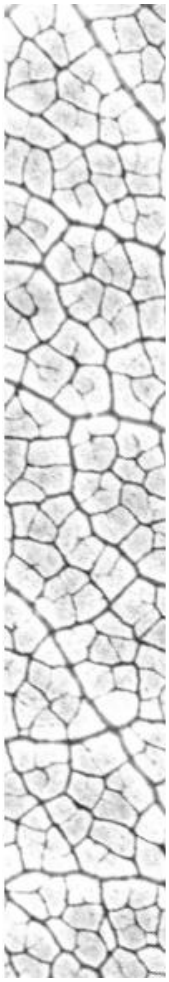

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G316A growth hormone polymorphism in pigs of various breeds of the Belgorod Region of Russia

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Abstract
Using the RFLP method, pigs were genotyped for the growth hormone gene (mutation G316A). The study involved 187 boars of four breeds (Duroc, Large White, Landrace and Yorkshire) from the Belgorod region of Russia. The AG genotype frequency for most breeds has the highest values equal to 0.426, 0.489, 0.413, and 0.468, respectively. In the Duroc, Large White and Landrace breeds, the G allele (0.383, 0.479, and 0.467, respectively) and the GG genotype (0.170, 0.234, and 0.261, respectively) have the lowest frequency. At the same time, in Yorkshires, allele A (0.298) and genotype AA (0.064) are characterized by the lowest frequency. We believe that a similar picture can be associated with the peculiarities of selection in various breeds. According to the χ^2 test, all studied breeds are in a state of Hardy-Weinberg genetic equilibrium.

Keywords: pigs, growth hormone, A316G polymorphism, RFLP

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INTRODUCTION

Growth hormone (somatotropin) is one of the key hormones in the regulation of growth processes and cell differentiation in pigs (Henricson, & Ullberg, 1960. Parr, et al. 2016). Thus, growth hormone stimulates various tissues to secret insulin-like growth factor 1 (IGF-I) (Velloso, 2008). promotes the development of skeletal muscles, regulates the growth of some bones [4], and controls fatty deposits (Parr, et al. 2016. Sillence, Munn, & Campbell, 2002. Dunshea, & D'souza2003). Growth hormone has been shown to have a significant effect on weight gain in pigs, reducing feed intake and reducing fat deposition (Henricson, & Ullberg, 1960, Etherton, et al. 1987). Currently, in some countries, growth hormone is approved for administration to pigs to improve their growth (Parr, et al. 2016).

In this regard, the growth hormone (GH) gene is considered as a promising marker of pig productivity (Knorr, Moser, Müller, & Geldermann, 1997. Cheng, et al. 2016. Faria, et al. 2006. Getmantseva, et al. 2017. Ferreira, et al. 2019).Currently, at least 17 single nucleotide polymorphisms have been identified in GH (Cheng, et al. 2016). And one of the promising markers can be SNP G316A (Faria, et al. 2006). Thus, a number of studies found a connection between this polymorphism and the productive qualities of pigs: the percentage of muscle mass(Bižienė, Miceikienė, Baltrėnaitė, & Krasnopiorova, 2011). carcass length (Faria, et al. 2006. Lyubov, et al. 2016) shoulder weight, pH 24h after slaughter, juice loss during thawing (Faria, et al. 2006). number of days, for which pigs reach a weight of 100 kg (Faria, et al. 2006. Lyubov, et al. 2016), average daily gain (Thuy et al., (2019)., feed consumption per 1 kg of gain (Pogorelsky, Serdyuklvanov, Yu. 2019). and the number of live pigs at birth (Kolosov, Leonova, Getmantseva, 2016). Moreover, the effects of the G316A growth hormone polymorphism are not universal and depend on the phenotypic trait and breed of pigs (Lyubov, et al. 2016; Ramar, et al, 2014). Thus, in sows of Brazilian interbreed crosses, the presence of the AA mutant genotype is associated with an increase in the number of correct teats and fat thickness, and the GG homozygote is associated with development indicators and carcass length, a faster increase in the average daily weight, lower losses due to juice leakage and a higher average pH 24 hours after slaughter. The average weight of pigs was higher in the heterozygous GA genotype (Faria, et al. 2006).Bižienė et al. (Bižienė, Miceikienė, Baltrėnaitė, & Krasnopiorova2011). studying the GH G316A polymorphism in pigs of various breeds from the State Pig Breeding Station of Lithuania, determined that animals with the GG genotype had less body fat and a

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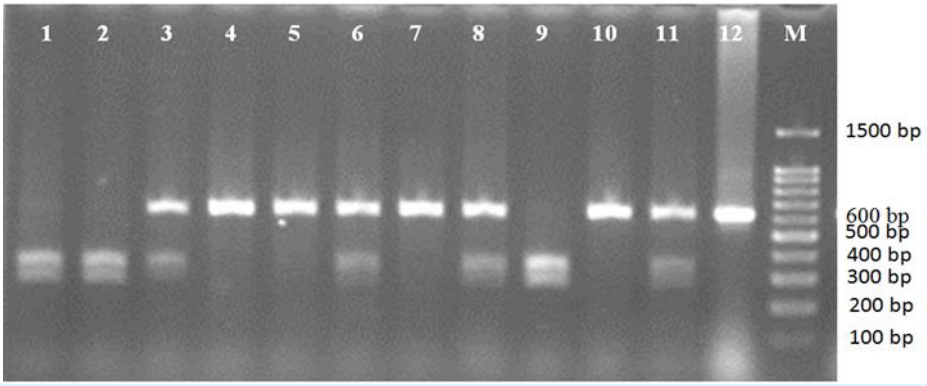

Fig. 1. Result of electrophoresis of GH PCR products after hydrolysis at the FokI site. 4, 5, 7, 10 - AA genotype, 3, 6, 8, 11 - AG genotype, 1, 2, 9 - GG genotypes, 12 - PCR product before hydrolysis, M - length marker 100+ (Evrogen, Russia)

higher percentage of musculature compared to other animals. In addition, pigs with the GG genotype reached a weight of 100 kg earlier than others. In Landrace pigs, the superiority of the GG genotype was revealed, which had a higher rate of multiplicity in comparison with individuals of other genotypes. However, polymorphism of the GH gene in pigs of this breed did not show a significant effect on fattening and meat qualities (Kolosov, Leonova,, Getmantseva, 2016). Hybrid Yorkshire × Landrace × Duroc pigs showed that those with the GG genotype, in comparison with pigs with the AA genotype, have a significantly greater weight and length of a half carcass, an average daily weight gain, a lower consumption of feed per 1 kg of gain and an age of attaining a live weight of 100 kg (Pogorelsky, Serdyuk, Ivanov, 2019).

The **objective** of this work is to assess the frequencies of alleles and genotypes based on the G316A polymorphism of the GH gene in pigs of the Duroc, Large White, Landrace and Yorkshire breeds 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 (47 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 G316A mutation of the GH gene was performed using RFLP. For PCR, the following primers were used

Forward: 5' – TTATCCATTAGCACATGCCTGCC – 3',

Reverse: 5' – CTGGGGAGCTTACAAACTCCTT – 3',

with their help a DNA fragment of 604 b.p. was amplified from the first GH intron containing the target mutation.

The volume of the PCR mixture was 20 μL, containing 20 ng of genomic DNA, 10 mM Tris-HCl (pH = 8.3), 50 mM KCl, 4 mM $MgCl_2$, 0.25 mM dNTP, 0.5 μM 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 μl was hydrolyzed with 4 units of the FokI restriction enzyme (SibEnzyme, Russia) for 16 hours. The hydrolysis products were separated by horizontal electrophoresis in 2% agarose gel (Mini-Sub Cell GT, BioRad, USA). To detect DNA fragments, the gel blocks were stained with ethidium bromide (0.5 μg/ml) and visualized on a UV transilluminator. Samples with *AA* genotype had a 604 b.p. DNA fragment, *AG* - 604, 345 and 259 b.p. fragments, *GG* - 345 and 259 b.p. (**Fig. 1**).

For statistical analysis of the data obtained, the Excel and GenAlEx 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 A316G polymorphism (**Table 1**). The highest frequency of the G allele (0.702) is characteristic of the Yorkshire pigs. The same breed is also characterized by the highest frequency of the GG genotype (0.468) with the lowest frequency of the AA genotype (0.064). In contrast, the lowest frequency of the G allele was noted for the Duroc pigs (0.383). Their frequency of the GG genotype is lower (0.170) and the frequency of the TT genotype is higher (0.404). The Large White and Landrace pigs have similar genotype and allele frequencies, intermediate between Duroc and Yorkshire.

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**). Moreover, for all breeds, except for the Yorkshire breed, positive values of the inbreeding coefficient were recorded. This may indicate the presence of selection factors in individual breed lines.

Table 1. The number of genotypes and allele frequencies for A316G polymorphism in boars of various breeds

Breed	Number of animals	Frequencies of genotypes			Frequencies of alleles	
		AA	AG	GG	A	G
Duroc	47	0.404	0.426	0.170	0.617	0.383
Large White	47	0.277	0.489	0.234	0.521	0.479
Landrace	46	0.326	0.413	0.261	0.533	0.467
Yorkshire	47	0.064	0.468	0.468	0.298	0.702
Region average	187	0.267	0.449	0.284	0.492	0.508

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

Breed	χ^2	F
Duroc	ns	0.100
Large White	ns	0.020
Landrace	ns	0.170
Yorkshire	ns	-0.119
Region average	ns	-0.043±0.062

Note: ns - no significant differences

Thus, the considered populations of pigs by the A316G polymorphism are in a state of genetic equilibrium with a limited influence of selection factors.

The review of the literature data on A316G polymorphism from other regions revealed the following situation. In cross-breeds obtained from the crossing of Brazilian wild boars with sows (Landrace × Large White × Pietrain), the frequency of the AA genotype was 0.323, the GG genotype - 0.567, and the AG genotype - 0.400. The G allele frequency was 0.767 (Faria, et al. 2006). For Duroc pigs from Vietnam, the highest frequency was in the AG genotype (0.504), and the lowest – in AA (0.150). The frequency of the G allele was also higher and amounted to 0.598. In this case, the studied population is in a state of genetic equilibrium according to Hardy-Weinberg ($\chi^2 = 1.07$, $p>0.05$) [16]. In the Large White and its crosses from Lithuania, the frequency of the AA genotype was the highest among other breeds (0.188), while the Landrace pigs had no homozygotes for the A allele. The frequency of the AG genotype was the lowest in the Yorkshire pig breed (0.094). On average, for breeds from Lithuania, the AG genotype had the highest frequency, and the AA genotype had the lowest one (0.474 and 0.121, respectively). The G allele (0.642) had the highest frequency (Bižienė, et al. 2011).

In pigs from Russian farms, the frequencies of genotypes and alleles also depend on the breed and region. A.Iu. Kolosov for the Landrace breed showed that the frequency of the AA genotype was the highest, and the lowest was the GG genotype (0.347 and 0.310, respectively). In this case, the A (0.52) allele had the highest frequency [18]. Testing this Landrace population for the Hardy-Weinberg ratio revealed a significant deviation of the observed genotype frequencies from the expected ones ($\chi2 = 9.08$, $p<0.05$). At the same time, for another population of the Landrace breed, excellent data were obtained in terms of genotype frequencies: the AA genotype was distinguished by the lowest frequency (0.075), and the highest - GG (0.575). The frequency of the A allele was 0.25 [19]. And in terms of the ratio of genotypes, this population corresponds to the Hardy-Weinberg distribution ($\chi2 = 1.6$, $p>0.05$). Similar frequencies were obtained for the Yorkshire × Landrace × Duroc hybrid pigs (Pogorelsky, Serdyuk, Ivanov, 2019).

L.V. Getmantseva et al. determined the presence of three genotypes of growth hormone in the Duroc pigs: AA (0.20), AG (0.60) and GG (0.20). The frequencies of the A and G alleles were found to be equal. Testing the Duroc population for the Hardy-Weinberg ratio revealed a deviation from equilibrium due to a deficit of homozygotes ($\chi^2 = 15$, $p<0.05$) (Lyubov, et al. 2016). For Large White, the following genotype frequencies were obtained: AA - 0.218, AG - 0.478, GG - 0.304. The G allele frequency was 0.54. And in terms of the ratio of genotypes, this population corresponds to the Hardy-Weinberg distribution ($\chi^2 = 0.37$, $p>0.05$) (Getmantseva, Bakoev, Kostyunina, Bakoev, 2019)

CONCLUSION

Thus, the frequencies of genotypes for A316G polymorphism depend both on the breed type of pigs and on a specific region or farm. In general, for various breeds and crosses of pigs, there is a tendency to a decrease in the frequency of the A allele and, accordingly, the *AA* genotype in the livestock. In most farms, either the heterozygous *AG* genotype or the *GG* genotype has the highest frequency. It is noteworthy that this trend is violated in farms that have a deviation in the Hardy-Weinberg distribution of genotype frequencies according.

The pig breeds of the Belgorod region, with the exception of Yorkshires, have the opposite situation. The frequency of the *A* allele in Duroc, Landrace and Large White pigs is higher than of the *G* allele. In addition, with the exception of Yorkshires, the heterozygous genotype occupies a greater frequency in pig breeds of the Belgorod region, while the *GG* genotype is the rarest. Probably, a similar picture has developed as a result of selection in Yorkshires and other breeds for different characteristics. In addition, local features of the breeding process in the Belgorod region could have played a role.

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