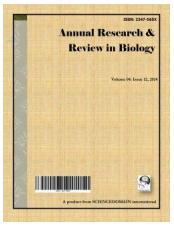

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Frequencies of Genotypes and Alleles of the K232A Substitution in the *DGAT1* Gene in Four Cattle Breeds of Russian Selection

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Authors' contributions

This work was carried out in collaboration between all authors. Author SK designed the study and wrote protocols. Author AA performed the experiments. Author EK performed the statistical analysis, managed the literature searches and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

The enzyme DGAT1 is involved in the synthesis of triglycerides. The most well-known polymorphic variant of the *DGAT1* gene is substitution of lysine with alanine at position 232 of the protein (K232A). The 232K allele is associated with increased enzyme activity and a higher content and yield of fat in milk. There is less data on the frequencies of alleles of this replacement in different breeds in literature. In our research work, we analyzed the frequencies of genotypes and alleles of the K232A substitution in the *DGAT1* gene in 4 breeds of Russian selection: Black-and-white Holsteinized, Kalmyk, Ayshire and Angus. We demonstrated that the K allele is minor in the populations of the analyzed cattle breeds.

Keywords: DGAT1; substitution K232A; polymorphism; cattle.

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1. INTRODUCTION

The *DGAT1* gene encodes acyl-CoA-diacylglycerol acyltransferase 1 (EC 2.3.1.20) involved in the final stage of triglyceride synthesis [1]. Impaired synthesis of both fatty acids and triglycerides results in a change in the qualitative composition of milk and meat fats.

Among many polymorphic variants of the *DGAT1* gene, dinucleotide substitution in exon 8 of GC→AA is the most well-known: positions 10433 and 10434 in the *DGAT1* gene sequence, (GenBank no. AJ318490) or positions 1802265 and 1802266 on chromosome 14 (substitutions rs109234250 and rs109326954, respectively). This dinucleotide substitution results in the substitution of lysine with alanine (K→A) at position 232 of the protein (substitution K232A).

The *DGAT1* gene is expressed in the small intestine, liver, adipose tissue and mammary gland [2,3]. The profile of its expression can explain the effect of its polymorphism on the characteristics of milk. The 232K allele is associated with increased enzyme activity and a higher content of fat in milk. The allele K is associated with increased in fat content (%), higher concentration of saturated fat and a lower concentration of unsaturated ones [4-10].

The literature describing the effect of the K232A polymorphism on the parameters of milk production, the ratio of the frequencies of genotypes and alleles in animals of different breeds is not sufficiently shown. With the proven effect of the K323A substitution alleles on the parameters of milk productivity, the estimation of allele frequencies can be used to carry out marker-assisted selection of cattle.

The objective of our study was to analyze the frequencies of genotypes and alleles of the K232A substitution in the *DGAT1* gene in four breeds of Russian selection.

2. METHODOLOGY

The research work analyzed DNA samples previously isolated from the whole blood of animals of the following breeds: black-and-white Holsteinized (n = 89, “Pravda N” LLC, Kaluga region, Dzerzhinsky district), Kalmyk (n = 77, “Agrofirma Uralan” LLC, Republic of Kalmykia), Ayshire (n = 62) and Angus (n = 71) both from “Russian Farmer” ANO Kaluga region, Medynsky district. Blood samples were obtained during regular collection of blood for veterinary control by a qualified specialist in accordance with the current rules for working with animals. DNA was isolated from whole blood using a Magna^{TM} DNA Prep 100 kit (Izogen Lab, Russia). The DNA isolation procedure was conducted as prescribed by the manufacturer, using special magnetic stands. A 100 µL sample of whole blood was used for the isolation. The DNA concentration was about 60 ng/µL (40-90 ng/µL). To assess the genotypes, we used our previously developed test system based on real-time PCR in accordance with the described methods [11]. To assess deviations from Hardy-Weinberg equilibrium, we used the online program Calculator of Hardy-Weinberg equilibrium (http://wpcalc.com/en/equilibrium-hardy-weinberg/). The calculation formulas are presented on the site.

3. RESULTS AND DISCUSSION

Our developed analysis of the genotyping results using the test system showed the following frequencies of genotypes and alleles (Table 1). The distribution of genotypes is consistent with the Hardy-Weinberg equilibrium (p value > 0.05, degree of freedom = 2), which indicates the absence of selection for this substitution in all studied populations of cattle.

15 years of review of literature on the allele frequencies of the K232A substitution in the *DGAT1* gene greatly vary. The variation of the

Table 1. The frequencies of genotypes and alleles of the A232K substitution in the *DGAT1* gene.

Breed	n	Genotypes, P			H-W, χ^2	Alleles, P		S(p)
		AA	AK	KK		A	K	
Black-and-white (Holsteinized)	89	0.62	0.30	0.08	1.47	0.77	0.23	±0.047
Kalmyk	77	0.63	0.37	0.00	0.97	0.82	0.18	±0.089
Ayrshire	62	0.75	0.17	0.08	1.92	0.83	0.17	±0.108
Angus	71	0.74	0.26	0.00	0.60	0.87	0.13	±0.065

n – number of animals studied, P is the frequency of the genotype/allele, S (p) – the standard error for allele frequencies, H-W, χ^2 – chi-square value for inconsistency with the Hardy-Weinberg equilibrium.

frequencies of this substitution alleles in different breeds is very greatly. Thus, the frequency of the allele K ranges from 0.09 [12] to 0.90 [13]. This may be due to the selection of animals based on the content of fats of different density, as well as the founder effect in the local population of the breed. The data we obtained indicate absence of selection for the studied substitution in breeds of beef cattle. The allele K frequencies of 0.28 in the dairy breed (black-and-white) are consistent with the published data for Russia [14]. For two Holstein cattle populations, the allele K frequencies are also close to those obtained by us, with the exception of the population from France, which can be explained by a more strict selection for fat content in milk [13].

4. CONCLUSION

Thus, we estimated the frequencies of genotypes and alleles of the K232A substitution in the DGAT1 gene in four breeds of cattle of the Russian selection viz., black-and-white Holsteinized, Kalmyk, Ayshire and Angus. The allele K is minor in populations of analyzed breeds. Meanwhile, the frequencies of the allele K allow selection in the studied cattle species for this DNA marker.

ETHICAL APPROVAL

As per international standard or university standard ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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