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Evaluation of the gene pool by *GH L127V* and *GHR F279Y* polymorphisms in Kazakh White-Headed cattle

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Abstract. **The aim of research i**s to monitor the genetic diversity of Kazakh White-Headed breed, taking into account the *GH L127V* and *GHR F279Y* polymorphisms. **Research methods.** The object of the study were cows (n = 57 heads) and young animals (calves and heifers, n = 50 heads) from the breeding farm "Krasnyy Oktyabr'", Volgograd region. Whole blood of animals served as the biomaterial; genotyping was carried out according to the polymorphisms *GH L127V* in growth hormone gene and *GHR F279Y* in growth hormone receptor gene by PCR-RFLP method. **Results.** The study of the gene pool by polymorphisms of the somatotropic axis genes in Kazakh White-Headed cattle showed that the representatives of mature herd and young animals had *L* (P_L = 0.660–0.728) and *V* (P_V = 0.272–0.340) alleles in the locus of growth hormone gene, as well as *F* (P_F = 0.412–0.550) and *Y* (P_Y = 0.450–0.588) in the locus of growth hormone receptor gene. Genotypes distribution in growth hormone gene polymorphism was more balanced according to the Hardy-Weinberg law, but the population significantly ($P < 0.05$) deviated from the equilibrium state according to the genetic frequencies in growth hormone receptor gene. Differences in genetic frequencies for the growth hormone gene did not reach a significant level (χ^2 = 4.451; P = 0.108) between cows and young animals. Тогда как по гену рецептора гормона роста отмечались значительные различия (χ^2 = 12,103; P = 0,002) по встречаемости носителей гомозиготных генотипов, что обусловливалось использованием в воспроизводстве стада гетерозиготного по этому полиморфизму быка-производителя. Whereas, there were significant differences (χ^2 = 12.103; P = 0.002) in the frequencies of homozygous genotypes carriers for the growth hormone receptor gene, which was due to the use of heterozygous sire for this polymorphism in the reproduction of the herd. **Scientific novelty.** For the first time, data on the assessment of the genetic structure of the Kazakh White-Headed mature herd and replacement young animals of Volgograd selection were obtained according to the polymorphisms *GH L127V* and *GHR F279Y*, associated with indicators of meat productivity. The results of the analysis of the genetic structure of the herd indicate the possibility of directed changes in the gene pool of the population in just one generation, which creates the prerequisites for the introduction of marker-assisted selection.

Keywords: Kazakh White-Headed breed, cows, young animal, genotype, allele, variability, polymorphism, *GH L127V*, *GHR F279Y*.

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Introduction

The genetic structure of any population of farm animals is variable under the breeding processes [1, p. 811]. For a long time, the characteristics of diversity within breeds, populations and herds were based mainly on morphological traits, exterior features and productive qualities in beef cattle. However, differentiation according to the animals' phenotype does not provide a complete assessment of intraspecific variability and is often limited by the fact that the expressiveness of particular traits and appearance is largely due to environmental factors [2, p. 268]. DNA polymorphisms have become the preferred biological markers for research in population genetics with the development of new technologies, which complement and take traditional approaches to genetic resource management to a new level [3, p. 376]. Thus, the use of molecular markers gives a detailed description of the individual and

group characteristics of animals, regardless of paratypical factors. The combination of genetic monitoring and traditional zootechnical methods increases the efficiency of selection in breeding herds [4, p. 86].

The selection of animals according to the intensity of average daily gain and meat traits is important in breeding work with cattle in high-quality beef production [5, p. 8]. These traits have a relatively high heritability according to numerous studies, which indicates the existence of genetic factors that determine the listed economic traits in cattle. Thus, single nucleotide polymorphisms (SNPs) in growth hormone (*GH*) and growth hormone receptor (*GHR*) genes involved in the somatotropic axis are associated with growth and development, carcass weight, rib eye area, and other selectable traits in beef cattle [6, p. 54]. Thereby, studying the state of the population gene pool for *GH* and *GHR* genes, evaluation and predicting its dynamics over time, and determining the limits of acceptable changes is of high economic importance in beef cattle breeding [7, p. 39].

These studies are especially relevant for domestic (Kalmyk and Kazakh White-Headed) beef breeds, because the improvement of their breeding traits for a long time took place due to the internal genetic resources. So, selection work with Kazakh White-Headed breed was based on breeding along lines that were founded on a limited number of outstanding prepotent sires with the large-scale use of artificial insemination in breeding herds [8, p. 207]. This means that sires had to stable pass on their heredity to progeny. At the next stage of breeding work, a strict selection of successors was carried out according to the results of a test by own productivity for further consolidation of the genealogical line. As a result, the current state of the genetic resources tends to decrease in diversity, which ultimately affects the decrease in phenotype variability in Kazakh White-Headed breed.

A large number of livestock (4th place among beef cattle) and a wide distribution area of Kazakh White-Headed breed, which occupies various natural and climatic zones of Russia, make it possible to minimize the negative effects of reducing genetic variability. In addition, ecological differentiation provided favorable conditions for the development of domestic cattle breeding, enriched its hereditary potential of productivity, and regular exchange of genetic material allows maintaining the unity of the breed using modern genetic and genomic methods [9, p. 9–10].

Information about the distribution of genetic variability between different generations of a breeding herd has important effects for selection and improving beef cattle breeds. Reliable assessment of population diversity for significant genes is critical for identifying the genetic relationships between different breeding groups and development an effective breeding system for breed improving. At the same time, selection for genes of the same physiological and biological pathway increases the efficiency of gene pool improving in beef cattle.

Due to the need for study the dynamic processes in the gene pool of Kazakh White-Headed cattle, **the purpose of our work** was to evaluate the genetic variability of the breed in two successive generations, taking into account the polymorphisms *GH L127V* of the growth hormone gene and *GHR F279Y* of the growth hormone receptor gene.

Methods

The object of the study was the population of Kazakh White-Headed cattle from the APC breeding farm "Krasnyy Oktyabr'" in Volgograd region. Cows (n = 57 heads) and young animals (bull-calves and heifers, n = 50 heads) were genotyped for polymorphisms *GH L127V* of the growth hormone gene and *GHR F279Y* of the growth hormone receptor gene. All young animals were obtained by artificial insemination from sire No. 3207 with GH^{LV} and GHR^{FY} genotype. Whole blood was taken from animals for genotyping, from which DNA was isolated using the "DIAtom^{tm}DNAPrep" kit (IsoGeneLab, Moscow). The "GenePakPCRCore" kits (IsoGeneLab, Moscow) were used for PCR. Genotyping was performed by PCR-RFLP on a "Tertsik" programmable thermal cycler (DNA-technology, Russia) to assess the polymorphisms of the growth hormone (*GH*) and growth hormone receptor (*GHR*) genes, using primers synthesized at the "Lytech" Co. Ltd.: *GH L127V* – (F: 5'-gct-gct-cct-gagcct-tcg-3' and R: 5'-gcg-gcg-gca-ctt-cat-gac-cct-3'), *GHR F279Y* – (F: 5'-ata-tgt-agc-agt-gac-aat-at-3'and R: 5'-acg-ttt-cac-tgg-gtt-gat-ga-3').

PCR program:

1) for *GH L127V* polymorphism: "hot start" – 5 minutes at +95 °C; 35 cycles: denaturation – 45 seconds at +94 °C, annealing – 45 seconds at +65 °C, extension – 45 seconds at +72 °C; final extension – 7 minutes at +72 °C;

2) for *GHR F279Y* polymorphism: "hot start" – 5 minutes at +95 °C; 35 cycles: denaturation – 30 seconds at +95 °C, annealing – 60 seconds at +60 °C, extension – 30 seconds at +72 °C; final extension – 10 minutes at +72 °C.

Restriction endonucleases were used to restrict amplified gene regions: for *GH L127V – AluI*; for *GHR F279Y – SspI*. The recognition site for *AluI* endonuclease is the nucleotide sequence AG↓CT. Restriction endonuclease *AluI* recognizes the allele containing C nucleotide (GH^{L}). If the allele contains a G nucleotide (GH^{V}), then the restriction site disappears. The recognition site for the *SspI* endonuclease is the AAT↓ATT nucleotide sequence. Restriction enzyme cuts the amplificate containing the T nucleotide (GHR^{F}). If the allele contains the A nucleotide (GHR^{Y}), then the restriction site disappears.

Restriction analysis was carried out at +37 °C. Identification of products for the growth hormone gene was:

GH^{VV} – 223 bp; *GH*^{LV} – 223, 171, 52 bp; *GH*^{LL} – 171, 52 bp; for the growth hormone receptor gene: *GHR*^{YY} – 182 bp; *GHR*^{FF} – 158, 24 bp; *GHR*^{FY} – 182, 158, 24 bp. The digested products were separated by horizontal electrophoresis in 1x Tris-borate buffer at a voltage of 80 V in a 2.5 % agarose gel with visualization in ethidium bromide. After that, the gel was analyzed in ultraviolet light on a "UVT-1" transilluminator, the "VITran v. 1.0" system was used for photographing. Fragment length was determined using the "GenePakR DNA Ladder M 50" (IsoGene Lab, Moscow) molecular weight marker.

The studies were carried out on the equipment of Laboratory of Immunogenetics and DNA Technologies of All-Russian Research Institute of Sheep Breeding and Goat Breeding - Branch FSBSI North Caucasian Federal Agricultural Research Center (certificate PZh-77 No. 008326 from 18.04.2018) and in Federal Research Centre of Biological Systems and Agrotechnologies RAS.

The genotypes frequency was determined by the formula:

$$p = n / N,$$

where p – genotype frequency;

n – the number of individuals that have a certain genotype,

N – the number of individuals.

The allelic frequency was determined by the formula:

$$P_A = (2nAA + nAB) \div 2N,$$

where P_A –frequency of A allele;

N – a total number of individuals.

The error of genotypic and allelic frequencies were determined by the formulas:

$$S_p = \sqrt{\frac{p(1-p)}{N}}, S_p = \sqrt{\frac{p(1-p)}{2N}}$$

where S_p – the error of frequencies;

p – frequency in sample;

N – number of individuals.

The expected frequencies of genotypes in the studied population were calculated according to the Hardy – Weinberg law.

Heterozygote excess value (Selender coefficient) was calculated by the formula:

$$D = \frac{H_o - H_e}{H_e},$$

where H_o and H_e – observed and expected heterozygosity.

The data analysis was carried out using "Statistica 10.0" ("Stat Soft Inc.", USA) program in algorithms of non-parametric statistics. The accordance of the observed and expected genotypes with genetic equilibrium was checked by the Chi-square method (χ^2). In this case, the deviation of observed from expected frequencies of genotypes is significant at $\chi^2 \geq 5.99$. The significance of differences in the allelic and genotypic frequencies was determined by the criterion χ^2 with the Yates amendment.

Results

Analysis of genetic structure of the herd by *GH L127V* polymorphism indicates the presence of 3 genotypes (LL-LV-VV) in the studied age categories of animals (Table 1), and the distribution of polymorphic variants in growth hormone gene corresponded to the expected distribution according to the Hardy – Weinberg equilibrium (χ^2 = 0.018 ... 0.571; $P > 0.05$). However, the genotypes frequencies varied both within groups and between generations of the Kazakh White-Headed herd. The general trend for cows and young animals was a low frequency of the homozygous *GH*^{VV} genotype, which varied from 0.070 in the mature part of the population to 0.140 in progeny. On the contrary, the homozygous *GH*^{LL} variant had a dominant distribution in the herd, unifying 46.0–52.6 % of individuals in studied age groups.

Cows differed by a high frequency of *LL* genotypes (by 0.066) and *LV* (by 0.004) relative to their progeny. While the young animals were superior by 0.070 in proportion of individuals with *VV* variant of the growth hormone gene compared to the mature part of the herd. However, differences between age groups did not reach a significant level (χ^2 = 4.451; P = 0.108).

Table 1

Genetic structure of the Kazakh White-Headed breed according to GH L127V and GHR F279Y polymorphisms

Group	Frequencies					χ^2
	Genotype			Allele		
GH L127V						
Cows	**LL**	**LV**	**VV**	**L**	**V**	
	0.526 ± 0.066	0.404 ± 0.065	0.070 ± 0.034	0.728 ± 0.042	0.272 ± 0.042	0.018
Young animals	0.460 ± 0.070	0.400 ± 0.069	0.140 ± 0.049	0.660 ± 0.047	0.340 ± 0.047	0.571
Average	0.495 ± 0.048	0.402 ± 0.047	0.103 ± 0.029	0.696 ± 0.031	0.304 ± 0.031	0.267
GHR F279Y						
Cows	**FF**	**FY**	**YY**	**F**	**Y**	
	0.246 ± 0.057	0.333 ± 0.062	0.421 ± 0.065	0.412 ± 0.046	0.588 ± 0.046	5.524
Young animals	0.360 ± 0.068	0.380 ± 0.069	0.260 ± 0.062	0.550 ± 0.050	0.450 ± 0.050	2.746
Average	0.299 ± 0.044	0.355 ± 0.046	0.346 ± 0.046	0.477 ± 0.028	0.523 ± 0.028	8.905

The allelic frequency also differed between generations of the herd, while the frequency of the prevailing *L*-allele of the growth hormone gene varied from 0.660 to 0.728 with a maximum value in mature part of the population. The highest distribution of the alternative *V*-allele was noted in the group of young animals, which exceeded the maternal contingent by 0.068 ($P = 0.0012$).

Genotyping of the Kazakh White-Headed herd by the *GHR F279Y* polymorphism of the growth hormone receptor gene showed a discrepancy between the gene pool and the equilibrium state according to the Hardy-Weinberg law. Thus, the deviation of the observed genotypes frequency from the expected one was significant ($\chi^2 = 8.905$; $P = 0.012$) for the whole herd. However, if we consider age categories separately, then the distribution of genotypes did not differ significantly from the optimal ratio ($\chi^2 = 2.746$; $P = 0.253$) in the group of young animals. On the contrary, the mature contingent was close to the non-equilibrium state of the gene pool ($\chi^2 = 5.524$; $P = 0.063$). In addition, heterozygous individuals were most widespread in the sample of young animals with a superiority of 0.020–0.120 compared to carriers of the homozygous GHR^{FF} and GHR^{YY} genotypes, respectively. On the contrary, the highest frequency (0.421) was found in GHR^{YY} genotype in the gene pool of cows, which exceeded the proportion of heterozygous carriers and GHR^{FF} variant of the growth hormone receptor gene by 8.8–17.5 %, respectively.

There were significant differences ($\chi^2 = 12.103$; $P = 0.002$) in the distribution of homozygous genotypes carriers when compare the gene pool structures of two age groups for the growth hormone receptor gene. So, individuals with GHR^{YY} variant (0.260) hold the minimum distribution among the young animals, while this genotype was predominant in the sample of the mature contingent with a superiority of 16.1 % relative to the progeny. The reverse trend was revealed when analyzing the frequency of GHR^{FF} variant of the growth hormone receptor gene, just as the proportion of young carriers exceeded the similar genotype by 11.4 % in cows. The differences were minimal (4.7 %) between the number of heterozygous individuals in two generations of Kazakh White-Headed cattle.

Features of the genetic structure according to the polymorphism in growth hormone receptor gene were associated with different allelic frequencies in a separate age categories of the population. Characteristically, there was a relatively low difference (0.100– 0.176) in distribution of alternative alleles in *GHR* gene compared to that recorded in *GH* gene (0.320–0.456) both for the dams' and for young animals' contingent. In addition, the rank according to the allelic frequency in growth hormone receptor gene was not the same in different generations of animals. Thus, there were individuals with the *Y*-allele with the highest frequency among the mature contingent, and the advantage was on the side of the *F*-allele carriers in the group of young animals. The difference in the distribution of the corresponding alleles was 0.138 between the studied age categories of the herd.

The studied Kazakh White-Headed herd was characterized by different levels of intrapopulation variability depending on the analyzed polymorphism and age group (Table 2). The observed heterozygosity (H_o) for the growth hormone gene averaged 0.402 and exceeded this parameter for the growth hormone receptor gene by 0.047 in the population. However, the expected heterozygosity (H_e) had an inverse distribution rank in the context of the studied genes. Thus, this indicator for *GHR F279Y* polymorphism was 0.499, which exceeded the theoretical genetic diversity for the growth hormone gene by 0.076 during herd genotyping.

A common feature for the studied age groups and the analyzed nucleotide substitutions was the excess of the expected heterozygosity over the observed one, except *GH L127V* polymorphism in the mature part of the herd, where the difference was 0.008 between H_o and H_e. This indicates a certain decrease in the genetic diversity level due to non-random crossing and the pressure of artificial selection, as well as an increase in population inbredness. A more significant difference between the observed and expected variability parameter was noted in growth hormone receptor gene according to the heterozygosity test, which varied in animals of different generations within –0.115 ... –0.152, and on average for the herd reached –0.144, which differed by –0.123 from the corresponding index in *GH L127V* polymorphism.

Table 2

Assessment of the genetic diversity in Kazakh White-Headed breed by GH L127V and GHR F279Y polymorphisms

Group	Heterozygosity		Heterozygosity test	Heterozygote excess value (D)
	Observed (H_o)	Expected (H_e)		
GH L127V				
Cows	0.404	0.396	0.008 $H_o > H_e$	0.020
Young animal	0.400	0.449	–0.049 $H_o < H_e$	–0.109
Average	0.402	0.423	–0.021 $H_o < H_e$	–0.050
GHR F279Y				
Cows	0.333	0.485	–0.152 $H_o < H_e$	–0.313
Young animal	0.380	0.495	–0.115 $H_o < H_e$	–0.232
Average	0.355	0.499	–0.144 $H_o < H_e$	–0.289

The deficiency of heterozygotes in Kazakh White-Headed population is also confirmed by the Selender coefficient (D), which had negative values in all cases (except for cows genotyped for *GH L127V* polymorphism). This indicates a deviation of the herd gene pool from the optimal state.

Discussion and Conclusion

Scientists and practitioners pay special attention to the gene pool state in domestic herds for genes that are reliably associated with meat qualities of animals to implement the program for the genetic improvement of beef cattle. Somatotropic axis genes (*GH*, *GHR*, *Pit-1*, *IGF-1*) fully satisfy this requirement, as they encode proteins that make up a single humoral chain and providing the growth and differentiation of organs and tissues [10, p. 58].

The bovine growth hormone gene is located on 19 chromosome and is approximately 1793 bp long, consists of 5 exons and 4 introns [11, p. 9]. The gene product (growth hormone) is secreted in somatotropic or acidophilic cells of the mammalian anterior pituitary gland [12]. Many researches have been carried out to study the structure of *bGH* as well as its SNP due to the critical role of *GH* in improving animal performance [13, p. 157; 14; 15, p. 5]. At least 10 single nucleotide polymorphisms have been identified in growth hormone gene: four in promoter region, one in region of the first exon, one in third intron, and four in fifth exon. Our studies was based in analysis of the genetic structure of Kazakh White-Headed herd according to *GH g.2141C > G* (*GH L127V*) polymorphism, which results in a non-synonymous replacement of leucine for valine (*Leu > Val*) amino acids in 127codon of the gene. This mutation is associated with different concentrations of growth hormone in the blood serum of bulls, what can affect unequal average daily gains in carriers of different genotypes. We identified three genotypes with different frequencies when genotyping Kazakh White – Headed cattle according to the *GH L127V* polymorphism. Carriers of LL genotype hold the largest proportion (49.5 %), and the VV variant (10.3 %) accounted for a smaller part of the herd. These data partially coincided with the results obtained on the Kazakh White-Headed breed in Russian (P_{LL} = 48.8% and P_{VV} = 3.7 %) and Kazakh populations (P_{LL} = 63.3 % and P_{VV} = 5.1%) [16, p. 1450]. The authors of the scientific work noted that the Kazakh selection is genetically different from domestic representatives of the breed. This is confirmed by the allelic distribution of the growth hormone gene in different populations. Thus, the frequencies of the minor *V*-allele were 0.275 and 0.304 (in our studies) in Russian herds, while it was 0.209 in the Kazakh population. In addition, data on the distribution of *L*-allele are presented in Herefords (P_L = 0.69) and Limousines (P_L = 0.71) [6, p. 55], which fully confirm the allelic ratio established in the herd of the breeding farm "Krasny Oktyabr'". Large-scale studies were carried out on 9 beef breeds in South East Asia, as a result the wide ranges of the *L*-allele (P = 0.423…0.719) and *V*-allele (P = 0.281…0.577) frequencies were revealed in different cattle [17, p. 5].

The bovine *GHR* gene is located on 20 chromosome and encodes a transmembrane growth hormone receptor belonging to a large superfamily of cytokine and hematopoietic growth factor receptors [18, p. 71; 19, p. 352]. The growth hormone receptor is a transducer of the growth hormone action, which plays a key role in lipid and carbohydrate metabolism [20, p. 35]. Analysis of mutations reveals ten polymorphic regions in the bovine *GHR* gene [21, p. 64]. Four mutations were SNPs in introns, one was in the 3'-untranslated region, three were synonymous mutations at the third position of codon, and two SNPs modified the amino acid sequence. The T→A nucleotide substitution in eighth exon causes a change in the amino acid sequence from phenylalanine to tyrosine at position 279 (*F279Y*). A new PCR-RFLP protocol was developed and 679 animals belonging to seven cattle breeds were genotyped in Italy to analyze this mutation [22, p. 417]. In their studies, all breeds were characterized by a high distribution (50.9–89.4 %) of the *FF*-genotype in the herds, while the proportion of GHR^{YY} had a minimal number (0.0–5.6 %) of individuals. On the contrary, in our work, the largest number of animals were carriers of the heterozygous (35.5 %) and homozygous *YY*-genotypes (34.6 %). The genotyping of two crossbred beef herds confirm the overwhelming advantage in distribution of GHR^{YY} and GHR^{FY} variants, which varied within 97.5–98.5 % in the aggregate [23, p. 6]. Thus, a wide range of genotype frequencies was observed, which is reflected in a different allelic distribution. Thus, the presence of *Y*-allele was 0.086-0.370 when genotyped the Kazakh White-Headed breed of Kazakh selection [24, p. 263]. In our work, the frequency of this allele reached 0.523 on average for the herd.

When studying the dynamics of the distribution in allelic frequencies for *GH L127V* and *GHR F279Y* polymorphisms in two different generations of animals, it is necessary to note a fairly high homogeneity of the gene pool for the growth hormone gene, and, on the contrary, a significant genetic differentiation of the herd for the growth hormone receptor gene. This is confirmed by the differences in allelic (F and Y) frequencies between particular age groups of Kazakh White – Headed cattle, which is expressed in significant deviations (χ^2 = 8.905; P = 0.012) of observed from expected genotype frequencies in accordance with the Hardy-Weinberg equilibrium in the general population [25, p. 167].

As a result, for the first time, data were obtained on the evaluation of the genetic structure of Kazakh white-headed mature herd and replacement young animals of Volgograd selection according to *GH L127V* and *GHR F279Y* polymorphisms, associated with indicators of meat productivity. The results of the herd gene

pool analysis indicate that *L*- and *V*-alleles are present in growth hormone gene locus, as well as *F* and *Y* in growth hormone receptor gene locus in two successive generations (mature herd and progeny). Moreover, if *V*-allele ($P = 0.272–0.340$) in *GH L127V* polymorphism was distinguished as minor in the studied age groups, then alternative alleles in *GHR F279Y* polymorphism had an unequal frequency rank in different categories of animals. The genotypes distribution in growth hormone gene polymorphism was more balanced according to the Hardy-Weinberg law, and the population deviated significantly ($P < 0.05$) from the equilibrium state in growth hormone receptor gene. Differences in particular genotypes frequencies for the growth hormone gene did not reach a significant level ($\chi^2 = 4.451$; $P = 0.108$) between cows and young animals. Whereas, there were significant differences ($\chi^2 = 12.103$; $P = 0.002$) in presence of homozygous genotypes carriers for growth hormone receptor gene, which was due to the use of a heterozygous sire for this polymorphism in herd reproduction. Thus, the presence of different allele's carriers of the studied genes creates the prerequisites for the introduction of marker-assisted selection in the herd aimed at increasing the meat productivity of Kazakh White-Headed cattle.

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