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Polymorphism in the *OLR1* gene and functional traits of dairy cattle

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ABSTRACT

The study analyzed the polymorphism of single nucleotide substitution A8232C, located in the 3'UTR region of the receptor of the oxidized low-density lipoprotein (OLR1) gene. The study was conducted in a herd of Polish Holstein-Friesian (Red-and-White strain) dairy cattle. Identification of genotypes of individuals was performed using PCR-RFLP. The study stated the following frequency of A8232C polymorphism alleles: *A* - 0.30 and *C* - 0.70. Statistical analysis showed that the AC genotype cows were characterized by higher milk yield, protein and fat and for fat yield, the difference was confirmed statistically ($P \leq 0.05$). The cows with genotype *AA* were characterized by the latest occurrence of first calving and the longest intercalving interval, but the results were not confirmed statistically.

Key words: O*LR1* gene; polymorphism; functional traits; cattle

Introduction

The receptor for oxidized low-density lipoproteins (Oxidized Low-density lipoprotein Receptor 1, OLR1) is a type II membrane protein with 50 kDa of weight, and with C-type extracellular lectin domain and short cytoplasmic tail. OLR1 mainly constitutes a multi-ligand binding protein that absorbs and decomposes low-density lipoproteins. Originally, OLR1 was identified as an oxidized endothelial receptor LDL (MEHTA and LI, 2002; CHEN et al., 2001). This protein can also be involved in regulation of induced apoptosis by Fas, as well as serving as a scavenger receptor (SR), classified as a molecular pattern recognition receptor. It has been shown that oxidized lipids can weaken glucose metabolism and significantly influence the metabolism of fats in the mammary glands and liver (RINGSEIS et al., 2007; LIAO et al., 2008). The OLR1 receptor, due to its significant function in the metabolism of oxidized low-density lipoproteins, can have an influence on the above-mentioned processes (KOMISAREK and DORYNEK, 2009). WATHES et

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al., (2007) showed in their study that the products of genes linked to the metabolism of fats and glucose, as well as metabolism pathways, may influence the energy balance of an organism, being in turn a factor influencing reproductive characteristics.

Therefore, we have undertaken research into the relationship between A8232C polymorphism in the *OLR1* gene and selected reproductive traits of Polish Holstein-Friesian (Red-and-White strain) cattle.

Materials and methods

The research included a herd of Polish Holstein-Friesian (Red-and-White strain) cattle consisting of 169 animals. The examined animals were all kept in similar environmental conditions in the south-western part of Poland. The entire herd completed three subsequent 305-day lactations. The animals were fed normalized food portions with the possibility of going out to pasture during the spring-summer period. In the documentation of milk performance assigned individually to each of the cows, the milking capacity, fat and protein content, and their percentage were included. Milking was done twice a day, while sample milkings were carried out according to the A4 method.

The material used for DNA isolation was peripheral blood taken from each of the cows via the external jugular vein into vacuum test-tubes containing anticoagulant K_3EDTA. The DNA isolation was conducted using a whole blood DNA isolation kit according to the manufacturer's instructions.

Polymorphism, classified as single nucleotide substitution (SNP) located in the 3'UTR region, was analysed, in position 8232 (GenBank NM_174132.2), and its C/A change. Genotyping was conducted using the PCR-RFLP method, according to the methodology of RYCHTAROVA et al., (2014). The product 582 bp in length was subject to digestion by the *Pst*I enzyme. The restriction fragments obtained were separated in 2% agarose gels and the genotyping results were analyzed statistically. In order to characterise the genetic structure of the population, calculations of A8232C genotype frequencies were made, as well as calculations of the frequency of each of the alleles.

In the next stage of the research, the correlations between the genotypes and the production and functional performance traits, such as milking yield, proteins and fat yield, age at first calving (AFC), and intercalving interval (CI) were analyzed. Statistical analysis of the relationship between A8232C polymorphism and milk performance traits and the selected reproductive parameters was performed using the STATISTICA 12.5 PL program. The mean values and standard deviation were calculated, and one-factor analysis of variance was conducted using Duncan's multiple range test.

Results

In the analysis of the fragment of the 3'UTR region of the *OLR1* gene, all three genotypes that were possible to determine were identified, that is, *AA* (uncut, 582 bp), *AC* (582, 337 and 245 bp) and *CC* (337, 245 bp). The presence of these genotypes is conditioned by two alleles - *A* and *C*. The frequency of individual *OLR1* genotypes and alleles is shown in Table 1.

Table 1. Frequency of genotypes and alleles of A8232C polymorphism in the *OLR1* gene

	Genotypes			Alleles	
	AA	*AC*	*CC*	*A*	*C*
Frequency	0.13	0.34	0.53	0.3	0.7

Frequency analysis of individual genotypes and alleles of A8232C polymorphism in the studied herd of Polish Holstein-Friesian (Red-and-White strain) cows showed that the most frequently occurring genotype is the *CC* homozygous genotype, while the allele with the highest frequency is the *C* allele.

Table 2. Mean values and standard deviation for milk production and functional traits for the A8232C genotypes *OLR1* gene

	AA		*AC*		*CC*	
	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD
Milk production traits						
MY (kg)	22	6350.59 ± 1289.52	58	6825.53 ± 1680.45	89	6763.13 ± 1129.18
FY (kg)	22	249.32[a] ± 40.22	58	280.19[a] ± 73.20	89	270.81 ± 59.43
PY (kg)	22	209.91 ± 32.16	58	228.87 ± 51.54	89	218.37 ± 35.60
Reproduction traits						
AFC (d)	22	858 ± 135	58	831 ± 100	89	831 ± 99
CI (d)	22	420 ± 80	58	413 ± 70	89	395 ± 57

Mean values in rows marked with the "a" letters differ significantly at P≤0.05; n - number of individuals, MY - milk yield, FY - fat yield, PY - protein yield, AFC - age at first calving, CI - calving interval, d - days

Table 2 shows the mean values and standard deviations of the selected milk production and reproductive traits in relation to individual genotypes of A8232C polymorphism. The

statistical analysis of the genotyping results showed that cows with the *AC* genotype are characterised by the highest milk yield, fat and protein yield, and for fat yield, the difference was confirmed statistically ($P \leq 0.05$) in reference to the *AA* genotype. When considering reproductive traits, it may be noted that cows with homozygous genotype *AA* were characterised by the latest occurrence of first calving and the longest intercalving interval, however, those differences were not statistically significant. Selection of the proper moment for the first submission shortens the reproductive period, influences the time cows can be maintained in the herd and lowers the occurrence of problems during birth.

Discussion

Progress in the field of molecular genetics over the last decade has resulted in better recognition of genomes of cattle and other breeding animals. The use of molecular markers has allowed for the design of precise gene maps for individual chromosomes, including traits related to milk and functional traits. The use of such gene maps has allowed the development of a method specified as the Marker-Assisted Selection (MAS) (ROTHSCHILD and PLASTOW, 2014). Because MAS assumes selection based on direct knowledge of individual animals, it can tip the scale of breeding benefits significantly. The connection between the presence of markers and milk production traits is shown inside chromosome 5, close to the *OLR1* gene *locus* (KHATKAR et al., 2004). A8232C polymorphism in the *OLR1* gene has been analysed by different authors involving different types of cattle. A higher frequency of the *C* allele was noted for Nelore beef cattle, divided into three selection groups, where its frequency ranged from 0.79 to 0.89 (FONSECA et al., 2015); for *Bos indicus* 0.83 and 0.95 for Brown Swiss (KHATIB et al., 2006); also for South Anatolian Red and East Anatolian Red 0.89 and 0.91, respectively (ATES et al., 2014). As for other studies, the researchers noted a lower frequency of the *C* allele than that observed in their own research: Holstein cattle 0.54 (KHATIB et al., 2006), Holstein-Friesian Black and White strain 0.57 (KOMISAREK and DORYNEK, 2009), Czech Fleckvieh 0.51 (RYCHTAROVA et al., 2014), Iranian Holstein 0.48, 0.47 and 0.53 (SOLTANI-GHOMBAVANI et al., 2013; REZAEI et al., 2016; MASHHADI et al., 2014), and Iranian Simmental 0.62 (REZAEI et al., 2016). The divergence in the distribution of particular genotypes may result from the different breeds for which individual research was conducted, as well as from the research of other authors involving individual, not very large herds of cattle.

A8232C polymorphism, until now, has mainly been studied in relation to the milk production traits of cattle. Numerous authors have shown a relationship between this polymorphism and mainly the fat yield and fat content in milk (KHATIB et al., 2006; KHATIB et al., 2007; KOMISAREK and DORYNEK, 2009; SOLTANI-GHOMBAVANI

et al., 2013). FONSECA et al., (2015) conducted research on the traits of Nelore beef cattle and found significant correlations ($P \leq 0.05$) between genotypes and the thickness of rump fat thickness and weaning weight.

KOMISAREK and DORYNEK, (2009), as well as RYCHTAROVA et al., (2014), also analysed reproductive coefficients. In the case of the study by KOMISAREK and DORYNEK, (2009) the estimated breeding value (EBV) of Holstein-Friesian Black and White strain bulls was analysed in relation to reproductive traits such as: age at first insemination (AFI, days), calving-to-conception interval after first calving (CCI, days), calving-to-first service interval after first calving (CFI, days), non-return rate at the 56th day after first insemination in heifers (NRH, %), and non-return rate at 56th day after first insemination in first-lactation cows (NRC, %). In that work, there were no statistically determined differences, but one can notice when investigating the result shown that animals with *CC* genotype were characterised by slightly higher values of the majority of the analysed coefficients. Whereas, in the work of RYCHTAROVA et al., (2014) an analysis of coefficients was performed, such as the days from calving to first insemination, days open, calving interval, days from first to last insemination, and number of inseminations in a herd of Czech Fleckvieh cows. In that study, no significant differences between individual genotypes were statistically determined, but it can be observed that cows with *CC* genotype were characterised by lower values of the coefficients days from calving to first insemination and days from first to last insemination, whereas animals with a heterozygous genotype had slightly lower values of the remaining coefficients.

The above individually-conducted research has pointed at a statistically significant relationship between polymorphism in the *OLR1* gene and fat yield in milk. In the case of the remaining traits, there were no statistically significant differences shown between individual genotypes of the examined cattle and the traits considered but, thanks to the analysis, it was possible to show certain tendencies for having lower or higher values of given traits manifested by individuals with a specified genotype. In conclusion, the results of the above research can be used in marker-assisted selection and can lead to economic benefits for milk producers. Of course, further analysis of polymorphic sites in the *OLR1* gene will be necessary in order to assess possible correlations between individual genetical variants and selected milk performance traits. Research conducted on much larger herds of cows and herds with greater variety of cow types could further verify the obtained results.

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SAŽETAK

Analiziran je polimorfizam pojedinačnog nukleotida na poziciji A8232C, smještenog u regiji 3'UTR, gena za receptor oksidiranog lipoproteina male gustoće (*OLR1*). U stadu poljskih holštajnsko-frizijskih (crveni i bijeli soj) krava za mlijeko genotipovi jedinki identificirani su PCR-RFLP metodom. Ustanovljena je sljedeća učestalost A8232 polimorfnih alela: A - 0,30 i C - 0,70. Statistička je analiza pokazala da su krave s genotipom AC imale veću proizvodnju mlijeka, bjelančevina i masti, pri čemu je razlika za mast potvrđena kao signifikantna ($P \leq 0,05$). Krave s genotipom AA imale su kasno prvo teljenje i dugo međutelidbeno razdoblje, no ti rezultati nisu potvrđeni kao statistički signifikantni.

Ključne riječi: *OLR1* gen; govedo; polimorfizam; funkcionalna svojstva