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EFFECT OF POLYMORPHISMS IN EXON 8 OF THE *PPARGC1A* GENE ON MILK PRODUCTION TRAITS IN CATTLE

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Abstract. The objective of this study was to investigate associations between genotypes of polymorphisms in exon 8 of the *PPARGC1A* gene and milk production traits in dairy cattle. The study was conducted in a herd of 959 Polish Holstein-Friesian of the black and white cows kept in western Poland. In this study, three polymorphisms within exon 8 of the *PPARGC1A* gene were analyzed: *rs445204772*, *rs109164431* and *rs133669403* and they are responsible for two missense and one synonymous type mutations. All cows were genotyped using the PCR-RFLP method. The *PPARGC1A* polymorphisms that were studied had the following major allele frequencies: *rs445204772* – allele *A* 0.523; *rs109164431* – allele *C* 0.607 and *rs133669403* – allele *A* 0.546. Statistical analysis was aimed at estimating the effect of individual genotypes on milk performance traits such as milk, protein, and fat yield as well as protein and fat content in milk. For *rs445204772* polymorphism, a statistically significant effect on milk yield ($P \leq 0.05$) and fat content ($P \leq 0.05$, $P \leq 0.01$) was observed. Polymorphism *rs109164431* significantly ($P \leq 0.05$, $P \leq 0.01$) affected milk, fat, and protein yield as well as milk fat content. In the case of polymorphism *rs133669403*, it was found that it affects to a different degree ($P \leq 0.05$, $P \leq 0.01$) most of the analyzed milk performance traits. The obtained results may contribute to the state of knowledge regarding the identification of the most important SNPs that could be used for the selection of marker assisted dairy cattle.

Key words: lactation, single nucleotide polymorphisms, Polish Holstein-Friesian cattle.

INTRODUCTION

Permanent modifications of animal performance traits may occur through gradual multistage genetic improvement, especially given that the heritability of individual dairy performance traits in cattle shows differences (Georges et al. 1995). Various types of meta-analyses including the entire dairy cattle genome allowed to identify numerous quantitative trait loci (QTL) of candidate genes responsible for milk production, which then can be used in marker-assisted selection programs to accelerate genetic improvement in dairy cattle (Khatkar et al. 2004; Smaragdov 2006; Ogorevc et al. 2009).

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PPAR (peroxisome proliferator-activated receptor) is a family of peroxisome proliferator-activated receptors. These include transcription factors responsible for the regulation of glucose and lipid metabolism, insulin sensitivity, inflammatory processes, immune response as well as cell proliferation and differentiation (Ferré 2004). There are three PPAR isoforms encoded by different genes and performing different functions, including peroxisome proliferator-activated receptor gamma coactivator 1-alpha (*PPARGC1A*, PGC-1α) encoded by the *PPARGC1A* gene, which plays a key role in energy regulation. *PPARGC1A* acts as nuclear receptors and transcription factors activator (Liang and Ward 2006) and influences the expression of genes involved in oxidative metabolism, adipogenesis and gluconeogenesis (Puigserver and Spiegelman 2003).

The bovine gene encoding *PPARGC1A* is located on chromosome 6, consists of 13 exons separated by introns with a total length of 6261 base pairs. The *PPARGC1A* gene is characterized by diverse expression in different tissue types (Weikard et al. 2005; Komisarek and Walendowska 2012).

Due to the location of the *PPARGC1A* gene near the BM143 marker, this gene has become the subject of many studies on the single nucleotide polymorphisms (SNPs) identification. Numerous experiments have shown that the *PPARGC1A* gene affects milk performance traits, such as milk yield, fat yield, fat content, protein yield and protein content in milk (Weikard et al. 2005; Khatib et al. 2007; Komisarek and Dorynek 2009; Schennink et al. 2009; Kowalewska-Łuczak et al. 2010; Boleckova et al. 2012).

However, at the moment there are no reports on SNP analysis in exon 8 of the *PPARGC1A* gene, which is the second largest exon of this gene or of their genetic impact on cattle milk performance traits. The aim of the study was to identify SNPs in the *PPARGC1A* gene in Polish Holstein-Friesian cattle using PCR-RFLP method. In addition, marker-trait linkage analysis in studied cattle herd was performed to find potential molecular markers in this gene, relevant to milk performance traits.

MATERIAL AND METHODS

The study was carried out in a herd of 959 Polish Holstein-Friesian cows of the black and white variety grazed in western Poland. All animals were reared in similar environmental conditions and fed with standardized feeding doses. Among the group of the animals studied, 958 cows completed their first lactation, 957 individuals completed the second lactation, 833 cows completed the third lactation. Each lactation lasted for 305 days. Cows were milked twice a day using a mechanical milking machine. The herd performance was assessed using the A4 method – a method compliant with International Committee for Animal Recording (ICAR) recommendations. For each individual milk performance documentation was kept, including milk yield, fat yield, fat content, protein yield and protein content.

SNP detection and genotyping

In order to identify and determine the attendance of genotypes and alleles of selected the *PPARGC1A* gene polymorphisms, the first step was to isolate the genetic material from peripheral blood collected from the external jugular vein of the studied individuals. DNA isolation was performed using a commercial reagent kit for DNA isolation MasterPure™ DNA Purification Kit for Blood Version II (Lucigen, Wisconsin, USA), according to the isolation protocol attached to the kit.

The analyzed polymorphisms were mapped in exon 8, and they are responsible for missense type mutations; Lys346Arg (*rs445204772*; A/G) and Ala438Thr (*rs133669403*; G/A) and synonymous His404= (*rs109164431*; T/C) mutation. Individual genotypes were determined by PCR-RFLP method. A pair of primers designed in the Primer3 (http://bioinfo.ut.ee/primer3-0.4.0/) program based on DNA sequences from the Ensembl database (https://www.

ensembl.org/index.html) were used to carry out the PCR reaction: forward 5'-AAGGCAGTA-ATTCCACCACGA-3', reverse 5'-AGCTCGGATTTCCTGGTCTTG-3'. The underlined C nucleotide in the forward starter was deliberate change introduced to create a restriction site (ACRS, amplification-created restriction site) for *Bss*SI enzyme for the *rs445204772* polymorphism. The amplification reaction was conducted in a mixture of a final volume of 25 µl containing the Forward primer (1.0 µl), Reverse primer (1.0 µl), PCR Mix (A&A Biotechnology, Gdynia, Poland) (12.5 µl), DNA template (2 µl) and Nuclease-Free Water (8.5 ml). The thermal conditions for PCR are: initial denaturation at 95°C for 5 minutes, followed by 94°C specific denaturation for 45 seconds, annealing at 54°C for 45 seconds, extension at 72°C for 45 seconds (repeated in 30 cycles) and final extension at 72°C for 5 minutes.

The resulting 365 bp product was then digested with individual restriction enzymes suitable for each SNPs: *Bss*SI (*rs445204772*), *Nla*III (*rs109164431*) and *Aci*I (*rs133669403*) (Thermo Scientific, Waltham, USA). The restriction enzyme digestion was carried out in a volume of 20 µl in 0.2 ml tubes at the time and temperature as recommended by the manufacturer. The obtained fragments were then separated using horizontal electrophoresis on 3% agarose gels stained with ethidium bromide and visualized under UV transilluminator.

Statistical analyses

The frequencies of genotypes and alleles and the Hardy-Weinberg equilibrium for individual SNPs were calculated using the POPGENE software (Yeh et al. 2000), and polymorphism information content (PIC) was evaluated according to Nei's methods (Nei and Roychoudhury 1974).

Statistical analysis was used for the obtained genotyping results. An analysis of the relationship between the various genotypes and selected traits of dairy cattle: milk yield (kg), fat and protein yield (kg), fat and protein content (%). Statistical analysis between *PPARGC1A* genetic variants and analyzed traits was conducted using the STATISTICA®12.0 PL program and General Linear Model (GLM) software packages. The significance of differences between polymorphisms was determined with Duncan's test.

The statistical analysis was performed using the following model:

$$Y_{ijkl} = \mu + a_i + b_j + c_k + d_l + e_{ijkl}$$

where:

Y_{ijklmn} – analyzed trait,

μ – overall mean of trait,

a_i – the effect SNPs on trait value in *PPARGC1A/1037 A>G*, *PPARGC1A/1209 T>C* and *PPARGC1A/1309 G>A* (i = 1, 2, 3, 4) for I, II and III lactation,

b_j – the effect of year calving (l = 1, 2, 3, ..., 7) for I, II and III lactation,

c_k – the effect of month calving (m = 1, 2, 3, ..., 12) for I, II and III lactation,

d_l – the effect of father's random (n = 1, 2, 3, …, 35) for I, II and III lactation,

e_{ijkl} – random residual effect (random error).

RESULTS

Genotyping of SNPS

Analysis of the genotyping results showed that three possible genotypes were identified for all tested polymorphic sites. In the case of *rs445204772* polymorphism, after digestion with *Bss*SI enzyme the genotypes *AA* (281, 84 bp), *AG* (281, 84, 65 bp) and *GG* (281, 65, 19 bp) were identified. Heterozygous genotype turned out to be the most common genotype (0.414), while the most common allele is the *A* allele (0.523). For the *rs109164431* polymorphism product PCR after the cut with *Nla*III enzyme, the genotypes *TT* (167, 135, 63 bp), *TC* (230, 167, 135,

63 bp) and *CC* (230, 135 bp) were identified. The genotype with the highest frequency was the *CC* genotype (0.440), and the allele with the highest frequency is the *C* allele (0.607); whereas for the *rs133669403* polymorphism after digestion with the *Aci*I enzyme the *GG* (295, 70 bp), *GA* (365, 295, 70 bp) and *AA* (365 bp, no cut) genotypes were found. Heterozygous genotype was the most common (0.633), and the most common allele – A allele (0.546). Information on the frequencies of genotypes and alleles for individual polymorphisms is presented in Table 1.

Table 1. Genotype and allele frequencies of analyzed polymorphisms in exon 8 of the *PPARGC1A* gene

Polymorphism	N	Genotypes frequency		Alleles frequency		χ^2 (HWE)	PIC
rs445204772	302	*AA*	0.316	*A*	0.523	27.5290**	0.358
	397	*AG*	0.414	*G*	0.477		
	259	*GG*	0.270				
rs109164431	216	*TT*	0.225	*T*	0.393	84.9001**	0.363
	321	*TC*	0.335	*C*	0.607		
	421	*CC*	0.440				
rs133669403	132	*GG*	0.138	*G*	0.454	72.9246**	0.373
	606	*GA*	0.633	*A*	0.546		
	220	*AA*	0.229				

N – number of cows; ** P ≤ 0.01

Table 1 also shows the effect of Hardy-Weinberg equilibrium and PIC analysis. The obtained results show a deviation of all three analyzed *loci* from HW equilibrium, which can probably be the result of inbreeding or long-term selection of dairy herds towards the desired milk production traits. The PIC analysis results show that all tested polymorphisms, according to Botstein et al. (1980) classification, show moderate polymorphism.

Table 2 shows the statistical analysis results of individual genotypes of tested polymorphisms in relation to milk performance traits such as milk, fat and protein yield and fat and protein content in milk.

Table 2. Mean values and standard deviations for analyzed milk yield traits

L	Genotype	N	Milk yield [kg]	Fat yield [kg]	Fat content [%]	Protein yield [kg]	Protein content [%]
				rs445204772			
I	*AA*	259	8577 ± 1478^{a}	337.76 ± 59.50	3.96 ± 0.45^{Aa}	299.00 ± 54.60	3.50 ± 0.39
	AG	397	8801 ± 1479	337.76 ± 58.27	3.86 ± 0.43^{a}	308.63 ± 54.43	3.45 ± 0.36
	GG	302	8986 ± 1512^{a}	338.13 ± 57.13	3.77 ± 0.48^{A}	305.38 ± 55.95	3.48 ± 0.39
II	*AA*	258	10200 ± 1682^{ab}	409.81 ± 73.15	3.93 ± 0.42^{AB}	363.03 ± 57.67	3.51 ± 0.25
	AG	397	10476 ± 1790^{a}	411.40 ± 75.11	4.05 ± 0.49^{Aa}	356.42 ± 56.33	3.48 ± 0.22
	GG	302	10471 ± 1826^{b}	394.98 ± 72.33	3.80 ± 0.50^{Ba}	360.73 ± 58.30	3.46 ± 0.23
III	*AA*	226	10286 ± 1801^{a}	424.51 ± 87.06	4.12 ± 0.47^{AB}	356.04 ± 66.35	3.46 ± 0.27
	AG	344	10575 ± 1969	418.22 ± 78.24	3.45 ± 0.25^{A}	363.70 ± 60.84	3.45 ± 0.25
	GG	263	11124 ± 2075^{a}	415.62 ± 83.22	3.90 ± 0.51^{B}	361.84 ± 58.86	3.40 ± 0.24
				rs109164431			
I	*TT*	321	8869 ± 1426^{B}	339.07 ± 57.43^{A}	3.84 ± 0.45	312.63 ± 60.52^{a}	3.48 ± 0.48
	TC	216	9051 ± 1484^{A}	348.79 ± 59.02^{a}	3.86 ± 0.49	307.43 ± 53.83^{A}	3.47 ± 0.36
	CC	421	8615 ± 1533^{AB}	331.37 ± 57.57^{Aa}	3.87 ± 0.45	298.48 ± 52.71^{Aa}	3.48 ± 0.33
II	*TT*	321	10342 ± 1653	399.84 ± 72.13^{Aa}	3.88 ± 0.48^{Aa}	358.36 ± 55.68	3.48 ± 0.24
	TC	216	10638 ± 1866	401.58 ± 71.31^{a}	3.92 ± 0.50^{a}	357.82 ± 56.86	3.48 ± 0.23
	CC	420	10322 ± 1812	421.79 ± 78.47^{A}	3.98 ± 0.42^{A}	368.98 ± 60.83	3.49 ± 0.23
III	*TT*	282	10455 ± 1859^{a}	413.25 ± 85.67^{Aa}	3.94 ± 0.49^{A}	357.51 ± 61.02^{a}	3.43 ± 0.26
	TC	179	11270 ± 1920	425.59 ± 82.63^{A}	4.03 ± 0.52^{A}	366.10 ± 61.57	4.46 ± 0.25
	CC	372	10544 ± 2021^{a}	420.39 ± 79.31^{a}	4.00 ± 0.49	361.27 ± 62.43^{a}	3.43 ± 0.24

L	Genotype	N	Milk yield [kg]	Fat yield [kg]	Fat content [%]	Protein yield [kg]	Protein content [%]
				rs133669403			
I	*GG*	321	8652 ± 1540	330.10 ± 62.70^{a}	3.83 ± 0.41	299.32 ± 58.10	3.47 ± 0.37
	GA	216	8741 ± 1522	335.56 ± 58.18	3.86 ± 0.45	302.12 ± 56.21	3.47 ± 0.40
	AA	421	9044 ± 1368	348.93 ± 54.04^{a}	3.86 ± 0.51	314.82 ± 49.23	3.49 ± 0.32
II	*GG*	132	9931 ± 1873^{Aa}	382.95 ± 80.65^{aB}	3.87 ± 0.49^{A}	346.38 ± 61.53^{Ba}	3.50 ± 0.22
	GA	606	10306 ± 1725^{ab}	439.41 ± 78.67^{aA}	4.02 ± 0.45^{a}	379.21 ± 56.97^{Aa}	3.48 ± 0.24
	AA	219	10942 ± 1734^{Ab}	398.25 ± 66.07^{AB}	3.90 ± 0.48^{Aa}	356.86 ± 55.27^{AB}	3.48 ± 0.23
III	*GG*	119	10331 ± 1795	405.04 ± 75.79^{A}	3.94 ± 0.54^{a}	355.11 ± 57.38	3.45 ± 0.23
	GA	536	10706 ± 1986	417.35 ± 82.34^{a}	3.98 ± 0.49	359.44 ± 61.65	3.43 ± 0.25
	AA	178	10787 ± 1934	433.74 ± 84.41^{Aa}	4.04 ± 0.48^{a}	369.84 ± 63.39	3.45 ± 0.28

L – lactation; n – number of cows; the values marked with the different letters (in columns) differ statistically: $^{A, B}$ ($P \leq 0.01$); $^{a, b}$ ($P \leq 0.05$).

RS445204772

In the *rs445204772* polymorphism case, a statistically significant effect on milk yield ($P \leq 0.05$) and fat content ($P \leq 0.05$, $P \leq 0.01$) was observed. As regards milk yield, it was demonstrated that significantly ($P \leq 0.05$) the lowest value of this trait was characteristic for cows with homozygous *AA* genotype in all three subsequent lactations, whereas the highest milk yield was noted for lactation I and III in cows with homozygous *GG* genotypes, and for lactation II in cows with heterozygous genotypes. Statistically significant ($P \leq 0.05$, $P \leq 0.01$) differences for all three lactations and all observed genotypes were showed for fat content. Thus, animals with the *GG* homozygous genotype had the lowest fat content in milk for I and II lactation, and milk of cows with heterozygous genotype had the lowest fat content for III lactation. The highest fat content in milk was characteristic for cows with *AA* genotype (I and III lactations) and heterozygous cows.

RS109164431

Analysis of outdated data in Table 2 indicates that the *rs109164431* polymorphism significantly ($P \leq 0.05$, $P \leq 0.01$) affects milk, fat and protein yield and fat content in milk. The highest milk yield in all three lactations had cows with heterozygous genotype, however significantly ($P \leq 0.05$, $P \leq 0.01$) higher yields were noted for lactation I and III. In lactation III significantly ($P \leq 0.05$) less milk was produced by cows with homozygous *TT* genotype, and in lactation I, it was demonstrated that cows with homozygous genotypes produced significantly ($P \leq 0.05$) less milk than cows with the heterozygous genotype. In the case of milk yield and fat content statistical analysis showed that cows with the *TT* genotype had significantly ($P \leq 0.05$, $P \leq 0.01$) the lowest milk yield in all three lactations and the lowest milk fat content for lactation II and III. The highest fat yield was observed in heterozygous cows (lactation I and III) an in cows with the homozygous *CC* genotype in lactation II. In the case of milk fat content statistically significant differences ($P \leq 0.05$, $P \leq 0.01$) were noted for lactation II and III – milk of cows with homozygous genotypes had the lowest fat content, while the highest milk fat content was observed in animals with *CC* (lactation II) and *TC* (lactation III) genotypes. Another trait for which statistically significant differences ($P \leq 0.05$, $P \leq 0.01$) were present is protein yield – differences were noted for lactation I and III. Thus, in the case of I lactation, cows with the *TT* genotype were characterized by the highest protein yield, and cows with *CC* genotype by the lowest. For lactation III it was shown that the highest protein yield was achieved by cows with heterozygous genotype and the lowest by animals with the *TT* genotype.

RS133669403

Considering the results of the statistical analysis for the *rs133669403* polymorphism, it was stated that this polymorphism affects the majority of analyzed milk performance traits to varying degrees ($P \leq 0.05$, $P \leq 0.01$). Cows with the *GG* homozygous genotype were shown to have significantly the lowest milk yield (lactation II), fat yield (all three lactations), protein yield (lactation II) and milk fat content (lactation II and III); whereas in the case of the highest average values of the examined traits in relation to individual genotypes, no consistent results were found in contrast to the lowest average results. Thus, the highest milk yield was characteristic for cows with the *AA* genotype for lactation I. The highest fat yield was noted for animals with the *AA* genotypes for lactation I and III and for animals with the heterozygous genotype for lactation II. Statistically significant highest milk fat content was found in individuals with *GA* (lactation II) and *AA* (lactation III) genotypes. In the case of protein yield statistically significant differences were noted only for lactation II, and here the highest yield was achieved by cows with heterozygous genotype.

DISCUSSION

Polymorphisms in the *PPARGC1A* gene are analyzed by various authors in relation to milk and meat performance traits, and even in terms of their effect on reproductive traits. As the *PPARGC1A* encoding gene is involved in lipid metabolism regulation as a transcription factor, the focus was primarily on the analysis of the influence of mapped polymorphisms in this gene on fat-related parameters (fat yield, milk fat content or fatty acid profile in milk or meat). The most frequently studied polymorphisms are c.1892+19*T>C* mapped in intron 9 and c.3359*A>C* mapped in 3'UTR region. Only in Weikard et al. (2005) work *rs109164431* polymorphism was studied, named as c.1209 *T>C*. In that study the most common allele was the *C* allele, and its frequency was 0.8–0.9, which is a much higher value than that obtained in this study (0.607).

In the same study (Weikard et al. 2005), an analysis of the relationship between individual genotypes and milk performance traits such as: milk yield, fat yield and fat content was carried out; however, no such relations for polymorphism were found.

In this study, two missense polymorphisms in the *PPARGC1A* encoding gene were studied. The *rs445204772* polymorphism being *A>G* transition causes Lys346Argamino acids substitution in the protein sequence, while *A>G* (*rs133669403*) transition causes the Ala438Thr amino acids substitutions. According to the work of Majewski and Ott (2003) amino acids, such as arginine and lysine, are among the least mutating amino acids, therefore when the substitution of these amino acids occurs, you can expect an impact on the protein structure and function. An analysis of the predicted changes in the protein secondary structure caused by the missense mutations in exon 8 of the *PPARGC1A* gene was also performed using the NPSA SOPMA Server (Combet et al. 2000). The results of such analysis indicate that some changes in the secondary protein structure can be expected in the case of the Ala438Thr amino acids substitution (Table 3).

Table 3. Results of predicting protein secondary structure variation caused by missense mutation in the coding region of the *PPARGC1A* gene

GenBank no.	Codon	Amino acid	Alpha helix [%]	Extended strand [%]	Beta turn [%]	Random coil [%[
rs445204772	AAG/	Lys	29.06	8.79	3.42	58.73
	AGG	Arg	29.06	8.79	3.42	58.73
rs133669403	GCG/	Ala	29.06	8.79	3.42	58.73
	ACG	Thr	28.82	8.79	3.42	58.97

CONCLUSIONS

These studies indicate that all three analyzed polymorphisms have a significant ($P \leq 0.05$, $P \leq 0.01$) effect on most of the analyzed milk performance, primarily on fat yield and content, and to a lesser extent on milk yield and protein yield. The results of performed tests for all three lactations for the *rs445204772* polymorphism indicated the appearance of certain tendencies occurring between a given genotype, and individual analyzed traits. Animals with the *AA* genotype had the lowest average values for milk yield, but the highest for milk fat content. In the case of *rs109164431* polymorphism, the combined analysis of all three lactations showed that cows with the *TT* genotype had the lowest average milk yield, fat yield and fat content values. Important information from the study seems to be the negative impact of the homozygous *GG* genotype of the *rs133669403* polymorphism on all traits for which statistically significant relationships have been demonstrated. This may be a clue for breeders carrying out selection to increase milk yield.

To our knowledge, this study is the first to analyze the effect of the polymorphism in exon 8 encoding the *PPARGC1A* gene on cattle milk performance traits such as milk, fat, and protein yield as well as fat and protein content in milk. The results obtained in this study seem to be a significant contribution to the state of knowledge regarding the identification of the most important SNPs that can be used for the marker-assisted dairy cattle selection. However, such studies should be continued, preferably based on the larger number of individuals or other cow breeds.

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WPŁYW POLIMORFIZMÓW W EKSONIE 8 GENU *PPARGC1A* W ODNIESIENIU DO CECH UŻYTKOWOŚCI MLECZNEJ BYDŁA

Streszczenie. Celem pracy było zbadanie związków pomiędzy genotypami polimorfizmów w eksonie 8 genu *PPARGC1A* a cechami użytkowości mlecznej bydła. Badania przeprowadzono w stadzie 959 krów rasy polskiej holsztyńsko-fryzyjskiej odmiany czarno-białej utrzymywanych w zachodniej Polsce. W niniejszym badaniu przeanalizowano trzy polimorfizmy w eksonie 8 genu *PPARGC1A*: *rs445204772*, *rs109164431* i *rs133669403*, które są odpowiedzialne za dwie mutacje zmiany sensu i jedną mutację synonimiczną. Wszystkie krowy genotypowano metodą PCR-RFLP. Badane polimorfizmy *PPARGC1A* miały następujące frekwencje głównych alleli: *rs445204772* – allel *A* 0,523; *rs109164431* – allel *C* 0,607 i *rs133669403* – allel *A* 0,546. Celem analizy statystycznej było oszacowanie wpływu poszczególnych genotypów na wybrane cechy użytkowości mlecznej bydła takie jak wydajność mleka, białka i tłuszczu oraz zawartość białka i tłuszczu w mleku. Dla polimorfizmu *rs445204772* zaobserwowano statystycznie istotny wpływ na wydajność mleka ($P \leq 0,05$) i zawartość tłuszczu ($P \leq 0,05$, $P \leq 0,01$). Polimorfizm *rs109164431* istotnie ($P \leq 0,05$, $P \leq 0,01$) wpłynął na wydajność mleka, tłuszczu i białka oraz zawartość tłuszczu w mleku. W przypadku polimorfizmu *rs133669403* stwierdzono, że wpływa on

w różnym stopniu (P ≤ 0,05, P ≤ 0,01) na większość analizowanych cech użytkowości mlecznej. Uzyskane wyniki mogą przyczynić się do poszerzenia stanu wiedzy dotyczącej identyfikacji najważniejszych SNP, które mogłyby być wykorzystane w selekcji bydła mlecznego wspomaganego markerami.

Słowa kluczowe: laktacja, polimorfizm pojedynczego nukleotydu, bydło rasy polskiej holsztyńsko-fryzyjskiej.