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ANALYSIS OF BETA-CASEIN GENE (CSN2) IN POPULATIONS OF GRAY UKRAINIAN BREED OF CATTLE

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АНАЛИЗ ГЕНА БЕТА-КАЗЕИНА (CSN2) В ПОПУЛЯЦИЯХ СЕРОЙ УКРАИНСКОЙ ПОРОДЫ КРУПНОГО РОГАТОГО СКОТА

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Ukrainian gray breed of cattle is one of the oldest resident breeds of cattle in Ukraine. Advances of modern molecular genetics make it possible to identify genes that control economically useful features, including milk productivity. One of the genes in milk proteins is beta-casein (CSN2), which has a direct effect on the quality of milk, in particular on the synthesis of β-casein in milk. The most common variants of β-casein in dairy breeds of cattle are A1 and A2. Option $CSN2^{A1}$causes serious abnormalities in the human body, namely a number of pathological disorders of the intestine, coronary heart disease, diabetes, autism in case of children. Analysis of scientific publications shows that the beta-casein gene (CSN2) has not been studied in aboriginal breeds of cattle in Ukraine, which are carriers of specific gene complexes and rarest alleles. We conducted molecular genetic studies of polymorphism at the locus of the beta-casein gene in populations of aboriginal gray Ukrainian breed from the main breeders of this breed in Ukraine.

Genomic DNA taken from the whole blood of 66 buffaloes was amplified using primers that are based on the basis of sequence of gene CSN2 of cattle. Amplified fragment of CSN2 with the length 121 bp was treated with DdeI restriction enzyme. All three genotypes were detected in the analyzed populations: homozygous $CSN2^{A2A2}$, $CSN2^{A1A1}$and heterozygous $CSN2^{A1A2}$. The analysis of the results showed that the cows of the SE DG "Markeevo" are dominated by carriers of the heterozygous genotype $CSN2^{A1A2}$ (94.2 %), the genotype $CSN2^{A2A2}$ was absent. The results of genotyping of SE Polyvanivka revealed that 20 % of animals are carriers of the $CSN2^{A2A2}$ genotype, which give A2 beta-casein milk. The $CSN2^{A1A2}$ genotype predominated in 53 % of cows. In general, the analyzed breed shows a fairly high level of the "desired" allele A2 - 0.471 (SE EF «Markeyevo») and 0.465 (SE «Polyvanivka»), the splitting of which does not produce β-casoformin 7 (BSM7).

***Key words**: Ukrainian gray breed, cows, PCR-RFLP, polymorphism, gene, A2-milk, allele, genotype.*

Серая украинская порода крупного рогатого скота – одна из старейших аборигенных пород крупного рогатого скота Украины. Достижения современной молекулярной генетики позволяют идентифицировать гены, контролирующие хозяйственно-полезные свойства, в том числе молочную продуктивность. Одним из генов молочных белков

является бета-казеин (CSN2), который оказывает прямое влияние на качество молока, в частности, на синтез β-казеина в молоке. Наиболее распространенными вариантами β-казеина у молочных пород крупного рогатого скота являются А1 и А2. Вариант CSN2A1 вызывает серьезные отклонения в организме человека, а именно ряд патологических нарушений кишечника, ишемическую болезнь сердца, диабет, аутизм у детей. Анализ научных публикаций показывает, что ген бета-казеина (CSN2) не изучался у аборигенных пород крупного рогатого скота в Украине, которые являются носителями специфических генных комплексов и наиболее редких аллелей. Нами проведены молекулярно-генетические исследования полиморфизма в локусе гена бета-казеина в популяциях аборигенной серой украинской породы от основных репродукторов этой породы в Украине.

Геномная ДНК, взятая из цельной крови 66 буйволов, была амплифицирована с использованием праймеров, основанных на последовательности гена CSN2 крупного рогатого скота. Амплифицированный фрагмент CSN2 длиной 121 п.н. обрабатывали рестриктазой DdeI. В анализируемых популяциях выявлены все три генотипа: $CSN2^{A2A2}$, гомозиготный $CSN2^{A1A1}$ и гетерозиготный $CSN2^{A1A2}$. Анализ результатов показал, что среди коров ГП ЭХ «Маркеево» преобладают носители гетерозиготного генотипа $CSN2^{A1A2}$ (94,2 %), генотип $CSN2^{A2A2}$ отсутствовал. По результатам генотипирования ГП «Поливановка» установлено, что 20 % животных являются носителями генотипа $CSN2^{A2A2}$, которые дают молоко с бета-казеином А2. Генотип $CSN2^{A1A2}$ преобладал у 53 % коров. В целом анализируемая порода показывает достаточно высокий уровень «желаемого» аллеля А2 – 0,471 (ГП ЭХ «Маркеево») и 0,465 (ГП «Поливановка»), при расщеплении которого не образуется β-казоформин 7 (БСМ7).

Ключевые слова: *украинская серая порода, коровы, ПЦР-ПДРФ, полиморфизм, ген, А2-молоко, аллель, генотип.*

Introduction.

Ukrainian gray breed of cattle is one of the oldest resident breeds of cattle in Ukraine characterized by extremely valuable economic and biological qualities: high resistance to diseases and extreme environmental factors, unpretentious to the keeping and feeding conditions, strength of the constitution, the long term of productive use, high fat and protein content in milk, high fattening and meat signs, high meat quality. This is a natural domestic gene pool object that is on the edge of extinction, which has been assigned the status of the first category of protection (Pic.1).

Pic.1. Ukrainian gray breed of cattle

Breed preservation is impossible without integrated systems of inspection, estimation and forecast of changes in the state of the genetic structure of the populations. The greatest attention deserves the genetic variability, which is ensured by the diversity of genes associated with the formation of economic-useful features.

Advances of modern molecular genetics make it possible to identify genes that control economically useful features, including milk productivity. One of the potential markers of milk productivity are milk protein genes: kappa-casein gene (*CSN3*), beta-lactoglobulin gene (*βLG*), beta-casein (*CSN2*). Analysis of scientific publications shows that the beta-casein gene (*CSN2)* has not been studied in aboriginal breeds of cattle in Ukraine, which are carriers of specific gene complexes and rarest alleles [1, 2].

Milk proteins casein are the predominant components of milk of cattle. Their part in the total amount of milk proteins is over 75 %. In fresh milk, casein is associated with calcium and is represented by a micellar form, which is destroyed during milk processing. There are four types of casein: *αS1*, *αS2*, *β* and *κ*. Their coding genes are located on chromosome 6 and are grouped into a *CN* cluster [3].

The *CSN2* gene is responsible for the synthesis of β-casein in milk, made up of 209 amino acids and accounting for 25–35 % of total milk protein **[**4, 5, 6, 7]. *CSN2* has 13 genetic variants: *A1, A2, A3, B, C, D, E, F, H1, H2, I, G, A4*, which differ by structures. The most common variants of β-casein among dairy breeds of cattle are A1 and A2.

It is believed that initially all domesticated cows produced milk that contained only A2 β-casein. However, probably due to a point mutation, 5000–10000 years ago in European herds Bos. Taurus codon of CAT, which encodes histin, was formed by changing the nucleotide base in the CST codon, which encodes Proline, at the 67th position of β-casein [7, 8, 9]. Thus, European cows have got one of the β-casein variants, variant A1 [10].

The high frequency of variant A1 is usually found in purebred breeds or crossed breeds of European origin [11]. The frequency of the $CSN2^{A1}$ allele in black-spotted cows is higher than in brown. Asian and African cattle do not have A1 beta-casein at all [12]. Animals of Holstein, Holstein-Friesian, Jersey breeds usually produce A1-milk [13]. One of the reasons for the high prevalence of the mutant A1 allele among cattle breeds is high milk yield, so all commercial cattle breeds are affected by the $CSN2^{A1}$ mutation. The main source of the mutant A1 allele is carrier bulls, although cows are also suppliers of the A1 allele, but to a lesser extent [14, 15].

A2-milk is milk obtained from cows carrying the $CSN2^{A2A2}$ genotype β-casein (beta-casein, *CSN2*). The *CSN2* gene has two common alleles: A1 and A2, so any cow can have a beta-casein genotype: $CSN2^{A1A1}$, $CSN2^{A1A2}$ and $CSN2^{A2A2}$ [16]. Protein A1 is increasingly called the main cause of intolerance to dairy products [17]. When the enzymes of the gastrointestinal tract split milk containing A1 β-casein (obtained from cows with genotypes $CSN2^{A1A1}$and $CSN2^{A1A2}$), the opioid peptide β-casomorphin 7 (BCM7) is formed in a much larger amount of milk than milk with A2 β-casein (from cows $CSN2^{A2A2}$). It is with the effect of BCM7 on the human body, various chronic inflammatory reactions are associated: allergies, skin manifestations, mucin secretion [18]. A1 β-casein can also cause type 1 diabetes, coronary heart disease and autism [19], while variant A2 lowers cholesterol and reduces the risk of inflammatory reactions in the gut [20].

In the present study, the PCR-RFLP technique was used to detect the genetic polymorphism of the CSN2 (beta-casein) gene which associated with milk trait in populations of Ukrainian gray breed of cattle.

Materials and methods of research. There were analyzed blood samples (n = 173) from cows of gray Ukrainian breed from the farms of State enterprise experimental farm «Markeyevo» «Institute of Animal steppe regions named after M.F. Ivanov» Askania Nova «National Science Selection and Genetics center vivchars» (133 goals) and State enterprise experimental farm «Polyvanivka» Institute of Agriculture steppe zone Academy of Agricultural Sciences Ukraine (40 heads). Molecular genetic research was conducted on the basis of the laboratory of genetics of the Institute of Breeding and Genetics of Animals named after M.V. Zubtsia of NAAS. Blood for DNA isolation was taken from the jugular vein in a volume of 5 ml in vacu-

um tubes with dry EDTA. Isolation of DNA from whole blood was performed using a standard commercial set «DNA-sorb-B» (manufactured by AmpliSens, Russia).

The polymorphism of the CSN2 gene was investigated by PCR-RFLP [21], using the method of Mc Lachlan (2006) [22]. The following primers were used for amplification:

5`- CCTTCTTTCCAGGATGAACTCCAG-3`;

5`- GAGTACGAGGAGGGATGTTTTGTGGGAGGCTCT-3`.

The PCR mixture consisted of: 2 μl of DNA polymerase buffer, 1 μl of a mixture of three phosphates, 1 μl of the appropriate primer, 0.2 μl of DNA polymerase ("Fermentas" Lithuania). Genomic DNA was added in an amount of 2 μl. The total volume of the DNA mixture was adjusted with H2O to 10 μl. Amplification of total DNA with primers was performed on a programmed four-channel thermocycle «Tertsyk» according to the following program: 95 °C, 5 minutes – 30 cycles: 95 °C for 10 seconds, 58 °C for 30 seconds, 72 °C for 30 seconds. The last step is 72 °C for 5 minutes. The PCR reaction products were electrophoresed on 2 % agarose gel stained with ethidium bromide to test the amplification success.

The 121 bp PCR product was treated with *DdeI* restriction enzyme according to the scheme: H2O – 3.5 μl, enzyme buffer – 1.0 μl, restriction enzyme – 0.5 μl and 10 μl of amplification per 15 μl of the working mixture. The reaction mixture was incubated at 37 °C in thermostat. After restriction digestion, visualization of the results was performed by electrophoretic distribution of DNA fragments in 3 % agarose gel with ethidium bromide in 1xTBE buffer [23] at 180 V for 15 minutes, followed by detection using a transilluminator TUV-1 in ultraviolet light 312 nm.

The size of the products obtained in PCR or as a result of restriction were detected using molecular weight markers: O'GeneRuler Ultra Low Range DNA Ladder, Ready-to-Use, «Thermo Scientific». Detection of the results was performed by photographing the gels with a digital camera.

Statistical analysis was performed using the software package Statistica 6.0 and Exel (Microsoft Office 2007).

Research results. The amplification obtained by us was analyzed by restriction analysis. After the split of the obtained amplification by the appropriate restriction endonuclease, in the presence or absence of restriction sites, the presence of two alleles A1 and A2 and three genotypes were detected: $CSN2^{A1A1}$ – 121 bp, $CSN2^{A1A2}$ – 121, 86 and 35 bp. and $CSN2^{A2A2}$ genotype – 86 and 35 bp. Figure 1 shows an example of an electrophoregram obtained by determining the genotypes of animals at the studied locus.

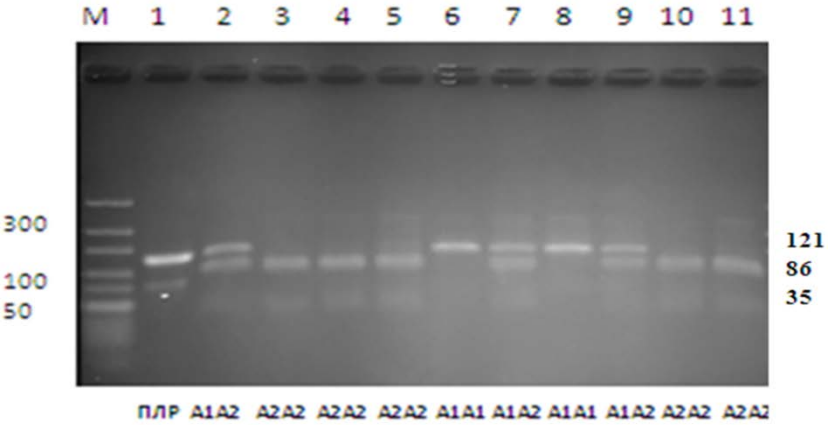

Fig.1. Electrophoretic analysis of restriction products in the determination of genotypes by CSN2: M – molecular weight marker; the genotypes of the samples are shown in the photo

Analysis of the obtained results of allele frequencies and genotypes of the beta-casein gene in cows of SE EF «Markeyevo» ITSR. M.F. Ivanov NAAS «Askania-Nova» – showed (Table 1) that among cows dominated by carriers of the heterozygous genotype $CSN2^{A1A2}$ (94.2 %) and only 8 animals were homozygous for the A1 allele (5.8 %).

Table 1. **Frequency of alleles and genotypes by the locus of the beta-casein gene amoung animals of the gray Ukrainian breed of cattle of SE RF « Markeyevo» (133 goals)**

n	Генотип	Частота генотипів	Частота алелів		H_0	H_e	χ^2	F_{is}
			A1	A2				
133	A1A1	0,058	0,529	0,471	0,942	0,498	0,4	– 0.890
	A1A2	0,942						
	A2A2	–						

Note: H_0 – actual heterozygosity; He – expected heterozygosity; χ^2 – criterion of conformity; F_{is} – wright fixation index.

The frequency of allele A1 was 0.529, allele A2 – 0.471. Animals with the $CSN2^{A2A2}$ genotype were absent. As a result, the actual heterozygosity (H_0 = 0.942) significantly exceeded the expected (He = 0.498), which indicates a shift in genetic equilibrium in the studied gene.

The results of genotyping of animals of SE «Polyvanivka» SI IZK NAAS (table 2) found that among cows in the locus of beta-casein was dominated by the following genotypes: $CSN2^{A1A2}$-53%, $CSN2^{A1A1}$–27 %. The fewest animals were carriers of the $CSN2^{A2A2}$ genotype (20 %), which

give A2 beta-casein milk.

Table 2. **Frequency of alleles and genotypes at the locus of the beta-casein gene in animals of the gray Ukrainian breed of cattle SE «Polyvanivka» (40 heads)**

n	Генотип	Частота генотипів	Частота алелів		H_0	H_e	χ^2	F_{is}
			A1	A2				
40	A1A1	0,270	0,535	0,465	0,530	0,498	0,002	-0,065
	A1A2	0,530						
	A2A2	0,20						

Note: H_0 – actual heterozygosity; He – expected heterozygosity; χ^2 – criterion of conformity; F_{is} – wright fixation index.

Accordingly, allele frequencies were determined at the level of A1-0.535 and A2-0.465. A small difference between the actual (H_0 = 0.530) and expected (He = 0.498) heterozygosity indicates a slight (F_{IS} = -0.065) predominance of heterozygotes.

The results of the study of the beta-casein gene (*CSN2*) indicate a high level of genetic diversity in the studied animals of the gray Ukrainian breed of cattle. The predominance of the $CSN2^{A1}$allele (0.529 and 0.535) and the $CSN2^{A1A}$

breed from the main breeders of this breed in Ukraine was determined.

A large genetic discrepancy was established for the beta-casein gene (*CSN2*) in the studied populations of the gray Ukrainian breed of cattle, which indicates a different system of selection measures of SE EF «Markeyevo» ITSR. M.F. Ivanova NAAS «Askania-Nova» – NNSGTsV and SE «Polyvanivka» SI IZK NAAS. In general, the studied breed shows a fairly high level of the «desired» allele A2 – 0.471 (SE « Markeyevo») and 0.465 (SE «Polivanivka»), the split of which does not produce β-casoformin 7 (BSM7).

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