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SLC27A1 GENE POLYMORPHISM ANALYSIS IN RELATION TO MILK PRODUCTION TRAITS IN JERSEY COWS

ANALIZA POLIMORFIZMU W GENIE SLC27A1 W ODNIESIENIU DO CECH UŻYTKOWOŚCI MLECZNEJ KRÓW RASY JERSEY

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Streszczenie. Celem badań było ustalenie ewentualnych zależności między polimorfizmami *g.14791C>T* oraz *g.14589A>G* w genie *SLC27A1* a cechami użytkowości mlecznej i liczbą komórek somatycznych w mleku krów rasy jersey. Nie stwierdzono istotnych zależności między genotypami a analizowanymi cechami. Kolejne badania na większej grupie osobników mogą pomóc w określeniu potencjalnego wpływu tego SNP na cechy wydajności tłuszczu i jego zawartości w mleku.

Key words: cattle, *SLC27A1*, milk production traits.

Słowa kluczowe: bydło, *SLC27A1*, cechy użytkowości mlecznej.

INTRODUCTION

The purpose of breeding programs is to achieve genetic progress in traits which are of economic importance. For that purpose breeders must determine which traits need to be improved and then describe their relative importance. By virtue of what dairy cattle is used for, the traits which are subject to continuous improvement relate to milk yield with a particular emphasis on improving milk quality because protein and fat contained in milk have a large impact on the nutritional, sensory and technological properties of milk products. Much attention is also paid to monitoring and reducing the number of somatic cells in milk. The increased number of somatic cells in milk accompanies mastitis, which contributes to economic losses in the dairy industry. It also reduces milk yield, changes milk composition, and is associated with incurring extra costs for treatment of animals.

Transport of fatty acids is supported by specific proteins. Transmembrane protein family SLC27 (solute carrier family 27), also known as FATP (fatty acid transport protein), consisting of six members SLC27A1-6 plays an important role here. Their primary function is to activate and facilitate the transport of long chain fatty acids across the cytoplasmic

membrane. The SLC27A1 protein, a member of that family, shows enzymatic activity similar to acyl-CoA synthetase (Hall et al. 2003). Insulin-stimulated translocation of the SLC27A1 from inside the cell to the cell membrane coincides with increased uptake of LCFA, indicating the important role of this protein in the maintenance of energy balance (Anderson and Stahl 2013). The *SLC27A1* gene expression was demonstrated in various tissues, particularly in those which are characterized by rapid metabolism of fatty acids, such as mammary tissue during lactation (Ordovás et al. 2006).

The bovine *SLC27A1* gene was mapped to chromosome 7 where QTLs for some milk production traits have been identified (Ordovás et al. 2005; Ogorevc et al. 2009). One of SNPs identified within the gene was significantly associated with fat yield in Chinese Holstein cattle (Ordovás et al. 2008; Lv et al. 2011) and with breeding value for protein content in Polish Holstein-Friesian cows (Kulig et al. 2013).

The effect of *SLC27A1* genotypes on milk production traits in Jersey cattle has not been reported so far. The aim of this study was to establish possible associations between the SNPs and milk production traits as well as somatic cell count in milk of Jersey cows.

MATERIALS AND METHODS

The study covered 133 Jersey cows belonging to one herd kept on a farm located in the western region of Poland. All the studied animals came from 16 sires and were kept in similar environmental conditions. All the 133 cows completed the first lactation, 80 animals completed the first and second lactations, and 38 animals completed the first, second, and third lactations. Milk yield was evaluated with the A4 method in compliance with the recommendations of the International Committee for Animal Recording (ICAR). SCC data was derived from breeding documentation drawn up on the basis of monthly test milking. SCC in the samples was determined according to PN - EN ISO / IEC 17025 standard. Data were collected over eight years.

The peripheral blood (approx. 3 ml) from each cow from the external jugular vein was collected into vacuum test tubes with the K₃EDTA anticoagulating factor. Genomic DNA was extracted from blood using MasterPure™ Genomic DNA Purification Kit (Epicentre®, USA). The study was conducted according to the Resolution No. 22/2006 of National Commission for the Ethical Experience on Animals (Poland).

Two exonic single nucleotide polymorphisms (SNPs) described by Ordovás et al. (2008) were analyzed: *g.14791C>T* and *g.14589A>G* (GeneBank acc. no. AAFC03051286). Genotypes were determined according to the earlier described method (Kulig et al. 2013). The restriction fragments were separated on 3% agarose gels and described using the software for photodocumentation of electrophoretic separation and image storage (Vilber Lourmat, France).

A statistical analysis of associations between the SNPs and milk traits (milk, fat and protein yield, kg; fat and protein content, %) in the first, second, and third lactations were performed. The following mixed effect model of multifactor analysis of variance (ANOVA), using the GLM (General Linear Model) procedure (Statistica®, 2010) was applied:

$$y_{ijkl} = \mu + a_i + b_j + c_k + d_l + f(DIM) + e_{ijkl}$$

where:

- y_{ijkl} – observed trait value in $ijkl$ -th animal;
- μ – mean trait value for herd;
- a_i – the effect of genotype ($i = 1, 2, 3$) or genotype combination ($i = 1, 2, 3, 4$);
- b_j – additive polygenic effect (sire) ($j = 1, 2, 3, \dots, 16$);
- c_k – the effect of year of calving ($k = 1, 2, 3, \dots, 8$);
- d_l – the effect of month of calving ($l = 1, 2, 3, \dots, 12$);
- $f(DIM)$ – regression coefficient on days in milk;
- e_{ijkl} – random error.

Afterwards, associations between SNPs and somatic cell count (SCC) in milk were performed. SCC was transformed into the natural logarithm scale (ln SCC). The following linear model was applied:

$$y_{ijklmn} = \mu + a_i + b_j + c_k + d_l + f_m + g_n(a_i) + e_{ijklmn}$$

where:

- y_{ijklmn} – observed trait value in $ijklmn$ -th animal;
- μ – mean trait value for herd;
- a_i – the effect of genotype ($i = 1, 2, 3$) or genotype combination ($i = 1, 2, 3, 4$);
- b_j – additive polygenic effect (sire) ($j = 1, 2, 3, \dots, 16$);
- c_k – the effect of parity ($k = 1, 2, \dots, 5$);
- d_l – the effect of lactation season ($l = 1, 2, 3, 4$);
- f_m – the effect of lactation stage ($m = 1, 2, \dots, 14$);
- $g_n(a_i)$ – environmental permanent effect (cow) nested within genotype ($n = 1, 2, \dots, 133$);
- e_{ijklmn} – random error.

RESULTS

In the studied herd of Jersey cows, all the possible genotypes were identified. The frequencies of the analyzed genotypes and alleles are presented in Table 1.

Out of the nine possible genotype combinations ($g.14791C>T/14589A>G$), seven were identified in the studied herd. Some genotype combinations were not included in the statistical analysis due to their low number (1 or 2 individuals).

Mean values and standard deviations of the milk production traits in relation to the *SLC27A1* genotypes are given in the table 2. Regarding both *SLC27A1* polymorphisms, no significant associations were found between the genotypes and the analyzed milk production traits of the studied cows (in case of $g.14791C>T$ data not shown). It is worth to note the association between $g.14589A>G$ genotype and fat yield in the first lactation, which, however, was not statistically confirmed ($p = 0.08$) (Table 2). The same refers to the combined genotype ($g.14791C>T/14589A>G$) analysis in the third lactation and it concerns the fat yield ($p = 0.09$) as well as the protein content ($p = 0.08$). No significant associations were also found between the analyzed polymorphisms and the somatic cell count in this study (data not shown).

Table 1. The genotype and allele frequencies of the studied polymorphisms
Tabela 1. Frekwencje genotypów i alleli badanych polimorfizmów

SNP	Genotype frequencies Frekwencja genotypów		Allele frequencies Frekwencja alleli	
<i>g.14791C>T</i>	<i>TT</i>	0.46	<i>T</i>	0.72
	<i>TC</i>	0.52	<i>C</i>	0.28
	<i>CC</i>	0.02		
<i>g.14589A>G</i>	<i>GG</i>	0.44	<i>G</i>	0.69
	<i>AG</i>	0.49	<i>A</i>	0.31
	<i>AA</i>	0.07		
<i>g.14791C>T/14589A>G</i>	<i>TT/GG</i>	0.32		
	<i>TT/AG</i>	0.10		
	<i>TT/AA</i>	0.04		
	<i>TC/GG</i>	0.12		
	<i>TC/AG</i>	0.37		
	<i>TC/AA</i>	0.03		
	<i>CC/AG</i>	0.02		

The *g.14791C>T* polymorphism – PCR product digestion with *Bsa*HI enzyme resulted in no cutting fragment (allele *T*) and cutting fragments of 106 and 54 bp (allele *C*). It resulted in three different genotypes: *CC* and *TT* homozygote as well as *CT* heterozygote – Polimorfizm *g.14791C>T* – trawienie produktu PCR enzymem *Bsa*HI daje w efekcie fragment nienaruszony (allel *T*) oraz fragmenty cięte o długości 106 and 54 pz (allel *C*). Efektem są trzy różne genotypy: homozygoty *CC* i *TT* oraz heterozygota *CT*.

The *g.14589A>G* polymorphism – PCR product digestion with *Ava*I enzyme results in no cutting fragment (allele *A*) and cutting fragments of 122 and 21 bp (allele *G*). It results in three different genotypes: *AA* and *GG* homozygote as well as *AG* heterozygote – Polimorfizm *g.14589A>G* – trawienie produktu PCR enzymem *Ava*I daje w efekcie fragment nienaruszony (allel *A*) oraz fragmenty cięte o długości 122 and 21 pz (allel *G*). Efektem są trzy różne genotypy: homozygoty *AA* i *GG* oraz heterozygota *AG*.

g.14791C>T/14589A>G – genotype combination analysis of both polymorphisms – analiza kombinacji genotypów obydwu polimorfizmów.

Table 2. Association analysis of *SLC27A1* polymorphism and milk production traits in Jersey cows
Tabela 2. Analiza zależności między polimorfizmem *SLC27A1* a cechami użytkowości mlecznej krów rasy jersey

Genotype	N	MY	FY	FC	PY	PC
I lactation – I laktacja						
<i>GG</i>	59	3942 ± 550	217.6 ± 28.7	5.54 ± 0.47	152.8 ± 19.1	3.88 ± 0.20
<i>AG</i>	65	3976 ± 597	227.0 ± 31.1	5.75 ± 0.56	154.5 ± 19.4	3.95 ± 0.40
<i>AA</i>	9	4152 ± 592	232.7 ± 21.9	5.70 ± 0.93	157.4 ± 18.5	3.81 ± 0.27
<i>p</i>		0.20	0.08	0.49	0.54	0.88
II lactation – II laktacja						
<i>GG</i>	33	4319 ± 545	249.4 ± 45.0	5.73 ± 0.55	174.3 ± 26.9	4.00 ± 0.21
<i>AG</i>	39	4366 ± 562	251.3 ± 30.6	5.79 ± 0.58	174.5 ± 21.3	4.01 ± 0.23
<i>AA</i>	8	4388 ± 937	242.1 ± 50.0	5.58 ± 0.72	170.1 ± 30.5	3.92 ± 0.35
<i>p</i>		0.36	0.23	0.63	0.19	0.58
III lactation – III laktacja						
<i>GG</i>	13	4695 ± 459	259.9 ± 29.5	5.54 ± 0.51	186.1 ± 23.0	3.91 ± 0.24
<i>AG</i>	22	4666 ± 718	266.0 ± 39.1	5.73 ± 0.56	185.5 ± 24.9	3.99 ± 0.22
<i>AA</i>	3	4305 ± 160	270.0 ± 33.2	6.26 ± 0.54	176.7 ± 4.5	4.11 ± 0.10
<i>p</i>		0.57	0.28	0.43	0.83	0.19
I lactation – I laktacja						
<i>TC/AG</i>	49	3933 ± 638	227.6 ± 33.4	5.83 ± 0.54	153.9 ± 21.0	3.99 ± 0.45
<i>TT/GG</i>	43	3945 ± 592	219.9 ± 30.6	5.59 ± 0.45	153.4 ± 20.9	3.89 ± 0.20
<i>TC/GG</i>	16	4934 ± 431	211.4 ± 22.5	5.39 ± 0.49	151.2 ± 13.7	3.85 ± 0.20
<i>TT/AG</i>	13	4178 ± 424	225.6 ± 24.6	5.43 ± 0.59	156.8 ± 13.3	3.76 ± 0.15
<i>TT/AA</i>	5	4377 ± 700	238.0 ± 21.2	5.55 ± 1.02	164.0 ± 23.2	3.77 ± 0.30
<i>TC/AA</i>	4	3871 ± 303	226.0 ± 23.9	5.88 ± 0.92	149.2 ± 5.7	3.87 ± 0.24
<i>TC/AG</i>	3	3790 ± 454	223.0 ± 23.1	5.90 ± 0.40	154.0 ± 18.5	4.05 ± 0.04
<i>p</i>		0.47	0.37	0.78	0.75	0.88

Table 2. Association analysis of *SLC27A1* polymorphism and milk production traits in Jersey cows (cont.)Tabela 2. Analiza zależności między polimorfizmem *SLC27A1* a cechami użytkowości mlecznej krów rasy jersey (cd.)

Genotype	N	MY	FY	FC	PY	PC
II lactation – II laktacja						
<i>TC/AG</i>	29	4317 ± 560	247.6 ± 30.4	5.77 ± 0.63	172.7 ± 20.4	4.01 ± 0.24
<i>TT/GG</i>	23	4160 ± 510	245.7 ± 50.0	5.82 ± 0.54	171.6 ± 29.8	4.06 ± 0.19
<i>TC/GG</i>	10	4687 ± 453	258.0 ± 31.0	5.51 ± 0.52	180.6 ± 18.4	3.86 ± 0.19
<i>TT/AG</i>	8	4443 ± 629	254.2 ± 28.6	5.76 ± 0.46	174.5 ± 24.3	3.93 ± 0.13
<i>TT/AA</i>	4	4562 ± 1187	241.7 ± 34.9	5.43 ± 0.75	171.7 ± 27.3	3.84 ± 0.43
<i>TC/AA</i>	4	4213 ± 748	242.5 ± 67.9	5.72 ± 0.78	168.5 ± 37.7	3.99 ± 0.28
<i>p</i>		0.26	0.75	0.78	0.68	0.31
III lactation – III laktacja						
<i>TC/AG</i>	18	4697 ± 762	265.3 ± 42.3	5.68 ± 0.55	187.1 ± 26.3	4.00 ± 0.24
<i>TT/GG</i>	9	4655 ± 476	258.6 ± 27.3	5.57 ± 0.48	183.3 ± 22.6	3.87 ± 0.26
<i>TC/GG</i>	4	4784 ± 473	263.0 ± 38.5	5.50 ± 0.66	192.2 ± 26.0	4.01 ± 0.18
<i>TT/AG</i>	3	4509 ± 659	262.0 ± 22.7	5.87 ± 0.73	176.3 ± 22.1	3.92 ± 0.13
<i>p</i>		0.53	0.09	0.71	0.55	0.08

Genotype symbols – as in Table 1 – Symbole genotypów – jak w tab. 1, N – number of cows – liczba krów, MY – milk yield – wydajność mleka [kg], FY – fat yield – wydajność tłuszczu [kg], FC – fat content – zawartość tłuszczu [%], PY – protein yield – wydajność białka [kg], PC – protein content – zawartość białka [%], *p* – probability value – poziom prawdopodobieństwa.

DISCUSSION

Candidate genes strategy is used for different genes whose products may affect the formation of performance traits in livestock. In cattle, genes encoding milk protein, certain hormones, transcription factors, among others, are intensively analyzed (Deb et al. 2014). Statistically significant associations between polymorphisms in these genes and milk traits have been observed. The list of candidate genes to be analyzed because of the functions of their products also includes genes encoding enzymes involved in the metabolism of fatty acids, proteins binding and transporting fatty acids and many others proteins (Ordovás et al. 2008; Zhao et al. 2015). However, in many cases, these studies are at the initial stage.

The gene encoding *SLC27A1* protein merit particular attention among the wide range of genes that could facilitate the explanation of variability of milk production traits in cattle and therefore be useful in the early selection of dairy cattle. The exonic polymorphisms within the *SLC27A1* gene, which were analyzed in study, have synonymous character. Their presence can affect mRNA splicing, stability and structure of the transcripts, as well as the processes related to transcription and post-translational processing (Woolfe et al. 2010).

Higher frequencies of alleles *g.14791*T* and *g.14589*G* found in Jersey cattle, were confirmed by the results obtained by Ordovás et al. (2008) and Kulig et al. (2013) in Holstein-Friesian breed. The results obtained in this study demonstrate that there are no significant associations between the *g.14791C>T* and *g.14589A>G* polymorphism and milk production traits as well as somatic cell count in Jersey breed cows. Few data are available on the research analyzing polymorphisms in the *SLC27A1* gene in terms of possibilities for improving the traits associated with bovine milk performance. No data on the analysis of the association between *SLC27A1* SNP somatic cell count were found. Ordovás et al. (2008) found no associations between the SNPs and estimated breeding value for milk fat yield in

the Holstein-Friesian population, but that study did not include an analysis of other milk production traits. Lv et al. (2011), however, associated the SNP *g.14791C>T* to the breeding value for milk yield. Animals with the *CC* genotype had significantly higher value of this trait than individuals with other genotypes. On the other hand, the results presented in the earlier study (Kulig et al. 2013) indicate the influence of *g.14791C>T* polymorphism on the breeding value for protein content in milk of Polish Holstein-Friesian cows, and its value was significantly higher in cows with genotype *CC*. These discrepancies may be explained among others by breed differences, herd size or different environmental conditions.

SLC27A1 gene polymorphism was also analyzed in beef cattle. Zhao et al. (2015) suggest an association between some SNPs and meat quality traits, such as backfat thickness and intramuscular fat, in Chinese Qinchuan cattle. SNPs were also identified in the porcine *SLC27A1*. Gallardo et al. (2009) reported the *c.49C>T* significant effects on the relative distribution of stearic acid and saturated fatty acid levels in muscles. Melo et al. (2013) found associations between the *c.441C>T* genotype and saturated fatty acid content in some muscles. Polymorphisms in the chicken *SLC27A1* gene were associated with carcass traits, among others live weight and carcass weight (Wang et al. 2010). *SLC27A1* gene polymorphisms were also analyzed in humans. Some studies suggest an association between the intronic polymorphism and changes in triglyceride metabolism (Gertow et al. 2003; Mairhaeghe et al. 2000).

CONCLUSIONS

The presented results suggest that further research involving much larger herd as well as herds of other cattle breeds is advisable. Continuing research in this area might facilitate a more in-depth interpretation of the results. Perhaps in the studies on a large number of individuals, the *SLC27A1 g.14589A>G* polymorphism could prove to be significant in determining fat yield.

REFERENCES

- Anderson C.M., Stahl A. 2013. SLC27 fatty acid transport proteins. *Mol. Aspects Med.* 34, 516–528.
- Deb R., Singh U., Kumar S., Singh R., Sengar G., Sharma A. 2014. Genetic polymorphism and association of kappa-casein gene with milk production traits among Frieswal (HF × Sahiwal) cross breed of Indian origin. *Iran. J. Vet. Res.* 15, 406–408.
- Gallardo D., Quintanilla R., Pena R.N. 2009. Characterization of the porcine fatty acid transport proteins (SLC27a/FATPs solute carrier family 27a), in: XXXIX Jornadas de Estudio, XIII Jornadas sobre Producción Animal, Zaragoza, España, 2009. [b.w.], 3–5.
- Gertow K., Skoglund-Andersson C., Eriksson P., Boquist S., Orth-Gomér K., Schenck-Gustafsson K., Hamsten A., Fisher R.M. 2003. A common polymorphism in the fatty acid transport protein-1 gene associated with elevated post-prandial lipaemia and alterations in LDL particle size distribution. *Atherosclerosis* 167, 265–273.
- Hall A.M., Smith A.J., Bernlohr D.A. 2003. Characterization of the Acyl-CoA synthetase activity of purified murine fatty acid transport protein 1. *J. Biol. Chem.* 278, 43008–43013.
- Kulig H., Kowalewska-Łuczak I., Żukowski K., Kunicka M., Kruszyński W. 2013. *SLC27A1* SNPs in relation to breeding value of milk production traits in Polish Holstein-Friesian cows. *Anim. Sci. Pap. Rep.* 4, 273–279.

- Lv Y., Wei C., Zhang L., Lu G., Liu K., Du L. 2011. Association between polymorphisms in the *SLC27A1* gene and milk production traits in Chinese Holstein cattle. *Anim. Biotechnol.* 22, 1–6.
- Meirhaeghe A., Martin G., Nemoto M., Deeb S., Cottel D., Auwerx J., Amouyel P., Helbecque N. 2000. Intronic polymorphism in the fatty acid transport protein 1 gene is associated with increased plasma triglyceride levels in a French population. *Arterioscler. Thromb. Vasc. Biol.* 20, 1330–1334.
- Melo C., Gallardo D., Quintanilla R., Zidi A., Castelló A., Díaz I., Amills M., Pena R.N. 2013. An association analysis between polymorphisms of the pig solute carrier family 27A (*SLC27A*), member 1 and 4 genes and serum and muscle lipid traits. *Livest. Sci.* 152, 143–146.
- Ogorevc J., Kunej T., Razpet A., Dovc P. 2009. Database of cattle candidate genes and genetic markers for milk production and mastitis. *Anim. Genet.* 40, 832–851.
- Ordovás L., Roy R., Zaragoza P., Hayes H., Eggen A., Rodellar C. 2005. Assignment of the *solute carrier family 27 member 1 (SLC27A1)* gene to bovine chromosome 7. *Anim. Genet.* 36, 352–353.
- Ordovás L., Roy R., Zaragoza P., Rodellar C. 2006. Structural and functional characterization of the bovine solute carrier family 27 member 1 (*SLC27A1*) gene. *Cytogenet. Genom. Res.* 115, 115–122.
- Ordovás L., Zaragoza P., Altarriba J., Rodellar C. 2008. Identification of 14 new single nucleotide polymorphisms in the bovine *SLC27A1* gene and evaluation of their association with milk fat content. *J. Dairy. Res.* 75, 129–134.
- Statsoft, Poland. 2010. Statistica 10.0. (Data Analysis Software System).
- Wang Y., Zhu Q., Zhao X.-L., Yao Y.G., Liu Y.P. 2010. Association of *FATP1* gene polymorphisms with chicken carcass traits in Chinese meat-type quality chicken populations. *Mol. Biol. Rep.* 37, 3683–3690.
- Woolfe A., Mullikin J.C., Elnitski L. 2010. Genomic features defining exonic variants that modulate splicing. *Genome Biol.* 11, R20.
- Zhao Z.D., Li A.N., Wei S.J., Wang M.M., Li S.J., Zan L.S. 2015. Genetic polymorphisms of the *FATP1* gene and their associations with meat quality traits in Chinese Qinchuan cattle. *Gen. Mol. Res.* 14, 17439–17446.

Abstract. The aim was to establish possible associations between the *g.14791C>T* and *g.14589A>G* SNPs in the *SLC27A1* gene and milk production traits as well as somatic cell count in milk of Jersey cows. No significant associations were found between the genotypes and the analyzed traits. Further study on a larger number of animals is necessary to determine the potential effect of this SNP on the fat yield and fat content in milk.

