



Assessment Of Pairwise Combinations' Association Of Polymorphic Variants Of The Genes Of Bpit-1, Bgh, Bghr Bigf Somatotropic Cascade With Meat Productivity Of The Cattle Bred In Kazakhstan

A. M. Nametov¹, I. S. Beishova², G. D. Chuzhebaeva², I. M. Tegza², A. V. Belaya³

¹Zhangir Khan West Kazakhstan Agrarian Technical University, Zhangir Khan St., 51, Uralsk, 090009, Kazakhstan

²Kostanai State University n. a. A. Baitursynov, A. Baytursynov St., 47, Kostanai, 110000, Kazakhstan

³GenLabService, Office 126, Birch Grove St., 106, Borovlyansky village, Minsk region, 223053, Belarus

Abstract

Assessment of the genetic potential in efficiency of agricultural animals by genetic markers is a modern, popular and rapidly developing area in breeding. Currently, effective genetic markers are searched for among the candidate genes for various traits in various breeds. It has been assumed that the phenotypic effect of genetic markers may be more pronounced if the genotype of the animal has genetic markers that potentiate the effect of each other. Therefore, genes of the somatotropic cascade have been taken for the study, the protein products of which are key links in the humoral chain involved in the processes of mammals' growth and development (bPit-1, bGH, bGHR, bIGF-1). In this case, expression of one gene affects expression of all other genes, and one polymorphism can potentiate the action of another one. The subjects of the study are polymorphic genes of the somatotropic cascade: bPit-1, bGH, bGHR and bIGF-1. Meat productivity of animals with various genotypes and their pairwise combinations was assessed based on live weight at the age of 18 and 24 months. Results of this work show that genetic markers that are diplotypes often have more pronounced phenotypic effect than individual marker genotypes. For example, the range of live weight at the age of 12 months for IGF-1^{BB} genotype is 325-331 kg, its pairwise combination with bGH-AluI^{LL} genotype potentiates this effect to 278-306 kg; genotypes that are individually associated with meat productivity in pairwise combinations have increased or decreased statistically significant phenotypic effect, compared to the total sample. Such combinations may be applied as genetic markers of productivity in breeding programs. An example is bGH-AluI polymorphism. Inversely, polymorphisms that individually show association with a symptom of productivity may be within the total sample in pairwise combination by the phenotypical effect.

Keywords: bPit-1, bGH, bGHR, bIGF-1, genes of the somatotropic cascade, polymorphism

INTRODUCTION

Global development of agriculture in general and livestock in particular is required for improving and ensuring food security of the country. The main products of animal husbandry that provide biologically complete substances to humans are meat and milk.

Over the years of reforms in agriculture, significant changes have occurred in the structure of agriculture, and in animal husbandry. The population of cattle has decreased twice, the flow of imported food is not reducing, and dependence on it increases [1].

In this context, the main factor of animal production, namely meat, is the development of beef cattle breeding.

The main task of long-term breeding is creating desired genotypes that could combine high production quality without reducing the reproductive potentiality and longevity, and show their valuable characteristics in the offered conditions of industrial technology.

In breeding, selection is of fundamental importance, since it finalizes identification of breeding value and choosing the best animals for farther use, based on preservation and increasing qualitative commercially valuable traits [2].

In breeding work in high productivity herds, one should envisage a program for using those animals that can transfer only useful qualities to their offspring [3].

Thus, genetic improvement is largely due to intense screening of highly productive animals and targeted selection.

A screening system may significantly change the basic constants that characterize the genetic status of the population, and, consequently, affect efficiency of further breeding. With that, acceleration of the genetic progress is achieved by using intralinear breeds and crosses of lines [4].

In animal breeding, breeding in lines and families is very important. This allows concentrating valuable hereditary qualities of the breed. Advantages of a breed are accumulated in

lines and families that are in structure, and ensure possibilities for improving the breed.

New lines are constantly formed, and old lines disappear in a breed. However, life duration of a line depends on the level of hereditary capacity of line founders and successors, and on efficiency of breeding work with this line.

Due to accumulation of hereditary properties (genotype) of the mother, over time each line loses its genetic similarity with the founder.

In this respect, in order to extend the life of the lines, prevent their "departure into ewes" and preserve the valuable properties, new breeding methods are to be used [5].

MATERIALS AND METHODS

The subject of the research was a sample population of cows of the Auliekol breed (n=284). Samples of the biological material and information about animals' productivity had been provided by LLC Karkyn, Republic of Kazakhstan.

The subjects of study were polymorphic genes of the somatotropic cascade: bPit-1, bGH, bGHR and bIGF-1. Meat productivity of animals with various genotypes and their pairwise combinations was assessed based on live weight at the age of 18 and 24 months.

Genotypes of the animals were determined by the PCR-RFLP method. Primers' sequences and PCR conditions for analysis of each polymorphism are shown in Table 1.

Analysis of the polymorphism of the lengths of restriction fragments included processing the amplificant with site-specific restrictase, followed by separating the obtained fragments with gel electrophoresis.

Analysis of HinFI of bPit-1 gene in exon 6 was performed using the method described by Moody D.E. [6]. The obtained pattern of electrophoresis is shown in Figure 1.

Analysis of polymorphism of the nucleotide sequence of bGH gene in exon 5 was performed according to Pawar R.S. [7] (Figure 2).

Analysis of polymorphism of the nucleotide sequence of bGHR gene in exon 08 was performed according to Skinkytė R. [8] (Figure 3).

Polymorphism of the nucleotide sequence of the insulin-like growth factor gene-1 bIGF-1 in the area P1 of the promotor region was identified according to Hines H.C [10] (Figure 4).

For all analyzed genes, genotype of the animal was documented and populated into a common database.

THE MATHEMATICAL MODEL OF THE EXPERIMENT

Assessment of polymorphisms of the genes of bPit-1-HinFI, bGH-AluI, bGHR-SspI, and bIGF-1-SnaBI somatotrophic cascade as genetic markers of meat productivity in Auliekol cows was performed in two directions.

The first one shows the traditional approach, which involves identification of preferred and alternative genotypes by comparing productivity in corresponding groups of animals.

The second approach has been proposed by Belorussian colleagues – in addition to the traditional approach, it involves comparing productivity in groups of animals with preferred and undesirable genotypes relative to the overall sample population, and assessment of the significance of observed differences [10]. Such additional analysis allows assessing appropriateness of selecting animals by the preferred genotype, or elimination of animals with an alternative genotype.

Quantitative traits were assessed with the use of the methods of nonparametric statistics.

Statistical assessment of the differences among the groups with 3 possible genotypes was performed using the Kruskal-Wallis ANOVA method for 3 and more independent groups. When the number of animals in a group with a rare genotype was less than 6, such a group was excluded from the statistical processing, and comparison was made with the use of the Mann-Whitney U-test for two independent groups. In all cases, the differences were considered statistically veracious when significance level P was less than 0.05 [11].

For the polymorphisms where the differences between the preferred and alternative genotypes were statistically veracious, and for the groups with pairwise combinations of genotypes, assessment of productivity was made in relation to the overall sample population by building a 95% confidence interval for the median of the analyzed group, followed by comparing it to the median of the overall sample population. This method allows assessing the differences between the group that is a part of the sample population and the sample population itself, the data are shown as a median of the lower and the upper limits of the 95% confidence interval. If the limits of confidence interval do not overlap, a conclusion is made that the analyzed group is veraciously different from the population. The data are analyzed and presented and discussed in the form of Me, [CI1; CI2] (25%; 75%).

The sequence numbers of sample population values, which are the lower (L) and the upper (U) limits, were determined by formulas 7 and 8:

$$L = n/2 - (Z_{1-\alpha} * \sqrt{n}/2) \quad (7)$$

$$U = 1 + n/2 + (Z_{1-\alpha} * \sqrt{n}/2) \quad (8)$$

where Z was the value of normal distribution for the selected probability. For the confidence probability of 95% Z = 1.96 [10];

n was the sample size.

The results were processed in Microsoft Excel 2010 and Statistica 6.0 (StatSoft, Inc. 1994 – 2001). Modules Basic Statistic/tables, Nonparametric Statistics were required [12].

RESULTS AND DISCUSSION

Table 2 shows the live weight of Auliekol calves with bPit-1-HinFI^{AA}, bPit-1-HinFI^{AB} and bPit-1-HinFI^{BB} genotypes by polymorphism of the gene of hypophysial growth factor-1 at the age of 18 and 24 months. It also shows the results of statistical assessment of the significance of the observed differences among these groups of animals.

The data in Table 2 show that in all ages, veraciously higher weight was characteristic of cows with bPit-1-HinFI^{AA} genotype compared to cows with bPit-1-HinFI^{AB} and bPit-1-HinFI^{BB} genotypes. Therefore, the bPit-1-HinFI^{AA} rarer genotype is preferred.

According to the data in the table for the age of 18 and 24 months, diagrams have been built that characterize the position of the groups relative to the total sample, Figures 5a and 5b.

As Figure 5a shows, at the age of 18 months, veracious difference was observed between the preferred bPit-1-HinFI^{AA} genotype and the alternative bPit-1-HinFI^{AB} one. The live weight of cows with bPit-1-HinFI^{AA} genotype was also statistically veraciously different from the total sample. In particular, the confidence interval of the median of the total sample was localized within 368-375 kg, while of bPit-1-HinFI^{AA} - within 375-411 kg.

As Figure 5b shows, at the age of 24 months, the difference between the animals with bPit-1-HinFI^{AA} genotype and bPit-1-HinFI^{AB} genotype was also preserved. The limits of confidence intervals for the group of cows with bPit-1-HinFI^{AA} and bPit-1-HinFI^{AB} genotypes were localized within 420-482 kg and 395-419 kg, respectively, and did not overlap. However, in relation to the total sample, the confidence intervals for the medians did overlap: 405-423 kg and 420-482 kg, respectively, for the total sample and the group with bPit-1-HinFI^{AA} genotype. Therefore, productivity of animals with the preferred genotype was within the limits of the total sample, and it was unreasonable to select the preferred genotype at the age of 24 months [13].

Table 3 shows the live weight of Auliekol calves with bGH-AluI^{LL}, bGH-AluI^{LV}, and bGH-AluI^{VV} genotypes in terms of polymorphism of the growth hormone gene and with bGHR-SspI^{FF}, bGHR-SspI^{FY}, and bGHR-SspI^{YY} genotypes according to the polymorphism of hormone receptor growth gene at the age of 18 and 24 months. It also shows the results of statistical assessment of the significance of the observed differences between these groups of animals.

The data in Table 3 show that there was a tendency to increasing the live weight in calves with bGH-AluI^{LV} genotype at the age of 12 and 18 months, and to lower weight at the age of 18 and 24 months in heterozygous calves with bGHR-SspI^{FY} genotype. However, these observations were not statistically veracious, therefore, assessment of the phenotypical effect of these polymorphisms in relation to the total sample was not made, either.

Table 4 shows the results of assessing association of SnaBI polymorphism of the insulin-like growth factor gene-1 with calves' weight at the age of 18 and 24 months.

The data in Table 4 show that at the age of 18 and 24 months there was a statistically veracious difference among the groups of cows with bIGF-1-SnaBI^{AA}, bIGF-1-SnaBI^{AB}, and bIGF-1-SnaBI^{BB} genotypes. In all age categories, the heterozygous bIGF-1-SnaBI^{AB} genotype was preferred, and the alternative one was homozygous bIGF-1-SnaBI^{BB} genotype.

The results of interval assessment of the association nature of bIGF-1-SnaBI^{AA}, bIGF-1-SnaBI^{AB}, and bIGF-1-SnaBI^{BB} genotypes relative to the productivity of the total sample are shown in diagrams in Figures 6 a, b.

The diagrams in Figure 6 show that veracious difference from the sample was observed at the age of 18 months not only for the preferred bIGF-1-SnaBI^{AA} genotype, but also for bIGF-1-

SnaBI^{BB} genotype, which was characterized by lower live weight of calves in all ages. In this case, the SnaBI polymorphism of the insulin-like growth rate gene-1 was associated with decreased productivity of the Auliekol cows, rather than with increased productivity in terms of the live weight at the age of 12 months.

Thus, the work with this genetic marker should be aimed not at selection by the preferred genetic marker, but at elimination of negative bIGF-1-SnaBI^{BB} genotype.

Table 1 – PCR modes for studied polymorphic loci of the genes of somatotrophic cascade

Polymorphism	Conditions of amplification	Primer sequences	References
bPit-1-HinI	94°C – 1 min; (95°C – 45 sec; 56°C – 45 sec; 72°C – 45 sec) x 35 cycles; 72°C – 1 min	HinFI-F: 5'-aaaccatcatctccctctt-3'	[6]
		HinFI-R: 5'-aatgtacaatgtctctgag-3'	
bGH-AluI	95°C – 5 min; (95°C – 30 sec; 64°C – 30 sec; 72°C – 1 sec) x 35 cycles; 72°C – 1 min	AluI –F: 5'-ccgtgtctatgagaagc-3'	[7]
		AluI-R: 5'-gtttctgagcagcgct-3'	
bGHR-SspI	95°C – 5 min; (95°C – 30 sec; 62°C – 30 sec; 72°C – 30 sec) x 35 cycles; 72°C – 1 min	SspI-F: 5'- aatactgggctagcagtgacaatat -3'	[8]
		SspI-R: 5'- acgttctactgggtgatga -3'	
bIGF-1-SnaBI	95°C – 5 min; (95°C – 30 sec; 64°C – 30 sec; 72°C – 30 sec) x 35 cycles; 72°C – 10 min	SnaBI-F: 5'-attcaagctgcctcctccc-3'	[9]
		SnaBI-R: 5'-acacgtatgaaaggaact-3'	

Table 2 – Nonparametric characteristics of live weight in groups of cows of the Auliekol breed with different genotypes in terms of bPit-1-HinFI polymorphism (Me, [CI1; CI2] (25%; 75%))

Age	Genotype	n	Me	95% confidence interval for the median		Interquartile range		p*
				CI 1	CI 2	25%	75%	
18 months.	bPit-1-HinFI ^{AA}	29	386	375	411	370	419	0.003
	bPit-1-HinFI ^{AB}	103	374	368	378	329	393	
	bPit-1-HinFI ^{BB}	103	368	354	372	329	387	
	Total sample	237	373	368	375	329	395	
24 months	bPit-1-HinFI ^{AA}	27	447	420	482	403	483	0.002
	bPit-1-HinFI ^{AB}	100	411	403	423	382	436	
	bPit-1-HinFI ^{BB}	101	405	395	419	377	437	
	Total sample	230	414	405	423	381	453	

a) groups were compared using the Kruskal-Wallis test (for 3 independent groups). The difference between the groups was veracious when P < α; α=0.05.

Table 3 – Nonparametric characteristics of live weight in groups of cows of the Auliekol breed with different genotypes in terms of bGH-AluI and bGHR-SspI polymorphism (Me, [CI1; CI2] (25%; 75%))

Age	Genotype	n	Me	95% confidence interval for the median		Interquartile range		P
				CI 1	CI 2	25%	75%	
18 months	bGH-AluI ^{LL}	98	371	365	378	343	387	0.74*
	bGH-AluI ^{LV}	110	375	368	378	327	402	
	bGH-AluI ^{VV}	28	371	331	393	329	396	
18 months	bGHR-SspI ^{FF}	219	373	368	378	329	397	0.84**
	bGHR-SspI ^{FY}	13	368	329	425	331	393	
	bGHR-SspI ^{YY}	3	384	284	401	284	401	
18 months	Total sample	237	373	368	375	329	395	
24 months	bGH-AluI ^{LL}	95	416	402	429	381	456	0.41*
	bGH-AluI ^{LV}	106	411	402	425	381	455	
	bGH-AluI ^{VV}	28	417	389	428	384	430	
24 months	bGHR-SspI ^{FF}	213	414	405	425	382	453	0.46**
	bGHR-SspI ^{FY}	12	396	365	447	373	431	
	bGHR-SspI ^{YY}	3	432	329	457	329	457	
24 months	Total sample	230	414	405	423	381	453	

a) groups were compared using the Kruskal-Wallis test (for 3 independent groups). The difference between the groups was veracious when P < α; α=0.05.

b) the groups were compared using the Mann Whitney U test (for 2 independent groups). The difference between the groups was veracious when P < α; α=0.05.

Table 4 – Nonparametric characteristics of live weight in groups of cows of the Auliekol breed with different genotypes in terms of bIGF-1-SnaBI polymorphism (Me, [CI1; CI2] (25%; 75%))

Age	Genotype	n	Me	95% confidence interval for the median		Interquartile range		p*
				CI 1	CI 2	25%	75%	
18 months	bIGF-1-SnaBI ^{AA}	45	372	358	386	327	402	0.009
	bIGF-1-SnaBI ^{AB}	100	377	372	382	362	395	
	bIGF-1-SnaBI ^{BB}	39	344	326	367	321	380	
	Total sample	237	373	368	375	329	395	
24 months	bIGF-1-SnaBI ^{AA}	45	414	397	447	376	462	0.005
	bIGF-1-SnaBI ^{AB}	100	423	414	429	397	454	
	bIGF-1-SnaBI ^{BB}	39	383	376	411	365	428	
	Total sample	230	414	405	423	381	453	

a) groups were compared using the Kruskal-Wallis test (for 3 independent groups). The difference between the groups was veracious when P < α; α=0.05.

Table 5 – Pairwise combinations of genotypes associated with weight at the ages of 12 to 24 months in Auliekol cows

Diplotype structure	Number of animals	Me	95% confidence interval		Interquartile range		
			CI 1	CI 2	25%	75%	
Weight at the age of 18 months							
bGH-AluI ^{LL} -bIGF-1-SnaBI ^{AA}	21	327	305	358	305	358	
bGH-AluI ^{LL} -bIGF-1-SnaBI ^{AB}	35	352	343	365	329	368	
bGH-AluI ^{LL} -bIGF-1-SnaBI ^{BB}	27	329	313	346	305	364	
bGH-AluI ^{LV} -bIGF-1-SnaBI ^{AA}	23	402	379	427	375	428	
bGH-AluI ^{LV} -bIGF-1-SnaBI ^{AB}	55	386	379	407	378	421	
bGH-AluI ^{LV} -bIGF-1-SnaBI ^{BB}	12	407	382	435	383	431	
bPit-1-HinFI ^{AB} -bIGF-1-SnaBI ^{AB}	36	378	377	382	377	385	
bPit-1-HinFI ^{AB} -bIGF-1-SnaBI ^{AA}	12	378	361	417	367	397	
Total sample	237	373	368	375	329	395	
Weight at the age of 24 months							
bGH-AluI ^{LL} -bIGF-1-SnaBI ^{AA}	21	376	361	397	361	397	
bGH-AluI ^{LL} -bIGF-1-SnaBI ^{AB}	35	389	383	402	381	404	
bGH-AluI ^{LL} -bIGF-1-SnaBI ^{BB}	27	379	365	383	346	399	
bGH-AluI ^{LV} -bIGF-1-SnaBI ^{AA}	23	462	429	487	423	489	
bGH-AluI ^{LV} -bIGF-1-SnaBI ^{AB}	53	436	429	459	427	477	
bGH-AluI ^{LV} -bIGF-1-SnaBI ^{BB}	11	467	428	513	432	488	
bPit-1-HinFI ^{AB} -bIGF-1-SnaBI ^{AB}	36	428	427	435	423	436	
Total sample	230	414	405	423	381	453	

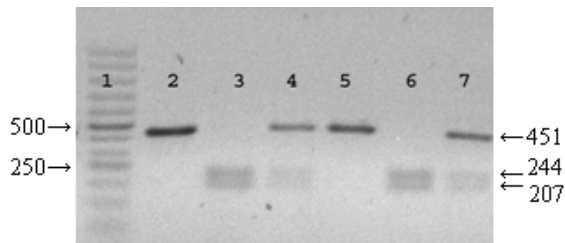


Figure 1 – Electrophoregram of DNA fingerprinting of bPit-1-HinFI polymorphism.

- a) Track 1 – molecular mass marker O’RangeRuler™ 50 bps DNA Ladder, Fermentas, Lithuania;
- b) track 2 – PCR product of 451 bps of bPit-1-HinFI gene fragment;
- c) track 3, 6 – restriction fragments of 244, 207 bps corresponding to bPit-1-HinFI^{BB} genotype;
- d) track 4, 7 – restriction fragments of 451, 244, 207 bps corresponding to bPit-1-HinFI^{AB} genotype;
- e) track 5 – restriction fragment of 451 bps corresponding to bPit-1-HinFI^{AA} genotype. Locations of specific bands on the gel are shown by arrows. Electrophoresis was performed in 2% agarose gel (Sea Kem LE Agarose, Lonza, USA)

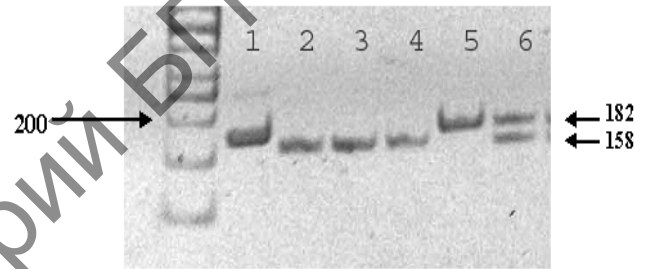


Figure 3 – Electrophoregram of DNA fingerprinting of bGHR-SspI polymorphism.

- a) Track 1 – PCR product of 182 bps of bGHR-SspI gene fragment;
- b) tracks 2, 3, 4 – restriction fragment of 158 bps corresponding to bGHR-SspI^{FF} genotype;
- c) track 5 – restriction fragment of 182 bps corresponding to bGHR-SspI^{YY} genotype;
- d) track 6 – restriction fragments of 182 and 158 bps corresponding to bGHR-SspI^{FY} genotype. Fragment of 24 bps is not visualized. Molecular mass marker O’RangeRuler™ 50 bp DNA Ladder, Fermentas, Lithuania, was used. Locations of specific bands on the gel are shown by arrows. Electrophoresis was performed in 2% agarose gel (Sea Kem LE Agarose, Lonza, USA)



Figure 2 – Electrophoregram of DNA fingerprinting of bGH-AluI polymorphism.

- a) Track 1 – molecular mass marker O’Range Ruler™ 50 bps DNA Ladder, Fermentas, Lithuania;
- b) tracks 2, 6 – restriction fragments of 208, 172, 35 bps corresponding to bGH-AluI^{LV} genotype;
- c) tracks 3, 4, 7 – restriction fragment of 172 bps corresponding to bGH-AluI^{LL} genotype;
- d) track 5 – restriction fragment of 208 bps corresponding to bGH-AluI^{VV} genotype;
- e) track 9 – PCR product of 208 bps of bGH-AluI gene fragment. Restriction fragment of 35 bps is not visualized. Locations of specific bands on the gel are shown by arrows. Electrophoresis was performed in 2% agarose gel (Sea Kem LE Agarose, Lonza, USA)

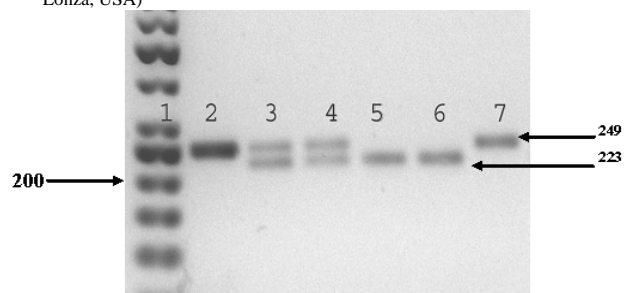


Figure 4 – Electrophoregram of DNA fingerprinting of bIGF-1-SnaBI polymorphism.

- a) Track 1 – molecular mass marker O’RangeRuler™50 bp DNA Ladder, Fermentas, Lithuania;
- b) track 2 – PCR product of 249 bps of bIGF-1-SnaBI gene fragment;
- c) tracks 3, 4 – restriction fragments of 249 and 223 bps corresponding to bIGF-1-SnaBI^{AV} genotype;
- d) tracks 5, 6 – restriction fragment of 223 bps corresponding to bIGF-1-SnaBI^{AA} genotype;
- e) track 7 – restriction fragment of 249 bp corresponding to bIGF-1-SnaBI^{BB} genotype. Fragment of 26 bps is not visualized. Locations of specific bands on the gel are shown by arrows. Electrophoresis was performed in 2% agarose gel (Sea Kem LE Agarose, Lonza, USA)

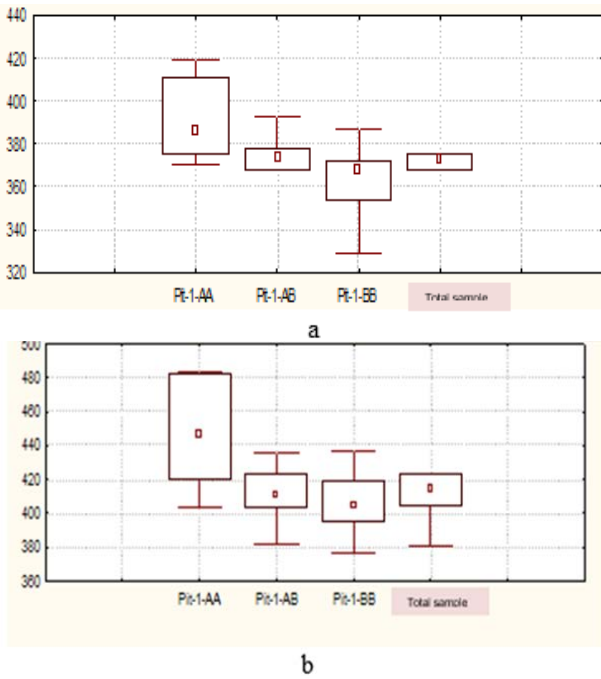


Figure 5 a, b - Live weight of Auliekol cows at the age of 18 and 24 months; bPit-1-HinFI polymorphism

The authors have created 54 possible pairwise compositions of polymorph genes of the somatotropic cascade.

By the result of DNA fingerprinting, animals with the appropriate pairwise genotype (diplotype) had been united into groups for analyzing their productivity relative to the total sample. Analysis of pairwise combinations involved genotypes regardless of whether there had been a separately identified weight association for them.

Table 5 shows nonparametric characteristics of the diplotypes associated with increased or decreased live weight, compared to the total sample, at the age of 6 to 24 months.

Table 5 demonstrated that Auliekol cows showed additional genetic markers among pairwise combinations of genotypes.

Thus, as a result of analyzing the live weight of Auliekol calves at the age of 18 and 24 months by 4

polymorphisms of genes of the somatotropic cascade (bPit-1, bGH, bGHR, bIGF-1), statistically veracious difference of a single genotype from of the total sample was detected only for bPit-1-HinFI and bIGF-1-SnaBI polymorphisms. In the first case, bPit-1-HinFI^{AA} genotype veraciously exceeded the live weight of the total sample at the age of 18 months; in the second case the animals with bIGF-1-SnaBI^{BB} genotype had veraciously lower weight compared to the total sample. In the pairwise combinations of genotypes, veracious phenotypical effects in relation to the total sample were identified in all ages, as well as the dyplotypes associated with both increased and decreased productivity compared to the total sample.

Figures 7 a and b show diagrams that allow assessing the nature (decreasing or increasing) and the strength of the phenotypic effect of live weight genetic markers in Auliekol cows at the age of 18 and 24 months.

The data in Figures 7 a, b show that at both ages, veraciously decreased live weight, compared to the total sample, was characteristic of bGH-AluI^{LL}-bIGF-1-SnaBI^{AA}, bGH-AluI^{LL}-bIGF-1-SnaBI^{AB}, and bGH-AluI^{LL}-bIGF-1-SnaBI^{BB} dyplotypes, the structure of which included bGH-AluI^{LL} genotype and 3 possible genotypes of bIGF-1 gene. This allowed suggesting that the L-allele of the growth hormone gene was, after all, associated with decreased weight of Auliekol cows. And while it itself was not the direct reason for the effect, it was possibly bonded to this part of the genome. At the same time, bGH-AluI^{LV} genotype was included into diplotypes with the gene of insulin-like growth factor 1. bGH-AluI^{LV} genotype was not found in the composition of diplotypes with increased weight at the age of 18 and 24 months, which was explained by the rarity of this genotype.

It should also be noted that bIGF-1-SnaBI^{AB} genotype, which separately showed association with decreased weight of calves, was found in the structure of diplotypes with both decreased and increased weight depending on the effect of the adjacent genotype in the pair.

bPit-1-HinFI^{AA} genotype associated with increased live weight had not been found in the composition of significantly weight-reducing or weight-increasing diplotypes. This was probably due to low occurrence rate of this genotype in the population.

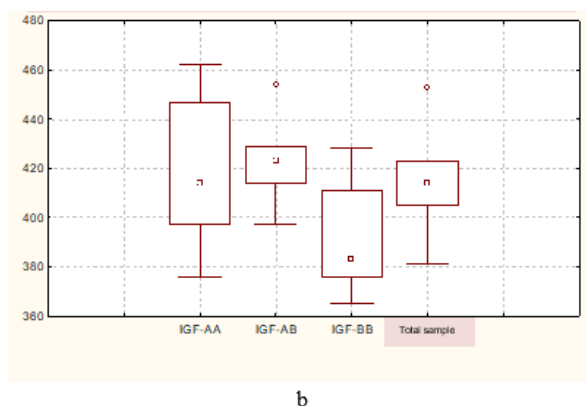
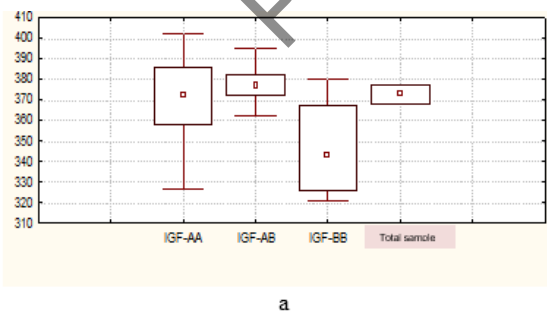


Figure 6 a, b - Live weight (kg) in Auliekol cows at the age of 18, 24 months; bIGF-1 polymorphism

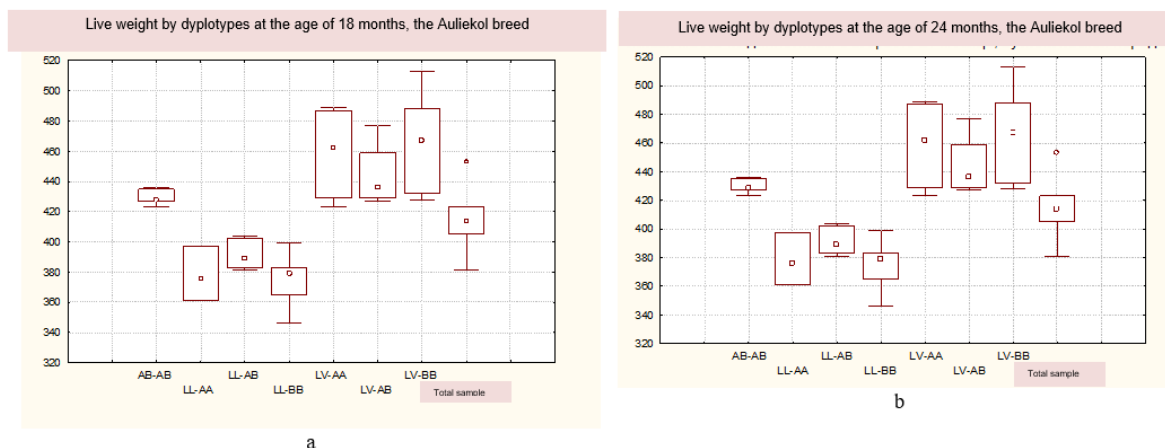


Figure 7 a, b – Diplotypes associated with increased and decreased live weight of Auliekol calves at the age of 18 and 24 months

CONCLUSIONS

Thus, the results of the research show the following:

– Diplotypes associated with increased or decreased productivity preserve their dynamics from age to age; in case of analyzing separate polymorphisms, the nature of the association is less stable and may disappear or even change at various ages. This observation allows suggesting that assessing the phenotypic effect by pairwise combinations is not only more efficient, but also more reliable.

– The genetic markers that are diplotypes often have more pronounced phenotypic effect than individual marker genotypes. For example, while the range of live weight at the age of 12 months for IGF-1^{BB} genotype was 325-331 kg, its pairwise combination with bGH-AluI^{LL} genotype potentiated this effect to 278-306 kg.

– Genotypes that are individually associated with meat productivity (but even homo- and heterozygotes are not different from each other) in pairwise combinations may have increased or decreased statistically significant phenotypic effect, compared to the total sample. Such combinations may be applied as genetic markers of productivity in breeding programs. An example is bGH-AluI polymorphism. Inversely, polymorphisms that individually show association with a symptom of productivity may be within the total sample in pairwise combination by the phenotypical effect.

– Analysis of pairwise combinations allows detecting more genetic markers, which allows expanding the range of animals that are carriers of the marker genotype for participation in breeding programs.

– In analyzing pairwise combinations, a larger set of traits is marked, which allows breeder-geneticist to more fully appreciate the genetic potential of the animal.

REFERENCES

- [1] Tsiulina, E.N., *Hozyaystvenno - poleznie priznaki korov cherno - pyostroi i golshinskoi porod v usloviyah yuzhnogo Urala* [Economically useful traits in Holstein and motley cows in the conditions of southern Urals], Troitsk 2009.
- [2] Kutrovsky, V., Ivanova, N., Fetisova, M., *Effektivnost podbora bikov dlya uluchsheniya molochnogo skota* [Efficiency selecting bulls for improving dairy cattle], *Zootekhnika* 2008, 2S, 16.
- [3] Bakay, A.I., *Vosproizvoditelnie kachestva golshinizirovannih korov s raznim urovнем karyotipicheskoi nestabilnosti* [Reproductive qualities of holsteinized cows with various levels of karyotypic instability], Moscow State Academy n.a. K.I. Skryabin, Moscow 2009.
- [4] Voronina, E., Strekozov, N., Abramplansky, F., *Vliyanie podbora korov na ih molochnyuyu produktivnost* [The effect of cows selection on their milk productivity], *Dairy and Beef Cattle Breeding* 2007, 4, 8.
- [5] Beyshova, I., Kikebayev, N., Kokanov, S., Chuzhebayeva, G., Nametov, A., *Genetic polymorphism of 17 microsatellite DNA loci in main lines of Kostanay breed horses*, *Life Science Journal* 2013, 10(10s), 85-91.
- [6] Moody, D.E., Pomp, D., Barendse, W., *Restriction fragment length polymorphism in amplification products of the bovine Pit-1 gene and assignment of Pit-1 to bovine chromosome 1*, *Animal Genetics* 1995, 26, 45-47.
- [7] Pawar, R.S., Joshi, C.G., Rank, D.N., *Growth hormone gene polymorphism and its association with lactation yield in dairy cattle*, *Indian Journal of Animal Science* 2007, 9, 884-888.
- [8] Skinkytė, R., Zwierzchowski, L., Riaubaitė, L., Baltėnaitė, L., Miceikienė, I., *Distribution of allele frequencies important to milk production traits in lithuanian black & white and lithuanian red cattle*, *Veterinarija ir Zootekhnika* 2005, 31(53), 93-97.
- [9] Belaya, E.V., Mikhailova, M.E., Batin, N.V., *Assessing the individual phenotypic effect of polymorphic variants of the genes of hypophysial growth factor-1 [bPit-1] and insulin-like growth factor-1 [bIGF-1] on milk productivity in motley Holsteinized cattle*, *Molecular and Applied Genetics* 2012, 13, 30-35.
- [10] Hines, H.C., Ge, W., Zhao, Q., Davis, M.E., *Association of genetic markers in growth hormone and insulin-like growth factor I loci with lactation traits in Holsteins*, *Animal Genetics* 1998, 29, 69.
- [11] Rebrova, O.Y., *Statisticheskiy analiz meditsinskih danyh. Primenenie paketa prikladnih programm STATISTICA* [Statistical analysis of medical data. The use of the STATISTICA software suite], MediaSphera, Moscow 2002.
- [12] Batin, N.V., *Kompyuternii statisticheskiy analiz danyh* [Computer analysis of statistical data], Institute of scientific personnel training of the Nat. Acad. of Sciences of Belarus, Minsk 2008.
- [13] Basova, I.S., Terletsky, V.P., Tyschenko, V.I., Belaya, E.V., Poddudinskaya, T.V., *Vliyanie allelei polimorfnyh genov bPIT-1, bGH i bGHR na pokazateli rosta u krupnogo rogatogo skota auliekolskoi porodi* [The influence of polymorphic alleles of bPIT-1, bGH and bGHR genes on the growth of Auliekol cattle], *Advances of Modern Natural Sciences* 2017, 4.