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Short communication

Effects of the *DGAT*1 K232A polymorphism on milk production traits in Holstein cattle

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*DGAT*1 gene polymorphism in exon 8 AA→GC which converts lysine to alanine at position 232 (K232A) was determined to have significant influence on bovine milk production characteristics like milk yield, protein content, fat content, and fatty acid composition. The aim of this study was to analyse the effect of *DGAT*1 gene polymorphism on dairy production traits [milk yield (kg), fat yield (kg), fat content (%), protein yield (kg) and protein content (%)]. Genotyping of 136 Holstein cows was performed using the ACRS-PCR method. The genotypes frequencies were as follows: homozygous genotype AA (80.88%), heterozygous genotype AK (16.91%) and homozygous genotype KK (2.21%). In the monitored population, allele A had a predominance with a frequency of 89.34% over allele K with a frequency of 10.66%. In the study was observed a statistically significant (P <0.0001) effect of *DGAT*1 K232A marker genotypes on breeding value variability for milk fat and protein content (%) as well as milk yield (kg) was observed.

Keywords: milk production, *DGAT*1, cattle, Diacylglycerol O-acyltransferase 1, Holstein cattle

1 Introduction

Milk is the first food for mammals, in addition to provide all the nutrients necessary for the proper growth and development of the body, it is also attributed to health benefits (Pereira, 2014). Through a wide range of milk components, it is involved in metabolism in various ways, or these components affect nutrient absorption (Haug et al., 2007). Bovine milk is considered important for human nutrition and is an important component of a healthy diet (Pereira, 2014; Palombo et al., 2018).

Enzyme diacylglycerol O-acyltransferase 1 is catalyses triglycerides synthesis by using diglycerides and acyl-coenzyme A (Lu et al., 2015). On chromosome 14 in the exon 8 of the *DGAT*1 gene was located dinucleotide substitution, which changes the amino acid sequence from lysine to alanine at position 232 in the mature protein (Tăbăran et al., 2015). It is known that the *DGAT*1 gene modulate milk composition. It was shown that gene *DGAT*1 has a strong effect on milk fat content (Marchitelli et al., 2013; Tăbăran et al., 2015; Čítek et al., 2018).

Milk fat is considered the most complex of all natural fats because contains approximately 400 different fatty acid (Månsson, 2008). Fat content is an important contributing to the nutritional quality of milk and dairy products (Ferlay et al., 2017). Diacylglycerol O-acyltransferase 1 (*DGAT*1) is one of the key elements involved in metabolic processes which can influence the quality of bovine milk (Babii et al., 2018). A polymorphic site at the K232A locus has been associated with significant influence on bovine milk production traits, including fatty acid composition in different cattle breeds (Lu et al., 2015; Vanbergue et al., 2016; Houaga et al., 2018). One way to alter the content of beneficial substances in milk fat is the monitoring of candidate genes and their polymorphisms associated with milk fatty acids biosynthesis, which could help to improve the quality of milk fat (Kala et al., 2016).

This study aimed to analyse the effect of *DGAT*1 gene polymorphism on milk production traits of Holstein cattle in Slovakia.

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2 Material and methods

2.1 Animals

In the study were analysed 136 Holstein cows. Samples of hair roots were taken from the highest, average and worst rated animals on the farm based on the Slovak production index. Genomic DNA was isolated using a commercial column kit QIAamp® DNA Mini Kit (Qiagen). The concentration and purity of DNA were measured using a spectrophotometer NanoPhotometer™ (Implen GmbH). All DNA samples were stored at -20 °C.

2.2 Genotyping

For genotyping *DGAT*1 gene polymorphism in exon 8 AA→GC, which converts lysine to alanine at position 232 (K232A) was used method artificially created restriction site (ACRS-PCR) described by Komisarek and Michalak (2008). The PCR reactions were optimised on a thermocycler C1000TM (Biorad) in total volume 25 µl. To identification of *DGAT*1 polymorphism was used specific restriction endonuclease. Primer sequences used in this study and conditions of used methods are shown in Table 1.

*DGAT*1 genotypes were determined using 2% agarose gel (Invitrogen) containing intercalating agent GelRedTM (Biotium) in 1 × SB buffer (Brody and Kern, 2004) at 180 V for 15 minutes. The gel was analysed with the UV rays and the results were subsequently recorded using Olympus C-7070 documentary system.

2.3 Genetic structure

Using molecular genetic analyses was determined the genotypic structure of the population and estimated the allelic frequencies of the *DGAT*1 gene in the monitored population. The significance of differences between experimental and theoretically expected frequencies of genotypes was verified with the χ2-test.

2.4 Association studies

To demonstrate the effect of different *DGAT*1 genotypes on the variability of average breeding values for milk, protein and fat yield in kilograms and contents of fat and protein in percentages were used the packed SAS version 9.3 (SAS Inc., 2011). One-way ANOVA evaluated the influence of individual genotypes of the *DGAT*1 gene on the variability of average breeding values:

$$y = \mu + G_i + e_{ij}$$

where:
y – breeding value (kg milk, kg protein, kg fat, % protein, % fat); µ – mean value; G_i – fixed effect of genotype, $i = 1, 2, 3$; e_{ij} – residual effect

3 Results and discussion

A total of 136 Holstein cows were analysed for *DGAT*1 K232A gene polymorphism. Genotyping was performed using the ACRS-PCR method. Allele K consists of 2 fragments of 282 bp and 96 bp in length, and allele A consists of 3 fragments of 254 bp, 96 bp and 28 bp in length (Figure 1). All three genotypes were observed in

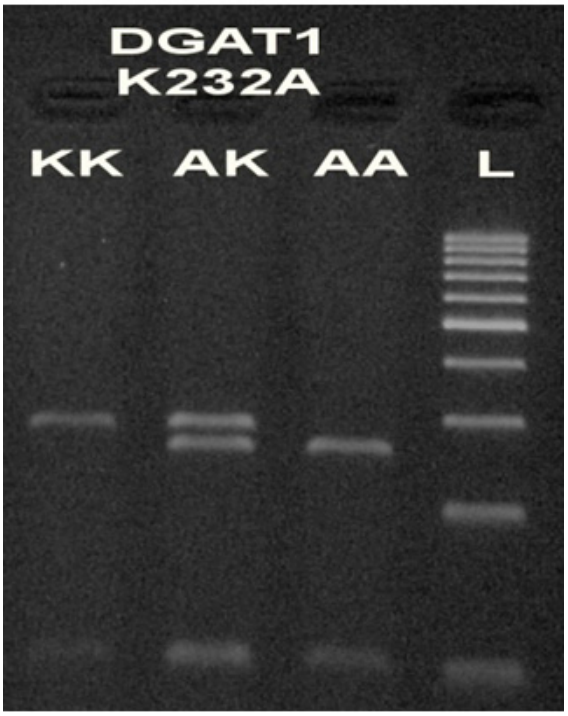

Figure 1 Representative results of the analyses *DGAT*1 K232A polymorphism
Genotype KK (282 bp, 96 bp), genotype AK (282 bp, 254 bp, 96 bp, 28 bp), genotype AA (254 bp, 96 bp, 28 bp). L – 100 bp ladder (Thermo Scientific BioScience)

Table 1 PCR primers and condition

Locus/genetic polymorphism	Primer sequences	Tm	Mg^{2+} (mM)	Restriction enzyme
DGAT1 exon 8 AA→GC GenBank AY065621	F: 5´ – TGCCGCTTGCTCGTAGCTTTGGCC – 3´ R: 5´ – CCTGGAGCTGGGTGAGGAACAGC – 3´ (Komisarek and Michalak, 2008)	66 °C	1.5	*Bg* I^{-1}

the population: 110 cows with homozygous genotype AA, 23 cows with heterozygous genotype AK and three cows with homozygous genotype KK. The frequencies of genotypes and alleles are listed in Table 2. The difference between genotypes' frequencies was not statistically significant, based on the χ^2-test.

The genotypes frequencies were as follows: homozygous genotype AA (80.88%), heterozygous genotype AK (16.91%) and homozygous genotype KK (2.21%). In the monitored population, allele A had a predominance frequency of 89.34% over allele K with a frequency of 10.66%. Our results are compatible with results by Tomka et al. (2016). In Holstein cattle's population, they found that allele A had a higher frequency (0.88) than the K allele (0.12). Also genotypes frequency were similar: AA (0.78), AK (0.20), KK (0.02). The other authors detected higher representation of homozygous genotype AA and higher frequency of allele A in different Holstein cows population like Carvajal et al. (2016); Bobbo et al. (2018) in Italian Holstein; Čítek et al. (2018) in German Holstein and Wang and Bovenhuis (2020) in Netherland Holstein Friesian cows. Ardicli et al. (2018) identified only two genotypes, genotype KK (4.17%) and genotype AK (95.83%), in purebred Holstein cattle. Tăbăran et al. (2015) observed predominance of the KK genotype (0.553), lower frequency of AK (0.359) genotype and the lowest frequency of genotype AA (0.088) in Romanian Holstein.

Breeding values for the monitored parameters are given in Table 3. The *DGAT*1 (GC/GC) A allele was associated with lower milk fat and protein content (Carvajal et al., 2016). The (AA/AA) K allele was associated with lower milk and protein yields and greater fat yield (Bovenhuis et al., 2015; Bobbo et al., 2018) and fat and protein per cent (Tomka et al., 2016; Bobbo et al., 2018). In our study was found a very highly statistically significant (P <0.0001) effect of *DGAT*1 K232A marker genotypes on breeding value variability for milk fat content (%). Genotypes AA and AK reduce the breeding value by an average of 0.45% and 0.3% compared to the genotype KK (Figure 2). According to Tomka et al. (2016), the replacement of one copy of the A allele by the K allele leads to a significant (P <0.01) increase of fat content by 0.31%. Tomka et al. (2016) and Mauriæ et al. (2017) recorded a significant difference (P <0.05) between AK genotype and AA

Table 2 Genotype and allelic frequencies of *DGAT*1 K232A polymorphism

Locus	Genotype frequencies			Allelic frequencies		χ^2	*P*
*DGAT*1	AA	AK	KK	A	K	1.701	0.4271
	0.8088	0.1691	0.0221	0.8934	0.1066		

Table 3 Average breeding values for milk production traits for *DGAT*1 K232A genotypes

Genotype	Number	Breeding value	Average	Standard error	Minimum	Maximum	*P*-value
AA	110	BVM	1,094.58+++	385.23	16.60	2,026.50	<0.0001
		BVF	14.75	13.88	-17.43	57.13	n.s.
		BVF%	-0.35+++	0.13	-0.61	-0.01	<0.0001
		BVP	24.99++	11.38	-0.51	63.89	<0.01
		BVP%	-0.14++	0.11	-0.39	0.11	<0.01
AK	23	BVM	801.55+++	378.40	180.80	1,474.70	<0.0001
		BVF	15.41	10.33	-5.94	36.28	n.s.
		BVF%	-0.21+++	0.18	-0.48	0.10	<0.0001
		BVP	18.90	8.85	2.77	39.93	n.s.
		BVP%	-0.10	0.13	-0.33	0.16	n.s.
KK	3	BVM	311.90	424.29	-14.90	791.40	n.s.
		BVF	19.99	18.66	7.06	41.39	n.s.
		BVF%	0.09	0.03	0.06	0.12	n.s.
		BVP	11.53	13.65	-0.81	26.20	n.s.
		BVP%	0.03	0.05	-0.01	0.08	n.s.

BVM – breeding values for the milk yield (kg), BVF – breeding values for the fat yield (kg), BVF% – breeding values for the fat content (%), BVP – breeding values for the protein yield (kg), BVP% – breeding values for the protein content (%), $P \leq 0.01$ – statistically highly significant (++), $P \leq 0.05$ – statistically significant (+), $P \geq 0.05$ – statistically non-significant (n.s.)

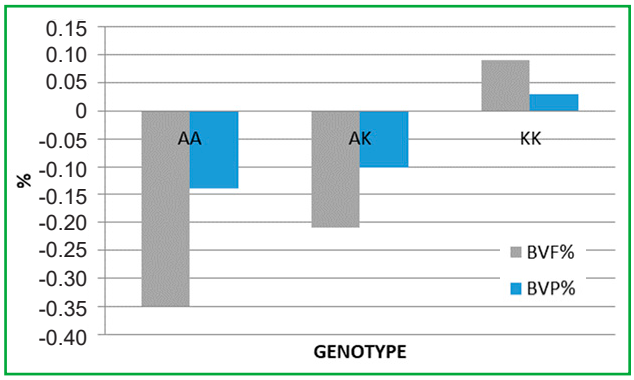

Figure 2 Influence of *DGAT*1 K232A marker genotypes on average breeding values for the fat and protein content in percent

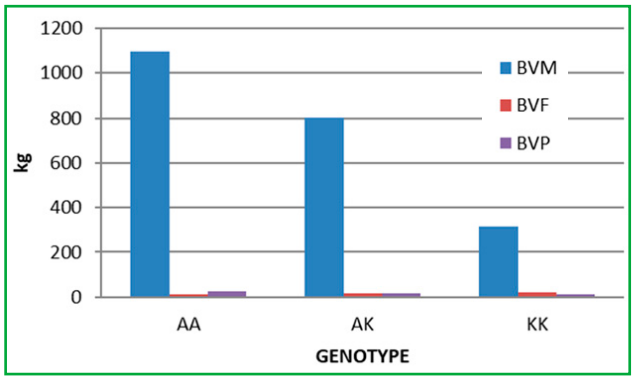

Figure 3 Influence of *DGAT*1 K232A marker genotypes on average breeding values for the amount of milk, fat and protein in kilograms

genotype for fat content, but not for protein content. Vanbergue et al. (2016) observed that milk protein and fat content are higher for genotypes KK and AK than genotype AA. The difference between KK and AA genotypes was 0.24% for protein content, and 0.63% for fat content. In genotype AK's case, it was higher by 0.18% for protein content and 0.49% for fat content than the genotype AA. We found that genotype AA reduces the breeding value for protein content by an average of 0.17% compared to genotype KK (Figure 2). Kadlecová et al. (2014) state in their study that the highest average daily milk production characterised AA homozygotes, which was significantly higher (P <0.05) than individuals with AK genotype and compared to KK homozygotes. Also, Čítek et al. (2020) found that cows with the AA genotype reported significantly higher milk, protein and fat yields. Our study, genotype AA was characterised by a significantly (P <0.0001) higher breeding value for the amount of milk in kg than genotype KK. The difference between genotypes was on average 782.68 kg. Also, the AA genotype increases the amount of protein by an average of 13.46 kg compared to genotype KK. The genotype AK demonstrably (P <0.0001) increases the breeding value for the amount of milk compared to the KK genotype by an average of 489.65 kg (Figure 3). In the study by Ardicli et al. (2018), their results indicated that genotype AK was associated with a higher peak milk yield than the KK genotype. Vanbergue et al. (2016) found significantly lower milk yield for KK (-5.3 kg) and AK (-5.0 kg) genotypes compared with the AA genotype. We did not find an association between genotypes and fat yields.

4 Conclusions

The *DGAT*1 gene encodes a key enzyme involved in fat metabolism in the mammary gland. The polymorphism of the *DGAT*1 K232A gene is characterised by a strong influence on milk fat content. The results of the present study confirm this statement. In addition to favourably affecting the milk fat content, the results show that it also has a demonstrable effect on milk production properties such as the amount of milk (kg), the amount of protein (kg) and the protein content (%). Monitoring effects of individual genotypes of the analysed gene on the production and nutritional properties of milk will contribute to recognising the relationships between performance indicators and DNA polymorphism and could have not only breeding but also economic benefits. The inclusion of individuals with genotypes that favourably affect the content and composition of milk in breeding programs can lead to an increased content of beneficial substances in milk and thus ensure the production of nutritionally valuable and at the same time potentially functional foods.

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