (expressed in g/100 g fat; Conte et al., 2010; Nafikov et al., 2013; Strillacci et al., 2014; Jiang et al., 2019). The gene *SREBF1*, known as a key player in FA synthesis (Bionaz and Loor, 2008), was shown to be associated with MIR milk spectral data in Danish Holstein and Danish Jersey (Zaalberg et al., 2020). Tiplady et al. (2021) identified a few QTL located between 42 to 57 Mb on BTA19 for milk MIR spectral in mixed-breed dairy cattle.

The genomic region located between 16.7 to 18.4 Mb on BTA22 was associated with C10:0, C12:0, and SFA. Bastin et al. (2013) reported that SFA is highly genetically correlated with C10:0 and C12:0, and that genetic correlation between C10:0 and C12:0 is also positive and close to 1. This region has been reported to be associated with MY (Cochran et al., 2013), PY (Ashwell et al., 2004), C10:0, and C14:0 (expressed in g/100 g fat) in Holstein cows (Bouwman et al., 2011), FP in DPBB cows (Atashi et al., 2022c). The genomic region located between 33.6 to 35.7 Mb on BTA24 was associated with C18:0. This region has been showed to be associated with FY and PY in Holstein cows (Daetwyler et al., 2008; Oliveira et al., 2019), and meat FA contents in Jersey and Limousin back-cross (Morris et al., 2010).

The region located between 20.4 to 22.9 Mb on BTA26 was associated with C18:1 *cis*-9, C18:1 *trans*, LCFA, MUFA, and PUFA. Bastin et al. (2013) reported that genetic correlations among C18:1 *cis*-9, LCFA, MUFA, and PUFA ranged from 0.91 to 0.97 in Holstein cows. Several studies have reported this region as an important region associated with milk production traits (Jiang et al., 2019) and individual and groups of milk FA (expressed in g/100 g fat) in dairy cattle (Bouwman et al., 2012; Li et al., 2014; Gebreyesus et al., 2019; Shi et al., 2019). Atashi et al. (2022c) reported that this region is associated with FP and PP in DPBB cows. Among functional genes located inside this region, *SCD (*also known as *SCD1*), *HIF1AN*, *WNT8B*, *DNMBP*, *LOC511498*, *PKD2L1*, *LZTS2*, *NFKB2*, *PAX2*, *CHUK*, *SLC25A28*, and *BTRC* have been reported to be associated milk FA (expressed in g/100 g fat) in Holstein cows (Li et al., 2014), and FP and PP in DPBB cows (Atashi et al., 2022c).

CONCLUSIONS

This is the first study to estimate genetic parameters and identify genomic regions associated with milk FA profile in the DPBB population. The genetic variability of milk FA in combination with their moderate to high heritabilities indicate that the milk FA profile could be changed by genetic selection; however, desirable direction of change in these traits needs to be defined. Several genomic regions were identified to be associated with milk FA profile in DPBB cows; however, the genomic regions identified differed between parities and lactation stages. It can be hypothesized that part of the changes in effects of genomic regions on milk FA traits during lactation may be the result of NEB in early lactation and pregnancy effects in late lactation, although exact mechanisms underlying the changing effects during lactation are still unknown. The number of records was not equal between the first and second parities, nor between different DIM for each parity, which may affect the power of QTL detection. Additional research about the relationships between milk FA profile and other important traits such as longevity, cheese-making traits, metabolic diseases, and fertility are needed.

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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. 51:28. 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 program. Paper presented at the Proceedings of the 10th World Congress on Genetics Applied to Livestock Production, Vancouver, BC, Canada.

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. Paper presented at the Proceedings of the 11th World Congress on Genetics Applied to Livestock Production, Auckland, New Zealand.

Ashwell, M. S., D. W. Heyen, T. S. Sonstegard, C. P. Van Tassell, Y. Da, P. M. VanRaden, M. Ron, J. I. Weller, and H. A. Lewin. 2004. Detection of quantitative trait loci affecting milk production, health, and reproductive traits in Holstein cattle. J. Dairy Sci. 87:468–475. https://doi.org/10.3168/jds.S0022-0302(04)73186-0.

Asselstine, V., F. Miglior, A. Suárez-Vega, P. Fonseca, B. Mallard, N. Karrow, A. Islas-Trejo, J. Medrano, and A. Cánovas. 2019. Genetic mechanisms regulating the host response during mastitis. J. Dairy Sci. 102:9043–9059. https://doi.org/10.3168/jds.2019-16504.

Atashi, H., C. Bastin, H. Soyeurt, F. Dehareng, H. Wilmot, S. Vanderick, and N. Gengler. 2021. Genetic parameters of butter softness and spreadability as new traits in Dual Purpose Belgian Blue. Interbull Bull. 56:167–171.

Atashi, H., C. Bastin, H. Wilmot, S. Vanderick, X. Hubin, and N. Gengler. 2022a. Genome-wide association study for selected cheese-making properties in Dual-Purpose Belgian Blue cows. J. Dairy Sci. 105:8972–8988. https://doi.org/10.3168/jds.2022-21780.

Atashi, H., M. Salavati, J. De Koster, J. Ehrlich, M. Crowe, and G. Opsomer. the GplusE Consortium, and M. Hostens. 2020. Genome-wide association for milk production and lactation curve parameters in Holstein dairy cows. J. Anim. Breed. Genet. 137:292–304. https://doi.org/10.1111/jbg.12442.

Atashi, H., H. Wilmot, and N. Gengler. 2022b. The pattern of linkage disequilibrium in Dual-Purpose Belgian Blue cattle. J. Anim. Breed. Genet. 139:320–329. https://doi.org/10.1111/jbg.12662.

Atashi, H., H. Wilmot, S. Vanderick, X. Hubin, and N. Gengler. 2022c. Genome-wide association study for milk production traits in Dual-Purpose Belgian Blue cows. Livest. Sci. 256:104831. https://doi.org/10.1016/j.livsci.2022.104831.

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.

Bartoloni, L., M. Wattenhofer, J. Kudoh, A. Berry, K. Shibuya, K. Kawasaki, J. Wang, S. Asakawa, I. Talior, B. Bonne-Tamir, C. Rossier, J. Michaud, E. R. B. McCabe, S. Minoshima, N. Shimizu, H. S. Scott, and S. E. Antonarakis. 2000. Cloning and characterization of a putative human glycerol 3-phosphate permease gene (SLC37A1 or G3PP) on 21q22. 3: Mutation analysis in two candidate phenotypes, DFNB10 and a glycerol kinase deficiency. Genomics 70:190–200. https://doi.org/10.1006/geno.2000.6395.

Bastin, C., F. Dehareng, N. Gengler, M. Sindic, E. Piraux, H. Soyeurt, H. Wilmot, C. Bertozzi, J. Leblois, and F. Colinet. 2020. Advanced monitoring of milk quality to address the demand of added-value dairy products. Paper presented at the Proceedings of the 71st Annual Meeting of the European Federation of Animal Science. Wageningen Academic Publishers.

Bastin, C., N. Gengler, and H. Soyeurt. 2011. Phenotypic and genetic variability of production traits and milk fatty acid contents across days in milk for Walloon Holstein first-parity cows. J. Dairy Sci. 94:4152–4163. https://doi.org/10.3168/jds.2010-4108.

Bastin, C., H. Soyeurt, and N. Gengler. 2013. Genetic parameters of milk production traits and fatty acid contents in milk for Holstein cows in parity 1–3. J. Anim. Breed. Genet. 130:118–127. https://doi.org/10.1111/jbg.12010.

Bionaz, M., and J. J. Loor. 2008. Gene networks driving bovine milk fat synthesis during the lactation cycle. BMC Genomics 9:366. https://doi.org/10.1186/1471-2164-9-366.

Bobe, G., J. Minick Bormann, G. Lindberg, A. Freeman, and D. Beitz. 2008. Estimates of genetic variation of milk fatty acids in US Holstein cows. J. Dairy Sci. 91:1209–1213. https://doi.org/10.3168/jds.2007-0252.

Bobe, G., E. Hammond, A. Freeman, G. Lindberg, and D. Beitz. 2003. Texture of butter from cows with different milk fatty acid compositions. J. Dairy Sci. 86:3122–3127. https://doi.org/10.3168/jds.S0022-0302(03)73913-7.

Bobe, G., S. Zimmerman, E. G. Hammond, A. Freeman, P. A. Porter, C. M. Luhman, and D. C. Beitz. 2007. Butter composition and texture from cows with different milk fatty acid compositions fed fish oil or roasted soybeans. J. Dairy Sci. 90:2596–2603. https://doi.org/10.3168/jds.2006-875.

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., H. Bovenhuis, M. H. Visker, and J. A. van Arendonk. 2011. Genome-wide association of milk fatty acids in Dutch dairy cattle. BMC Genet. 12:43. https://doi.org/10.1186/1471-2156-12-43.

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. 13:93. https://doi.org/10.1186/1471-2156-13-93.

Buitenhuis, B., N. A. Poulsen, L. B. Larsen, and J. Sehested. 2015. Estimation of genetic parameters and detection of quantitative trait loci for minerals in Danish Holstein and Danish Jersey milk. BMC Genet. 16:52. https://doi.org/10.1186/s12863-015-0209-9.

Chen, S., G. Bobe, S. Zimmerman, E. G. Hammond, C. M. Luhman, T. D. Boylston, A. E. Freeman, and D. C. Beitz. 2004. Physical and sensory properties of dairy products from cows with various milk fatty acid compositions. J. Agric. Food Chem. 52:3422–3428. https://doi.org/10.1021/jf035193z.

Cochran, S. D., J. B. Cole, D. J. Null, and P. J. Hansen. 2013. Discovery of single nucleotide polymorphisms in candidate genes associated with fertility and production traits in Holstein cattle. BMC Genet. 14:49. https://doi.org/10.1186/1471-2156-14-49.

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 12:408. https://doi.org/10.1186/1471-2164-12-408.

Conte, G., M. Mele, S. Chessa, B. Castiglioni, A. Serra, G. Pagnacco, and P. Secchiari. 2010. Diacylglycerol acyltransferase 1, stearoyl-CoA desaturase 1, and sterol regulatory element binding protein 1 gene polymorphisms and milk fatty acid composition in Italian Brown cattle. J. Dairy Sci. 93:753–763. https://doi.org/10.3168/jds.2009-2581.

Couvreur, S., C. Hurtaud, C. Lopez, L. Delaby, and J.-L. Peyraud. 2006. The linear relationship between the proportion of fresh grass in the cow diet, milk fatty acid composition, and butter properties. J. Dairy Sci. 89:1956–1969. https://doi.org/10.3168/jds.S0022-0302(06)72263-9.

da Cruz, A. S., D. C. Silva, L. B. Minasi, L. K. de Farias Teixeira, F. M. Rodrigues, C. C. da Silva, A. S. do Carmo, M. V. G. B. da Silva, Y. T. Utsunomiya, J. F. Garcia, and A. D. da Cruz. 2021. Single-nucleotide polymorphism variations associated with specific genes putatively identified enhanced genetic predisposition for 305-day milk yield in the Girolando crossbreed. Front. Genet. 11:573344. https://doi.org/10.3389/fgene.2020.573344.

Daetwyler, H. D., F. S. Schenkel, M. Sargolzaei, and J. A. B. Robinson. 2008. A genome scan to detect quantitative trait loci for economically important traits in Holstein cattle using two methods and a dense single nucleotide polymorphism map. J. Dairy Sci. 91:3225–3236. https://doi.org/10.3168/jds.2007-0333.

de las Heras-Saldana, S., S. A. Clark, N. Duijvesteijn, C. Gondro, J. H. van der Werf, and Y. Chen. 2019. Combining information from genome-wide association and multi-tissue gene expression studies to elucidate factors underlying genetic variation for residual feed intake in Australian Angus cattle. BMC Genomics 20:939. https://doi.org/10.1186/s12864-019-6270-4.

Fink, T., K. Tiplady, T. Lopdell, T. Johnson, R. G. Snell, R. J. Spelman, S. R. Davis, and M. D. Littlejohn. 2017. Functional confirmation of PLAG1 as the candidate causative gene underlying major pleiotropic effects on body weight and milk characteristics. Sci. Rep. 7:44793. https://doi.org/10.1038/srep44793.

Fleming, A., F. Schenkel, F. Malchiodi, R. Ali, B. Mallard, M. Sargolzaei, J. Jamrozik, J. Johnston, and F. Miglior. 2018. Genetic correlations of mid-infrared-predicted milk fatty acid groups with milk production traits. J. Dairy Sci. 101:4295–4306. https://doi.org/10.3168/jds.2017-14089.

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.

Freitas, P. H. F., H. R. Oliveira, F. R. Silva, A. Fleming, F. S. Schenkel, F. Miglior, and L. F. Brito. 2020. Time-dependent genetic parameters and single-step genome-wide association analyses for predicted milk fatty acid composition in Ayrshire and Jersey dairy cattle. J. Dairy Sci. 103:5263–5269. https://doi.org/10.3168/jds.2019-17820.

Gebreyesus, G., A. Buitenhuis, N. Poulsen, M. Visker, Q. Zhang, H. 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.

Gengler, N., P. Mayeres, and M. Szydlowski. 2007. A simple method to approximate gene content in large pedigree populations: Application to the myostatin gene in dual-purpose Belgian Blue cattle. Animal 1:21–28. https://doi.org/10.1017/S1751731107392628.

Georges, M., D. Nielsen, M. Mackinnon, A. Mishra, R. Okimoto, A. T. Pasquino, L. S. Sargeant, A. Sorensen, M. R. Steele, and X. Zhao. 1995. Mapping quantitative trait loci controlling milk production in dairy cattle by exploiting progeny testing. Genetics 139:907–920. https://doi.org/10.1093/genetics/139.2.907.

Gonzalez, S., S. Duncan, S. O'Keefe, S. Sumner, and J. Herbein. 2003. Oxidation and textural characteristics of butter and ice cream with modified fatty acid profiles. J. Dairy Sci. 86:70–77. https://doi.org/10.3168/jds.S0022-0302(03)73585-1.

Grisart, B., W. Coppieters, F. Farnir, L. Karim, C. Ford, P. Berzi, N. Cambisano, M. Mni, S. Reid, P. Simon, R. Spelman, M. Georges, and R. Snell. 2002. Positional candidate cloning of a QTL in dairy cattle: identification of a missense mutation in the bovine DGAT1 gene with major effect on milk yield and composition. Genome Res. 12:222–231. https://doi.org/10.1101/gr.224202.

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

Han, Y., and F. Peñagaricano. 2016. Unravelling the genomic architecture of bull fertility in Holstein cattle. BMC Genet. 17:143. https://doi.org/10.1186/s12863-016-0454-6.

Hein, L., L. P. Sørensen, M. Kargo, and A. J. Buitenhuis. 2018. Genetic analysis of predicted fatty acid profiles of milk from Danish Holstein and Danish Jersey cattle populations. J. Dairy Sci. 101:2148–2157. https://doi.org/10.3168/jds.2017-13225.

Ibeagha-Awemu, E. M., S. O. Peters, K. A. Akwanji, I. G. Imumorin, and X. Zhao. 2016. High density genome wide genotyping-by-sequencing and association identifies common and low frequency SNPs, and novel candidate genes influencing cow milk traits. Sci. Rep. 6:31109. https://doi.org/10.1038/srep31109.

Iung, L. H. S., J. Petrini, J. Ramírez-Díaz, M. Salvian, G. A. Rovadoscki, F. Pilonetto, B. D. Dauria, P. F. Machado, L. L. Coutinho, G. R. 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.

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

Kemper, K. E., M. D. Littlejohn, T. Lopdell, B. J. Hayes, L. E. Bennett, R. P. Williams, X. Q. Xu, P. M. Visscher, M. J. Carrick, and M. E. Goddard. 2016. Leveraging genetically simple traits to identify small-effect variants for complex phenotypes. BMC Genomics 17:858. https://doi.org/10.1186/s12864-016-3175-3.

Klucken, J., C. Büchler, E. Orsó, W. E. Kaminski, M. Porsch-Özcürümez, G. Liebisch, M. Kapinsky, W. Diederich, W. Drobnik, M. Dean, R. Allikmets, and G. Schmitz. 2000. ABCG1 (ABC8), the human homolog of the Drosophila white gene, is a regulator of macrophage cholesterol and phospholipid transport. Proc. Natl. Acad. Sci. USA 97:817–822. https://doi.org/10.1073/pnas.97.2.817.

Knutsen, T. M., H. G. Olsen, I. A. Ketto, K. K. Sundsaasen, A. Kohler, V. Tafintseva, M. Svendsen, M. P. Kent, and S. Lien. 2022. Genetic variants associated with two major bovine milk fatty acids offer opportunities to breed for altered milk fat composition. Genet. Sel. Evol. 54:35. https://doi.org/10.1186/s12711-022-00731-9.

Knutsen, T. M., H. G. Olsen, V. Tafintseva, M. Svendsen, A. Kohler, M. P. Kent, and S. Lien. 2018. Unravelling genetic variation underlying de novo-synthesis of bovine milk fatty acids. Sci. Rep. 8:2179. https://doi.org/10.1038/s41598-018-20476-0.

Kolbehdari, D., Z. Wang, J. Grant, B. Murdoch, A. Prasad, Z. Xiu, E. Marques, P. Stothard, and S. Moore. 2009. A whole genome scan to map QTL for milk production traits and somatic cell score in Canadian Holstein bulls. J. Anim. Breed. Genet. 126:216–227. https://doi.org/10.1111/j.1439-0388.2008.00793.x.

Langley, M. R., E. M. Triplet, and I. A. Scarisbrick. 2020. Dietary influence on central nervous system myelin production, injury, and regeneration. Biochim. Biophys. Acta Mol. Basis Dis. 1886:165779. https://doi.org/10.1016/j.bbadis.2020.165779.

Lanier, J. S., and B. A. Corl. 2015. Challenges in enriching milk fat with polyunsaturated fatty acids. J. Anim. Sci. Biotechnol. 6:26. https://doi.org/10.1186/s40104-015-0025-0.

Li, C., D. Sun, S. Zhang, S. Wang, X. Wu, Q. Zhang, L. Liu, Y. Li, and L. Qiao. 2014. Genome wide association study identifies 20 novel promising genes associated with milk fatty acid traits in Chinese Holstein. PloS One 9:e96186. https://doi.org/10.1371/journal.pone.0096186.

Lock, A. L., and D. E. Bauman. 2004. Modifying milk fat composition of dairy cows to enhance fatty acids beneficial to human health. Lipids 39:1197–1206. https://doi.org/10.1007/s11745-004-1348-6.

Lu, H., and H. Bovenhuis. 2019. Genome-wide association studies for genetic effects that change during lactation in dairy cattle. J. Dairy Sci. 102:7263–7276. https://doi.org/10.3168/jds.2018-15994.

Lu, H., and H. Bovenhuis. 2020. Phenotypic and genetic effects of pregnancy on milk production traits in Holstein-Friesian cattle. J. Dairy Sci. 103:11597–11604. https://doi.org/10.3168/jds.2020-18561.

Lu, H., Y. Wang, and H. Bovenhuis. 2020. Genome-wide association study for genotype by lactation stage interaction of milk production traits in dairy cattle. J. Dairy Sci. 103:5234–5245. https://doi.org/10.3168/jds.2019-17257.

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. https://doi.org/10.1186/1297-9686-40-2-177.

Marques, E., J. Grant, Z. Wang, D. Kolbehdari, P. Stothard, G. Plastow, and S. Moore. 2011. Identification of candidate markers on bovine chromosome 14 (BTA14) under milk production trait quantitative trait loci in Holstein. J. Anim. Breed. Genet. 128:305–313. https://doi.org/10.1111/j.1439-0388.2010.00910.x.

Mele, M., R. Dal Zotto, M. Cassandro, G. Conte, A. Serra, A. Buccioni, G. Bittante, and P. Secchiari. 2009. Genetic parameters for conjugated linoleic acid, selected milk fatty acids, and milk fatty acid unsaturation of Italian Holstein-Friesian cows. J. Dairy Sci. 92:392–400. https://doi.org/10.3168/jds.2008-1445.

Morris, C. A., C. D. K. Bottema, N. G. Cullen, S. M. Hickey, A. K. Esmailizadeh, B. D. Siebert, and W. S. Pitchford. 2010. Quantitative trait loci for organ weights and adipose fat composition in Jersey and Limousin back-cross cattle finished on pasture or feedlot. Anim. Genet. 41:589–596. https://doi.org/10.1111/j.1365-2052.2010.02058.x.

Mota, R. R., P. Mayeres, C. Bastin, G. Glorieux, C. Bertozzi, S. Vanderick, H. Hammami, F. Colinet, and N. Gengler. 2017. Genetic evaluation for birth and conformation traits in dual-purpose Belgian Blue cattle using a mixed inheritance model. J. Anim. Sci. 95:4288–4299. https://doi.org/10.2527/jas2017.1748.

Nafikov, R. A., J. P. Schoonmaker, K. T. Korn, K. Noack, D. J. Garrick, K. J. Koehler, J. Minick-Bormann, J. M. Reecy, D. E. Spurlock, and D. C. Beitz. 2013. Sterol regulatory element binding transcription factor 1 (SREBF1) polymorphism and milk fatty acid composition. J. Dairy Sci. 96:2605–2616. https://doi.org/10.3168/jds.2012-6075.

Narayana, S. G., F. Schenkel, A. Fleming, A. Koeck, F. Malchiodi, J. Jamrozik, J. Johnston, M. Sargolzaei, and F. Miglior. 2017. Genetic analysis of groups of mid-infrared predicted fatty acids in milk. J. Dairy Sci. 100:4731–4744. https://doi.org/10.3168/jds.2016-12244.

Ning, C., D. Wang, X. Zheng, Q. Zhang, S. Zhang, R. Mrode, and J. F. Liu. 2018. Eigen decomposition expedites longitudinal genome-wide association studies for milk production traits in Chinese Holstein. Genet. Sel. Evol. 50:12. https://doi.org/10.1186/s12711-018-0383-0.

Oliveira, H. R., J. P. Cant, L. F. Brito, F. L. B. Feitosa, T. C. S. Chud, P. A. S. Fonseca, J. Jamrozik, F. F. Silva, D. A. L. Lourenco, and F. S. Schenkel. 2019. Genome-wide association for milk production traits and somatic cell score in different lactation stages of Ayrshire, Holstein, and Jersey dairy cattle. J. Dairy Sci. 102:8159–8174. https://doi.org/10.3168/jds.2019-16451.

Olsen, H. G., T. M. Knutsen, A. Kohler, M. Svendsen, L. Gidskehaug, H. Grove, T. Nome, M. Sodeland, K. K. Sundsaasen, M. P. Kent, H. Martens, and S. Lien. 2017. Genome-wide association mapping for milk fat composition and fine mapping of a QTL for de novo synthesis of milk fatty acids on bovine chromosome 13. Genet. Sel. Evol. 49:20. https://doi.org/10.1186/s12711-017-0294-5.

Pausch, H., R. Emmerling, B. Gredler-Grandl, R. Fries, H. D. Daetwyler, and M. E. Goddard. 2017. Meta-analysis of sequence-based association studies across three cattle breeds reveals 25 QTL for fat and protein percentages in milk at nucleotide resolution. BMC Genomics 18:853. https://doi.org/10.1186/s12864-017-4263-8.

Pedrosa, V. B., F. S. Schenkel, S.-Y. Chen, H. R. Oliveira, T. M. Casey, M. G. Melka, and L. F. Brito. 2021. Genome-wide 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.

Pegolo, S., A. Cecchinato, J. Casellas, G. Conte, M. Mele, S. Schiavon, and G. Bittante. 2016. Genetic and environmental relationships of detailed milk fatty acids profile determined by gas chromatography in Brown Swiss cows. J. Dairy Sci. 99:1315–1330. https://doi.org/10.3168/jds.2015-9596.

Petrini, J., L. H. S. Iung, M. A. P. Rodriguez, M. Salvian, F. Pértille, G. A. Rovadoscki, L. D. Cassoli, L. L. Coutinho, P. F. Machado, G. Wiggans, and G. B. Mourão. 2016. Genetic parameters for milk fatty acids, milk yield and quality traits of a Holstein cattle population reared under tropical conditions. J. Anim. Breed. Genet. 133:384–395. https://doi.org/10.1111/jbg.12205.

Rischkowsky, B., and D. Pilling. 2007. List of breeds documented in the Global Databank for Animal Genetic Resources. The state of the world's animal genetic resources for food and agriculture. FAO.

Sajdakowska, M., J. Gębski, and K. Gutkowska. 2021. Directions of changes in the health values of dairy products in the opinion of consumers. Nutrients 13:1945. https://doi.org/10.3390/nu13061945.

Sasago, N., T. Abe, H. Sakuma, T. Kojima, and Y. Uemoto. 2017. Genome-wide association study for carcass traits, fatty acid com-

position, chemical composition, sugar, and the effects of related candidate genes in Japanese Black cattle. Anim. Sci. J. 88:33–44. https://doi.org/10.1111/asj.12595.

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 18:386. https://doi.org/10.1186/s12864-017-3754-y.

Shi, L., L. Liu, X. Lv, Z. Ma, Y. Yang, Y. Li, F. Zhao, D. Sun, and B. Han. 2019. Polymorphisms and genetic effects of PRLR, MOGAT1, MINPP1 and CHUK genes on milk fatty acid traits in Chinese Holstein. BMC Genet. 20:69. https://doi.org/10.1186/s12863-019-0769-1.

Shi, L., X. Wu, Y. Yang, Z. Ma, X. Lv, L. Liu, Y. Li, F. Zhao, B. Han, and D. Sun. 2021. A post-GWAS confirming the genetic effects and functional polymorphisms of AGPAT3 gene on milk fatty acids in dairy cattle. J. Anim. Sci. Biotechnol. 12:24. https://doi.org/10.1186/s40104-020-00540-4.

Shingfield, K. J., M. Bonnet, and N. D. Scollan. 2013. Recent developments in altering the fatty acid composition of ruminant-derived foods. Animal 7(Suppl 1):132–162. https://doi.org/10.1017/S1751731112001681.

Shingfield, K. J., C. K. Reynolds, G. Hervás, J. M. Griinari, A. S. Grandison, and D. E. Beever. 2006. Examination of the persistency of milk fatty acid composition responses to fish oil and sunflower oil in the diet of dairy cows. J. Dairy Sci. 89:714–732. https://doi.org/10.3168/jds.S0022-0302(06)72134-8.

Silva, A. A., D. A. Silva, F. F. Silva, C. N. Costa, H. T. Silva, P. S. Lopes, R. Veroneze, G. Thompson, and J. Carvalheira. 2020. GWAS and gene networks for milk-related traits from test-day multiple lactations in Portuguese Holstein cattle. J. Appl. Genet. 61:465–476. https://doi.org/10.1007/s13353-020-00567-3.

Soyeurt, H., P. Dardenne, F. Dehareng, C. Bastin, and N. Gengler. 2008. Genetic parameters of saturated and monounsaturated fatty acid content and the ratio of saturated to unsaturated fatty acids in bovine milk. J. Dairy Sci. 91:3611–3626. https://doi.org/10.3168/jds.2007-0971.

Soyeurt, H., P. Dardenne, A. Gillon, C. Croquet, S. Vanderick, P. Mayeres, C. Bertozzi, and N. Gengler. 2006. Variation in fatty acid contents of milk and milk fat within and across breeds. J. Dairy Sci. 89:4858–4865. https://doi.org/10.3168/jds.S0022-0302(06)72534-6.

Soyeurt, H., F. Dehareng, N. Gengler, S. McParland, E. Wall, D. Berry, M. Coffey, and P. Dardenne. 2011. Mid-infrared prediction of bovine milk fatty acids across multiple breeds, production systems, and countries. J. Dairy Sci. 94:1657–1667. https://doi.org/10.3168/jds.2010-3408.

Staniewski, B., D. Ogrodowska, K. Staniewska, and J. Kowalik. 2021. The effect of triacylglycerol and fatty acid composition on the rheological properties of butter. Int. Dairy J. 114:104913. https://doi.org/10.1016/j.idairyj.2020.104913.

Stoop, W. M., J. A. M. Van Arendonk, J. M. L. Heck, H. J. F. Van Valenberg, and H. Bovenhuis. 2008. Genetic parameters for major milk fatty acids and milk production traits of Dutch Holstein-Friesians. J. Dairy Sci. 91:385–394. https://doi.org/10.3168/jds.2007-0181.

Strillacci, M. G., E. Frigo, F. Canavesi, Y. Ungar, F. Schiavini, L. Zaniboni, L. Reghenzani, M. Cozzi, A. Samoré, Y. Kashi, E. Shimoni, R. Tal-Stein, M. Soller, E. Lipkin, and A. Bagnato. 2014. Quantitative trait loci mapping for conjugated linoleic acid, vaccenic acid andΔ 9-desaturase in Italian Brown Swiss dairy cattle using selective DNA pooling. Anim. Genet. 45:485–499. https://doi.org/10.1111/age.12174.

Suwannasing, R., M. Duangjinda, W. Boonkum, R. Taharnklaew, and K. Tuangsithtanon. 2018. The identification of novel regions for reproduction trait in Landrace and Large White pigs using a single step genome-wide association study. Asian-Australas. J. Anim. Sci. 31:1852–1862. https://doi.org/10.5713/ajas.18.0072.

Thorning, T. K., A. Raben, T. Tholstrup, S. S. Soedamah-Muthu, I. Givens, and A. Astrup. 2016. Milk and dairy products: good or bad for human health? An assessment of the totality of scientific evidence. Food Nutr. Res. 60:32527. https://doi.org/10.3402/fnr.v60.32527.

Tiezzi, F., K. L. Parker-Gaddis, J. B. Cole, J. S. Clay, and C. Maltecca. 2015. A genome-wide association study for clinical mastitis in first parity US Holstein cows using single-step approach and genomic matrix re-weighting procedure. PLoS One 10:e0114919. https://doi.org/10.1371/journal.pone.0114919.

Tiplady, K. M., T. J. Lopdell, E. Reynolds, R. G. Sherlock, M. Keehan, T. J. Johnson, J. E. Pryce, S. R. Davis, R. J. Spelman, B. L. Harris, D. J. Garrick, and M. D. Littlejohn. 2021. Sequence-based genome-wide association study of individual milk mid-infrared wavenumbers in mixed-breed dairy cattle. Genet. Sel. Evol. 53:62. https://doi.org/10.1186/s12711-021-00648-9.

Toledo-Alvarado, H., A. I. Vazquez, G. de Los Campos, R. J. Tempelman, G. Gabai, A. Cecchinato, and G. Bittante. 2018. Changes in milk characteristics and fatty acid profile during the estrous cycle in dairy cows. J. Dairy Sci. 101:9135–9153. https://doi.org/10.3168/jds.2018-14480.

Vanhatalo, A., K. Kuoppala, V. Toivonen, and K. J. Shingfield. 2007. Effects of forage species and stage of maturity on bovine milk fatty acid composition. Eur. J. Lipid Sci. Technol. 109:856–867. https://doi.org/10.1002/ejlt.200700023.

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

Vohra, V., S. Chhotaray, G. Gowane, R. Alex, A. Mukherjee, A. Verma, and S. M. Deb. 2021. Genome-wide association studies in Indian Buffalo revealed genomic regions for lactation and fertility. Front. Genet. 12:696109. https://doi.org/10.3389/fgene.2021.696109.

Wang, Q., and H. Bovenhuis. 2018. Genome-wide association study for milk infrared wavenumbers. J. Dairy Sci. 101:2260–2272. https://doi.org/10.3168/jds.2017-13457.

Wiggans, G. R., T. S. Sonstegard, P. M. VanRaden, L. K. Matukumalli, R. D. Schnabel, J. F. Taylor, F. S. Schenkel, and C. P. 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.

Wilmot, H., G. Glorieux, X. Hubin, and N. Gengler. 2022. A genomic breed assignment test for traceability of meat of Dual-Purpose Blue. Livest. Sci. 263:104996. https://doi.org/10.1016/j.livsci.2022.104996.

Yang, S.-H., X.-J. Bi, Y. Xie, C. Li, S.-L. Zhang, Q. Zhang, and D.-X. Sun. 2015. Validation of PDE9A gene identified in GWAS showing strong association with milk production traits in Chinese Holstein. Int. J. Mol. Sci. 16:26530–26542. https://doi.org/10.3390/ijms161125976.

Zaalberg, R. M., L. Janss, and A. J. Buitenhuis. 2020. Genome-wide association study on Fourier transform infrared milk spectra for two Danish dairy cattle breeds. BMC Genet. 21:9. https://doi.org/10.1186/s12863-020-0810-4.

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