

Effect of *DGAT1* gene variants on milk quantity and quality in Holstein, Simmental and Brown Swiss cattle breeds in Croatia

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Abstract

The aim of this study was to determine allele frequency at the diacylglycerol-O-transferase 1 gene (*DGAT1*) K232A polymorphic site and effect of allelic variants *K* and *A* on milk production traits of Holstein (dairy), Simmental and Brown Swiss (dual purpose) cattle breeds in Croatia. The DNA was extracted from hair of 371 cows, while coding region of *DGAT1*p.K232A was amplified using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique. Dominant frequency of *K* allele was determined in all three analysed breeds: Holstein (0.775), Simmental (0.616) and Brown Swiss (0.651). The most Holstein cows were homozygous for *KK* variant (0.637), while dominant genotype in other two breeds was *KA* (Simmental 0.647; Brown Swiss 0.644). Positive effect of *KK* genotype on milk yield was detected in Holstein and Brown Swiss breeds, while in Simmental breed was negative (-220.8 kg; -463.6 kg) in regard to *KA* and *AA* genotype. Milk of cows with *KK* genotype in Holstein breed, *KA* in Simmental and *AA* in Brown Swiss were found as the most favourable in terms of fat content. Cows of *AA* genotype in all the three analysed breeds have had higher milk protein content. Statistical analysis was performed using the GLM procedure, including genotype and breed effects as independent variables. The effects of *DGAT1* genotypes on milk quantity and quality were not significant ($P > 0.05$).

Key words: *DGAT1*, polymorphism, production traits, milk, cattle

Introduction

The gene diacylglycerol-O-transferase 1 (*DGAT1*) is mapped in the centromere region of *BTA14* and encodes the diacylglycerol-O-transferase enzyme which catalyses the final step in triglyceride synthesis (Grisart et al., 2002). The allele frequencies and effects of polymorphic variants of *DGAT1* gene have been characterized in many dairy cattle population: in New Zealand (Spelman et al., 2002), Germany (Thaller et al., 2003), France (Gautier et al., 2007), Italy (Scotti et al., 2010), Czech Republic (Hanusova et al., 2014) and many other

countries. In dairy cows, *DGAT1* gene is considered to be a candidate gene for milk fat content. A non-conservative lysine to alanine substitution (K232A) in this gene has been proved to have a major effect on milk production trait. In particular, the lysine encoding allele (allele *K*) is associated with increased fat content of milk compared to the alanine encoding allele, allele *A* (Winter et al., 2002; Gautier et al., 2007). Berry et al. (2010) found that the *K* allele was associated with reduced milk and protein yield, but higher yield of milk fat, and concentration of milk fat and milk protein in Irish Holstein cows. The *DGAT1* polymorphism creates great

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possibilities to accomplish a relatively rapid change in milk composition using genotyped bulls (Ikonen et al., 2004). The aim of presented study was determination of the polymorphic allelic variants of *DGAT1* gene and their interactions with milk production traits (milk yield, fat and protein content) in Holstein, Simmental, and Brown Swiss cattle in Croatia.

Materials and methods

The study included 371 cows belonging to Holstein (182), Simmental (116) and Brown Swiss (73) breeds from nine farms in Croatia. DNA was extracted from the hair using Sigma-Aldrich™ Gen-Elute Mammalian Genomic DNA Miniprep Kit. Amplification of *DAGT1* gene was determined by PCR-RFLP technique according to Winter et al. (2002). PCR reaction was consisted in a total volume of 20 μ L using DNA as template, 10 \times PCR buffer, 25 mM MgCl₂, 10 mM dNTP, 5 U Taq Polymerase and 10 pmol of each primers (GenBank Acc., No. AJ318490; forward: 5'-GCACCATCCTCTTCCTCAAG-3'; reverse 5'-GGAAGCGCTTTCG-GATG-3'). The PCR profile included an initial denaturation step 94 °C for 4 min, 35 amplification cycles of 60 s at 94 °C, 30 s at 59 °C, 30 s at 72 °C, and final extension step 10 min at 72 °C. The amplified fragments of 411 bp (5.4 μ L of PCR product) were digested with 5 U *CfrI* restriction enzyme for 2 h at 37 °C in reaction mixture (4.0 MiliQ water, 1.2 μ L buffer R, 5.4 μ L of PCR product and 0.15 μ L *CfrI*). The PCR products and digestion products were visualized and evaluated on 1 % EtBr agarose gels with TBE buffer. Genotypes were determined based on number/length DNA fragments: (KK 411 bp), KA (411 bp, 208 bp, 203 bp) and AA (208 bp, 203 bp). Data for milk yield, milk fat and protein content in lactation (first three lactations were averaged) were obtained from central laboratory for milk control of Croatian Agricultural

Agency. Frequencies of allele and genotype, level of heterozygosity, Hardy-Weinberg equilibrium and *F* statistics were calculated using the Arlequin ver. 3.0 (Excoffier et al., 2005). Statistical analysis of the experimental data was performed using the GLM procedure of SAS V9.2 (2008) including genotype and breed effects as independent variables. The level of significance of the treatment was set at $P < 0.05$.

Results and discussion

Analysis of *DGAT1* polymorphisms revealed the presence of three genotypes (KK, KA and AA), i.e. two allelic variants (*K* and *A*) of *DGAT1* gene in three studied cattle populations. Allele and genotype frequencies are reported in Table 1.

Dominant frequency of *K* allelic variant was determined in all three analysed breeds, thus KA genotype was predominant in Simmental and Brown Swiss, and KK in Holstein breed. The Holstein breed showed the highest frequency for allele *K* (0.775), followed by Brown Swiss (0.651) and Simmental cattle (0.616). Previous studies have showed a wide interval frequency of *K* and *A* allelic variants *K* (0.37) and *A* (0.63) (Grisart et al., 2002; Gautier et al., 2007); *K* (0.25) and *A* (0.75) (Fontanesi et al., 2007); *K* (0.59) and *A* (0.41) (Patel et al. 2009); *K* (0.32) and *A* (0.68) (Berry et al. 2010); *K* (0.19) and *A* (0.81) (Manga and Riha, 2011); *K* (0.79) and *A* (0.21) (Mashhadi et al., 2012). As reported by Spelman et al. (2002) frequency of *K* allele in New Zealand Holstein population was increased from 30 to 60 % due to import of Holstein cattle from the USA, Canada and Netherlands. Opposite to this study, Thaller et al. (2003), Kaupe et al. (2004), and Scotti et al. (2010) have found domination of *A* allelic variant of *DGAT1* gene in Simmental breed. In Croatian population of Brown Swiss cattle was observed significant higher frequency of allele *K* (0.651) to compared allele *A*,

Table 1. Genotype and allele frequencies of *DGAT1* gene, *Chi* and *p* values, observed (H_O) and expected heterozygosity (H_E), and coefficient of inbreeding (F_{IS}) in analysed cattle breeds

Breed	Genotype frequencies			Allele frequencies		<i>Chi</i>	<i>p</i> value	H_O	H_E	F_{IS}
	AA	KA	KK	<i>A</i>	<i>K</i>					
Simmental	0.060	0.647	0.293	0.384	0.616	15.64	0.0001	0.647	0.475	-0.361
Holstein	0.088	0.275	0.637	0.225	0.775	8.25	0.0054	0.274	0.350	0.234
Brown Swiss	0.027	0.644	0.329	0.349	0.651	12.65	0.0007	0.644	0.458	-0.412

while in other study on Brown Swiss cattle (Kaupe et al., 2004; Scotti et al., 2010) was established domination of *A* allelic variant. Results from this and other studies imply that the frequencies of alleles and genotypes of *DGAT1* in different breeds are diverse worldwide (Lacorte et al., 2006; Ripoli et al., 2006).

Fixation index in Holstein population (Table 1) indicate inbreeding, probably as results of long term using advanced breeding technologies and the proliferation of a small group of closely related sires. Hansen et al. (2003) report that only 10 bulls of their respective breed sired 61 % of the Canadian, 21 % Brown Swiss, 29 % Jersey cattle populations, and 41 % of the Holstein bulls in the bull studs. More and more breeders are demanding proven sires to increase milk production from fewer cows resulting in slow but expected decreasing level of genetic diversity.

Effect of *DGAT1* genotypes on milk yield, fat and protein content in analysed breeds are presented in Table 2. Cows with *KK* genotype produced 256.5 kg more milk compared to cows *KA* genotype and 381.9 kg more milk than cows *AA*, but the observed differences were not significant ($P > 0.05$). Holstein and Brown Swiss cows of *KK* genotype in regard to *KA* and *AA* had higher milk production, but differences not significant ($P > 0.05$). Opposite from results of this study, many authors (Oshima-Hori and Serrano-Barreras, 2003; Thaller et al., 2003; Sanders et al., 2006; Oikonomou et al.,

2008) observed a positive effect *A* allelic variant of *DGAT1* gene on milk yield of Holstein cows. A possible reason for the negative impact of the *AA* genotype could be explained by the extremely small number of cows with the mentioned genotype and the effects of housing. Kaupe et al. (2004) have found negative effect of *K* allelic variant on the amount of milk yield (-258 kg) and the positive effect on the fat content (+0.28 %) and protein content (+0.06 %) in population of Holstein cows. Hradecka et al. (2008) also reported a positive effect *A* allelic variant on milk yield (+548 kg) and the protein yield (+12.6 kg) of Holstein homozygous individuals and negative effect on the fat yield (-15.4 kg). In Simmental breed *AA* genotype was associated with the highest milk yield, but also differences were not significant ($P > 0.05$).

The results of this study indicated that the genotype *KA* of *DGAT1* gene was related to higher average fat content in milk (+0.13 %; +0.17 %) compared to *KK* and *AA* genotypes. Considering the average milk protein content, milk of cows with *AA* genotype had more favourable values (+0.05 %; +0.03 %) than cows with *KK* and *KA* genotypes. Although there were evident differences in the average fat and protein content in milk, they were not significant ($P > 0.05$). Thaller et al. (2003) and Hradecka et al. (2008) have observed in the Holstein population negative effect *K* allelic variant of *DGAT1* gene on the protein yield, and a positive effect mentioned variant on fat yield. Considering the

Table 2. Effect of *DGAT1* genotypes on milk yield, fat and protein content in Holstein, Simmental and Brown Swiss breeds

Breed	Genotype (n)	Milk yield (kg/305 days)	Fat content (%)	Protein content (%)
Holstein	<i>AA</i> (16)	8825.8±608.8	3.93±0.15	3.33±0.06
	<i>KA</i> (50)	8866.2±405.0	4.09±0.10	3.26±0.04
	<i>KK</i> (116)	9224.6±259.5	4.14±0.06	3.30±0.02
Simmental	<i>AA</i> (7)	6840.0±529.2	4.16±0.21	3.43±0.08
	<i>KA</i> (75)	6597.2±224.8	4.39±0.09	3.46±0.03
	<i>KK</i> (34)	6376.4±305.1	4.14±0.12	3.42±0.04
Brown Swiss	<i>AA</i> (2)	6050.6±930.2	4.00±0.34	3.62±0.16
	<i>KA</i> (47)	7293.4±318.0	3.84±0.11	3.47±0.05
	<i>KK</i> (24)	7496.0±396.0	3.70±0.14	3.45±0.06
All breeds	<i>AA</i> (25)	7356.5±378.8	3.98±0.12	3.44±0.05
	<i>KA</i> (172)	7481.9±173.8	4.15±0.05	3.41±0.02
	<i>KK</i> (174)	7738.4±205.5	4.02±0.06	3.39±0.02

average values for milk fat and milk protein content, milk of Holstein cows with *AA* genotype in regard to *KK* and *KA* genotypes had less fat content (-0.21 %; -0.16 %) and more protein content (+0.03 %; +0.07 %), but the observed differences were not significant ($P>0.05$). Simmental cows of *KA* genotype in regard to *KK* and *AA* genotypes had higher average fat content in milk (+0.25 %; +0.23 %), also the observed differences were not significant ($P>0.05$). The average content of milk fat in Brown Swiss cows of *KK* genotype of *DGAT1* gene in regard to *KA* and *AA* genotypes was lower (-0.14 %; -0.30 %), although not significant ($P>0.05$). The results presented in this study are consistent with previous studies where *KK* genotype of *DGAT1* gene was associated with the largest milk fat content (Spelman et al., 2002; Thaller et al., 2003; Leskova et al., 2013). The average content of milk protein from Simmental cows of *KA* genotype in regard to *KK* and *AA* genotypes was higher (-0.04 %; -0.03 %), but differences were not significant ($P>0.05$). Although in milk of Brown Swiss cows with *AA* genotype compared with milk of *KK* and *KA* genotypes was found favorable milk protein content (+0.17 %; +0.15 %) without significant differences ($P>0.05$). In some of previous studies (Spelman et al., 2002; Thaller et al., 2003; Sanders et al., 2006) it was observed that the allelic variant *K* of *DGAT1* gene has a negative effect on the milk protein content and mentioned effect was partly found in this study.

Conclusions

In three cattle breed from Croatia was observed dominance of *K* allelic variant of *DAGT1* gene. Association between genotypes of *DGAT1* gene and milk production traits (milk yield, fat and protein content) was found between breeds, but the observed differences were not significant. Across all three breeds, *KK* genotype was responsible for higher milk production, *KA* genotype for higher amount of milk fat and *AA* genotype for higher protein content. Since the results of studies carried out are different in terms of the impact of polymorphism of *DGAT1* gene on the milk production traits, it is necessary to conduct studies on a larger number of animals, especially dairy cattle breeds.

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Utjecaj DGAT1 genetskih varijanti na količinu i kvalitetu mlijeka holstein, simentalске i smeđe pasmine goveda u Hrvatskoj

Sažetak

Cilj ovog istraživanja bio je utvrditi frekvenciju alela diacilglicerol-0-transferase 1 gena (*DGAT1*) K232A polimorfnog mjesta i utjecaja alelnih varijanti *K* i *A* na količinu i kvalitetu mlijeka tri pasmine krava, mliječnog i dvojnog tipa proizvodnje u Hrvatskoj. Istraživanje je uključivalo 371 jedinku holstein, simentalске i smeđe pasmine krava. DNA je izolirana iz dlake, kodogena regija *DGAT1*p.K232A gena umnožena je korištenjem PCR-RFLP metode. Dominantna frekvencija alela *K* utvrđena je kod sve tri istraživane pasmine: holstein (0,775), simentalске (0,616) i smeđe pasmine (0,651). Većina krava holstein pasmine bila je homozgotna za *KK* varijantu (0,637), dok je dominantan genotip za druge dvije pasmine bio *KA* (simentalска 0,647; smeđa pasmina 0,644). Pozitivan utjecaj *KK* genotipa na proizvodnju mlijeka zabilježen je populacijama u holstein i smeđe pasmine, dok je u simentalскоj populaciji bio negativan (-220,8 kg; -463,6 kg) u odnosu na *KA* i *AA* genotipove. Mlijeko krava *KK* genotipa holstein, *KA* genotipa simentalске i *AA* genotipa u smeđe pasmine imalo je veći sadržaj mliječne masti. Krave *AA* genotipa u sve tri istraživane pasmine proizvodile su mlijeko s većim sadržajem proteina. Statistička analiza provedena je uz pomoć GLM procedure, uvažavajući genotip i pasminu kao neovisne varijable. Utjecaj *DGAT1* genotipova na istraživane kvantitativne i kvalitativne osobine mlijeka nisu bile signifikantne ($P>0,05$).

Ključne riječi: *DGAT1*, polimorfizam, proizvodne osobine, mlijeko, krava

References

1. Berry, D.P., Howard, D., O'Boyle, P., Waters, S., Kearney, J.F., McCabe, M. (2010): Association between the K323A polymorphism in the diacylglycerol-O-transferase 1 (DGAT1) gene and performance in Irish Holstein, *Irish Journal of Agricultural and Food Research* 49, 1-9.
2. Excoffier, L., Laval, G., Schneider, S. (2005): Arlequin ver. 3.0: an integrated software package for population genetics data analysis, *Evolutionary Bioinformatics Online* 1, 47-50.
3. Fontanesi, L., Scotti E., Schiavini, F., La Mattina, V., Davoli, R., Russo, V., Bagnato, A. (2007): Investigation of the DGAT1 K323A and VNTR mutations in dairy and dual purpose cattle breeds, *Italian Journal of Animal Science* 6, 137.
4. Gautier, M., Capitan, A., Fritz, S., Eggen, A., Boichard, D., Druet, T. (2007): Characterization of the DGAT1 K323A and variable number of tandem repeat polymorphisms in French dairy cattle, *Journal of Dairy Science* 90, 2980-2988. doi: 10.3168/jds.2006-707
5. Grisart, B., Coppieters, W., Farnir, F., Karim, L., Ford, C., Berzi, P., Combisano, N., Mni, M., Reid, S., Simon P., Spelman R., Georges M., Snell R. (2002): Positional 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 Research* 12, 222-231. doi: 10.1101/gr.224202
6. Hansen, C., Shrestha, J.N.B., Parker, R.J., Crow G.H., Derr, J.N. (2003): Genetic base and inbreeding of Canadian, Brown Swiss, Holstein and Jersey cattle in Canada, *FAO Animal Genetic Resources Information* 33, 11-20. doi: 10.1017/S1014233900001590
7. Hanusova, L., Mikova, L., Vecerek, D., Schroeffelova, D., Rehout, V., Tothova, L., Vernerove, K., Holsnedlova, B., Citek, J. (2014): Effect of DGAT1 polymorphisms on the estimated breeding values of Czech Simmental sires, *Czech Journal of Animal Science* 59, 365-373.
8. Hradecka, E., Citek, J., Panicke, L., Rehout, V., Hanusova, L. (2008): The relation of *GHI*, *GHR* and *DGAT1* polymorphisms with estimated breeding values for milk production traits of German Holstein sires, *Czech Journal of Animal Science* 53, 238-245.
9. Ikonen, T., Morri, A., Tyriseva, A.M., Ruottinen, O., Ojala, M. (2004): Genetic and phenotypic correlations between milk coagulation properties, milk production traits, somatic cell count, casein content and pH of milk, *Journal of Dairy Science* 87, 458-467. doi: 10.3168/jds.S0022-0302(04)73185-9
10. Kaupe, B., Winter, A., Fries, R., Erhardt, G. (2004): DGAT1 polymorphism in *Bos indicus* and *Bos taurus* cattle breeds, *Journal of Dairy Research* 71, 182-187. doi: 10.1017/S0022029904000032
11. Lacorte, G.A., Machado, M.A., Martinez, M.L., Campos, A.L., Maciel, R.P., Verneque, R.S., Teodoro, R. L., Peixoto, M.G.C.D., Carvalho, M.R.S., Fonseca, C.G. (2006): DGAT1 K323A polymorphism in Brazilian cattle breeds, *Genetics and Molecular Research* 5, 475-482.
12. Leskova, L., Bauer M., Chrenek, P., Lackova, Z., Sorocinova, J., Petrovic, V., Kovac, G. (2013): Detection of DGAT1 gene polymorphism and its effect on selected biochemical indicators in dairy cows after calving, *Acta Veterinaria Brno* 82, 265-296. doi: 10.2754/avb201382030265
13. Manga, I., Ríha, H. (2011): The DGAT1 gene K232A mutation is associated with milk fat content, milk yield and milk somatic cell count in cattle, *Archiv Tierzucht* 54, 257-263.
14. Mashhadi, H., Nassiri, M.R., Mahmoudi, M., Rastin, M., Kashan, N.E.J., VaezTorshizi, R., Tabasi, N., Nooraee, S.E. (2012): Polymorphism and sequencing of DGAT1 gene in Iranian Holstein Bulls, *Iranian Journal of Applied Animal Science* 2, 63-67.
15. Oikonomou, G., Angelopoulou, K., Arsenos, G., Zygoiannis, D., Banos, G. (2008): The effect of polymorphisms in the DGAT1, leptin and growth hormone receptor gene loci on bodyenergy, blood metabolic and reproductive traits of Holstein cows, *Animal Genetics* 40, 10-17. doi: 10.1111/j.1365-2052.2008.01789.x
16. Oshima-Hori, S., Serrano-Barreras, A. (2003): Relationships between DGAT1 and PIT-1 genes polymorphism and milk yield in Holstein cattle. *Western Section American Society of Animal Science*, Proceedings, p. 54.
17. Patel, R.K., Chauhan, J.B., Soni, K.J., Singh, K.M. (2009): Genotype and allele frequencies of DGAT1 gene in Indian Holstein bulls, *Current trends in biotechnology and pharmacy* 3, 385-388.
18. Ripoli, M. V., Corva, P., Giovambattita, G. (2006): Analysis of a polymorphism in the DGAT1 gene in 14 cattle breeds through PCR-SSCP methods, *Research Veterinary Science* 80, 287-290. doi: 10.1016/j.rvsc.2005.07.006
19. Scotti, E., Fontanesi, L., Schiavini, F., La Matina, V., Bagnato, A., Russo, V. (2010): DGAT1 p.K323A polymorphism in dairy and dual purpose Italian cattle breeds. *Italian Journal of Animal Science* 9, 79-82.
20. Spelman, R.J., Ford, C.A., McElhinney, P., Gregory, G.C., Snell, R.G. (2002): Characterization of the DGAT1 gene in the New Zealand dairy population, *Journal of Dairy Science* 85, 3514-3517. doi: 10.3168/jds.S0022-0302(02)74440-8
21. Sanders, K., Bennewitz, J., Reinsch, N., Thaller, G., Prinzeberg, E.M., Kuhn, C., Kalm, E. (2006): Characterization of the DGAT1 mutations and the CSN1S1 promoter in the German Angeln dairy cattle population, *Journal of Dairy Science* 89, 3164-3174. doi: 10.3168/jds.S0022-0302(06)72590-5
22. SAS (2008): The Statistical Analysis System. Cary, NC: SAS Institute.
23. Thaller, G., Kramer, W., Winter, A., Kaupe, B., Erhardt, G., Fries, R. (2003): Effect of DGAT1 variants on milk production traits in German cattle breeds, *Journal of Animal Science* 81, 1911-1918.
24. Winter, A., Kramer, W., Werner, F.A.O., Kollers, S., Kata, S., Durstewitz, G., Buitkamp, J., Womack, J.E., Thaller, G., Fries, R. (2002): Association of a lysine-232/alanine polymorphism in bovine gene encoding acyl-CoA:diacylglycerolacyltransferase (DGAT1) with variation at a quantitative trait locus for milk fat content, *Proceedings of the National Academy of Sciences of the USA* 99, 9300-9305. doi: 10.1073/pnas.142293799