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Evaluation of Polymorphic Variants of the Genes bPit-1, bGH, bGHR as Genetic Markers of Meat Productivity in Cows of Auliekol Breed

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Abstract: At present, there is an acute need in the Republic of Kazakhstan for modernization of the beef cattle breeding industry. The solution of this problem assumes, along with the importation of highly productive foreign selection breeds, the intensification of selection of local breeds such as Auliekolskaya, that are well adapted to climate conditions, the forage base and has a stable immunity to diseases prevalent in the territory of the Republic of Kazakhstan through the introduction of modern highly effective scientifically sound technologies. One of these DNA technologies is the Marker-Assisted Selection (MAS) which allows accelerating the rates of breeding and reducing financial costs in the implementation of classical breeding activities. MAS selection uses information on the phenotypic manifestation of gene alleles responsible for quantitative traits (candidate genes) in order to assess the genetic potential of animal productivity in the early stages of postnatal development.

INTRODUCTION

Of great interest from the point of view of the search for genetic markers of milk productivity are the genes of the somatotropin cascade, the protein products of which are the key links of one humoral chain involved in the growth and development of mammals (bPit-1, bGH, bGHR)[1-3].

Synthesis of somatotropin and the realization of its physiological effects is a chain of successive protein-receptor interactions (somatotropin cascade). The key links of this chain are: pituitary transcription factor-1 (bPit-1), triggering the expression of the somatotropin and prolactin, prolactin and growth hormone, regulating lactation, the growth hormone receptor (bGHR), transmitting the somatotropin humoral signal to target cells and the insulin-like factor growth-1 (bIGF-1), triggering intracellular responses to the action of somatotropin[4-5].

Genes of the somatotropin cascade are polymorphic, a wide range of their alleles which are of interest for MAS-selection as genetic markers of economically useful

traits, has been revealed in cattle. However, in a number of cases the published data on the association of the alleles of genes of the somatotropin cascade (bPit-1-HinFI, bPRL-RsaI and bGH-AluI) with the signs obtained on different rocks are difficult to compare and contradict each other[6-8], there are data on the fact that the same polymorphism in representatives of closely related breeds may have the opposite phenotypic effect and for a large part of the alleles identified, associations with signs of productivity (meat and milk) were not conducted at all.

Earlier, for these polymorphism, studies were conducted on various rocks in several countries but information on the Auliekol breed which is strategically important for the breeding of the Republic of Kazakhstan, is not available today.

Based on the foregoing, the aim of this study was to study the phenotypic effect of polymorphisms of bPit-1-HinFI, bGH-AluI and bGHR-SspI genes to assess the prospects for their use as markers for increased meat productivity of Auliekol breed cattle.

MATERIALS AND METHODS

The object of the study was a sample of cows of Auliekol breed (n = 296). Subject of the study: polymorphic genes of the somatotropin cascade: bPit-1, bGH, bGHR.

The material of the study is DNA samples isolated from the blood of Auliekol breed cows. Blood samples were provided by LLP Karkyn in Kostanai oblast. The source of information on the productivity of animals served as tribal maps of animals represented by the farm. The method of DNA-typing of animals includes the following operations:

- Selection and preparation of samples for analysis (carried out by employees of the economy that provides samples
- Extraction of DNA from the test samples. Genomic DNA was isolated from the blood of cows using the DiatomTMPrep200 kit (Isogen Laboratory, Moscow), according to the manufacturer's instructions
- Amplification of DNA with appropriate primers (PCR)
- Treatment of the amplificate with a restriction enzyme
- Separation of restriction products by gel electrophoresis
- Identification of the animal's genotype
- Documenting and entering information into a common database

Identification of animal genotypes was carried out using the PCR-RFLP method. The primer sequences and

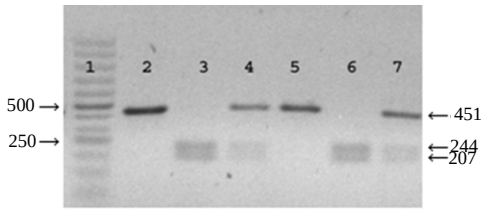

Fig. 1: Electrophoregram of DNA typing; Genotype bPit-1-HinFIAB

PCR conditions for the analysis of each polymorphism are shown in Table 1. Analysis of the polymorphism of the length of the restriction fragments of the genes bPit-1-HinFI, bGH-AluI and bGHR-SspI.

An analysis of restriction fragment length polymorphism included the processing of the ampli fi er with a site-specific restriction enzyme and subsequent separation of the fragments obtained by gel electrophoresis.

The analysis of the polymorphism of the nucleotide sequence of the bPit-1 gene in exon 6 was carried out using the HinFI restriction enzyme. Polymorphism is caused by A→G nucleotide substitution which does not lead to a change in the amino acid sequence. The recognition site for the HinFI restriction enzyme is the sequence G↓ANTC. The fragment cut during fermentation contains the nucleotide A corresponding to the allele bPit-1-HinFIB[9]. In the case of the presence of the G nucleotide, the restriction site disappears such an allele is designated as bPit-1-HinFIA.

The length of the amplified fragment of the βPit-1 gene is 451 bp. The length of fragments after restriction is 244 and 207 bp. On the electrophoregram, variants of bands of a certain length, characteristic of genotypes, are visualized: one band of 451 bp. (genotype bPit-1-HinFIAA); two bands of 244 and 207 bp. (genotype bPit-1-HinFIBB); three bands 451, 244 and 207 bp. (genotype bPit-1-HinFIAB) (Fig. 1).

Lane 1 molecular weight marker O'RangeRulerTM 50 bp DNALadder, Fermentas, Lithuania; lane 2 PCR product 451 bp a fragment of the bPit-1-HinFI gene; lane 3, 6-restriction fragment 244, 207 bp corresponding to genotype bPit-1-HinFIBB; lane 4, 7-restriction fragments 451, 244, 207 bp corresponding to the genotype bPit-1-HinfIAB; lane 5 is a 451 bp restriction fragment corresponding to the bPit-1-HinFIAA genotype. The position on the gel of specific bands is shown by arrows. Electrophoresis was performed in a 2% agarose gel (SeaKemLEAgarose, Lonza, USA).

Polymorphism bPit-1-HinFI: Analysis of polymorphism of the nucleotide sequence of the bGH gene in exon 5 is carried out using the AluI restriction enzyme. Polymorphism is caused by the transition C→G, leading

Table 1: Individual characteristics of PCR conditions for the polymorphic loci of the genes of the somatotropin cascade

Polymorphism	Amplification conditions	Sequences of primers	References
bPit-1-HinI	94°C - 1 min; (95°C- 45 sec; 56°C-6° sec; 72°C-6C° sec)×35 cycles; 72°C-1° min	HinFI-F: 5'-aaaccatcatctcccttctt-3'	[9]
bGH-AluI	95°C-5 min; (95°C-3° sec; 64°C-3° sec; 72°C-6° sec)×35° cycles; 72°C-1° min	AluI-F: 5'-ccgtgtctatgagaagc-3' AluI-R: 5''-gttcttgagcagcgcgt-3'	[6]
bGHR-SspI	95°C-5 min; (95°C-3° sec; 64°C-3° sec; 72°C-6° sec)×35° cycles; 72°C-1° min	SspI-F: 5'-aatacttgggctagcagtgacaatat -3' SspI-R: 5'-acgtttcactgggttgatga -3'	[10]

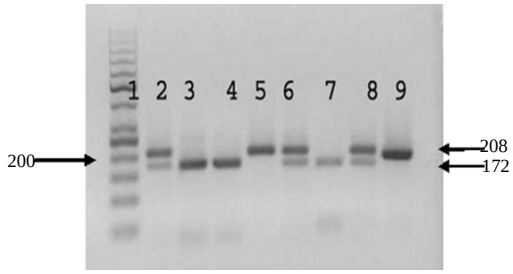

Fig. 2: Electrophoregram of DNA typing; Polymorphism bPit-1-HinFI

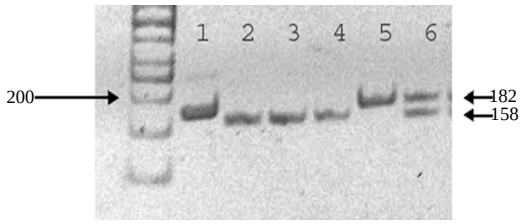

Fig. 3: Electrophoregram of DNA typing; Polymorphism bGH-AluI

to the replacement of the amino acid leucine with valine in the protein sequence. The recognition site for the restriction enzyme AluI is the sequence AG↓CT. The enzyme recognized by the enzyme contains nucleotide C and is designated as bGH-AluIL. In the case of the presence of the G nucleotide, the restriction site disappears such an allele is designated as bGH-AluIV (Fig. 2).

Lane 1-molecular weight marker O'Range Ruler TM 50 bp DNA Ladder, Fermentas, Lithuania; lanes 2, 6-restriction fragments 208, 172, 35 bp corresponding to the genotype bGH-AluILV; lanes 3, 4, 7, a 172 bp restriction fragment corresponding to the bGH-AluILL genotype; lane 5 is a 208 bp restriction fragment corresponding to the bGH-AluIVV genotype; lane 9-PCR product 208 bp a fragment of the bGH-AluI gene. A restriction fragment of 35 bp. not visualized. The position on the gel of specific bands is shown by arrows. Electrophoresis was performed in a 2% agarose gel (SeaKem LE Agarose, Lonza, USA).

Polymorphism bGH-AluI: The length of the amplified fragment of the bGH gene is 208 bp. The length of fragments after restriction is 172 and 35 bp. On the electrophoregram, variants of bands of a certain length characteristic of genotypes can be seen: one band of 208 bp. (genotype bGH-AluIVV); two bands, 172 and 35 bp. (genotype bGH-AluILL); three bands 208, 172 and 35 bp. (genotype bGH-AluILV). A restriction fragment of 35 bp on an agarose gel is not visualized[6].

The polymorphism of the nucleotide sequence of the bGHR gene in exon 8 was carried out using the SspI restriction enzyme. Restrictase SspI recognizes T→A transcription in exon 8. This SNP causes substitution of a polar, albeit uncharged, tyrosine residue in place of neutral phenylalanine at position 279 of the protein. The recognition site for the restriction enzyme is the sequence AAT↓ATT. The enzyme cut enzyme contains the nucleotide T corresponding to the allele bGHR-SspIF [10]. In the case of the presence of an A-nucleotide, the restriction site disappears such an allele is designated as bGHR-SspIY. The length of the amplified fragment of the bGHR gene is 182 bp. The length of fragments after restriction 158 and 24 bp. On the electrophoregram, variants of bands of a certain length characteristic for genotypes can be seen: one band of 182 bp. (genotype bGHR-SspIYY), two bands158 and 24 bp. (genotype bGHR-SspIFF); three bands-182, 158 and 24 bp. (genotype bGHR-SspIFY). Fragment of 24 bp. on an agarose gel is not visualized (Fig. 3).

Lane 1-PCR product 182 bp a fragment of the bGHR-SspI gene; lanes 2, 3, 4, a 158 bp restriction fragment corresponding to the bGHR-SspIFF genotype; lane 5-a 182 bp restriction fragment corresponding to the bGHR-SspIYY genotype; lane 6-restriction fragments 182 and 158 bp corresponding to the genotype bGHR-SspIFY. Fragment of 24 bp. not visualized. The molecular weight marker O'RangeRulerTM 50 bp DNALadder, Fermentas, Lithuania was used. The position on the gel of specific bands is shown by arrows. Electrophoresis was performed in a 2% agarose gel (SeaKemLEAgarose, Lonza, USA).

Polymorphism bGHR-SspI: The animal genotype for all analyzed genes is documented and entered in a common database.

Mathematical model of the experiment: An assessment of the prospects for using the polymorphic variants of the bPit-1, bGH, bGHR genes as DNA markers of increased

meat productivity in the breeding programs of the Auliekol breed of cattle included two stages. The first is the analysis of the genetic structure of the breeding stock of Auliekol breed cows over the polymorphic genes of the somatotropin cascade; the second is the evaluation of the phenotypic effect of polymorphisms on the live weight of animals at the age of 6, 12, 18 and 24 months.

The study of the genetic structure of the population included the determination and analysis of the frequencies of alleles and genotypes, in particular the frequency counting of allelic variants of the genes of the somatotropin cascade as well as an assessment of the correspondence of the frequency distribution of genotypes theoretically expected in accordance with the Hardy-Weinberg law.

The frequencies of genotypes are determined by direct counting. Relative frequencies of alleles of the studied genes according to Eq. 1:

$$Q(A) = (2N1+N2)/2n \quad (1)$$

Where:

N1 = The number of homozygotes for the allele under investigation
N2 = The number of heterozygotes
n = The sample size[11]

The statistical error of the relative allele frequencies is calculated by Eq. 2:

$$SQ = \sqrt{(Q(1-Q)/2n)} \quad (2)$$

Where:

Q = The relative frequency of the allele under investigation
n = The sample size

The correspondence between the actual distribution of genotypes theoretically expected in accordance with the Hardy-Weinberg law is estimated using the χ^2 criterion, (Eq. 4). The number of degrees of freedom is 1 (the number of genotypes minus the number of alleles).

$$\chi^2 = \sum (But-He)\ 2/He \quad (3)$$

where, He are the observed frequencies of genotypes. Non-expected frequencies of genotypes:

- AA = p2
- AB = 2 pq
- BB = q2

In the event that the expected values of the population in at least one of the classes are less than five, the calculation of χ^2 is carried out with the Yates correction:

$$\chi^2 = \sum (But-He)-0.5\ 2/He \quad (4)$$

The permissible value of χ^2 for one degree of freedom and 5% significance level is 3.84[12]. Assessment of the association of the sign of meat production and genotype was carried out in several stages. For the processing of data used the program "Microsoft Excel 2010" and "Statistica 6.0".

Previously, for the choice of statistical processing methods, the character distribution of the trait in groups was tested using the Shapiro-Wilk's W test. Since, in some groups the nature of the distribution of the trait was different from the normal one, further analysis was performed by nonparametric statistics, the data are presented in the form of a median and interquartile range (Me, (25, 75%)).

At the first stage, preferred and alternative genotypes were established by comparing the performance of groups with different genotypes among themselves.

A statistical assessment of the reliability of the differences was carried out with the help of the Kraskel-Wallis test which tests the hypothesis of whether the studied groups are from the same population or from different populations with equal medians.

In the event that a significant difference was found between three groups of animals with three different genotypes for a given polymorphism, the genotype with the highest value of the indicator was preferred for this attribute, the other two were taken as alternative ones.

At the second stage, after revealing the preferred and undesirable genotypes for the given polymorphism, a comparison was made of the productivity indices of these groups of animals with the productivity indicators of the total sample.

We assessed the reliability of observed differences using the 95% confidence interval for the median which makes it possible to estimate the differences between the group that is part of the sample and the sample itself.

For the median interval estimation, the lower and upper limits of the confidence interval were determined. The ordinal numbers of the sample values which are the Lower (L) and Upper (U) boundaries were determined by Eq. 5 and 6:

$$L = n/2-(Z1-a*\sqrt{n}/2) \quad (5)$$

$$U = 1+n/2+(Z1-a*\sqrt{n}/2) \quad (6)$$

where, Z is the value of the normal distribution for the chosen probability. For the confidence probability of 95% Z = 1.96[12], n is the sample size. To evaluate the statistical significance of the observed differences, P was calculated for the Wilcoxon test.

RESULTS AND DISCUSSION

The analysis of the genetic structure of the populations under study included a comparison of the allele frequency distribution as well as an analysis of the correspondence of the observed genotype frequencies to the theoretically expected equilibrium distribution in accordance with the Hardy-Weinberg law.

The frequencies of the alleles of the studied genes in the population of Auliekol cattle are shown in Table 2. The statistical evaluation was carried out by finding the calculated level of significance of P by the value of the t-test and the number of degrees of freedom from the tables of the student's distribution.

According to the data given in Table 2, it can be noted that for the polymorphism bPit-1-HinFI allele bPit-1-HinFIA. These data correspond to the results of Zhao *et al*.[13] Obtained in the study of Angus beef cattle. In particular, in his works the frequency of allele bPit-1-HinFIA is 0.33. According to Yang *et al*.[14], the ratio of A/B alleles in Nanyang cattle, Qinchuan cattle, Jiaxianhong cattle, izhen cattle, Luxi cattle and Holstein cattle was 0.444/0.556.0.477/0.523, 0.538/0.462, 0.421/ 0.579, 0.523/0.477, 0.475/0.525, respectively.

At the same time, the bPit-1-HinFIA allele frequency is slightly lower in the dairy breeds, in comparison with the data of meat breeds. Thus, Renaville *et al*.[9] The frequencies of the alleles bPit-1-HinFIA and bPit-1-HinFIB were 0.18 and 0.82, respectively, when studying Holstein cattle. According to our Belarusian colleagues, the frequency of this allele among representatives of Holstein and black-and-white breeds is 0.21 and 0.23, respectively[3, 15].

Thus, it can be noted that the frequencies of occurrence of the allele bPit-1-HinFIA differ in different populations of the same breed.

The distribution of allele frequencies bGHAluIL and bGH-AluIV of the growth hormone gene according to our data is 0.648/0.352 in the Auliekol breed population. These data are correlated with the results of a study of the Lithuanian cattle population of black and motley breed: the allele ratio of bGHAluIL and bGH-AluIV is 0.7 and 0.3, respectively[10]. At the same time, in different populations of Holstein cattle this ratio varies from 0.74-0.93 for the bGH-AluIL allele and from 0.07-0.26 for the bGH-AluIV allele[5, 6].

The frequency of the rare allele of polymorphism bGHR-SspI according to our data was bGHR-SspIY 0.040 in representatives of Auliekol breed of cattle. It is noted that the frequency of this allele varies considerably according to the data of different authors. Thus, according to Hosner *et al*.[16], the frequencies of allele bGHR-SspIY in representatives of Jersey, Holstein-Frisian and Simmental breeds are 0.05, 0.27 and 0.10, respectively. The frequency of the allele

Table 2: Estimation of the relative frequencies distribution of the alleles of the investigated genes (Q±SQ) in the Auliekol population (n = 284 cattle (n = 296)

Polymorphism	Allele	Observed frequencies alleles	Relative frequencies alleles
bPit-1-HinFI	bPit-1-HinFI^{A}	154	0,341±0,002
	bPit-1-HinFI^{B}	298	0,659±0,002
bGH-AluI	bGH-AluI^{V}	159	0,352±0,002
	bGH-AluI^{L}	452	0,648±0,002
bGHR-SspI	bGHR-SspI^{F}	434	0,960±0,000
	bGHR-SspI^{Y}	18	0,040±0,000

bGHR-SspIF in the representatives of these rocks reaches, respectively 0.95, 0.73 and 0.90. According to the data of Viitala *et al*., in the Finnish Aurshire livestock the ratio of the allele frequencies bGHR-SspIF and bGHR-SspIY is 0.89 and 0.11[17]. Thus, our data is within the information published earlier by other authors. It should be noted that according to the distribution of allele frequencies bGHR-SspIY and bGHR-SspIF, the rocks under analysis differ significantly from each other. The estimation of the correspondence of the observed frequencies of genotypes theoretically expected by the Hardy-Weinberg law.

We also analyzed the correspondence of the distribution of genotypes for the studied polymorphic genes of the somatotropin cascade to the theoretically expected, according to the Hardy-Weinberg law, among the animals of the Auliekol and Kazakh white-headed breeds. The significance of the observed deviations was estimated using the χ^2 criterion. The data obtained are presented in Table 3.

Table 3 shows that in the population of Auliekol cattle according to the polymorphisms bPit-1-HinFI, the value of χ^2 is 0.05 which supports the deviation of the observed number of genotypes from the equilibrium ones. According to the polymorphism bGH-AluI, the observed frequencies of the genotypes correspond to the equilibrium distribution theoretically expected by the Hardy-Weinberg law. At the same time, according to the polymorphism bGHR-SspI, the value of χ^2 is 21.12 which is in favor of a statistically significant deviation of the observed number of genotypes from the equilibrium ones. This indicates the presence of pressure of artificial selection in the investigated populations and the possible association of this polymorphism with signs of productivity in cattle of the Auliekol breed. Thus, based on the studies carried out, the following is established.

First, the established allele frequencies for all the polymorphisms studied are correlated with the data of other authors; alleles, claimed as rare in representatives of other breeds are rare in the same way according to the results of our study.

Secondly, it has been shown that, according to the polymorphisms bPit-1-HinFI and bGHR-SspI, the observed frequencies of the genotypes deviate from

Table 3: Frequency distribution of genotypes of polymorphic genes of somatotropin cascade in populations of Auliekol and Kazakh white-headed cattle

		Auliekol breed (n = 286)		
Polymorphism	Genotypes	n observable	n expected	χ^2
bPit-1-HinFI	bPit-1-HinFI^{AA}	27	26	0,05
	bPit-1-HinFI^{AB}	100	102	
	bPit-1-HinF1^{BB}	99	98	
bGH-AluI	bGH-AluI^{VV}	28	28	0,00
	bGH-AluI^{LV}	103	103	
	bGH-AluI^{LL}	95	95	
bGHR-SspI	bGHR-SspI^{FF}	211	208	21,12
	bGHR-SspI^{FY}	12	17	
	bGHR-SspI^{YY}	3	0	

Deviation of the observed genotype frequencies from the theoretically expected Hardy-Weinberg law is significant for $\chi^2 \geq 3.84$

theoretically expected Hardy-Weinberg equilibrium equilibria. This, in turn, indicates the presence of artificial selection pressure in the studied populations and the possible association of these polymorphisms with the productivity of animals.

Association of polymorphic genes of somatotropin cascade with signs of meat productivity in cows of Auliekol breed. Evaluation of polymorphisms of the genes of the somatotropin cascade bPit-1-HinFI, bGH-AluI and bGHR-SspI as genetic markers of milk productivity in Auliekol breed cows took into account two aspects. The first-reflects the traditional approach which involves the identification of preferred and alternative genotypes by comparing the performance indicators of the respective groups of animals. Preferred is the genotype, the owners of which are characterized by the highest productivity on the test feature. The second, proposed by our Belarusian colleagues[15, 16]. In addition, to the traditional approach suggests a subsequent comparison of productivity in groups of animals with preferred and alternative genotypes relative to the total sample and an assessment of the significance of the observed differences. Such additional analysis makes it possible to evaluate the expediency of selecting animals on a preferred genotype or eliminating individuals with an alternative genotype.

Meat characteristics of animals were estimated by such indicators as live weight at birth and also at the age of 6, 12, 18 and 24 months; weight gain for 6 months of maintenance: from the 1st to the 6th, from the 7th to the 12th, from the 13th to the 18th and from the 19th to the 24th; daily increase in the 1 st, 2 nd, 3 rd and 4 th half-year content.

Meat characteristics of animals were estimated by such indicators as live weight at birth and also at the age of 6, 12, 18 and 24 months and daily increase in live weight in the 1 st, 2 nd, 3 rd and 4 th half of the year.

The results of the assessment of live weight at birth as well as at the age of 6, 12, 18 and 24 months are presented in Table 4.

From the data in Table 4 it follows that the calves of the Auliekol breed selected for the experiment have approximately the same weight, regardless of the genotype. According to the polymorphism of bPit-1-HinFI, the group of animals with the genotype bPit-1-HinFIAA is characterized by an increased live mass already by the end of the first half of the year. Later, by the age of 12 months, this trend persists and at the age of 18 and 24 months the observed differences become statistically reliable.

There are no clear trends on polymorphisms of bGH-AluI and bGHR-SspI. Thus, animals with genotype bPit-1-HinFIAA statistically significantly differ from animals with genotypes bPit-1-HinFIAB and bPit-1-HinF1BB on the basis of live weight at the age of 18 and 24 months.

The observations made suggest that in the Auliekol breed cows, the bPit-1-HinFIAA genotype in these age groups is preferable to the bPit-1-HinFIAB and bPit-1-HinF1BB genotypes based on the live weight at the age of 18 and 24 months.

There were no significant differences in the live weight among the animals with the bGH-AluILL, bGH-AluILV and bGH-AluIVV polymorphisms of the bGH-AluI polymorphism and among the animals with the genotypes bGHR-SspIFF, bGHR-SspIFY and bGHR-SspIYY by bGHR-SspI polymorphism.

For the polymorphism bPit-1-HinFI, the daily growth rates in the 1st, 2nd, 3rd and 4th half were also analyzed. The data are given in Table 5.

From Table 5 it follows that, beginning with the first half of the year, heifers with the genotype bPit-1-HinFIAA are characterized by a higher daily gain in live weight, both in relation to animals with the genotypes bPit-1-HinFIAB and bPit-1-HinF1BB. Although the results of the calculation of the Kruskel-Wallis criterion, observations can not be considered statistically significant.

Since, the frequency of the allele bPit-1-HinFIA is 0.345±0.002 and the genotype bPit-1-HinFIAA in the studied population is rare, it can be considered as a potential genetic marker for increased meat production for selecting such animals during breeding programs with Auliekol breed.

Table 4: Characteristics of live weight by age in groups of cows with different genotypes by the polymorphisms bPit-1-HinFI, bGH-AluI and bGHR-SspIauliekol breed, kg (Me, (25, 75%)

Age groups	At birth	6 months	12 months	18 months	24 months
Total sample	26 (26; 26)	207 (182; 218)	325 (295; 348)	373 (329; 398)	414 (381; 447)
PolymorphismbPit-1-HinFI					
Genotype					
bPit-1-HinFI^{AA}	26 (26; 26)	215 (204; 218)	332 (321; 364)	386 (370; 423)	447 (403; 483)
bPit-1-HinFI^{AB}	26 (26; 26)	208 (179; 218)	325 (299; 346)	375 (329; 394)	411 (382; 436)
bPit-1-HinF1^{BB}	26 (26; 26)	204 (179; 216)	324 (289; 334)	368 (329; 387)	405 (377; 437)
P*	0,3819	0,1695	0,0794	0,0342	0,0406
PolymorphismbGH-AluI					
Genotype					
bGH-AluI^{LL}	26 (26; 26)	204 (179; 216)	324 (298; 343)	371 (341; 387)	416 (381; 456)
bGH-AluI^{LV}	26 (26; 26)	209 (182; 218)	326 (302; 348)	374 (327; 399)	409 (381; 447)
bGH-Alu^{VV}	26 (26; 26)	214 (177; 218)	326 (293; 338)	371 (329; 396)	417 (384; 430)
P	0,9940	0,4577	0,6220	0,9325	0,8337
PolymorphismbGHR-SspI					
Genotype					
bGHR-SspI^{FF}	26 (26; 26)	208 (182; 218)	325 (299; 348)	373 (329; 398)	414 (381; 453)
bGHR-SspI^{FY}	26 (26; 26)	195 (164; 208)	308 (278; 345)	357 (330; 401)	396 (373; 431)
bGHR-SspI^{YY}	26 (24; 26)	204 (154; 221)	322 (226; 375)	384 (284; 401)	432 (329; 457)
P	0,4459	0,1194	0,5693	0,7722	0,5812

The calculated level of significance for estimating variance variance. Allows you to assess the significance of the difference in the indicator in groups with different genotypes. The difference is significant at $p<0.05$

Table 5: Characteristics of the daily increase in live weight in the semi- year in groups of cows with different genotypes of the bPit-1-HinFI polymorphism of the Auliekol breed, in grams, (Me, (25, 75%)

1-th half-year	2-th half-year	3-th half-year	4-th half-year
1033 (973; 1049)	694 (607; 803)	295 (262; 333)	246 (197; 361)
1003 (836; 1049)	658 (566; 784)	270 (227; 320)	251 (194; 0,292)
967 (831; 1038)	0,639 (557; 776)	268 (208; 317)	262 (197; 311)
0,705	0,4102	0,1299	0,3460

The calculated level of significance for estimating variance variance. Allows you to assess the significance of the difference in the indicator in groups with different genotypes. The difference is significant at $p<0.05$

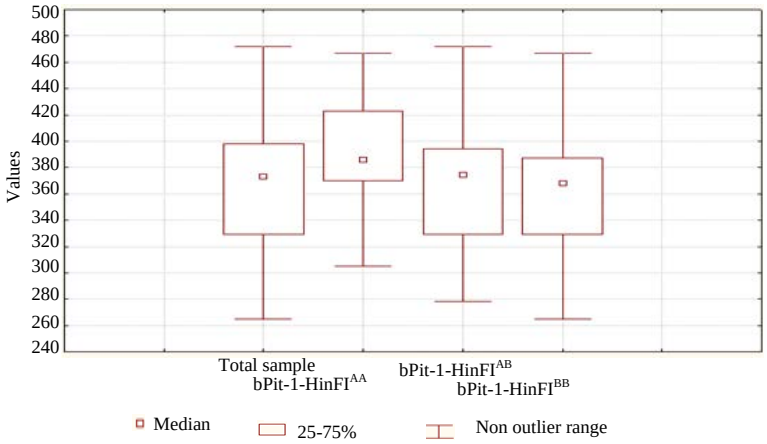

Fig. 4: Animal age 24 months

To assess the degree of phenotypic effect of the preferred and alternative genotypes by polymorphism, a comparison was made between the indices of the productivity of these groups of animals relative to the total sample.

In Table 4 above, data on the live weight of animals with different genotypes for the polymorphism of bPit-1-HinFI is given in the sample average. Figures 4 show graphically the ratio of cow's productivity to the genotypes bPit-1-HinFI^{AA}, bPit-1-HinFI^{AB} and bPit-1-HinF1^{BB} relative to the total sample at 12 and 24 months of age when the bPit genotypes-1-HinFI^{AA}, bPit-1-HinFI^{AB} and bPit-1-HinF1^{BB} are statistically significantly different in terms of live weight.

Figure 4 Graphic correlation of cows' productivity with genotypes relative to the total sample of bPit-1-HinFI^{AA}, bPit-1-HinFI^{AB} and bPit-1-HinF1^{BV} at the age of 18 and 24 months.

Table 6: Characteristics of live weight in groups of cows of Auliekol breed with different genotypes according to polymorphism bPit-1-HinFI in relation to the total sample, (Me, [CI 95%])

Group/parameter	18 months	24 months
Total sample	373 [377; 368]	414 [418; 404]
bPit-1-HinFI^{AA}	386 [419; 372]	447 [480; 421]
bPit-1-HinFI^{AB}	375 [378; 367]	411 [423; 395]
bPit-1-HinF1^{BB}	368 [377; 357]	405 [423; 404]

From the graphs shown in Fig. 4, it is clear that the live weight of the Alykelek breeds with the genotype bPit-1-HinFI^{AA}, at the age of 18 and 24 months, exceeds not only the weight of animals with alternative genotypes but also the total sample. While the weight of animals with the genotypes bPit-1-HinFI^{AB} and bPit-1-HinF1^{BB} is within the total sample.

Statistical evaluation of the reliability of observed differences was carried out by the interval estimation method, as described in the section "Materials and Methods". The results are shown in Table 6.

From the data shown in the table it is clear that at the age of 18 months with 95% of the probability, the median of the sign of live weight in the total sample of cows of Auliekol breed is 373 kg and is in the range from 368 to 377 kg. At the same age in cows with the genotype bPit-1-HinFI^{AA}, the median of the sign of live weight is 386 kilograms and lies in the range of 372-419 kg.

Therefore, in cows with the genotype bPit-1-HinFI^{AA} at the age of 18 months, the lower limit of the confidence interval for the median is 372 kg and overlaps with the upper confidence interval value for the median sample of 377 kg. It follows that it is impossible to assert with 95% of the probability that at the age of 18 months the cows with the genotype bPit-1-HinFI^{AA} exceed the total sample by live weight.

But at the age of 24 months, as follows from the table with 95% of the probability, the median of the sign of live weight in the total sample of cows of the Auliekol breed is 414 kg and ranges from 404-418 kg. At the same age, in cows with the genotype bPit-1-HinFI^{AA}, the median of the sign of live weight is 447 kg and ranges from 421-480 kg. That is, in cows with the genotype bPit-1-HinFI^{AA}, the lower limit of the confidence interval for the median is 421 kg and does not overlap with the upper confidence interval value for the median sampling of 418 kg.

It follows that with 95% of the probability of asserting that at the age of 24 months the cows with the genotype bPit-1-HinFI^{AA} exceed the total sample by the sign of live weight.

Thus, in the presence of a significant difference in the live weight in cows with the genotypes bPit-1-HinFI^{AA}, bPit-1-HinFI^{AB} and bPit-1-HinF1^{BV} between themselves at the age of 18 and 24 months relative to the total sample, of the total sample for cows with the genotype bPit-1-HinFI^{AA} is detected only at the age of 24 months. This means that the genotype bPit-1-HinFI^{AA}, despite a significant excess of the live weight at the age of 18 and 24 months can be used as a marker for increased productivity only for the age of 24 months. Since, at the age of 18 months, the live weight of this group is still within the values of the total sample.

CONCLUSION

As a result of the work the following was established: the detected frequencies are correlated with the data of other authors; alleles, claimed as rare in representatives of other breeds are rare in the same way according to the results of our study.

It is shown that according to the polymorphisms bPit-1-HinFI and bGHR-SspI, the observed frequencies of genotypes deviate from theoretically expected Hardy-Weinberg equilibrium equilibria which in turn indicates the presence of artificial selection pressure in the studied populations and the possible association of data The relationship of genotypes of polymorphisms of polymorphisms bPit-1-HinFI, bGH-AluI and bGHR-SspI with signs of live weight at the age of 6, 12, 18 and 24 months and daily increment in the 1st, 2nd, 3rd and 4th half-lives was studied. The absence of significant differences in live weight among animals with genotypes bGH-AluI^{LL}, bGH-AluI^{LV} and bGH-AluI^{VV} of bGH-AluI polymorphism and among animals with the genotypes bGHR-SspI^{FF}, bGHR-SspI^{FY} and bGHR-SspI^{YY} by bGHR-SspI polymorphism was shown.

For bPit-1-HinFI polymorphism, animals with the bPit-1-HinFI^{AA} genotype are statistically significantly different from animals with the bPit-1-HinFI^{AB} and bPit-1-HinF1^{BB} genotypes on the basis of live weight at the age of 18 and 24 months. Therefore, in the cows of the Auliekol breed, the bPit-1-HinFI^{AA} genotype in these age groups is preferable to the bPit-1-HinFI^{AB} and bPit-1-HinF1^{BB} genotypes based on live weight in the age group and it can be considered as a potential genetic marker for increased meat productivity for the selection of animals during breeding programs with Auliekol breed.

The degree of phenotypic effect of the genotype bPit-1-HinFI^{AA} in relation to the productivity of the total sample was studied. It was shown that the genotype bPit-1-HinFI^{AA}, despite the significant excess of the live weight at the age of 18 and 24 months with respect to the genotypes bPit-1-HinFI^{AB} and bPit-1-HinF1^{BB} can be used as a marker for increased productivity only for of the age of 24 months, since at the age of 18 months, the live weight of this group is still within the values of the total sample.

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