



## Polymorphism in the leptin gene (T3469C) of Different Pig Breeds of the Belgorod Region of Russia

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### Abstract

Pigs were RFLP-genotyped for the leptin gene (mutation T3469C). The study examined 185 boars of four breeds (Duroc, Large White, Landrace, and Yorkshire) from the Belgorod region of Russia. In all breeds, the wild T allele is the most frequent, which is responsible for leaner meat and lower fat content. The highest frequency of the C mutant allele was found in the Large White (0.223), and the lowest – in Yorkshire pigs (0.011). At the same time, no homozygotes were found for this allele in Landrace and Yorkshire meat breeds. In general, the allele frequencies of the studied breeds correspond to the frequencies of European and Canadian selection. According to the  $\chi^2$  test, all studied breeds are in a state of genetic equilibrium according to Hardy-Weinberg.

**Keywords:** pigs, leptin, 3469 mutation, polymorphism, RFLP

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### INTRODUCTION

One of the main consumer parameters of pork is the thickness of the bacon and the fat content in the meat, in connection with which the selection according to these characteristics is of great interest. (Oreshin, Teltsov, Trofimov, 2011). An important role in lipid metabolism, which affects the level of adipose tissue development in pigs, is played by the main adipose tissue hormone – leptin. (Switonski, Stachowiak, Cieslak, Bartz, & Grzes, 2010; Rikhtegarzadeh, et al, 2016). Leptin is a 16 kDa protein secreted by white adipocytes and acts as a saturation signal to the hypothalamus, thereby regulating body weight and energy expenditure. (Campfield, et al. 1995). The secretion of leptin leads to a decrease in feed intake, loss of body weight and fat depots, and an increase in energy metabolism (Maffei, et al. 1995 Houseknecht, et al. 1998. Liu, Zhang, Yang, & Zhang, 2007. Garcia-Galiano, Allen, & Elias, 2014). Park, & Ahima, 2014). It is also involved in the induction of insulin resistance, which further leads to the modification of the metabolic effects of insulin (Tritos, & Mantzoros, 1997. Urbanski, 2001).

The leptin gene (LEP) in pigs is located on chromosome 18 (Sasaki, Clutter, & Pomp, 1996). and acts as a promising candidate gene for assessing the growth and obesity of pigs (Roehe, Plastow, & Knap, 2003. Pérez-Montarelo et al. 2012. Mankowska, Szydlowski, Salamon, Bartz, & Switonski, 2015. Bauer, Babelová, Omelka, & Bauerova, 2006). In particular, the

leptin gene (LEP) in pigs is associated with feed intake, growth and thickness of fat (Bauer, Babelová, Omelka & Bauerova, 2006). and sperm capacity and viability. (Aquila, Rago, Guido, Casaburi, Zupo, & Carpino, 2008).

In total, 98 polymorphisms were found for the LEP gene, including 89 single nucleotide polymorphisms (SNPs) located mainly either in introns or in the 3' UTR region. (D'Andrea, et al. 2008. Liu, et al. 2007)

One of the most well-studied polymorphisms in the LEP gene is a point mutation in the third exon at position 3469, where the thymine base characteristic of the wild variant is replaced by cytosine. (Stratil, Peelman, VanPoucke, & Cepica, 1997). As a result of the substitution, the HinfI site is formed in the mutant allele, which makes it possible to genotyping pigs for the T3469C polymorphism using the restriction fragment length polymorphism (RFLP) method (Bauer, Babelová, Omelka, & Bauerova, 2006. Jiang, & Gibson, 1999, Chao, et al. 2012, Kennes, Murphy, Pothier, & Palin, 2001). The T → C substitution itself at position 3469 results in the replacement of the TTG codon encoding leucine with the synonymous CTG codon. As a result, the mutation does not lead to a change in the primary

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sequence of the leptin protein.(Liu, Zhang, Du, Yang, & Zhang, 2007).

Nevertheless, a number of authors have revealed a significant relationship between the T3469C polymorphism and economically significant traits. Thus, in Landrace pigs, a relationship ( $P < 0.0078$ ) with feed consumption and growth was revealed (Kennes, Murphy, Pothier, & Palin, 2001. Kulig, Grzesiak, & Szatkowska, 2001). Similar results were obtained for Brazilian herds of Landrace, Large White and Pietrain breeds: there was a correlation with piglet weight ( $P < 0.02-0.05$ ), feed intake ( $P < 0.01$ ), with average daily gain ( $P < 0.01$ ), with feed conversion ( $P < 0.01$ ) and with a slaughter weight ( $P < 0.03$ ).(Facioni, et al. 2006). Similar data were obtained for the Chinese Landrace, Yorkshire and Meishan pigs.(Chao, et al.2012). At the same time, it should be noted that a number of works did not reveal any reliable relationship between any phenotypic traits of pigs and genotypes for the considered polymorphism: for the nature of growth in Landrace, Large White and synthetic lines of pigs from Poland (Szydłowski, et al. 2004. Kolodziej, et al. 2009) reproductive qualities of sows.(Kolodziej, et al. 2009).

A number of studies on various breeds has shown that pigs with a mutant C allele in a homozygous (CC) and, to a lesser extent, heterozygous (CT) state, are characterized by a higher fat content, a greater fat thickness, a large average daily gain during the feeding period, greater live mass, faster weight gain due to the accumulation of fat in the sacral spine, while the TT genotype is associated with a lower fat content in the carcass (Jiang, & Gibson, 1999. Chao, et al. 2012. Kulig, et al. 2001. Tempfli, Simon, Kovács, Posgay, & Bali Papp, 2015. Kurył, et al. 2003). Studies in Poland on sows (Large White × Landrace) with different LEP genotypes showed that animals with the CC genotype by the number of live piglets born, they favorably differed from TT.(Wierzchowska, Kołodziej-Skalska, Napierała, Kawęcka, & Jacyno, 2013). The carriers of the mutant allele were superior in the number of piglets and in the weight of the nest at weaning. However, sows with the TT genotype gave birth to larger piglets than the mutant allele carriers.(Giniyatullin, 2017). At the biochemical level, it has been established that the blood plasma of boars that are carriers of the point mutation C3469T have an initially high level of secondary, most toxic products of lipid peroxidation, and there is a violation of the regulation of lipid metabolism.(Oreshin, Teltsov, Trofimov, 2011). In contrast, Kennes et al. (Kennes, Murphy, Pothier, & Palin, (2001). reported T-associated higher mean daily weight gain for landrace.

In general, we consider it expedient to carry out genotyping of pigs of various breeds for the T3469C polymorphism during breeding activities at pig breeding farms.

The **objective** of this work is to assess the frequencies of alleles and genotypes based on the

polymorphism of the leptin T3469C gene in the Duroc, Large White, Landrace and Yorkshire pigs in the farms of the Belgorod region of Russia.

## MATERIAL AND METHODS

The object of the study is boars of the main breeds of pigs from farms of the Belgorod region of Russia: Duroc (45 animals), Large White (47 animals), Landrace (46 animals) and Yorkshire (47 animals).

Genomic DNA was isolated from alcoholized earmarks using a DNA-Extran-2 reagent kit (SINTOL, Russia) according to the protocol. Pig genotyping for the T3469C mutation was performed using RFLP. For PCR, the following primers were used.(Jiang, & Gibson, 1999).

Forward: 5' – GAGCCAACATCTCTCTCGCTGAG – 3',

Reverse: 5' – GACTCCTGGAAGCTCAGGTTTCTTC – 3',

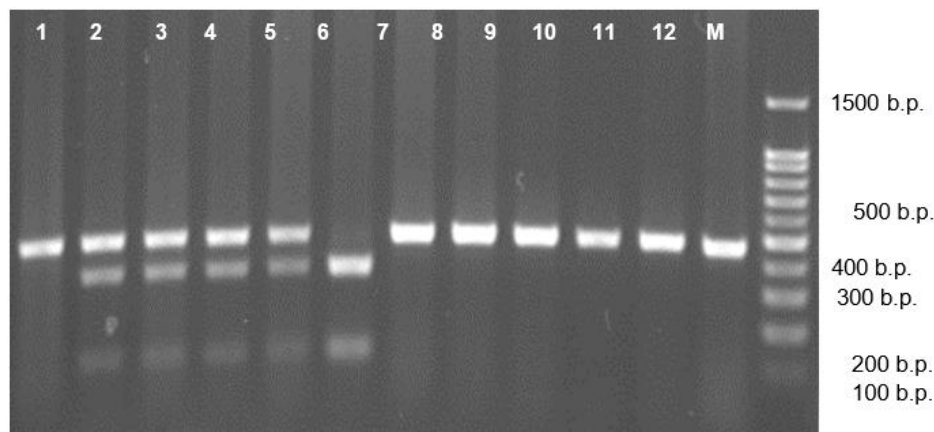
with their help a DNA fragment of 468 b.p. was amplified from the third exon of the leptin gene containing the target mutation.

The volume of the PCR mixture was 20  $\mu$ L, containing 20 ng of genomic DNA, 10 mM Tris-HCl (pH = 8.3), 50 mM KCl, 4 mM MgCl<sub>2</sub>, 0,25 mM dNTP, 0,5  $\mu$ M primer, 1-unit Taq DNA polymerase. The reaction was carried out on a Veriti amplifier (Applied Biosystems, USA) under the following conditions: hot start - 5 min/94°C, 35 cycles (denaturation - 20 s/94°C, primer annealing - 20 s/59°C, synthesis - 20 s/72°C), additional synthesis - 2 min/72°C. The PCR product in an amount of 10  $\mu$ l was hydrolyzed with 4 units of the HinfI restriction enzyme (SibEnzyme, Russia) for 16 hours. The hydrolysis products were separated by horizontal electrophoresis in 4% agarose gel (Mini-Sub Cell GT, BioRad, USA). To detect DNA fragments, the gel blocks were stained with ethidium bromide (0,5  $\mu$ g/ml) and visualized on a UV transilluminator. Samples with TT genotype had a 465 and 3 b.p. DNA fragments, TC - 465, 347, 118 and 3 b.p., CC – 347, 118 and 4 b.p. (**Fig. 1**).

For statistical analysis of the data obtained, the Excel and GenAEx software package was used.

## RESULTS

According to the data obtained, pig breeds in the Belgorod region are characterized by different frequencies of genotypes and alleles for the T3469C polymorphism (**Table 1**). The large white breed has the highest frequency of the C mutant allele (0.223). This is consistent with the opinion that this breed is prone to obesity. On the other hand, the lowest frequencies of the C allele were noted for meat breeds of Landrace and Yorkshire pigs, 0.065 and 0.011, respectively. At the same time, no individuals with the CC genotype were found in these breeds.



**Fig. 1.** Result of electrophoresis of Lep PCR products after hydrolysis at the *Hinf*I site. 1, 7-11 - TT genotypes, 2-5 - CT genotypes, 6 - CC genotype, 12 - PCR product before hydrolysis, M - 100+ length marker (Evrogen, Russia)

**Table 1.** The number of genotypes and allele frequencies for Lep T3469C polymorphism in boars of various breeds

Breed	Number of animals	Number of genotypes			Frequencies of alleles	
		TT	CT	CC	T	C
Duroc	45	32	11	2	0.833	0.167
Large White	47	28	17	2	0.777	0.223
Landrace	46	40	6	0	0.935	0.065
Yorkshire	47	46	1	0	0.989	0.011
Region average	185	146	35	4	0.884	0.116

**Table 2.** The  $\chi^2$  test results for the correspondence of genotype frequencies to the Hardy-Weinberg distribution in pig breeds and the inbreeding factor (F)

Breed	$\chi^2$	F
Duroc	ns	0.120
Large White	ns	-0.042
Landrace	ns	-0.070
Yorkshire	ns	-0.011
Region average	ns	-0.001±0.042

Note: ns - no significant differences

In all investigated breeds, the empirical frequencies of genotypes do not differ ( $p > 0.05$ ) from the theoretically expected distribution according to Hardy-Weinberg (Table 2). Positive values of the inbreeding factor were recorded for the Duroc pigs only. Thus, the considered populations of pigs by the T3469C polymorphism are in a state of genetic equilibrium without a clear effect of selection factors.

The data obtained are consistent with the literature data, according to which the C mutant allele is also found unevenly in pig breeds and regions. Its highest frequency is observed in the Brazilian Piau race with a strong tendency to accumulate fat, as well as for the Chinese Bamey and Meishan, 0.75, 0.60 and 1.0-0.725, respectively [19, 21, 32]. In the countries of Central and Eastern Europe, the highest frequencies were recorded for the Czech Duroc and the Slovak Large White with frequencies of 0.35 and 0.29, respectively (D'Andrea, et al. 2008. Urban, Kuciel & Mikolasova, 2002) At the same time, for the Hungarian and Polish populations of Large White, the frequencies of the C allele were lower and amounted to 0.13 and 0.11 (Szydowski, et al. 2004. Hunyadi-Bagi, Balogh, Nagy, & Kusza, 2016) A similar level of frequency of this allele was noted for most of the

studied populations of Duroc, Landrace, Large White, and their crosses from this region. It is noteworthy that the complete absence of the C allele was recorded only in Pietrain pigs from Poland.(Kurył, Kapelański, Pierzchała, Bocian, & Grajewska, 2003). Study by scientists from Canada reveal lower frequencies of the mutant allele for the Duroc and Landrace pigs – 0.09 and 0.06, respectively.(Kennes, Murphy, Pothier & Palin, 2001). Similar frequencies were noted for Landrace populations from China (Jiang, & Gibson, 1999. Chao, et al. 2012). In this case, the frequencies of the C allele for the Yorkshire pigs of Canada and China are slightly higher and amounted to 0.15 and 0.12, respectively (Chao, et al. 2012)., Kulig, Grzesiak, & Szatkowska, 2001).For the large white in China, the frequency of the C allele was 0.28 (Jiang, & Gibson, 1999) which is comparable to some populations of this breed from Central Europe. For Landrace, Large White, and Brazilian Pietrain pigs, the frequency of the C allele is 0.19, 0.09–0.12, and 0.06, respectively, which is generally similar to the frequencies for pigs from Central and Eastern Europe (Borges, & Goulart, 2002.Silveira, et al. 2008). In Russia, the frequencies of 0.304, 0.300, and 0.308 were noted for the Duroc, Landrace, and Yorkshire pigs (Oreshin, Teltsov, Trofimov, 2011). for the Large White breed — 0.31 (Getmantseva, et al. 2017).and for Landrace × Yorkshire crosses — 0.45.(Giniyatullin, 2017). These frequencies are higher than those of similar breeds from other regions of the world, which may be due to the peculiarities of regional selection.

## CONCLUSION

Thus, the obtained frequencies of the C mutant allele for the Large White pigs from the Belgorod region are closer to the populations of this breed from Slovakia and China. The Duroc pigs of the Belgorod region had the frequency of the C allele slightly higher than in Central and Eastern Europe, Landrace - closer to the populations of Canada and China. And the Yorkshire pigs from the Belgorod region had one of the lowest frequencies of the C allele in this breed recorded, which is an order of magnitude lower than in other regions, and brings it closer to the population of the Pietrain pigs from

Poland with a complete absence of the C allele. (Kurył, et al. 2003). In general, the frequencies of the C allele obtained are significantly lower than those previously obtained for breeds and crosses of pigs from Russia and are more consistent with Central European ones. Perhaps this is the result of breeding work in Russia, aimed, among other things, at reducing the fat thickness and content.

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