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## GENETIC ANALYSIS OF THE MONTBELIARD BREED FOR THE SUITABILITY OF A2 MILK USE IN FOOD TECHNOLOGIES

**Olha Yakubenko** <sup>1</sup>, **Nataliia Mokhnachova** <sup>2</sup>, **Svitlana Danylenko** <sup>1</sup>,  
**Ostap Zhukorskyi** <sup>2</sup>, **Hanna Kozlovskaya** <sup>3</sup>, **Oleksandr Verheles** <sup>3</sup>

<sup>1</sup> Institute of Food Resources, NAAS of Ukraine, 4 Ye. Sverstiuk St., Kyiv 02002, Ukraine

<sup>2</sup> Institute of Animal Breeding and Genetics named after M. V. Zubets, NAAS of Ukraine  
1 P. Pohrebniak St., vil. Chubynske, Kyiv Region, Boryspil District 08321, Ukraine

<sup>3</sup> National University of Life Resources and Environmental Management of Ukraine  
16 Vystavkova St., Kyiv 03041, Ukraine

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**Background.** Cow milk has long been a source of protein and certain micronutrients, such as calcium. A2 milk is gaining popularity among consumers who are constantly looking for healthy foods. One factor that affects the quality of milk proteins is the breed of cattle. Over the last decade, countries such as the USA, the EU, India, China, and others have been widely assessing the frequency of  $\beta$ -casein genotypes in dairy cattle of various breeds. Research has shown that the desirable A2 allele of milk  $\beta$ -casein exhibits a broad occurrence range across countries and breeds, varying from 24 to 80.9 %.

The aim of this study was to conduct a genetic and biochemical assessment of the Montbeliard breed to determine its suitability for the use of A2 milk in food technologies.

**Materials and Methods.** The polymorphism of the *CSN2* gene was investigated by the ACRS-PCR method following the McLachlan protocol. The frequencies of alleles and genotypes of the *CSN2* gene ( $\beta$ -casein) in the Montbeliard breed of cattle were determined. The *DdeI* restriction enzyme was used. The physicochemical parameters of milk were determined through conventional methods.

**Results.** According to the physical and chemical parameters, the milk of Montbeliard cows meets the standards of this breed, namely the ratio between fat and protein in milk is about 1.2:1, which is the norm, the average lactose content in milk was 5.23 %, the average protein content was 3.23 %, and the average fat content was 3.23 %.



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and the average protein content was 3.35 %. An analysis of  $\beta$ -casein gene polymorphism in Montbeliarde cows revealed that 27 % of the animals carried the homozygous  $CSN2^{A1A1}$  genotype, 56 % had the  $CSN2^{A1A2}$  genotype, and 17 % possessed the “desirable” homozygous  $CSN2^{A2A2}$  genotype for obtaining A2-milk.

**Conclusions.** Among the Montbeliarde cows studied at LLC “Inter” in the Chernihiv Region, 17% of the tested cows carried this genotype, making them suitable for producing milk free from the “harmful” A1  $\beta$ -casein. It has been confirmed that A2 milk is obtained from cows carrying the  $CSN2^{A2A2}$   $\beta$ -casein ( $CSN2$ ) genotype. In terms of all biochemical parameters, the herd with the A2A2  $\beta$ -casein genotype demonstrated high-quality milk characteristics.

In terms of protein content, there is almost no difference between the groups by homozygous genotype, while the fat content in cows with the homozygous genotype  $CSN2^{A2A2}$  is higher by 0.78 % compared to the homozygous genotype  $CSN2^{A1A1}$  and 0.62 % –  $CSN2^{A1A2}$ .

**Keywords:** A2-milk, Montbeliarde breed, fat content, protein content, genotype, allele

## INTRODUCTION

Milk from cows has long served as a source of protein and certain micronutrients, such as calcium. However, in recent years, increasing attention in the dairy industry has been directed toward the composition of milk – specifically, the polymorphism of milk proteins, which is associated with the technological properties of milk (Mayer *et al.*, 2021). Among consumers who are constantly seeking health-promoting food products, A2 milk is gaining widespread popularity. One of the factors influencing the qualitative composition of milk proteins is the breed of cattle (Kamiński *et al.*, 2007).

Modern literature on dairy farming highlights a variety of biologically active components, including lactate, whey protein, and  $\beta$ -casein protein. There is a global trend toward breeding cows that produce A2-type milk, and this milk is already available on the market. It is important to note that A2 milk is not a substitute for individuals with cow milk allergy. Specifically, cow milk contains two main variants of the  $\beta$ -casein protein, A1 and A2, which differ by a single nucleotide substitution that changes the codon at position 67. While the A2 variant is unlikely to undergo enzymatic cleavage during digestion, the A1 variant is more prone to such cleavage, leading to the release of the peptide  $\beta$ -casomorphin-7, a known  $\mu$ -opioid receptor agonist (Kay *et al.*, 2021).

The primary protein in milk is casein. It is present in combination with calcium, that is, in the form of calcium caseinate. In fresh milk, casein exists as fine particles in the liquid. This form of casein is sometimes referred to as caseinogen. When milk sours, it coagulates as casein precipitates in the form of curd. It accounts for up to 80 % of the total protein content in milk. Casein belongs to the group of proteins known as phosphoproteins.  $\beta$ -casein is one of the six milk proteins, consisting of 209 amino acids and accounting for up to 35 % of the total protein in milk (Farrell *et al.*, 2004). The synthesis of  $\beta$ -casein protein is controlled by the  $CSN2$  gene. Twelve genetic variants of  $CSN2$  are known, which cause changes in certain amino acids in the  $\beta$ -casein protein and alter its properties. Based on the amino acid at position 67, these variants can be classified into two groups – A1 and A2. Variants belonging to the A1 group (His67) include A1, B, C, F, and G. Variants belonging to the A2 group (Pro67) include A2, A3, H1, H2, I, J, K, and L (Cieślińska *et al.*, 2012).

Numerous studies maintain that A1 milk from cattle can be a possible cause (risk factor, dietary trigger) of cardiovascular diseases, type 1 diabetes, sudden infant death syndrome, and neurological disorders such as autism and schizophrenia (McLachlan, 2001; Elliott *et al.*, 1999; Jianqin *et al.*, 2016).

S. S. Kay *et al.* (2021) noted that the consumption of A1 milk was associated with elevated markers of inflammation. It has also been reported to trigger an opioid-like response that may lead to clinical symptoms of neurological disorders such as autism spectrum disorder (Kay *et al.*, 2021). A potential cause of these conditions is the biologically active peptide  $\beta$ -casomorphin-7 (BCM-7), which exhibits significant opioid activity and is formed during the enzymatic digestion of milk containing A1  $\beta$ -casein in the gastrointestinal tract (Massella *et al.*, 2017).

A renowned global expert in child health, Honorary Professor at the University of Auckland, and recipient of the 2020 New Zealand Senior of the Year title, Bob Elliott, in an interview with the online portal *Farmers Weekly* in July 2019, called on New Zealand farmers to fully transition to A2 milk. The professor cited recent research by Chinese medical scientists showing that the problems Chinese people experience with drinking milk are less related to lactose intolerance and more to the effects of milk from animals with the A1A1  $\beta$ -casein genotype on digestive disorders and certain other health issues (Elliott *et al.*, 1999).

The genetic structure of the Holstein cattle population bred in Ukraine was studied with respect to the alpha loci of  $\beta$ -casein and tumor necrosis factor. Using allele-specific PCR (AS-PCR) and PCR-restriction fragment length polymorphism analysis (PCR-RFLP), polymorphism of the  $\beta$ -casein gene (*CSN2*) was identified for the A1 and A2 allelic variants, and of tumor necrosis factor-alpha (*TNF- $\alpha$* ) for the *SacI* polymorphism in the promoter region of the gene (marker mutation –824 A>G) and the *RsaI* polymorphism in the fourth exon. The polymorphic nature of both loci in the studied cattle population was confirmed. Based on the analysis of milk production traits in individuals with different *CSN2* genotypes, it was found that animals with the A2A2 genotype had higher standard milk yield values compared to those with the A1A1 genotype ( $p = 0.042$ ). For the *SacI* and *RsaI* polymorphisms of the *TNF- $\alpha$*  gene, no significant differences in standard milk production over two lactations were observed between individuals with different genotypes (Kulibaba *et al.*, 2024).

In this context, scientists and practitioners in the field of dairy cattle breeding and selection in many countries have begun to pay special attention to the evaluation and selection of cows, particularly potential dams of breeding bulls, taking into account their milk  $\beta$ -casein genotypes (Fuerer *et al.*, 2020).

The Montbeliarde breed is a dual-purpose (dairy and beef) cattle breed. It was developed in France in 1883 by crossing local Alsatian (Abundance-type) cattle with Swiss Simmental. The breed is common in the rural regions of France near the Swiss border and is considered one of the high-yielding French breeds.

The Montbeliarde heifer has a large, bowl-shaped udder. Montbeliarde cattle are renowned for their longevity and strong health. Careful selective breeding has enabled French farmers to develop highly productive animals. The milk is characterized by an optimal balance of protein and fat, along with an increased concentration of vitamins and minerals. The lactation period in heifers of this breed is long and quite generous. Bulls of this breed are also valued for their meat quality. Experts have recognized the high standard of their beef.

Montbeliarde heifers produce milk with excellent taste qualities. It is widely used for the industrial production of premium cheeses and yogurts. Dairy products from Montbeliarde cattle are in high demand due to their outstanding flavor and nutritional value.

Cows of this breed are well-known for their strong health, which contributes to the high quality of their meat. Meat characteristics of this breed include: a high slaughter yield of up to 65–68 % (ratio of carcass weight to mass).

Montbeliarde cattle are not demanding in terms of feed. They can digest even coarse forage, but to ensure healthy herd growth, the diet should be rich in vitamins, trace elements, and an adequate amount of carbohydrates.

Therefore, the evaluation and selection of breeding cows and their testing for  $\beta$ -casein milk genotypes are relevant for the domestic dairy industry.

The aim of this study was to perform a genetic and biochemical evaluation of the Montbeliarde breed to determine its suitability for the use of A2 milk in food technologies.

## MATERIALS AND METHODS

Samples of blood and milk ( $n = 30$ ) were collected from Montbeliarde cows of the LLC „INTER” agricultural enterprise, located in Ichnia, Pryluky District, Chernihiv Region, Ukraine (Fig. 1).



Fig. 1. Montbeliard breed animals, LLC “INTER”

Blood for DNA extraction was collected from the jugular vein in a volume of 5 mL using vacuum tubes containing dry EDTA. DNA was extracted from whole blood using the standard *DNA-sorb-B* commercial kit (AmplifySens).

Polymorphism of the *CSN2* gene was analyzed using the PCR-ACRS method, following the protocol described by McLachlan (2006). The following primers were used for amplification:

*CSN2f*: 5'-CCTTCTTTCCAGGATGAACTCCAG-3' and

*CSN2r*: 5'-GAGTACGAGGAGGGATGTTTTGTGGGAGGCTCT-3'.

PCR was carried out using a four-channel programmable thermal cycler “Tercyk” under the following conditions: 95 °C for 5 min; 30 cycles of 95 °C for 10 sec., 58 °C for 30 sec., and 72 °C for 30 sec.; followed by a final extension at 72 °C for 5 min. The PCR

mixture contained: 2.5 µL of 10× PCR buffer, 2.5 µL of a 2 mM dNTP mix, 0.2 µL of each primer at a concentration of 50 µM, and 5 U/µL of DNA polymerase. Genomic DNA was added in the amount of 1.5 µL (50 pg–1 µg). The total volume of the DNA mixture was adjusted to 25 µL with PCR-grade water.

The PCR products were digested with the *DdeI* restriction endonuclease according to the following protocol: 3.5 µL of water, 1.0 µL of 10× enzyme buffer, 0.5 µL of restriction enzyme, and 10 µL of PCR product were combined to prepare a 15 µL working mixture.

For visualization of PCR-ACRS fragments, DNA samples were loaded into wells of a gel containing 3% high-resolution agarose, 0.5 µg/mL ethidium bromide, and subjected to horizontal electrophoresis at 15 V/cm in 1× TBE buffer for 60 min. The electrophoresis gel was then examined under UV light at a wavelength of 312 nm using a transilluminator, where genotypes were identified based on the number and size of the fragments. The sizes of the PCR or restriction digestion products were determined using molecular weight markers: GeneRuler™ 50 bpDNA Ladder, GeneRuler™ 100 bpDNA Ladder ("Fermentas").

The obtained experimental results were processed using population-genetic and biometric analysis methods with GEN Alex 6 and Statistica software.

The genotype frequency was calculated using the formula:

$$p = \frac{n}{N},$$

where  $p$  – genotype frequency;  $n$  – the number of individuals with a specific genotype;  $N$  – the total number of individuals.

The allele frequency was calculated using the formula:

$$p = \frac{2n_{AA} + n_{AB}}{2N} \text{ and } q = \frac{2n_{BB} + n_{AB}}{2N},$$

where  $p$  – the frequency of allele A;  $q$  – the frequency of allele B;  $n_{AA}$ ,  $n_{AB}$ ,  $n_{BB}$  – the number of individuals with specific genotypes;  $N$  – the total number of individuals.

The actual heterozygosity was calculated using the formula:

$$H_0 = \frac{N_2}{n},$$

where  $N_2$  – number of heterozygotes for the studied allele;  $n$  – sample size.

The actual heterozygosity was calculated using the formula:

$$H_e = 1 - \sum_{i=1}^n p_i^2,$$

where  $p_1, p_2, \dots, p_n$  – allele frequencies.

To assess the genetic differentiation of the studied populations, the individual Wright's fixation index ( $F_{IS}$ ) was used, which quantitatively reflects the deviation from panmixia:

$$F_{IS} = \frac{(H_e - H_0)}{H_e},$$

where  $H_0$  – the actual heterozygosity in the population;  $H_e$  – the expected heterozygosity in the population ( $H_0 \neq H_e$ ).

The correspondence between the actual and expected genotype distribution was tested using Pearson's chi-square test ( $\chi^2$ ) with the following formula:

$$\chi^2 = \frac{\sum (A - T)^2}{T},$$

where  $A$  – the actual number of genotypes;  $T$  – the theoretical number of genotypes.



Total solids content – according to DSTU 8552:2015 Milk and dairy products. Methods for the determination of moisture and total solids and DSTU ISO 5534:2005 Cheese and processed cheese. Determination of total solids content (reference method) (ISO 5534:2004, IDF 4:2004, IDT);

Total protein content was determined by the Kjeldahl method under the requirements of DSTU EN ISO 8968-1:2022 Milk and milk products. Determination of nitrogen content. Part 1. Kjeldahl principle and calculation of crude protein (EN ISO 8968-1:2014, IDT; ISO 8968-1:2014, IDT);

Density – DSTU 6082:2009 Milk and dairy products. Methods for the determination of density;

Mass fraction of fat – in accordance with DSTU ISO 1211:2002 “Milk. Gravimetric method for determination of fat content” (reference method) and DSTU ISO 11870:2007 Milk and milk products. Determination of fat content. General guidance on the use of methods involving the butyrometer (ISO 11870:2000, IDT);

The content of solid non-fat milk residue (SNF) was determined using the following formulas:

$$\text{SNF} = \left( \frac{F}{5} + \frac{D}{4} \right) + 0.76;$$

$$\text{SNF} = S - F,$$

where  $S$  – total solids content in milk, %; 4.9; 4; 0.5; 1.31; 26.5; 0.1; 5; 0.76 – constant coefficients;  $F$  – fat content in milk, %;  $D$  – milk density, °;  $A$ ,  $G$  – milk density, kg/m<sup>3</sup>; SNF – solid non-fat milk residue content, %.

Active acidity (pH) – potentiometrically according to DSTU 8550:2015 Milk and milk products. Measurement of pH by the potentiometric method;

Titrate acidity – in accordance with DSTU ISO 6091:2007 Dried milk. Determination of titrate acidity (reference method) (ISO 6091:1980, IDT);

Ash content was determined by the gravimetric method using a muffle furnace according to DSTU ISO 5544:2014 “Caseins. Determination of bound ash content (reference method)”;

Lactose content – in accordance with DSTU 8059:2015 Dairy products. Determination of lactose and galactose content by spectrometric method.

All international, national, and/or institutional principles for the care and use of animals were followed. The studies were approved by the Commission on the Treatment of Animals in Scientific Research of the M.V. Zubets IRGT (Protocol No. 2 dated 04 April, 2025; Chubynske, Ukraine).

## RESULTS AND DISCUSSION

Milk samples from Montbeliarde cows of the agricultural enterprise LLC „INTER”, Chernihiv Region, Pryluky District, the town of Ichnia, were examined.

The average fat content in the milk of Montbeliarde cattle was 3.91 %. The ratio between fat and protein in the milk obtained from 30 Montbeliarde cows is approximately 1.2:1, which is considered normal. The average lactose content in the milk was 5.23 %, and the average protein content was 3.35 % (**Table 1**).

Milk density – one of the most important indicators of its quality – was 1031 kg/m<sup>3</sup>.

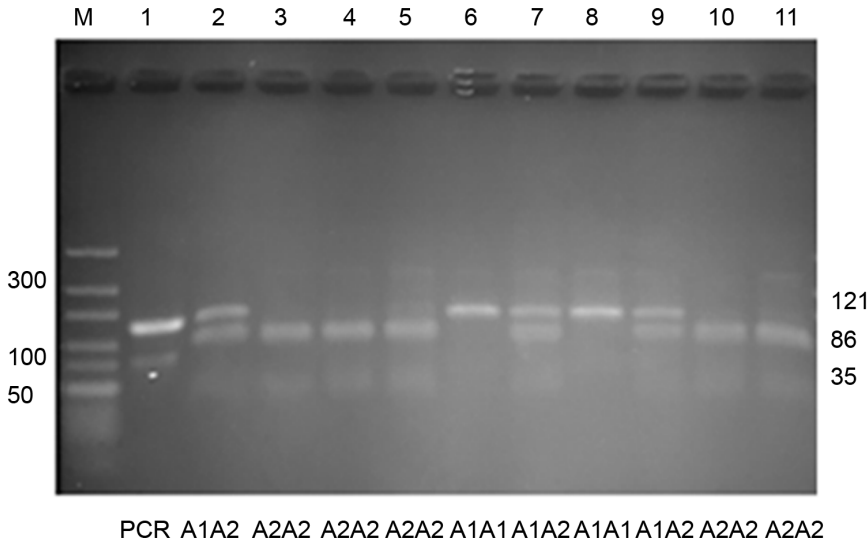
Thus, the average composition values of Montbeliarde cow milk meet the breed standards. This breed demonstrates higher levels of fat, protein, and lactose.

**Table 1. Physicochemical parameters of milk from Montbeliarde cows of LLC „INTER” (n = 30)**

Indicators	Experimental group of cows
Dry matter	13.38±0.21
Fat, %	3.91±0.11
Protein, %	3.35±0.02
Lactose, %	5.23±0.12
Dry skim milk residue, %	9.25±0.23
Ash, %	0.68±0.01
Density, g/cm <sup>3</sup>	1.031±0.001
Titrateable acidity, °T	16.94±0.50
Active acidity (pH)	6.10±0.11

The alleles of milk protein (caseins) genes are considered potential DNA markers for milk productivity and quality in cattle. Blood samples (n = 30) were collected from Montbeliarde cows. DNA was extracted from whole blood using the “DNA-sorb-B” kit.

The amplification product obtained was analyzed using restriction analysis. The 67th triplet sequence of the *CSN2* gene – CCT, which encodes proline – overlaps with the recognition site of the restriction enzyme *DdeI*. Accordingly, the PCR product is cleaved by the restriction endonuclease. A single nucleotide substitution in this codon, from CCT -> CAT, causes the *DdeI* recognition site to disappear, so this amplicon is not digested by the enzyme. After digestion of the amplification product with the *DdeI* restriction endonuclease, based on the presence or absence of restriction sites, two alleles – A1 and A2 – and three genotypes were identified: *CSN2*<sup>A1A1</sup> – 121 bp, *CSN2*<sup>A1A2</sup> – 121, 86, and 35 bp, and *CSN2*<sup>A2A2</sup> – 86 and 35 bp. An example of an electrophoregram obtained during genotyping of animals at the studied locus is shown in **Fig. 2**.



**Fig. 2.** Electrophoretic analysis of restriction products for *CSN2* genotyping: M – molecular weight marker; genotypes of the samples are indicated in the photo

Thus, the study demonstrated the applicability of the *CSN2f* and *CSN2r* primers, the *DdeI* restriction enzyme, and the corresponding PCR-ACRS protocol as a whole for genotyping cattle by the *CSN2* gene.

Based on the analysis of  $\beta$ -casein gene polymorphism in 30 Montbeliarde cows, it was established that 8 animals, or 27 %, carried the homozygous genotype *CSN2*<sup>A1A1</sup>, 17 animals, or 56 %, had the *CSN2*<sup>A1A2</sup> genotype, and 5 cows, or 17 %, exhibited the “desirable” homozygous genotype *CSN2*<sup>A2A2</sup> for A2 milk production. The allele frequencies of A1 and A2 were 0.55 and 0.45, respectively (**Table 2**).

Table 2. Genetic structure characteristics of Montbeliarde cattle by the  $\beta$ -casein gene in the “Inter” agricultural enterprise, Chernihiv Region, Pryluky District, Ichnia

Breed	Sample size	Genotype frequency		Allele frequency		Heterozygosity		$\chi^2$	$F_{is}$
				A1	A2	$H_o$	$H_e$		
Montbeliarde	30	A1A1	0.27	0.55±0.017	0.45±0.017	0.560	0.495	0.52	-0.131
		A1A2	0.56						
		A2A2	0.17						

Notes:  $H_o$  – observed heterozygosity;  $H_e$  – expected heterozygosity;  $\chi^2$ – goodness-of-fit test;  $F_{is}$ – Wright’s fixation index

When comparing the actual and theoretically expected heterozygosity values for the  $\beta$ -casein (*CSN2*) gene in Montbeliarde cattle, it was noted that the actual heterozygosity of 0.065 exceeded the expected value. The negative value of Wright’s fixation index indicates an excess of heterozygotes among the studied animals.

The next stage involved analyzing the fat and protein content in the milk of Montbeliarde cows (**Table 3**).

Based on the presented data, the fat content in cows with the homozygous *CSN2*<sup>A2A2</sup> genotype was 0.78 % higher compared to those with the homozygous *CSN2*<sup>A1A1</sup> genotype and 0.62 % higher than those with the heterozygous *CSN2*<sup>A1A2</sup> genotype. As for protein content, there was almost no difference between the groups.

It should be noted that, across all biochemical parameters, the herd with the A2A2  $\beta$ -casein genotype demonstrated high and favorable milk quality indicators.

Table 3. Average values of protein and fat content in the milk of Montbeliarde cows by homozygous genotype

Homozygous genotype	Number of cows, heads	Protein	Fat
<i>CSN2</i> <sup>A1A1</sup>	8	3.39±0.105	3.32±0.125
<i>CSN2</i> <sup>A1A2</sup>	17	3.41±0.123	3.48±0.110
<i>CSN2</i> <sup>A2A2</sup>	5	3.38±0.068	4.10±0.109

Holstein cattle are well known for their high average milk yield, but are more susceptible to diseases and have lower fertility compared to other cattle breeds. Nevertheless,



Holsteins still hold significant value and purpose (Chang *et al.*, 2023). They exhibit excellent traits and characteristics, and despite health and reproductive challenges, they may still be the most suitable choice for specific market demands or particular farms.

The consumption of A2 milk has been associated with beneficial effects and is more easily digested by sensitive individuals. Further research is needed to investigate the short- and long-term effects of consuming A1  $\beta$ -casein compared to milk containing A2  $\beta$ -casein proteins (Kay *et al.*, 2021).

The milk fat content of Montbeliarde heifers is 4 %, with a protein content of 3.45 %. On average, a Montbeliarde cow produces up to 8000 kg of milk per year. Milk yield indicators are stable and may vary slightly with changes in the feeding ration. However, milk fat content remains consistently stable (Hazel *et al.*, 2017; Kuczynska *et al.*, 2012; Pires *et al.*, 2025).

Our research showed that the fat content in Montbeliarde cows is  $(3.91 \pm 0.11)$  %. No significant differences in protein content were observed between the groups.

The proteins in cow's milk consist of almost 80% caseins (CN), which corresponds to 2.5–2.8 % mass/vol. (Maurmayr *et al.*, 2018). Our results indicate that milk protein content does not vary depending on the homozygous genotype.

The analysis of the frequency of the desirable  $CSN2^{A2A2}$  allele in animals from three Ukrainian indigenous cattle breeds (Ukrainian Grey, Ukrainian Whiteheaded, and Carpathian Brown) and buffaloes showed that buffaloes and the Ukrainian Grey breed lead with allele frequencies of 1.0 and 0.468, respectively. The valuable milk properties of indigenous cattle breeds and buffaloes should help prevent the decline of these unique animal populations (Mokhnachova, 2021).

Genotyping of animals from the Ukrainian Black-and-White dairy breed for the  $\beta$ -casein gene was conducted. It was found that the allele frequencies at the  $\beta$ -casein locus were A1 – 0.421 and A2 – 0.579. Accordingly, the frequencies of the genotypes A1A1, A1A2, and A2A2 were different, amounting to 25 %, 35 %, and 40 %, respectively. Genetic-statistical analysis revealed an excess of homozygous variants A1A1 and A2A2 and a deficiency of the heterozygous A1A2 genotype at the  $\beta$ -casein locus (Ladyka *et al.*, 2021).

The studies conducted by Yu. I. Sklyarenko established that the quality indicators of milk productivity in Ukrainian Brown dairy cattle vary significantly across different farms. Specifically, the fat content in milk ranges from 3.43 % to 3.98 %; protein content – from 3.10 % to 3.55 %; casein content – from 2.83 % to 3.31 %; total solids – from 12.4 % to 13.1 %; and solid non-fat content – from 8.95 % to 9.13 % (Skliarenko *et al.*, 2017).

According to biochemical indicators, the herd with the A2A2  $\beta$ -casein genotype demonstrates high-quality milk characteristics. Among the studied Montbeliarde cows from LLC “Inter” in the Chernihiv Region, 17 % of the tested animals possess the A2A2 genotype and can be used for producing milk free from the “harmful”  $\beta$ -casein A1.

## CONCLUSIONS

Overall, A2 milk is the milk obtained from cows carrying the  $CSN2^{A2A2}$  genotype of the  $\beta$ -casein ( $CSN2$ ) gene. It should be noted that, according to all biochemical parameters, the herd with the A2A2  $\beta$ -casein genotype demonstrates high-quality milk indicators. Among the studied Montbeliarde cows from LLC “Inter” in the Chernihiv Region, 17 % of the tested cows possess this genotype and can be used to produce milk free from the “harmful”  $\beta$ -casein A1.

## COMPLIANCE WITH ETHICAL STANDARDS

**Animal studies:** all international, national, and institutional guidelines for the care and use of laboratory animals were followed.

**Conflict of interest:** the authors declare the absence of any conflicts of interest.

**Human Rights:** this article does not contain any research involving human subjects by any of the authors.

All authors have read and approved the published version of the manuscript.

## AUTHOR CONTRIBUTIONS

Conceptualization, [D.S.; M.N.]; methodology, [Zh.O.]; research, [M.N.; Ya.O.]; resources, [V.O.; M.N.; D.S.]; data presentation, [Ya.O.; M.N.; K.H.]; writing – original draft preparation, [Ya.O.; K.H.]; writing – review and editing, [M.N.; D.S.]; visualization, [Ya.O.; V.O.]; supervision, [Zh.O.; D.S.]; project administration, [D.S.; M.N.].

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## ГЕНЕТИЧНИЙ АНАЛІЗ МОНБЕЛЬЯРДСЬКОЇ ПОРОДИ НА ПРИДАТНІСТЬ ДЛЯ ВИКОРИСТАННЯ А2-МОЛОКА В ХАРЧОВИХ ТЕХНОЛОГІЯХ

Ольга Якубенко<sup>1</sup>, Наталія Мохначова<sup>2</sup>, Світлана Даниленко<sup>1</sup>,  
Остап Жукорський<sup>2</sup>, Ганна Козловська<sup>3</sup>, Олександр Вергелес<sup>3</sup>

<sup>1</sup> Інститут продовольчих ресурсів НААН України  
вул. Євгена Сверстюка, 4А, Київ 02002, Україна

<sup>2</sup> Інститут розведення і генетики тварин ім. М. В. Зубця НААН України  
вул. Погребняка, 1, с. Чубинське, Бориспільський р-н, Київська обл. 08321, Україна

<sup>3</sup> Національний університет біоресурсів і природокористування України  
вул. Виставкова, 16, Київ 03041, Україна

**Обґрунтування.** Коров'яче молоко здавна слугувало джерелом білка та деяких мікроелементів, наприклад, кальцію. Серед споживачів, які постійно шукають корисні для здоров'я продукти харчування, молоко А2 набуває широкої популярності. Одним із чинників, що впливають на якісний склад білків молока, є порода великої рогатої худоби. В останнє десятиліття у США, ЄС, Індії, Китаї та ін. широко здійснюють оцінку частоти народження генотипів β-казеїну у молочної худоби різних порід. Дослідженнями встановлено, що бажаний алель А2 β-казеїну молока має широкий діапазон народження у розрізі країн і порід – від 24 до 80,9 %. Метою роботи було провести генетичну та біохімічну оцінку монбельярдської породи на придатність для використання А2-молока в харчових технологіях.

**Матеріали та методи.** Методом ACRS-PCR досліджували поліморфізм гена CSN2, використовуючи методику McLachlan. Визначили частоти алелів і генотипів гена CSN2 (β-казеїну) в монбельярдській породи великої рогатої худоби. Використовували рестриктазу DdeI. Фізико-хімічні показники молока визначали загальноновживаними методиками.

**Результати.** Фізико-хімічні показники молока корів монбельярдської породи відповідали стандартам цієї породи, а саме: співвідношення між жиром і білком у молоці становило близько 1,2:1, що є нормою; середнє значення вмісту лактози в молоці – 5,23 %, середній вміст білка перебував на рівні 3,35 %. На основі аналізу поліморфізму гена β-казеїну корів монбельярдської породи встановлено, що 27 % тварин несуть гомозиготний генотип CSN2<sup>A1A1</sup>, 56 % – генотип CSN2<sup>A1A2</sup>, а у 17 % виявлено “бажаний” для отримання А2-молока гомозиготний генотип CSN2<sup>A2A2</sup>.

**Висновки.** Серед досліджених корів монбельярдської породи СТОВ “Інтер” Чернігівської області такий генотип мають 17 % протестованих корів, які можуть бути використані для отримання молока, яке не містить “шкідливого” β-казеїну А1. Доведено, що А2-молоко – це молоко, отримане від корів носіїв генотипу CSN2<sup>A2A2</sup> β-казеїну (CSN2). За всіма біохімічними показниками стадо з генотипом А2А2 за β-казеїном має високі та якісні показники молока.

За вмістом білка різниці між групами з гомозиготним генотипом майже не виявлено, тоді як вміст жиру у корів із гомозиготним генотипом CSN2<sup>A2A2</sup> вищий на 0,78 %, порівняно з гомозиготним генотипом CSN2<sup>A1A1</sup> та 0,62 % – CSN2<sup>A1A2</sup>.

**Ключові слова:** молоко А2, монбельярдська порода, вміст жиру, вміст білка, генотип, алель