Bulgarian Journal of Agricultural Science, 30 (No 6) 2024, 1090–1096

GWAS analysis of milk composition traits in Ayrshire cattle breed

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Abstract

Dysin, A., Pozovnikova, M., Tulinova, O. & Scherbakov, Y. (2024). GWAS analysis of milk composition traits in Ayrshire cattle breed. *Bulg. J. Agric. Sci., 30*(6), 1090–1096

This research report presents a comprehensive analysis of the genetic basis of milk composition traits in the Ayrshire cattle breed using genome-wide association studies (GWAS) and single nucleotide polymorphisms (SNPs). Through GWAS analysis, significant SNPs associated with milk composition traits in Ayrshire cattle were identified, many of which were located near genes known to be involved in various biological processes associated with milk production. The study focused on traits such as basic fatty acids, somatic cell count, differential somatic cell counts, lactose, protein, pH, and Solid-Non-Fat Content. The highest number of identified SNPs was associated with somatic cell count in milk and fatty acids. These findings provide valuable insights into the genetic mechanisms underlying milk production and quality in Ayrshire cattle, with the potential to contribute to the development of tools for improving milk production and quality in the dairy industry. The study highlights the importance of understanding the complex genetic mechanisms involved in milk production and quality, specifically in the Ayrshire breed. Further research is warranted to explore the functional roles of the identified genes and their potential applications in breeding programs for enhanced milk production and quality.

Keywords: dairy cows; fatty acids; milk protein; lactose; SNP

Introduction

Dairy production is a critical part of the global economy, providing food, employment and income for millions of people (Peterson & Mitloehner, 2021). Milk characteristics such as milk yield, composition and quality are of great importance to the profitability of the industry (Nickerson, 1995). The Ayrshire cow breed is one of the most popular breeds in the global dairy industry due to its high productivity and milk quality. In Russia, this breed ranks third in terms of productivity – from 7600 to 8800 kg of milk per year, with fat content from 4.08% to 4.45% – after Holstein Black-Beast and Red-Beast breeds (Tulinova et al., 2021). Ayrshire cows are characterized by a moderate build, strong limbs, red-mottled color, high longevity, easy calving, and fairly high milk yields with excellent milk quality and high fat content (Gutierrez-Reinoso et al., 2021).

The study of cattle genetics and dairy traits has been a subject of interest to many researchers over the years. Several studies have investigated the genetic basis of dairy traits in dairy cattle, providing valuable insights into the complex genetic mechanisms underlying milk production and quality (Gutierrez-Reinoso, Aponte, & Garcia-Herreros, 2021; Liu et al., 2022; Peñagaricano, 2020; Yan et al., 2020;). Genome-wide association studies (GWAS) are widely used to identify genomic regions associated with mammary traits (Ariyarathne et al., 2021; Freebern et al., 2020; Jiang et al., 2019; Singh et al., 2022; Yan et al., 2020; Zhou et al., 2019), and single nucleotide polymorphisms (SNPs) are widely used as genetic markers in GWAS analyses (Ateya, Ibrahim, & Al-Sharif, 2022; da Cruz et al., 2020; Jayawardana et al., 2023; Teng et al., 2023; Ricardo Zamorano-Algandar et al., 2023). SNP genotyping allows the development of tools to

improve milk production and quality, for example, to better methods to select the most productive animals and to improve the efficiency of selection in the dairy industry (Koopaee & Koshkoiyeh, 2014; Worku et al., 2022).

As we know, milk is a complex mixture of different components, each of which plays a different role and function in animal physiology and human health. Therefore, it is important to study not only the total amount of milk produced by an animal, but also its individual components that determine its nutritional and technological value. For example, milk protein provides essential amino acids for human nutrition and fulfils various biological functions (Gross, 2022). Milk protein also affects milk cost and profitability, milk quality and processing, and animal health and welfare (Buchanan, 2002).

Fatty acids are also an important component of cattle milk as they provide energy, flavour and health benefits to consumers. Monounsaturated and polyunsaturated fatty acids are beneficial for blood lipid levels and cardiovascular health (Abdoul-Aziz, Zhang, & Wang, 2021; Bionaz, Vargas-Bello-Pérez, & Busato, 2020). In addition, cow's milk contains long-chain polyunsaturated essential fatty acids, eicosapentaenoic acid, arachidonic acid, and docosahexaenoic acid, which are important for the proper development of organs, tissues, and nervous system of calves (Anitaş & Göncü, 2018).

Somatic cell count (SCC) is the main indicator of milk quality and udder health in dairy cows. Most somatic cells are white blood cells that increase in response to pathogens that cause mastitis (Sharif & Muhammad, 2008). A high SCC indicates a high level of infection and inflammation in the udder, which can reduce milk yield, alter milk composition, impair milk processing and affect human health (Farschtschi, Mattes, & Pfaffl, 2022; Li et al., 2014; Wickström et al., 2009). Therefore, SCC is used as a criterion to determine the suitability of milk for human consumption.

In this study, we plan to perform GWAS analysis and SNP genotyping to determine the genetic factors affecting a number of milk parameters from the first lactation of Ayrshire cows.

Materials and Methods

Phenotype gathering

Samples were taken during scheduled control milkings of 600 cows, conducted in six farms (5 in the Leningrad and 1 in the Moscow region) during 10 months of the first lactation. The volume of each sample was 40-50 ml, and Broad Spectrum Microtabs II – micro tablets for preservation of milk samples (8 mg of bronopol and 0.3 mg of natanitsin) were used for preservation. Samples were stored in a refrigerator at +4°C. Samples were delivered to the laboratory no later than 4 days from the time of sampling. Raw milk samples were analysed on the basis of RRIFAGB — Branch of the L. K. Ernst Federal Science Centre for Animal Husbandry Collaboration Centre on 23 indicators using infrared analyser FOSS 7 DSCC (Denmark). The following parameters were evaluated: basic fatty acids (g/100 g milk): saturated FA (SFA), monounsaturated FA (MUFA), long-chain FA (LCFA), medium-chain FA (MCFA); SCC (ths. units/ml); differential somatic cell counts (DSCC) (the share of lymphocytes and PMN in the total cell count) (%); Lactose (%); Protein (%); active acidity (pH); Solid-Non-Fat Content (SNF) (%).

SNP genotyping

DNA samples were genotyped using an Illumina BovineSNP50v2 high-density chip (Illumina Inc., USA). DNA samples with genotyping quality of more than 95% at SNP loci were selected for further study. SNP filtering was performed using PLINK 1.9 software (Chang et al., 2015) and minor allele frequency (MAF) > 0.05. After SNP filtering, 45470 variants were available for further analysis.

GWAS

GWAS was performed using EMMAX software and a state-wise kinship matrix generated by EMMAX (Kang et al., 2010). For GWAS analyses, the obtained data for 10 milk parameters for each individual (SFA, MUFA, LCFA, MCFA, SCC, DSCC, lactose, total protein, pH, SNF) were used. The following model was implemented to calculate the effect of SNPs on the trait:

$$Y = Xb + u + e,$$

where Y is the vector of phenotypes; b is the SNP effect; X is the SNP genotype design matrix; u is the vector of additive genetic effects, which is assumed to be normally distributed with mean equal to 0 and (co)variance σ2aG, where σ2a is the additive genetic variance and G is the genomic relationship matrix; e is the vector of random residual effects.

Significance and estimated levels for SNP effects were set using Bonferroni correction to exclude false positives ($1.09962*10^{-6}$ (0.05/45470) and $2.199252*10^{-5}$ (1.00/45470), respectively. Whole-genome significance was assessed using the simpleM in R method, and the calculation of the effective number of independent tests was performed using M_{eff} (Gao, 2011).

Manhattan and quantile-quantile (Q-Q) plots were constructed based on GWAS results and using the qqman package in R software (Figure 1 and Figure 2) (Turner, 2014). Genes matching or close to a candidate SNP candidate genomic region were identified using the ARS-UCD1.2 ge-

nome assembly (GCA_002263795.2). SNP information for the corresponding genes was obtained using NCBI and Ensemble genomic browsers.

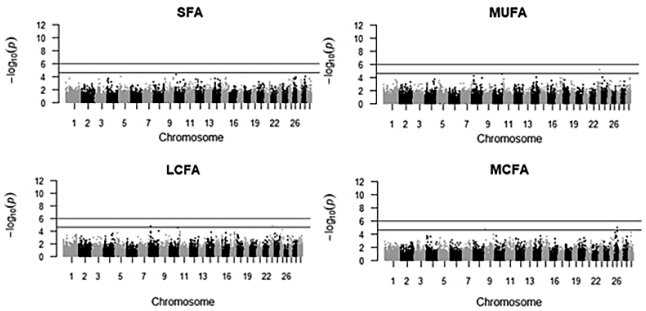

Fig. 1. Manhattan plots of Ayrshire milk fatty acids GWAS

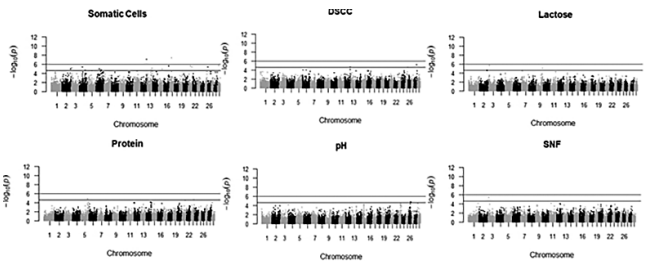

Fig. 2. Manhattan plots of Ayrshire milk other traits GWAS

Results and Discussion

In this study, we aimed to investigate the genetic factors affecting various milk parameters in Ayrshire cows during their first lactation. We performed a whole genome association study and genotyping of single nucleotide polymorphisms to identify regions of the genome associated with milk composition traits in this breed. Our GWAS analysis identified several significant SNPs associated with milk composition traits in Ayrshire cattle (Table 1) (Figure 1 and Figure 2).

It is worth noting that the identified SNPs are located in or near genes known to be involved in various biological processes associated with milk production. For example, rs42098380, associated with SFA levels in milk, is located in an intergenic region next to the NRAP gene, which encodes a muscle-specific protein belonging to the nebulin family and may be involved in the attachment of actin filament ends in myofibrils to the membrane and the transfer of tension from myofibrils to the extracellular matrix (Buggiotti et al., 2021). Our data suggest that this gene may also play a role in regulating milk composition in Ayrshire cows. This finding is consistent with a recent study by Tiplady et al. (2021) who performed GWAS on 895 individual FT-MIR wave number phenotypes using conditional whole genome sequencing for 38 085 New Zealand mixed breed dairy cows, including Ayrshire. They also found a significant association between rs42098380 and SFA levels in milk, as well as other FT-MIR wave number phenotypes related to lipid content and composition. They also found that NRAP was one of the co-localised and co-segregating eQTLs for FT-MIR wave number phenotypes, suggesting that NRAP expression may affect milk lipid synthesis or secretion (Tiplady et al., 2021).

Another significant SNP, rs41588600, was associated with MUFA ($p<0.01$). Also rs41588600 showed significant association with MUFA and LCFA levels. This SNP is located in an intergenic region next to the PROX1 gene, which encodes a transcription factor involved in developmental processes such as cell fate determination, regulation of gene transcription and regulation of progenitor cells in a number of organs including the liver and pancreas (Shen et al., 2018). This suggests that PROX1 may also influence fatty acid synthesis in Ayrshire cows. This finding is novel and has not been reported in previous studies investigating milk composition traits in dairy cattle. However, PROX1 has been implicated in lipid metabolism and homeostasis in other species such as mice and humans (Kretowski et al., 2015; Truman et al., 2012). Hence, it is possible that PROX1 may fulfil a similar function in the bovine mammary gland and influence the FA profile in milk.

Also, we found an association between SNPs and MCFA levels. For example, rs110748053 located in the LMBRD1 gene showed significant association with MCFA levels. The LMBRD1 gene encodes a lysosomal membrane protein that is involved in the transport and metabolism of cobalamin (vitamin B12), which is essential for the normal functioning of enzymes involved in the metabolism of amino acids, lipids and cholesterol (Rutsch et al., 2009). This suggests that LMBRD1 may also influence MCFA levels in Ayrshire cow's milk. This finding is consistent with a previous study by Gebreyesus et al. (2019) who conducted a multi-population GWAS on FA composition in milk samples from 16 823 Holstein cows from China, Denmark and the Netherlands using HD genotypes. They also found a significant association between rs110748053 and MCFA levels in milk as well as other FA indicators such as C10:0 and C12:0 (Gebreyesus, Buitenhuis, et al., 2019). They also performed pathway and GO analyses to identify promising candidate genes in new areas and found that LMBRD1 was rich in terms related to lipid transport and metabolism (Gebreyesus, Bovenhuis, et al., 2019). Our results suggest that these genes may play a

Table 1. Main results of GWAS of some milk parameters of Ayrshire breeds

Milk parameter	*SNP name*	Chromosome	SNP position	p-value	SNP localization	SNP (Ensembl)	Gene ID	SNP ID	Variance
SFA	*rs42098380*	26	34358100	2.23E-05	NRAP	A/G	ENSBTAG00000019327	rs42098380	–
	BTA-100341-no-rs	26	34821636	1.40E-05	intergenic variant	A/C	–	rs41567905	–
	BTA-113150-no-rs	17	43762526	1.10E-05	intergenic variant	A/C	–	rs41618252	–
	BTB-01212667	14	52216536	2.18E-05	intergenic variant	A/G	–	rs42372922	–
MUFA	*ARS-BFGL-NGS-32980*	10 (7)	87523687	2.49E-05	CAMK4	A/G	ENSBTAG00000035662	rs3423093341	–
	BTA-56450-no-rs	23	35706450	8.39E-06	intergenic variant	A/C	–	rs41588600	intron variant
LCFA	*BTA-56450-no-rs*	23	35706450	1.67E-05	intergenic variant	A/C	–	rs41588600	intron variant
	ARS-BFGL-NGS-2625	8	21328935	2.07E-05	intergenic variant	T/C	–	rs110892039	–
MCFA	*BTA-100341-no-rs*	26	34821636	1.26E-05	intergenic variant	G/T	–	rs41567905	–
	ARS-BFGL-NGS-72307	9	8933985	2.11E-05	LMBRD1	A/G	ENSBTAG00000016164	rs110748053	–
SCC	*ARS-BFGL-NGS-23063*	17	28862421	4.38E-08	intergenic variant	A/G	–	rs109897445	–
	BTB-01661144	12	88018471	9.86E-08	MYO16	T/C	ENSBTAG00000018237	rs42775315	–
	BTB-02076946	29	24014819	2.26E-06	intron variant	A/C	ENSBTAG00000050228	rs43178042	–
	ARS-BFGL-NGS-72766	21	35809110	2.84E-06	intergenic variant	A/G	–	rs109664122	intron variant
	ARS-BFGL-NGS-42703	16	71431541	2.87E-06	PROX1	T/C	ENSBTAG00000010978	rs109659498	–
	ARS-BFGL-NGS-98526	26	6692455	4.19E-06	intergenic variant	A/G	–	rs110879043	–
	ARS-BFGL-NGS-108622	21	57870643	4.46E-06	RIN3	A/G	ENSBTAG00000010416	rs109146048	intron variant
	BTB-01995313	4	42395110	5.19E-06	intergenic variant	A/C/T	–	rs43098703	–
	BTA-91502-no-rs	3	11770282	7.20E-06	intergenic variant	A/C	–	rs41661537	intron variant
	ARS-BFGL-NGS-37900	3	3424295	8.54E-06	LRRC52	T/C	ENSBTAG00000001686	rs109004361	–
	ARS-BFGL-NGS-113928	13	80583992	8.91E-06	ZFP64	T/C	ENSBTAG00000001512	rs109909102	–
	Hapmap39531-BTA-101781	13	72610173	9.94E-06	intergenic variant	A/C	–	rs42657117	intron variant
	BTA-37234-no-rs	15	61893000	1.21E-05	intergenic variant	T/C	–	rs41583263	intron variant
	ARS-BFGL-NGS-118090	2	124821120	1.23E-05	PTPRU	A/G	ENSBTAG00000012848	rs108942230	–
	BTB-01201475	6	50826657	1.32E-05	intergenic variant	A/G	–	rs42360562	–
	BTB-00264565	6	79705920	2.12E-05	intergenic variant	T/C	–	rs43469330	splice polypyrimidine tract variant
	ARS-BFGL-NGS-43812	4	84080999	2.30E-05	intergenic variant	G/C	–	rs110040170	–
DSCC	*ARS-BFGL-NGS-58728*	28	41921923	7.59E-06	upstream gene variant	T/C	ENSBTAG00000017946	rs109651956	–
Lactose	*BTA-91502-no-rs*	3	11770282	2.24E-06	intergenic variant	A/C	–	rs41661537	intron variant
	ARS-BFGL-NGS-25568	9	87429492	1.03E-05	UST	A/G	ENSBTAG00000051913	rs43605625	–
Protein	*ARS-BFGL-NGS-14939*	5	101788321	1.65E-05	DPPA3	A/T	ENSBTAG00000046609	rs109054828	intron variant
	Hapmap42255-BTA-56826	23	41313003	2.50E-05	JARID2	A/G	ENSBTAG00000012938	rs41589759	upstream gene variant
pH	*ARS-BFGL-NGS-94552*	26	34955110	2.11E-05	TDRD1	A/C	ENSBTAG00000006855	rs110792748	–
SNF	*ARS-BFGL-NGS-20009*	3	12917444	4,26E-06	intergenic variant	G/T	ENSBTAG00000019327	rs109373837	intron variant

role in the regulation of fatty acid synthesis and metabolism in milk.

In addition to FA-related traits, we identified SNPs associated with other milk composition parameters. For example, our analysis identified SNPs associated with SCC, which is an important indicator of udder health and milk quality. SNP rs42657117 was found to be significantly associated with SCC. SCC is often used as an indicator of mastitis, an inflammatory disease of the mammary gland. Identification of genetic markers associated with SCC may facilitate the development of strategies to improve udder health and reduce the incidence of mastitis in Ayrshire cows. The rs109909102, located next to the ZFP64 gene, also showed significant association with SCC. The ZFP64 gene encodes a zinc finger protein that may be involved in the regulation of mesenchymal cell differentiation and milk protein synthesis (Sakamoto et al., 2008). Our data suggest that ZFP64 may also play a role in modulating udder health and susceptibility to mastitis in Ayrshire cows. The rs42775315 located in the MYO16 gene was significantly associated with SCC in

milk ($P < 0.05$). The MYO16 gene encodes a myosin protein that is involved in cell motility and cytoskeleton organisation (Roesler et al., 2019). Previous studies have shown that myosin proteins play a role in leukocyte migration and phagocytosis, which are important for the immune response to pathogens (Mansfield, Shayman, & Boxer, 2000; Reville et al., 2006).

In our work, rs42775315, located in the MYO16 gene, was significantly associated with SCC in milk ($p < 0.05$). Hence, we suggest that the MYO16 gene may influence SCC in milk by modulating leukocyte recruitment and activity in the mammary gland. Another RIN3 gene, found at rs109146048, encodes a Ras and Rab interactor protein that is involved in endocytosis and vesicle transport (Shen et al., 2022). Previous studies have shown that RIN3 regulates the internalisation and degradation of epidermal growth factor receptor (EGFR), which is a key factor in cell proliferation and survival (Wu et al., 2021). Hence, we hypothesise that the RIN3 gene may affect SCC in milk by modulating EGFR turnover and signaling in mammary epithelial cells. The LRRC52 gene, found at rs109146048, encodes a leucine-rich protein containing repeats that is involved in calcium signaling and cell adhesion (Yang et al., 2011). The PTPRU gene encodes a receptor-type protein tyrosine phosphatase that is involved in cell adhesion, migration and differentiation (Liu et al., 2014). To our knowledge, this is the first report of a link between the above genes and SCC in milk.

DSCC is also an important indicator of udder health and is commonly used to assess mastitis, which is an inflammatory disease of the mammary gland. The only SNP associated with DSCC is rs109651956, located in an intron variant next to the ENSBTAG00000017946 gene. These results are partially consistent with a previous study by Sanchez et al. (2019), who performed a GWAS on milk cheese-forming properties and milk composition traits in 1011 Montbeliarde cows from France using conditional whole genome sequence data. They also found a significant association between rs42657117 and SCC as well as other CMP and milk composition traits such as rennet curdling time, curd hardness, protein and casein content. However, they found no significant association between rs109651956 and DSCC or any other trait (Sanchez et al., 2019). Similar studies have also identified other SNPs associated with DSCC in other dairy cattle breeds. For example, a study by da Cruz et al. (2021) identified SNPs associated with DSCC in the Girolando dairy cattle breed. These SNPs were located near genes involved in immune response and inflammation pathways, suggesting their potential role in mastitis susceptibility (da Cruz et al., 2021).

Measurement of milk pH is important to ensure milk safety, quality and shelf life. It can also help in the diagnosis and prevention of mastitis in dairy cows, which can improve their welfare and productivity (Kandeel et al., 2019). Our analysis confirmed the association between rs110792748 located in the TDRD1 gene and milk pH in Ayrshire breed. This converges with the results of a similar study by Wang et al. (2022) conducted on the Holstein breed. However, in their case this SNP, although located in the same gene, was associated with a different milk characteristic, milk fat percentage (Wang et al., 2022).

Conclusion

In conclusion, our study utilized GWAS and SNPs to investigate the genetic factors influencing milk composition traits in Ayrshire cattle. Through our analysis, we identified several significant SNPs associated with milk composition traits in this breed, many of which were located near genes known to be involved in various biological processes associated with milk production. These findings provide valuable insights into the genetic mechanisms underlying milk production and quality in Ayrshire cattle. Further research and validation of these genetic markers could contribute to the development of tools for improving milk production and quality in the dairy industry.

Acknowledgements

The study was funded by the Russian Science Foundation grant No. 21-16-00049.

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Received: **August, 17, 2023**; *Approved*: **October, 10, 2023;** *Published:* **December, 2024**