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Evaluation of the Phenotypic Effect of Polymorphic Variants of Kappa-Casein Gene on Milk Productivity in Belarusian Black-and-White Cattle Breed

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Summary. Productivity of cattle with different genotypes of kappa-casein gene was evaluated. Prerferable and undesirable genotypes were revealed by comparing among cow groups. Productivity evaluation of the groups with different genotypes with respect to the total sample has shown that the genotype BB can be used as a genetic marker.

Key words: Belarusian black-and-white cattle, kappa-casein.

Modern DNA-technologies, such as marker-assisted selection, can greatly accelerate breeding rate and reduce financial costs for implementaion of classical breeding activities. However animal selection with preferable genotypes associated with milk productivity of cattle does not always bring such a quick and significant result as would be expected. Many authors pay attention to the necessity to improve methods for evaluation of phenotypic effects of genetic markers [1-3]. Therefore development of methods for estimating the association of polymorphic variants of candidate genes with quantitative traits of productivity is of particular interest.

At present studies on the association of polymorphic candidate genes with cattle milk productivity traits are reduced primarily to revealing a preferable allele and a genotype by comparing productivity indices among animals with various genotypes [2, 4-5]. This approach allows us to identify genotypes showing an increased and decreased productivity levels for the trait under study, however it does not represent the degree of their superiority in productivity traits over all

investigated livestock. Therefore we have suggested that the conventional comparative analysis of genotypes should be accompanied by evaluating a phenotypic effect of preferable and undesirable genotypes with respect to the productivity level of the total sample [6, 7]. This made it possible to compare and quantify the degree of manifestation of raising effects for preferable genotypes and lowering ones for undesirable genotypes. During investigations of polymorphic candidate genes belonging to the group of somatotropin cascade, we have previously revealed that the productivity of animals with a preferable and undesirable genotype can be located within the average values in the total sample. There were also cases when the productivity of a preferable genotype was located within the average values in the sample and an undesirable genotype was significantly associated with the trait. In such cases selection of animals with a preferable genotype will be ineffective [8, 9].

At present a great number of potential candidate genes, polymorphic variants of which are associated with cattle milk productivity, were identified and kappa-casein gene (*bCSN3*) is of great interest for breeding of Belarusian cattle livestock. Casein is the main milk protein presented in several forms — α, β, γ and others. As of today seven alleles of gene *bCSN3* are described: A, B, C, D, E, F, G, H. A and B allelic variants of kappa-casein are most frequently encountered in cattle. They differ in two amino acid substitutions in 136 *и* 148 positions of the polypeptide chain caused by corresponding point mutations in positions 5309 (C→T) and 5345 (A→C) [10]. The allele B of kappa-casein is shown to correlate positively to a higher content of the total milk protein, an increased kappa-casein content, as well as to the best cheese-making characteristics of milk. Therefore the allele B of kappa-casein gene was suggested to be used as a genetic marker of cattle milk productivity [10, 11].

The task of the present study was to estimate the association of polymorphic variants of kappa-casein gene with milk yield and protein content by comparing genotypes and with respect to the total sample**.**

The object of research was bull producing cows from Nesvizh branch of RUSE "Minsk Plempredpriyatie" of Belarusian Black-and-White breed with a conditional heritability proportion for Holstein breed to 69,1% (n = 197). Information on animal productivity was taken from tribal cards.

Research methods. Genomic DNA was isdolated from cow blood using the Diatom^{TM} Prep^{200} kit (Laboratory Isogen, Moscow), in compliance with the instructions of the manufacturer. The genotype was identified by the PCR-RFLP method.

Primers Bocas A (5´- atg tgc tga gca ggt atc cta gtt atg g -3) and Bocas B (5´- cca aaa gta gag tgc aac aac act gg - 3´) were used for genotyping of animals under study by kappa-casein gene locus. PCR mode**:** 94^0 - 1 min; (95^0- 45 c; 56^0-60 c; 72^0 - 60 c) x 35; 72^0-10 min. Amplified fragment length 883 bp [4].

Upon restrictase Pst1-treatment in 2% agarose gel the fragments of 471, 306 and 106 bp in length, corresponding to the AA genotype; those of 777, 471, 306 and 106 bp, corresponding to the AB genotype and 777, 106 bp, corresponding to the BB genotype, were visualized by staining with ethidium bromide.

Results. The study on the genetic structure of the analysed population included the analysis of the compliance of genotype frequency distribution with theoretically expected one according to the Hardy-Weinberg law and evaluation of the significance of the observed deviations using the criterion χ^2. The results are shown in Table 1.

Table 1 – Relative Frequencies of Alleles and Genotypes of Kappa-Casein Gene

Allele frequencies	Genotype frequencies*

A	$\pm S_Q$	B	$\pm S_Q$	AA		AB		BB		χ^2
				n_H	n_O	n_H	n_O	n_H	n_O	
0,835	0,002	0,165	0,002	137	137	55	54	5	6	0,005

* n_H - observed genotype frequency; n_O - expected genotype frequency

As seen from Table 1, the observed genotype frequencies are consistent with the expected ones according to the Hardy-Weinberg law. . Hence, an artificial selection was not directed at an increase of the allele B frequency in the population.

The average values of milk yield and milk protein content of animals with various genotypes are given in Table 2 which shows that the group of cows with the genotype BB is a leader for both milk yield and milk protein content.

Table 2 – Productivity of Bull-Producing Cows with Various Genotypes of Kappa-Casein Gene ($Q \pm S_Q$), (n=197).

Trait	Average value of the sample	Genotype		
		AA	AB	BB
Milk yield (L. for 305 days)	9667±303	9717±91	9401±150	11232±732
Milk protein content (kg for 305 days)	302,93±2,12	302,37±2,45	300,69±4,10	343,00±15,00

Statistical evaluation of the difference among the groups with various genotypes as well as comparison of the animal productivity with a certain

genotype with respect to the total sample was carried out using single-factor analysis of variance. If t-statistic is higher than t critical two-way one (and P(T<=t)), two-way value is less than the specified significance level α), we can assume that the compared groups differ significantly in the average value of the trait. The results of milk yield analysis are shown in Table 3.

Table 3 – Statistical Evaluation of the Difference in the Average Values of Milk Yield among the Groups with Various Genotypes and in Relation to the Total Sample

Statistical values	Comparison groups					
	AA-AB	AA-BB*	AB-BB*	AA-выборка	AB-выборка	BB-выборка*
t-statistic	1,82	3,04	3,37	0,40	-1,54	3,02
P(T<=t) two-way	0,07	0,00	0,00	0,68	0,12	0,00
t critical two-way	1,97	1,97	2,00	1,96	1,97	1,97

*-The difference in the average values in these groups is statistically significant.

The data given in Table 3 indicate that animals with the genotype BB differ significantly from animals with the genotype AA (milk yield is higher than the average value in the sample) and from the group with the genotype AB (milk yield is lower than the average value in the sample). This suggests that the genotype BB is preferable, and the genotype AB – undesirable when selecting for this trait. However comparison of the groups with the genotypes BB, AA and AB with respect to the average value in the sample reveals a significant difference only in

the genotype BB. It means that milk yield in the groups with the genotypes AA and AB is within the average values in the sample. Hence these genotypes are neither preferable nor desirable for the breeding process.

The results of protein content analysis are shown in Table 4. The data given in Table 4 show the pattern similar to that observed during analysis of milk yield. As seen from Table 4, the protein content of cows with the genotype BB differs significantly from the groups with the genotypesAA and AB (milk content of which is practically equal to the average value in the sample).

Table 4 – Statistical Evaluation of the Difference in the Average Values of Milk Protein Content for 305 Days among the Groups with Preferable and Undesirable Genotypes and in Relation to the Total Sample

Statistical Values	Comparison groups					
	AA-AB	AA-BB*	AB-BB*	AA-sample	AB- sample	BB- sample *
t-statistic	0,36	3,09	2,95	-0,17	-0,48	2,95
P(T<=t) two-way	0,72	0,00	0,00	0,86	0,62	0,00
t critical two-way	1,97	1,98	2,00	1,96	1,96	1,97

*-The difference in the average values in these groups is statistically significant.

Comparison with the sample confirms that out of three groups differing among themselves only the milk protein content value in the group with the genotype BB differs significantly from the sample. This indicates that only the

genotype BB is a genetic marker of productivity (in this case, higher milk protein content).

Conclusions. Thus, evaluation of the average values of milk yield and milk protein content has revealed statistically significant differences of animal groups with the genotype BB of kappa-casein gene from the groups with the genotypes AA and AB. This has made it possible to designate the genotype BB as preferable and the genotypes AB and AA as undesirable ones for these traits. However comparison of productivity in these groups with the average value in the sample has shown that out of three genotypes only the genotype BB differs significantly in productivity from the sample. Productivity of the genotypes AA and AB is within the normal range of this sample. So, out of three genotypes only one genotype BB may be recommended as a productivity marker for milk yield and milk protein content. In the case of considered polymorphism, this is a preferable genotype associated with increased productivity in the studied group.

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