Scientific Papers Series Management, Economic Engineering in Agriculture and Rural Development
Vol. 25, Issue 1, 2025
PRINT ISSN 2284-7995, E-ISSN 2285-3952

RESEARCH OF COMPLEX MILK PROTEINS GENOTYPES IN THE CONTEXT OF THEIR QUALITY IMPROVEMENT

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Abstract

Recently, scientists increasingly use the achievements of genetics in the practical selection of dairy cattle. Special attention is paid to genes associated with indicators of cows' milk productivity. These include genes for CSN2, CSN3, BLG and others. The CSN2 gene genotype of animals is being studied especially intensively today, which is explained by the assumption of its influence on human health. Two groups of animals derived from local Lebedyn cattle has been studied. The difference in frequencies of complex genotypes CSN2/CSN3/BLG has been found. Brown breeds characterized by a high frequency of complex genotypes CSN2A2A2BLGBB, CSN3BBBLGAB, CSN2A2A2CSN3AB, CSN2 A2A2CSN3BB. Black and white breeds characterized by a high frequency of complex genotypes CSN2A1A2BLGAB, CSN3AABLGAB, CSN2A1A2CSN3AA, CSN2A2A2CSN3AA. According to the distribution of individual loci, no cows with complex genotypes CSN2A1A1BLGAA, CSN2A1A2BLGAA, CSN2A1A1CSN3AB found among brown breeds of animals, among black and white breeds of cows there were no animals with CSN2A1A2BLGBB, CSN2A1A1CSN3AB complex genotypes. A statistically significant difference in terms of milk productivity found between several genotypes. We consider the number of livestock recommended by the FAO for studies of the genetic structure by individual loci does not allow to fully research the genetic structure according to complex genotypes.

Key words**:** *beta-casein, kappa-casein, beta-lactoglobulin, breed, genotype, milk productivity*

INTRODUCTION

Modern cattle breeding widely uses the achievements of genetics [6, 15]. Thanks to its rapid development, breeders have the opportunity to research and use the polymorphism of individual genes in practical breeding. Scientists believe that among all agricultural animals, cattle are the most studied in terms of molecular genetics. This is because these animals have the highest number of productivity markers. Among the large number of genes that have an impact on the milk productivity of cows, scientists single out a certain group of genes that have the greatest impact on this productive trait. Among them, the genes of kappa-casein (CSN3), beta-lactoglobulin (BLG), prolactin, pituitary-specific transcription factor and others can be distinguished [4, 16].

Researchers debate about the possibility of improving the quality characteristics of milk using animal selection based on molecular markers. In this regard, scientists recommend using population parameters (genotype and allele frequencies) as a tool for improving selection. Thus, studies of polymorphism of milk protein genes are increasingly used in individual breeding programs for dairy cattle. The advantage of DNA technologies, according to scientists, can be considered the possibility of determining the genotype of animals regardless of their age, physiological state, and sex. In turn, they make it possible to significantly increase the accuracy and efficiency of breeding, while simultaneously reducing the generation of intervals, which allows to accelerate the effect of breeding [13, 17]. Among the genetic markers associated with the technological properties of milk,

scientists are conducting research on CSN3, BLG and other genes. However, more and more often, scientists do not study individual genes, but complex genotypes. It should be noted that such studies conducted on local dairy cattle are few [14].

The use of any genetic material can also be attributed to the advantages of using molecular genetic markers. It can be blood, urine, pus, semen, wool with hair follicles, and others. Scientists pay attention to the value of the information obtained from the genetic assessment, which consists in the early (even immediately after birth) determination of the animal's genetic potential [12].

In countries with developed dairy farming, molecular genetic methods have been used together with mathematical modeling for a long time. Thanks to this, marker-associated selection has been implemented in leading agricultural enterprises. It has been proven that the identification of certain genes and their mutations, which determine the degree of development of a certain economically useful trait, allows to accelerate breeding progress, and as a result increases the profits of the industry [12]. Genes such as CSN2, CSN3, and BLG considered to be components associated with the technological properties of milk [2].

Therefore, scientists believe that the selection of dairy cattle, focused on the maximum realization of its productive potential, should include molecular genetic research of animals. The absence of the latter will not allow fixation of the "desired" alleles of the corresponding genes [1, 7].

In this context, the study of the genetic structure of complex genotypes of cattle milk proteins is relevant. This will help to improve the productive characteristics of dairy cattle and will allow increasing the quality characteristics of cows' milk. This was the main aim of the work.

MATERIALS AND METHODS

The experiment conducted on the production facilities of the State Enterprise "Experimental Farm of the Institute of Agriculture of the North East of the National Academy of Sciences of Ukraine" in the Sumy oblast. In accordance with the purpose of the work, we used the firstborn of two domestic dairy breeds, respectively Ukrainian brown dairy (UBD) and Ukrainian black and white dairy (SITUBWD) breeds. The population of experimental animals consisted of 30 heads, which met the requirements of the FAO for genetic research [3]. The genotypes of the following genes were determined in experimental animals: kappa-casein (CSN3), beta-casein (CSN2) and beta-lactoglobulin (BLG). Cows were genotyped according to kappa-casein (CSN3), beta-casein genes (CSN2) and beta-lactoglobulin (BLG).

The selection of biological samples and the study of CSN2 and CSN3 gene polymorphisms were carried out according to the method described in our publications [9,10, 11].

Genetic studies conducted on DNA samples taken from hair bulbs of cows. To study the single nucleotide polymorphism of the BLG gene of cattle (chromosome 11, GenBank: X14710.1, exon 4, rs458095482 (Gcc/Ccc, 270A>P) and rs109625649 (gCc/gTc, 270A>V) we used the PCR-RFLP method with specific primers and HaeIII restriction endonuclease. DNA was isolated from hair follicles using the commercial kit "DNK-Sens"). Amplification of BLG gene fragment conducted in thermocycler “Tertsick” (DNA-techologies) using primers: F-5'-TGTGCTGGACACCGACTACAAAAAG-3' IR-5'-CTCCCGGTATATGACCACCCTCT-3" [5].

The PCR mixture (10 ml) contained: 5 μl master mix (10x buffer for DNA polymerase (1 μl), DNA polymerase (Fermentas, Lithuania, 0.25 units), 2.5 mM DNATP (1 μl), deionized H2O (3 μl)), 1 μμ mixture of primers (5 μl) and DNA (5 μl). Temperature regime: initial denaturation — 2.5 min at 94°C, next 38 cycles — 94°C 20 sec., 64°C 30 sec., 72°C 1 min., final elongation at 72°C 7 min. The size of the amplicon is 247 bps. The studied fragment has one monomorphic restriction site for HaeIII (GG↓CC) and one polymorphic one. Expected restriction patterns for genotypes: AA (HaeIII-) – 148/99

bps; BB (HaeIII+) –74/74/99 bps; AB - 148/99/74 bps.

Amplification products were treated with HaeIII endonuclease according to the manufacturer's instructions (Fermentas, Lithuania). The number and length of the restriction products were determined by electrophoresis in a 3% agarose gel (with the addition of 0.5 μg/ml ethidium bromide) in Tris-borate buffer (TBE: 0.089 M Tris, 0.089 M boric acid, 0.002 M EDTA pH 8.0) with using a molecular weight marker (100 bps Ladder, Simgen). Electrophoresis results visualized on a transilluminator in the UV spectrum (312 nm).

The polymorphism of the locus of the pituitary-specific transcription factor PIT 1 (chromosome 1, GenBank: Y15995.1, exon 6, synonymous transition 1256 G>A, ctG>ctA) studied by the PCR-PDRF method using HinfI endonuclease (G↓ANTC restriction site). Primers: Forward: 5'-CAATGAGAAAGTTGGTGC-3'; Reverse: 5'-TCTGCATTCGAGAT GCTC-3') [5].

The conditions for PCR amplification of the PIT 1 gene are as follows: initial denaturation — 2.5 min at 94°C, the next 35 cycles — 94°C 20 sec., 52°C 30 sec., 72°C 1 min., final elongation at 72 °C 7 min. The size of the amplicon is 1301 bp. The studied fragment has two monomorphic and one polymorphic (1256 G>A) restriction sites for HinfI (G↓ANTC). Restriction fragments with a length of 617, 424 and 260 bp. correspond to allele A (NinfI), fragments 617, 379, 260 and 45 bp. indicate the B allele (NinfI+) [5].

The electronic database of Dairy stock management system "Orsek" was used to assess productive characteristics. Indicators of reproductive capacity and milk productivity were assessed. A counter - indicator "*ИУ*-1" was used to take milk samples. The milk sample was stored in a plastic container (25 ml) and preserved with a 0.2 ml solution of potassium dichromate (concentration 10%). The content of milk components was determined in the laboratory of the Sumy National Agrarian University using the Ultrasonic milk analyzer Master Classic.

Statistical data processing was performed using the licensed software STATISTICA 10.0 (StatSoft) for Windows.

RESULTS AND DISCUSSIONS

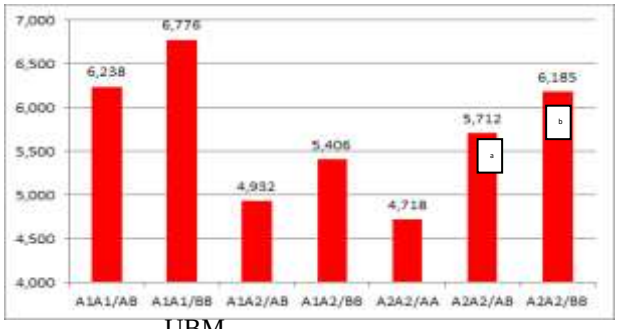
Having analyzed the results of genetic research, we can note that the majority of UBD cows had the A2A2 CSN2 gene genotype (74%). Animals with other genotypes A1A1 and A1A2 accounted for 6% and 20%, respectively. Most of the animals of this breed had the heterozygous AB BLG gene genotype (60%). Animals with other AA and BB genotypes accounted for 9% and 31%, respectively. According to the distribution of these genotypes, the CSN2/BLG complex genotype was represented by seven genotypes out of nine possible ones (Table 1).

Table 1. Frequency distribution of the studied combinations of CSN2 and BLG milk protein genotypes

CSN2	BLG		
	AA	AB	BB
	UBM		
A1A1	0.00	0.03	0.03
A1A2	0.00	0.10	0.10
A2A2	0.09	0.47	0.18
	SITUBWD		
A1A1	0.03	0.18	0.03
A1A2	0.00	0.26	0.10
A2A2	0.13	0.20	0.07

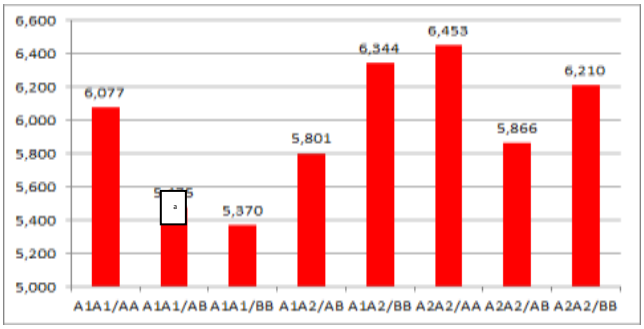
Source: Own research.

Genotypes CSN2A1A1BLGAA and CSN2A1A2BLGAA were absent in UBD breed cows. The highest frequency was the complex genotype CSN2A2A2BLGAB, the share of which was almost half. Among the animals of the SITUBWD, the majority had the CSN2 gene genotype A1A2 and A2A2, the share of which was 36% and 40%, respectively. Most animals of this breed having BLG gene, had the AB genotype, the share of which was 64%. The complex genotype CSN2A1A2BLGAA was not found in SITUBWD cows, and most animals had genotypes CSN2A1A2BLGAB and CSN2A2A2BLGAB, the share of which was 26 and 20%, respectivelyAnimals with different complex genotypes had a statistically significant difference in milk yield (Fig. 1).



UBM

Probability: a – to genotype A2A2/AA (P<0.05); b – to the A2A2/AA genotype (P<0.01)



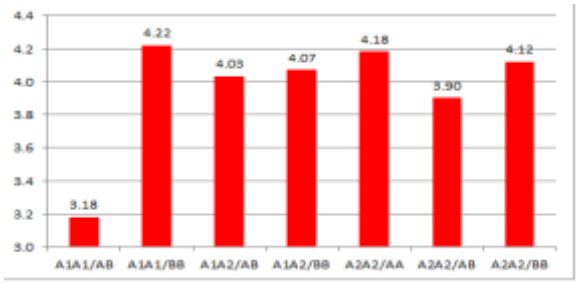
SITUBWD

Probability: a – to genotype A2A2/AA (P<0.01)

Fig. 1. Milk yield of cows with different CSN2-BLG complex genotypes, kg
Source: Own research.

Thus, the first time calving animals of UBD with complex genotypes $CSN2^{A2A2}BLG^{AB}$ and $CSN2^{A2A2}BLG^{BB}$ with different degrees of probability prevailed in terms of average milk yield over cows with complex genotype $CSN2^{A2A2}BLG^{AA}$ by 994 kg and 1467 kg, respectively. Between animals with complex genotypes $CSN2^{A1A1}BLG^{AB}$ and $CSN2^{A2A2}BLG^{AA}$ there is a significant difference in favor of the genotype $CSN2^{A2A2}BLG^{AA}$.

There is no significant difference in the fat content of milk between the animals of the experimental breeds (Fig. 2).



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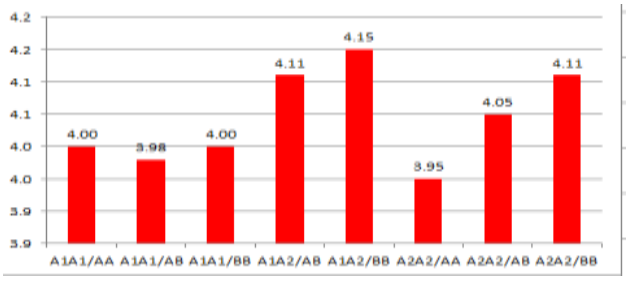
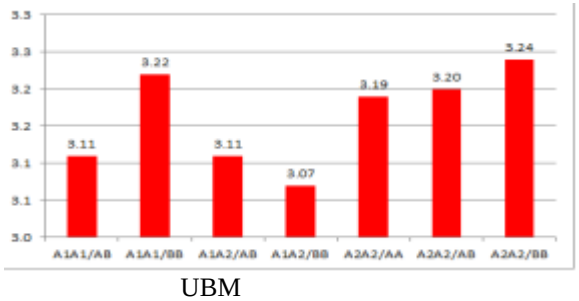


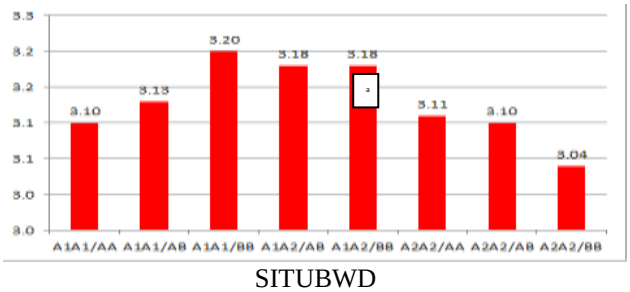
SITUBWD

Fig. 2. Study of fat content in milk in animals with different complex genotypes CSN2-BLG, %
Source: Own research.

There is a likely difference in milk protein content between SITUBWD animals with complex genotypes $CSN2^{A1A2}BLG^{BB}$ and $CSN2^{A2A2}BLG^{BB}$. Animals with the $CSN2^{A2A2}BLG^{BB}$ genotype predominated. There is no significant difference between animals (UBD) (Fig. 3).



UBM



SITUBWD

Probability: a – to genotype A2A2/BB (P<0.01)

Fig. 3. Milk protein content of cows with different complex CSN2-BLG genotypes, %
Source: Own research.

Among the animals of the UBD, having CSN3 gene, the majority had the heterozygous AB genotype, the share of which was 51% (Table 2).

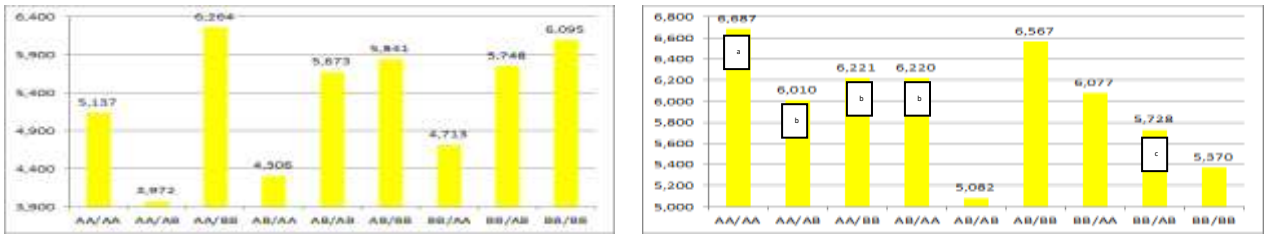
Table 2. Frequency distribution of the studied combinations of CSN3 and BLG milk protein genotypes

CSN3	BLG		
	AA	AB	BB
	UBM		
AA	0.03	0.03	0.07
AB	0.03	0.31	0.17
BB	0.03	0.26	0.07
	SITUBWD		
AA	0.06	0.40	0.14
AB	0.07	0.17	0.03
BB	0.03	0.07	0.03

Source: Own research.

The share of homozygous AA and BB genotypes was 13% and 36%, respectively. Among the first time calving animals of both breeds, all possible nine complex genotypes according to the studied genes were present. Accordingly, the majority of animals of this breed had the complex genotype $CSN3^{AB}BLG^{AB}$ and $CSN3^{BB}BLG^{AB}$, the shares of which were 31% and 26%, respectively. Among the animals of SITUBWD, the majority had the complex genotype $CSN3^{AA}BLG^{AB}$

While animals of the UBD having different complex genotypes, showed no statistically significant difference in the amount of milk yield, cows of the SITUBWD with the complex genotype $CSN3^{AB}BLG^{AB}$ were inferior in terms of milk yield to animals with the genotypes $CSN3^{AA}BLG^{AA}$, $CSN3^{AA}BLG^{AB}$, $CSN3^{AA}BLG^{BB}$, and $CSN3^{AB}BLG^{AA}$ with different degree of probability. A probable difference between $CSN3^{AA}BLG^{AA}$ and $CSN3^{BB}BLG^{AB}$ genotypes was also established in favor of the first animals (Fig. 4).

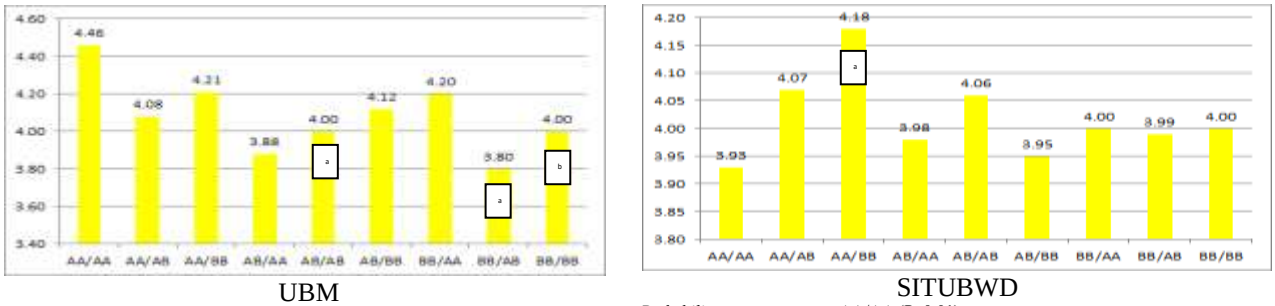


UBM SITUBWD

Probability: a – to genotype AB/AB (P<0.01); b – to the AB/AB genotype (P<0.05); c – to genotype AA/AA (P<0.05);

Fig. 4. Milk yield in cows with different CSN3-BLG complex genotypes, kg
Source: Own research.

According to the content of fat in milk among the first time calving animals of UBD, a statistically significant difference was established between animals with genotypes $CSN3^{AA}BLG^{BB}$ and $CSN3^{AB}BLG^{AB}$, $CSN3^{BB}BLG^{AB}$ in favor of the first genotype, $CSN3^{BB}BLG^{BB}$ and $CSN3^{AB}BLG^{BB}$ in favor of the latter (Fig. 5).



UBM SITUBWD

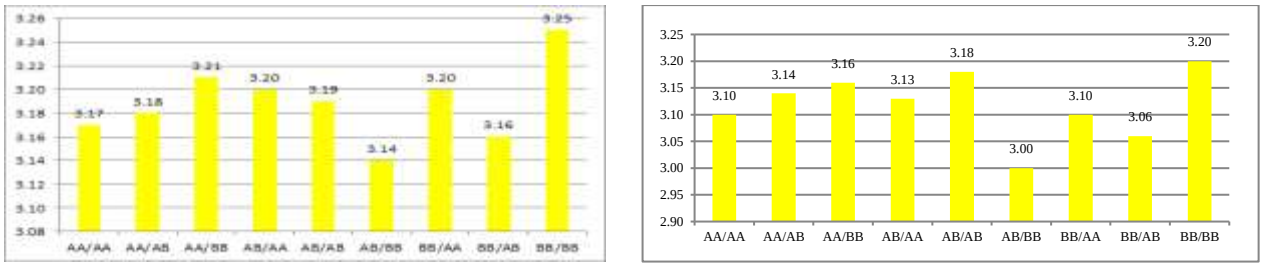
Probability: a – to genotype AA/BB (P<0.05); b – to genotype AB/BB (P<0.05);

Probability: a – to genotype AA/AA (P<0.01);

Fig. 5. Milk fat content in cows with different complex CSN3-BLG genotype
Source: Own research.

Among UBD, a probable difference was established between the first time calving animals with genotypes $CSN3^{AA}BLG^{AA}$ and $CSN3^{AA}BLG^{BB}$ in favor of the latter The studied genes of complex genotype did not have a

statistically significant effect on the protein content in milk (Fig. 6).



UBM SITUBWD

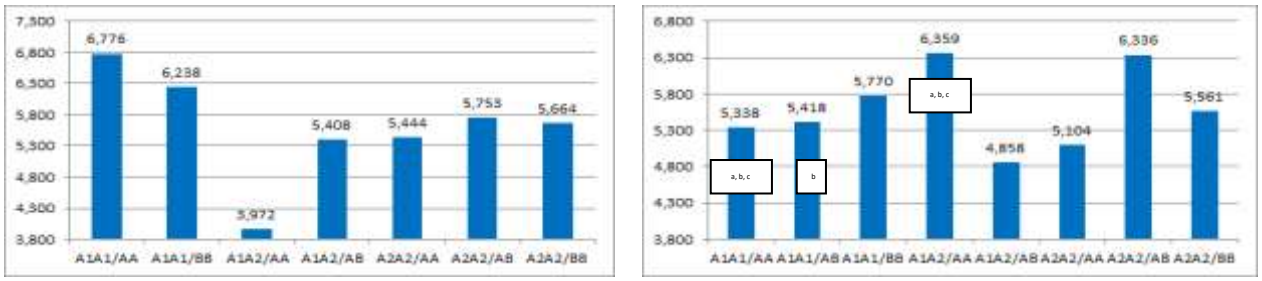
Fig. 6. Milk protein content in cows with different complex CSN3-BLG genotypes
Source: Own research.

Assessing the animals of the test breeds having CSN2/CSN3 complex genes, we found that out of nine possible complex genotypes, only seven were found among UBD cows, and eight among SITUBWD cows. Among animals of the UBD, there were no animals with complex genotypes $CSN2^{A1A1}$ $CSN3^{AB}$ and $CSN2^{A1A2}$ $CSN3^{BB}$, and among cows of the SITUBWD - $CSN2^{A1A2}$ $CSN3^{BB}$. The majority of the first time calving animals of UBD breed had complex genotypes $CSN2^{A2A2}$ $CSN3^{AB}$ and $CSN2^{A2A2}$ $CSN3^{BB}$, respectively 34% and 33%. The majority of the SITUBWD cows had $CSN2^{A1A2}$ $CSN3^{AA}$ and $CSN2^{A2A2}$ $CSN3^{AA}$ genotypes (Table 3).

Table 3. Study of frequencies of complex genotypes CSN2/CSN3

CSN2	CSN3		
	AA	AB	BB
	UBM		
A1A1	0.03	0.00	0.03
A1A2	0.03	0.17	0.00
A2A2	0.07	0.34	0.33
	SITUBWD		
A1A1	0.07	0.07	0.10
A1A2	0.26	0.10	0.00
A2A2	0.27	0.10	0.03

No statistically significant difference in milk yield was found among the first time calving animals of UBD having different complex genotypes. (Fig. 7).



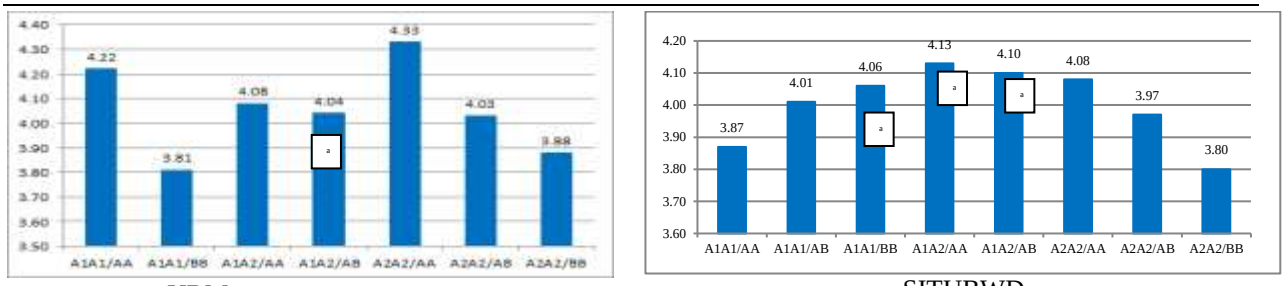
UBM SITUBWD

Probability: a – to genotype A1A2/AA (P<0.05); c – to the A2A2/AA genotype (P<0.05), c – to the A2A2/AB genotype (P<0.05)

Fig. **7.** Milk yield in cows with different CSN2-CSN3 complex genotypes, kg
Source: Own research.

Whilst, the first time calving animals of the SITUBWD having the complex genotype $CSN2^{A1A2}$ $CSN3^{AA}$, $CSN2^{A2A2}$ $CSN3^{AA}$, $CSN2^{A2A2}$ $CSN3^{AB}$ prevailed over animals with the genotypes $CSN2^{A1A1}$ $CSN3^{AA}$ and $CSN2^{A1A2}$ CSN3AA.

The complex genotype of the studied genes had a statistically significant effect on the fat content in milk. Among the first time calving animals of UBD, a probable difference was found between animals with genotypes $CSN2^{A1A2}$ $CSN3^{AB}$ and $CSN2^{A1A2}$ $CSN3^{BB}$. Among SITUBWD animals, cows with complex genotypes CSN2A1A1CSN3BB, CSN2A1A2 CSN3AA, CSN2A1A2 CSN3AB showed the higher content of fat in milk (Fig. 8).



UBM

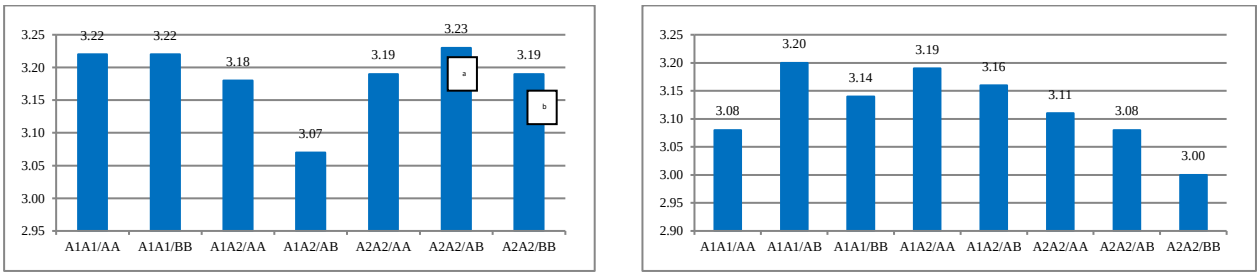
Probability: a – to genotype A2A2/AB (P<0.05)

SITUBWD

Probability: a – to genotype A1A1/AA (P<0.05)

Fig. 8. Fat content in milk of cows with different complex genotype CSN2-CSN3, %
Source: Own research.

According to the content of protein in milk, among the animals of UBD, animals with complex genotypes $CSN2^{A2A2}$ $CSN3^{AB}$ and $CSN2^{A2A2}$ $CSN3^{BB}$ had higher values (Fig. 9).



UBM

Probability: a – to genotype A1A2/AB (P<0.05); b – to genotype A1A2/AB (P<0.01)

SITUBWD

Fig. 9. Protein content in milk of cows with different complex genotype CSN2-CSN3, %
Source: Own research.

They probably prevailed in this feature over animals with the $CSN2^{A1A2}$ $CSN3^{AB}$ genotype. Among the SITUBWD animals, no probable difference was found

CONCLUSIONS

At the first stage of our research, it was planned to research separately the polymorphism of each of the studied genes: CSN2, CSN3 and BLG among animals of the UBD and SITUBWD, which originates from the local Lebedyn breed. This aim determined the study of thirty animals from each breed, according to the minimum recommendation of FAO. Recently, in Ukraine, much attention has been paid to the formation of dairy herds for the production of A2 [10] milk. Therefore, in our opinion, for this purpose it was desirable to conduct an analysis of the possibility of using complex genotypes for the needs of marker selection. A similar practice has already been encountered by other researchers on other local Ukrainian breeds of dairy cattle [7, 8].

Research of complex genotypes of the local breed included CSN2 and CSN3 genes genotypes. Research of the BLG gene was not conducted on the stock of these breeds. According to the results, the majority of brown cattle had complex genotypes $CSN2^{A2A2}CSN3^{AB}$, $CSN2^{A2A2}CSN3^{BB}$ and $CSN2^{A1A2}CSN3^{AB}$ [9]. These results fully correspond to our results. The only difference is the absence of animals with two complex genotypes in our studies. According to the results of previous studies, animals of SITUBWD have a higher frequency of complex genotypes $CSN2^{A1A1}CSN3^{AB}$, $CSN2^{A1A2}CSN3^{AA}$, and $CSN2^{A2A2}CSN3^{AA}$ [9].

Two complex genotypes in animals of the experimental breeds had an advantage over the others in terms of their frequency. These are such genotypes as $CSN2^{A1A2}CSN3^{AA}$ and $CSN2^{A2A2}CSN3^{AA}$. Interbreed differentiation is also established by this feature. There is a certain difference in genotypes that do not occur among experimental animals. Thus, according to the results of our research,

genotype $CSN2^{A1A2}CSN3^{BB}$ is not found. Other authors claim the absence of the following genotypes ($CSN2^{A1A2}CSN3^{AB}$ and $CSN2^{A2A2}CSN3^{BB}$). The results of our research on the frequency of the CSN2 and CSN3 genes also do not correspond to the results of other researchers to some extent. Thus, according to the A2A2 genotype and the CSN2 gene, the difference among animals of the UBD was - 20%, and SITUBWD - 13%. According to the BB genotype of the CSN3 gene, the difference between brown animals was 6%, and SITUBWD - 3%.

Most of the animals of the UBD breed have the genotype of the BLG gene BB, and the SITUBWD - AB. According to the distribution of the studied genes, the majority of the animals of the UBD had the $CSN2^{A2A2}BLG^{AB}$ genotype; $CSN3^{AB}BLG^{AB}$, $CSN3^{BB}BLG^{AB}$ and SITUBWD - $CSN2^{A1A2}BLG^{AB}$; $CSN3^{AA}BLG^{AB}$.

Accordingly, among the animals of the UBD breed, regardless of the CSN2 gene genotype, animals with the BB BLG gene genotype, and animals of SITUBWD with the AA genotype, respectively, had a greater amount of milk yield. For the latter, a similar trend was preserved when the CSN3 and BLG genes were combined.

The content of milk components (fat and protein) showed no clear dependence on complex genotypes.

In our opinion, when working on the formation of a breeding nucleus in a dairy herd, it is desirable to increase the number of animals for genotyping. Number of animals recommended by FAO is appropriate to use with one locus. The use of the recommended number of animals when studying complex genotypes and selection based on its results is insufficient. Similar conclusions are made by other researchers [7, 8].

It is worth noting, that in order to increase efficiency of herd creating work with the desired genotype based on a single gene, or a complex genotype based on a group of loci, it is necessary to perform herd bulls genotyping. This will make it possible to use sperm production from animals with the desired genotype, which in turn will allow in subsequent generations to obtain replacement animals with the necessary complex genotype.

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