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POLYMORPHISM OF THE KAPPA-CASEIN GENE AND ITS RELATIONSHIP WITH MILK PRODUCTIVITY AND MILK QUALITY OF COWS OF DIFFERENT LINEAR ORIGIN

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Background. The efficiency of dairy farming is largely determined by the increase in cow productivity and depends on the quality of milk, which has significantly deteriorated in recent years.

Materials and Methods. Genomic DNA was extracted from individual samples of biological material (hair samples with bulbs) taken from cows using a commercial set of reagents “DNA Sorb B” (AmpliSens). Polymerase chain reaction was performed on an Amply-4-2 amplifier (Biokom); detection was performed using a UVD transilluminator (Biokom). Milk productivity was recorded according to the results of individual monthly control milkings of cows.

Results. Genetic analysis has proven the polymorphism of the kappa-casein locus in the experimental array of cows of the Ukrainian Black-and-White dairy breed, represented by three genotypes: AA (63.1 %), AB (31.6 %), BB (5.3 %), with a predominance of the frequency of the A allele (0.79). Cows with the AA genotype of the Starbuck line demonstrate maximum milk productivity, surpassing animals with the AB genotype by 361.6 kg or 5.8 % against the background of a probably significant advantage over representatives with the BB genotype – by 727.6 kg or 12.3 % ($p < 0.01$). At the same



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time, individuals with the AB genotype of the Chifa line in terms of milk yield per lactation exceeded animals with the AA genotype by only 25.8 kg or 0.4 %, while in the group of cows with the AB genotype of the Valiant line, the difference in the corresponding indicator, on the contrary, was lower compared to animals with the AA genotype of the same line by 559.0 kg or 9.2 %. During laboratory analysis, it was found that cows with the BB genotype of the Starbuck and Elevation lines had higher values of the mass fraction of fat in milk by 0.01 and 0.20 %, compared to animals with the AB and AA genotypes of the Starbuck line and by 0.09 and 0.18 % in the Elevation line. These animals are also characterized by higher values of the mass fraction of protein by 0.39 and 0.25 %, and 0.45 and 0.49 %.

Conclusion. The results of the research indicate the polymorphism of the experimental array of cows of the Ukrainian Black-and-White dairy breed, which is represented by three genotype: AA (63.1 %), AB (31.6 %), and BB (5.3 %), with the prevalence of the frequency of the A allele (0.79). Cows-descendants with the AA genotype of the Starbuck line are characterized by a higher milk yield by 5.8 and 12.3 % than animals with the AB and BB genotypes, while cows with the BB genotype of the Elevation line have a higher content of mass fractions of protein by 0.42 and 0.49 % and fat by 0.18 and 0.09 % compared to animals with the AA and AB genotypes.

Keywords: polymorphism, kappa-casein, cows, milk, line

INTRODUCTION

From the point of view of organizational, technological and economic solutions, dairy farming is considered the most complex technological link in livestock production (Paliy *et al.*, 2020; Henchion *et al.*, 2022). Under these conditions, there is a primary need to produce high-quality milk as a biologically valuable food product that would allow us to compete in the global market (Nguyen *et al.*, 2022). In addition, the development of processing facilities that produce a wide range of dairy products is directly dependent on the level of intensification of the dairy farming industry (Moschopoulou, 2021; Linehan *et al.*, 2024).

Milk, meat and their processed products are integral components of the consumer market and serve as raw materials for numerous related food-processing industries. The growing importance and uniqueness of these components in a balanced human diet highlight the need to increase production and improve their quality, which remain priority objectives traditionally included among the main tasks of the livestock industry (Abegaz, 2022; Gross, 2023; Khalak *et al.*, 2023). However, the ever-increasing demands of the food market for milk quality, particularly in terms of fat and protein content, together with the traditional selection of dairy herds based on quantitative traits, require the use of advanced molecular-genetic methods that allow the identification of gene polymorphisms and further in-depth work to improve milk protein and fat content, taking into account the desired alleles (Thomasen *et al.*, 2014; Adamov *et al.*, 2020; Pishchan & Sylychenko, 2021; Ardiçlı Su *et al.*, 2024; Korkh *et al.*, 2025). The main fractions in cow's milk protein are represented by α S1-casein, α S2-casein, β -casein, and κ -casein as insoluble components (78–80 %) and whey proteins β -lactoglobulin and α -lactoalbumin as soluble components (20–22 %). They are characterized by a variety of structures, distinctive physical and chemical properties, and specific technological and biological functions (Sebastiani *et al.*, 2020; Jaiswal & Worku, 2022; Demirel & Çak, 2025).

The kappa-casein gene is the most studied gene, the frequency of which directly affects milk production, its chemical composition, technological and coagulation properties, which play a key role in the processing and manufacture of dairy products, including cheese (Manguy & Shields, 2019; Khastayeva *et al.*, 2021; Rahmatalla *et al.*, 2022; Jiménez-Montenegro *et al.*, 2022; Runthala *et al.*, 2023).

The most common alleles in this gene are A and B, which differ in the substitution of two amino acids in the position of the polypeptide chain: 136 threonine to isoleucine and 148 aspartic acid to alanine and are caused by point mutations at positions 5309 (C/T) and 5345 (A/C) of the nucleotide sequence of the fourth exon of kappa-casein, other alleles are considered rare in most cattle breeds (Ullah *et al.*, 2025). Genetic variants of kappa-casein show significant variability in the frequency of occurrence in different populations of dairy cattle (Ladyka *et al.*, 2022). In particular, taking into account the genotypic characteristics of the Holstein breed, a group of researchers drew attention to the increase in the frequency of the CSN3A allele compared to the CSN3B allele. In particular, its value in dairy herds in Serbia was 0.70 (Lukač *et al.*, 2015), Croatia – 0.76 (Ivanković *et al.*, 2011), Mexico – 0.79 (Duifhuis-Rivera *et al.*, 2014), Turkey – 0.82 (Akyüz *et al.*, 2013), Palestine – 0.75–0.84 (Khaizaran *et al.*, 2014), while crossbreeding of this breed with the Zebu breed was accompanied by a slight increase in the frequency of the A allele in crossbreds to 0.85 (Botaro *et al.*, 2009). Based on the quantitative assessment of the cattle, the Czech experts also found a clear trend towards an increase in the frequency of the A allele of kappa-casein in the tested animals compared to the group of animals in which the B allele was isolated (Bartoňová *et al.*, 2012; Lavon *et al.*, 2024). Results from studies of milk from Simmental and Aberdeen Angus cows showed a similar increase in the frequency of the kappa-casein A allele (Curi *et al.*, 2005). At the same time, the A allele of kappa-casein is associated with an increase in milk yield in cows, and the B allele is associated with a high content of fat and protein mass fractions in milk and better technological characteristics (Huiam, 2018). Milk from cows with the BB and AB kappa-casein genotypes is characterized by a higher mass fraction of protein that coagulates faster under the action of rennet than in milk from cows with the AA genotype (Pazzola *et al.*, 2020). Similar results were reported by C. Cipolat-Gotet *et al.* (2018). However, contradictory research materials are cited in the publication. Currently, as the authors of the article state, cows with the AA genotype compare favorably with other genotypes in terms of higher milk production but shorter milk coagulation time.

The relevance of this research is supported by an analysis of the literature on state-of-the-art approaches to increasing milk productivity and improving milk quality in cows with different kappa-casein genotypes. However, the available studies do not always provide a comprehensive framework for the objective evaluation of modern dairy cattle breeds with respect to these parameters. The inconsistency of scientific evidence concerning the relationship between the kappa-casein gene and milk productivity and quality traits in dairy cows underscores the importance of revising breeding stock in accordance with the growing requirements of dairy production. This would provide a basis for defining the desired animal type and implementing targeted selection of replacement heifers to establish a genetically superior breeding nucleus.

Currently, in Ukraine, efforts are being made to improve the Ukrainian Black-and-White dairy breed through various breeding techniques. The goal is to maximize milk

production in cows while enhancing milk quality, body conformation, health, stress resistance, and productive longevity. This study is part of a comprehensive research program that aims to identify productive traits and technological properties of milk from cows of this breed with different kappa-casein genotypes. Although there are individual studies on the relationship between the kappa-casein genotype and milk production, as well as analyses of herd genetic structure, comprehensive research in the domestic literature remains lacking. Currently, some researchers studying the polymorphism of quantitative traits focus on the relationship between the kappa-casein genotype and milk yield in cows, as well as the composition of individual milk components. Meanwhile, others only characterize the genetic structure of the herd based on this specific locus. However, a key aspect of breeding programs aimed at ensuring high milk quality is studying productive and technological traits, particularly cheese-making suitability, in Ukrainian Black-and-White dairy cows with different kappa-casein genotypes, as determined by lineage, which is the scientific novelty and practical significance of the present study.

The aim of this study is to determine the polymorphism of the kappa-casein gene and its relationship with milk production and milk quality in cows of different linear origin.

MATERIALS AND METHODS

Location of the experiment. The work was carried out at the Institute of Animal Science of the National Academy of Agrarian Sciences of Ukraine. The experimental part of the work was carried out at the breeding station of the Ukrainian Black-and-White dairy breed of the base farm in the Kharkiv region.

Experimental animals. The experiments conducted on animals do not contradict the current legislation of Ukraine (Article 26 of the Law of Ukraine 5456-VI of 16.10.2012 „On the Protection of Animals from Cruelty”) as amended as of 04.08.2017 and the „General Ethical Principles for Animal Experiments” adopted by the First National Congress on Bioethics (Kyiv, 2001) and international bioethical standards (Materials of the IV European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Purposes (Strasbourg, 1985) (Simmonds, 2017). The research program was reviewed and approved by the Bioethics Committee of the Institute of Animal Husbandry of the National Academy of Sciences of Ukraine in accordance with the current procedure.

A total of 95 dairy cows were selected for the experiment. Based on the results of the DNA test for the kappa-casein gene, the selected cows were divided into three groups with genotypes: AA, AB and BB. The criteria for completing the groups were based on the principle of analogous pairs by breed, live weight, and last calving. The duration of the experiment was 730 days.

During the research period, the experimental groups of cows were housed in the same way: in summer – in groups, untethered in the farm camp; in winter – individually, tethered in typical two- and four-row stalls. During the same period, according to the daily routine of the farm, the animals were given two hours of daily exercise in the walking and feeding areas next to the stables. Chopped cereal straw was used as bedding. Milking – twice a day, in the milk pipeline; drinking – at will, from group drinkers; feed consumption – from the feed table.

Nutrition. The basis of the cows' diet was a mixture of maize silage and alfalfa haylage in winter, and green fodder of cereals and legumes of the field crop rotation

in summer, which was balanced in terms of basic nutrients, the amount of which was adjusted to the physiological state and the level of the average daily milk yield. In addition, sainfoin or vetch-oat hay was fed as part of the feed mixture. During the lactation period, fodder beet molasses was added to the main feed. The fodder mixture was prepared using a Triolet mobile mixer, and transported and distributed using a KTU-10. As concentrated feed, we used compound feed of our own production, which contained in various proportions a homogeneous mixture of cereal and legume grains crushed to medium size, sunflower cake, and mineral additives. Diets were weighed daily and fed in two doses per day during morning and evening milking. Mineral deficiencies in the diet were replenished by adding 1 % premix and salt to the feed components. The veterinary drug Trivitamin was used to provide vitamin support to the cows' bodies. The feed supply was 53–62 c feed units per cow per year. The total nutritional value of the diet was on average 212 MJ of metabolizable energy; 20.4 kg of dry matter; 2815 g of crude protein at an energy concentration of 10.4 MJ per kg of dry matter.

Preparation of samples. Genomic DNA was isolated from individual samples of biological material collected from the experimental cows using the commercial DNA-Sorb B reagent kit manufactured by AmpliSens (NeoPrep100 (NEOGENE, Ukraine)). Hair follicle samples served as the biological material. Polymorphism of marker genes was determined using the PCR-RFLP method. The polymerase chain reaction was performed on the Amply-4-2 amplifier (Biokom), and the detection was performed using the UVD transilluminator (Biokom). PCR amplification was performed according to the following protocol: initial denaturation at 94 °C for 5 min followed by 35 cycles, each consisting of denaturation at 94 °C for 30 s, primer annealing at 51 °C for 30 s, and extension at 72 °C for 45 s; final extension at 72 °C for 10 min. The final volume of the reaction mixture was 20 µL; the concentration of each primer in the reaction was 0.2 µM.

The size of the restriction fragments was determined using the pUC19 molecular marker (Thermo Fisher Scientific, USA). To amplify a 271-bp fragment of the kappa-casein (*CSN3*) gene (Sequence ID: MK455075.1), the following oligonucleotide primers were used:

F: 5'GAAATCCCTACCATCAATACC-3';

R: 5'CCATCTACCTAGTTTAGATG-3'.

The *CSN3* gene polymorphism (chromosome 6, exon 4, position 85656772, rs43703016 A>C, Thr148Ala) was investigated using the restriction endonuclease HinfI (restriction site 5'-G↓ANTC-3', Thermo Fisher Scientific, USA) according to the manufacturer's protocol.

Restriction fragment analysis was performed using horizontal electrophoresis in a 2.5 % agarose gel at a voltage of 15 V/cm. The AA genotype corresponded to three fragments measuring 131, 91, and 49 base pairs (bp), the AB genotype corresponded to four fragments measuring 222, 131, 91, and 49 bp, and the BB genotype corresponded to two fragments measuring 222 and 49 bp. Electropherograms were recorded using a Canon digital camera.

The genotype and individual allele frequencies, as well as the genetic equilibrium between the actual and theoretically expected frequency distributions, were calculated using GenAlex 6.5 software with built-in formulas. In particular, the frequencies of kappa-casein genotypes were determined by formula 1:

$$P = \frac{2 \cdot n}{N}, \quad (1)$$

where P is the frequency of occurrence of the corresponding genotype of kappa-casein; n – the number of animals with the corresponding genotype of kappa-casein, heads; N – the total number of animals in the sample, heads.

The frequency of occurrence of individual kappa-casein alleles was determined by the formulas 2 and 3 of G. M. Shangin-Berezovsky:

$$P_A = \frac{2n \cdot AA + nAB}{2N}, \quad (2)$$

$$P_B = \frac{2n \cdot BB + nAB}{2N}, \quad (3)$$

where P_A is the frequency of the A allele of kappa-casein; P_B – the frequency of the B allele of kappa-casein; n – the number of animals with the available genotype of kappa-casein, heads; N is the total number of individuals in the experimental group.

Genetic equilibrium was calculated using the Hardy–Weinberg formula 4:

$$p^2 = 2pq + q^2 = 1, \quad (4)$$

where p is the frequency of occurrence of the A allele of kappa-casein; q is the frequency of occurrence of the B allele of kappa-casein.

The χ^2 criterion (chi-square) was used to assess the compliance of the actual frequencies of kappa-casein alleles with the expected ones. The χ^2 value was calculated by the formula 5:

$$\chi^2 = \sum (O - E)^2 / E, \quad (5)$$

where O is the actual frequency of occurrence of the kappa-casein genotype; E is the expected frequency of occurrence of the kappa-casein genotype.

Evaluation of cow milk productivity. Milk productivity was recorded based on the results of individual monthly control milkings of cows, with subsequent calculation for each month and for the entire lactation period (305 days). The amount of milk fat and protein (kg) was calculated by the formula 6:

$$K = \frac{H \cdot C}{100}, \quad (6)$$

where K is the amount of milk fat or protein, kg; N – milk yield, kg; C – a weighted average mass fraction of fat or protein, %.

Chemical analysis of milk. The mass fractions of fat, protein, total protein (Pro. total), lactose, and dry matter were analyzed monthly in average milk samples using a Bentley milk analyzer (USA). The determination of the mass fractions of fat, protein, lactose, and dry matter was performed by infrared spectrometry. The content of skimmed milk powder was estimated by calculation (by the difference between the mass fraction of dry matter and fat). The number of somatic cells in milk was measured by fluorescence-optical laser flow cytometry using a combined model Somacount 150 device (USA). Milk density was determined using an areometer. The determination of titratable acidity in milk and cottage cheese in Turner degrees (T°) was carried out by the acid-base titration method.

The energy value of milk was calculated taking into account that one gram of protein contains 4 kcal, fat – 9 kcal, and lactose – 3.75 kcal, and then multiplied by a factor of 4.184 to convert to megajoules.

Statistical analysis. The results were processed by variational statistics methods using the software package Stat Plus 5 (6.7.0.3) (Analyst Soft Inc., USA). The analysis of genotype frequency distribution at the kappa-casein locus was performed using the chi-square test. Statistical analysis of milk yield and quality data was performed using a two-factor analysis of variance (ANOVA). The factors included the genotype at the kappa-casein locus, with three levels, and the animal lines, with four levels. The normality of the data distribution was verified using the Shapiro–Wilk test. A posteriori analysis in the case of parametric ANOVA was performed using the Tukey–Kramer test. In cases of deviation from normality or significant differences in variance between groups, the nonparametric Kruskal–Wallis test (with a posteriori comparisons using Dunn's test) was used.

The results are presented as mean $\pm$ standard error ($\bar{x} \pm SE$). The difference between the groups was considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

The genotyping results of the experimental group of cows showed that the *CSN3* locus is polymorphic and represented by three genotypes (**Table 1**).

An evaluation of the amplification results revealed a clear trend favoring carriers of the homozygous AA genotype for kappa-casein (60 animals) within the studied population. This distribution suggests long-term, intensive breeding efforts involving the targeted use of AA-genotype breeding bulls, a genotype linked to higher milk production. In the total sample, thirty animals were heterozygous for the AB genotype, while only five animals possessed the more desirable homozygous BB genotype, which is linked to higher technological properties of milk, particularly its suitability for cheese making. Theoretically expected frequencies of the AA and BB genotypes were slightly lower than the observed frequencies, by 0.7 % and 0.9 %, respectively. Conversely, among animals with the AB genotype, there was a 1.6 % increase in the proportion of heterozygous animals. Thus, the studied sample exhibited a shift toward decreased homozygous genotype frequencies and increased heterozygote frequencies, which combine traits of increased milk productivity and improved milk cheese-making suitability.

Table 1. Genetic structure of the experimental group of cows by the *CSN3* locus

Distribution	n, heads	Genotype frequency, %			Allele frequency		χ^2
		AA	AB	BB	A	B	
F	95	63.1	31.6	5.3	0.79	0.21	0.23
E		62.4	33.2	4.4			

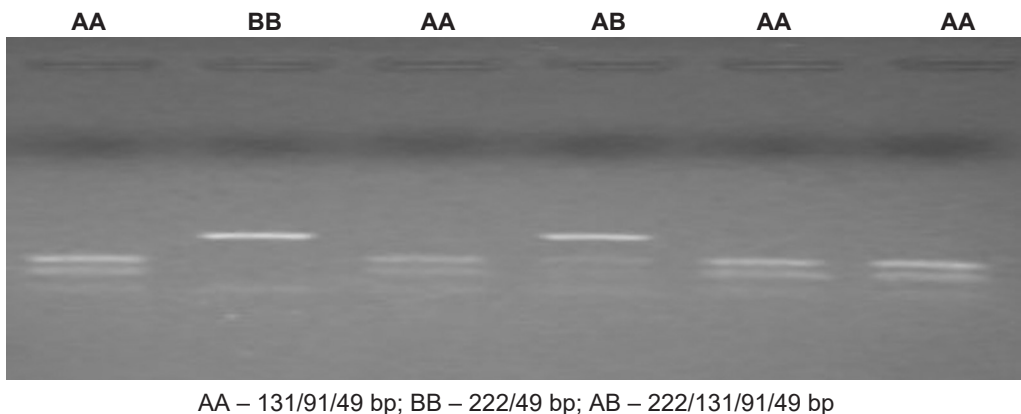
Note: F – actual distribution of genotypes; E – theoretically expected distribution of genotypes

In the actual genetic structure of the experimental herd of cows, homozygotes with the AA genotype for kappa-casein were predominant, accounting for 63.1 %. The proportion of AB heterozygotes was slightly lower – 31.6 %. Meanwhile, carriers of the rare BB homozygous genotype accounted for 5.3 % of the total sample size. The theoretic-

cally expected population structure was close to the actual one, amounting to 62.4 %, 33.2 %, and 4.4 %, respectively. This indicates the relative stability of the gene pool and underscores the importance of further breeding efforts to promote the broader use of breeding bulls, primarily those with the AB and BB genotypes, in planned matings. This will help consolidate the herd in terms of milk quality and cheese-making suitability.

A comparison of the actual genotype frequency distribution with the theoretically expected distribution, calculated using the Hardy–Weinberg formula, revealed a balanced genotype ratio and the absence of a statistically significant deviation from genetic equilibrium for the studied kappa-casein polymorphism. The χ^2 value obtained during the assessment of genetic diversity ($\chi^2 = 0.23$) was significantly lower than the critical level, confirming that the population is in a state of genetic equilibrium. Calculation of the allele frequencies of the kappa-casein locus showed that in the studied herd, allele A occurred with a frequency of 0.79. At the same time, the frequency of the technologically more valuable allele B was only 0.21, which is almost four times lower compared to the prevalence of allele A among the animals in the sample. Similar variability in the genetic structure of cattle herds has been reported in studies (Khaizaran *et al.*, 2014; Duifhuis-Rivera *et al.*, 2014; Mitioglo, 2021).

The polymerase chain reaction (PCR) yielded a 271-bp amplicon. Further electrophoretic analysis of the amplicon following restriction with Hind I allowed us to identify differences based on both the presence or absence of specific bands. Depending on the genotype, the size of the DNA fragments ranged from 49 to 131 bp (see **Figure**).



Results of molecular genetic typing of DNA samples obtained from cows carrying different kappa-casein genotypes

The range of cows in terms of origin is represented by seven lines descended from 27 sire bulls. All sire bulls are listed in the catalogues of dairy and beef sires approved for reproduction in Ukraine. Only semen from bulls that have given their daughters an average milk yield of more than 8–10 thousand kilograms of milk is used to produce offspring. From these sires, the herd's heifers and calves are descended. The lines of Starbuck 352790, Elevation 1491007, Chifa 1427381, and Valiant 1650414, which have been used to improve the herd, are of considerable value in the breeding and technological sense. In addition, the base farm uses semen from bulls of the lines Cavalier 1620273, Bell 1667366, and Josco Besna 5694028588.

Given that one of the most important levers for improving the productive and breeding qualities of animals in livestock is the method of breeding by lines, and the information on the influence of the lineage of bulls on milk production and milk quality of cows of different genotypes of kappa-casein is contradictory, it became necessary to focus on a more detailed study of this issue. Despite the genotype of kappa-casein among the experimental cows, 52 heads (54.7 %) were sired by bull Starbuck 352790, 15 heads (15.8 %) by bull Elevation 1491007, 22 heads (23.2 %) by bull Chifa 1427381 and 6 heads (6.3 %) by bull Valiant 1650414.

A thorough study of the data of the breeding records and the evaluation of the productive traits made it possible to establish that the genealogical composition of the array of breeding stock with the genotype of kappa-casein AA and AB involved in the research was mainly represented by the line Starbuck 352790 (31 and 18 heads, respectively, or 53 % and 60.0 % of the total number), the line Chifa 1427381 (16 and 6 heads, respectively, or 26.7 and 60.0 %), the line Elevation 1491007 (10 and 3 heads, respectively or 16.7 and 10.0 %), and the line Valiant 1650414 (3 heads respectively or 3.3 and 10.0 %), while animals with the BB genotype belonged to the line Starbuck 352790 (3 heads or 60.0 %) and the line Elevation 1491007 (2 heads or 40.0 %).

Given that the total number of cows with the BB genotype of the Elevation line was only two, this did not allow us to determine the probability of a difference between the groups within it. However, the descendants of this line are characterized by a high ability to form productive traits inherent in other animals carrying the B allele.

The analysis of the evaluation data of different bulls involved in the reproductive process on the farm revealed a number of differences in the manifestation of hereditary potential in terms of milk yield of cows assigned to them, but in most cases the difference between the groups was statistically insignificant.

At present, representatives of the Starbuck line, regardless of the genotype at the kappa-casein locus, are characterized by the highest level of productivity; animals of the Valiant line stood out with the lowest level of milk yield, and the descendants of the Chifa and Elevation lines occupied an intermediate position in this indicator (**Table 2**).

It was found that, in general, milk yields for 305 days of lactation in cows of all groups ranged from 5915.50 ± 131.66 kg to 6643.00 ± 180.29 kg. However, the intergroup rates of increase in milk yield during lactation were different. In particular, they increased most significantly in animals with the AA genotype of the Starbuck line, which exceeded animals with the AB genotype by 361.6 kg or 5.8 % and representatives with the BB genotype by 727.5 kg or 12.3 % ($p < 0.01$). In animals with the AB genotype of the Chifa line, compared to the AA genotype, milk production was only 25.8 kg or 0.4 % higher, while in cows with the AB genotype of the Valiant line, the difference in this trait during lactation, on the contrary, was lower than in the latter by 559.0 kg or 9.2 %. Regarding the animals of the Elevation line, milk yields in animals with different kappa-casein genotypes were almost identical and approached the value of 6000 kg. The dependence of milk production on the genotype of cows by the kappa-casein gene of other breeds is confirmed by the conclusions of other experts (Petrovska *et al.*, 2017; Čítek *et al.*, 2021).

Cows with the BB genotype of the Starbuck and Elevation lines differed from those with AB and AA genotypes by higher values of the mass fraction of fat in milk, which amounted to 0.01 and 0.20 % compared to animals of the Starbuck line and 0.09 and 0.18 % of the Elevation line. The difference between the maximum and minimum

Table 2. Level of milk productivity and milk quality of cows with different kappa-casein genotypes and origin (x ± SE)

Indicator	Line			
	Starbuck	Chifa	Elevation	Valiant
Kappa-casein AA genotype				
Number of animals, heads	31	16	10	3
Milk yield, kg	6643.0 ± 180.29***	6130.8 ± 21.10	5962.4 ± 222.10	6087.0 ± 491.28
Mass fraction, %				
including: fat	3.76 ± 0.06	3.77 ± 0.09	3.89 ± 0.13	3.94 ± 0.23
protein	3.12 ± 0.04	3.15 ± 0.09	3.19 ± 0.05	3.13 ± 0.08
Amount per lactation, kg				
including: milk fat	249.78 ± 8.91	231.13 ± 11.53	231.94 ± 8.60	239.83 ± 5.56
milk protein	207.26 ± 7.12	193.12 ± 7.90	190.20 ± 8.05	190.52 ± 10.28
Kappa-casein AB genotype				
Number of animals, heads	18	6	3	3
Milk yield, kg	6281.4 ± 189.36	6156.6 ± 29.99	5953.5 ± 240.95	5528.0 ± 571.01
Mass fraction, %				
including: fat	3.95 ± 0.06#	3.97 ± 0.19	3.98 ± 0.09	3.94 ± 0.07
protein	3.26 ± 0.13	2.89 ± 0.37	3.15 ± 0.14	3.65 ± 0.65
Amount per lactation, kg				
including: milk fat	248.12 ± 7.77	244.42 ± 12.71	236.95 ± 10.69	217.80 ± 8.62
milk protein	204.77 ± 10.66	177.93 ± 13.56	187.54 ± 23.61	201.77 ± 14.91
Kappa-casein BB genotype				
Number of animals, heads	3	–	2	–
Milk yield, kg	5915.5 ± 131.66	–	5963.7	–
Mass fraction, %				
including: fat	3.96 ± 0.10	–	4.07	–
protein	3.51 ± 0.24	–	3.64	–
Amount per lactation, kg				
including: milk fat	234.25 ± 8.98	–	242.72	–
milk protein	207.63 ± 17.78	–	217.08	–

Note: **p < 0.01 – the probability of difference was calculated for cows with the BB genotype of the corresponding line; #p < 0.05 – for cows with the AA genotype of the corresponding line

values of this component of milk in representatives of AA and AB genotypes of the Chifa and Elevation genealogical groups was 0.09 and 0.20 % in favor of the latter, although the difference between these genotypes of the Starbuck line had lower values than the BB genotype, but it was at the first level of statistical significance. At the same time, intergroup differences in Valiant cows by the mass fraction of fat in milk were leveled.

It should be noted that cows with the BB genotype of the Starbuck and Elevation lines also had the maximum milk protein content by 0.39 and 0.25 % and 0.45 and 0.49 % higher compared to animals of the AA and AB genotypes of these lines. This indicates a certain specificity of the manifestation of biological characteristics of the descendants of these lines (deviation towards increased milk protein content) in the breeding stock with the BB genotype of kappa-casein. Among the representatives of AA and AB genotypes of the Valiant line, the advantage in the mass fraction of protein was on the side of the latter by 0.52 %, but in animals of the Chifa line, the difference between the respective groups was reversed in favor of individuals with the AA genotype – by 0.26 %.

The maximum amount of milk fat was obtained from the milk of cows with the AA genotype of the Starbuck line, which had the highest milk yields but the lowest mass fraction of fat in milk, prevailed over representatives of the AB genotypes by 1.66 kg or 0.7 % and BB – by 15.53 kg, or 6.6 %. At the same time, the differences between animals of AA and AB genotypes of the Valiant line were also greater and amounted to 22.03 kg, or 10.1 % in favor of the first ones. As for the animals of the Chifa line, they were smaller by 13.29 kg, or 5.4 %. At the same time, cows with the BB genotype of the Elevation line exceeded both the animals with the AB genotype by 5.77 kg, or 2.4 % and animals with the AA genotype by 10.78 kg, or 4.6 %.

When comparing the results obtained, a tendency was noted to increase the amount of milk protein in the milk of cows with the BB genotype of the Starbuck and Elevation lines relative to the other kappa-casein genotypes, which was formed by increasing their productivity and the mass fraction of protein in milk. In particular, according to the data presented in the table, the amount of milk protein in these cows of the Starbuck line was 0.37 kg, or 0.2 % higher than the same indicator in animals with the AA genotype, and 2.86 kg, or 1.4 %, with the AB genotype.

The increased content of milk protein in the milk of animals of this genotype of the Elevation line was manifested to a greater extent and the difference between them and individuals with the AA genotype was 26.88 kg, or 14.1 %, and the AB genotype – 29.54 kg or 15.8 %. This difference was slightly less between cows with genotypes AA and AB of the Chifa line, namely in terms of the quantitative composition of protein in milk; experimental animals with the AA genotype prevailed over those with the AB genotype by 15.19 kg, or 8.5 %, but representatives of this genotype of the Valiant line were inferior to the latter by 11.25 kg, or 5.6 %.

The content of mass fractions of protein, lactose, dry matter, and dry skimmed milk in the milk of cows with genotype BB of Starbuck and Elevation lines was higher and, in the statistical processing of the results of cow evaluation, the difference, in most cases, compared to animals of genotypes AA and AB of these lines was significant (**Table 3**).

It is worth noting that the differences in the mass fraction of protein in milk between cows with the BB genotype of the Starbuck and Elevation lines and representatives of the other evaluated genotypes fully reflected the recorded difference in the mass fraction of protein: Starbuck line animals were characterized by 0.40 and 0.25 % higher

values of the corresponding trait, respectively, and Elevation animals were also noticeably distinguished, with a difference of 0.37 % exceeding AA and 0.41 % exceeding AB genotypes. In experimental cows with the AA genotype of the Chifa line, a similar trend was found in relation to individuals with the AB genotype, but its value was lower and was at the level of 0.16 %. In addition, the belonging of the animals to the Valiant line had a greater impact on the growth of the latter's advantage in terms of protein mass fraction by 0.44 %.

Table 3. Mass fractions of protein, lactose, dry matter, dry skimm residue, and somatic cells in milk of cows with different kappa-casein genotypes and origin (x ± SE)

Indicator	Line			
	Starbuck	Chifa	Elevation	Valiant
Kappa-casein AA genotype				
Number of animals, heads	31	16	10	3
Mass particle content, %.				
including: protein	3.32 ± 0.04	3.36 ± 0.09	3.40 ± 0.05	3.39 ± 0.15
lactose	4.88 ± 0.02	4.89 ± 0.02	4.79 ± 0.03	4.88 ± 0.06
dry matter	12.92 ± 0.05	12.73 ± 0.08	13.06 ± 0.12	12.78 ± 0.21
dry skimmed residue	9.16 ± 0.06	8.96 ± 0.07	9.17 ± 0.10	8.84 ± 0.02
Number of somatic cells, thousand/cm ³	424.77 ± 68.24	282.52 ± 34.78	432.15 ± 135.93	493.90 ± 120.10
Kappa-casein AB genotype				
Number of animals, heads	18	6	3	3
Mass particle content, %.				
including: protein	3.47 ± 0.12	3.20 ± 0.33	3.36 ± 0.14	3.83 ± 0.58
lactose	4.89 ± 0.06	4.96 ± 0.05	4.94 ± 0.03 ^{##}	4.87 ± 0.08
dry matter	13.28 ± 0.10	13.13 ± 0.27	12.97 ± 0.18	13.29 ± 0.24
dry skimmed residue	9.33 ± 0.13	9.16 ± 0.20	8.99 ± 0.14	9.35 ± 0.15
Number of somatic cells, thousand/cm ³	395.42 ± 57.82	342.10 ± 35.79	236.97 ± 44.95	358.30 ± 15.70
Kappa-casein BB genotype				
Number of animals, heads	3	—	2	—
Mass particle content, %.				
including: protein	3.72 ± 0.23	—	3.77	—
lactose	5.08 ± 0.03 ^{###/00}	—	4.97	—
dry matter	13.57 ± 0.11 ^{###/00}	—	13.57	—
dry skimmed residue	9.61 ± 0.29	—	9.50	—
Number of somatic cells, thousand/cm ³	231.93 ± 46.12	—	484.55	—

Note: ^{##}p < 0.01; ^{###}p < 0.001 – the probability of difference was calculated for cows with AA genotype of the corresponding line; ⁰⁰p < 0.01 – for cows with AB genotype of the corresponding line

The belonging of cows to certain lines caused more significant changes in the mass fraction of milk sugar. The milk of cows with the BB genotype of the Starbuck line contained the highest mass fraction of lactose, and the differences in this trait relative to the AA genotype were the largest – 0.20 % ($p < 0.001$), while in comparison with the representatives of the AB genotype, the differences had a lower level of statistical significance ($p < 0.01$) and amounted to 0.19 %. Against this background, animals with genotypes AB and BB of the Elevation line were characterized by significantly better hereditary qualities, which is confirmed by a significant difference and a tendency to increase the mass fraction of lactose in their milk by 0.15 % ($p < 0.01$) and 0.18 % compared to animals with the AA genotype. However, the differences between the AA and AB genotypes of the Chifa line in terms of milk sugar content remained statistically insignificant.

The leading position in terms of the mass fraction of dry matter was also occupied by cows with the BB genotype of Starbuck and Elevation lines. It has been found that the nutritional value of milk of these animals is significantly different from those with AA and AB genotypes by 0.65 % ($p < 0.001$) and 0.29 % ($p < 0.01$), and 0.51 and 0.60 %. Despite the significant difference in improving the quality of milk from cows of these lines, in animals with the AB genotype of the Starbuck line, the manifestation of heredity for increasing the mass fraction of dry matter does not have a significant effect on the representatives of the AA genotype, although it still increased by 0.36 %. A higher value of the studied parameter was also recorded in cows with the AB genotype of the Chifa and Valiant lines, although the difference in animals with the AA genotype by 0.40 and 0.51 %, respectively, was also statistically insignificant.

The mass fraction of milk dry matter from cows with the BB genotype of Starbuck and Elevation lines contained more dry skimmed residue. However, they showed only a tendency to increase it by 0.45 and 0.28 % and 0.33 and 0.51 %, respectively, compared to these indicators in animals with AA and AB genotypes. In turn, the descendants of sires of the Chifa and Valiant lines had a positive effect on the increase in the mass fraction of dry skimmed milk in the milk of cows with the AB genotype by 0.20 and 0.51 %, respectively, in contrast to animals with the AA genotype.

The level of somatic cells in the milk of linear cows with different genotypes was within the regulated requirements of DSTU 3662:2018 “Raw cow’s milk. Specifications” and ranged from 231.93 ± 46.12 thousand/cm³ to 493.90 ± 120.10 thousand/cm³. However, the differences between the groups were not statistically significant.

The density, titrated and active acidity of milk were almost constant, both in absolute values and in terms of indicators, among genotypes of cows of different origins: 1028.00 ± 0.10 – 1028.50 kg/m³, 17.30 ± 0.10 – 18.10 ± 0.17 °T, and 6.64 ± 0.02 – 6.78 ± 0.06 pH. These parameters were within the regulatory requirements, which indicates the freshness and „extra” quality of the selected samples (**Table 4**).

It was found that if the milk of cows with different genotypes of kappa-casein and origin is characterized by a certain coincidence in these parameters, this cannot be said about the ratio of its main components. In particular, in animals of the Starbuck line, one part of protein accounted for 1.13 to 1.21 parts of fat, the Chifa line – from 1.22 to 1.26, Elevation – from 1.12 to 1.38, and Valiant – from 1.08 to 1.26. The ratios between the mass fraction of protein and fat were not constant either, they were: in Starbuck line animals from 0.83 to 0.88 : 1; Chifa – from 0.79 to 0.82 : 1; Elevation – from 0.73 to 0.89:1, Valiant – from 0.79 to 0.93 : 1. Other scientists also emphasize the improvement of technological characteristics of milk in cows with different genotypes of kappa-casein (Cipolat-Gotet *et al.*, 2018; Huiam, 2018; Pazzola *et al.*, 2020; Bangar *et al.*, 2021).

Table 4. Technological indicators and the ratio of main components in the milk of cows with different genotypes of kappa-casein and origin ($\bar{x} \pm SE$)

Indicator	Line			
	Starbuck	Chifa	Elevation	Valiant
Kappa-casein AA genotype				
Number of animals, heads	31	16	10	3
Density, kg/m ³	1028.06 $\pm$ 0.07	1028.00 $\pm$ 0.10	1028.09 $\pm$ 0.14	1028.01 $\pm$ 0.15
Titrated acidity, °T	17.97 $\pm$ 0.14	17.64 $\pm$ 0.18	17.87 $\pm$ 0.25	17.30 $\pm$ 0.10
Active acidity, pH	6.70 $\pm$ 0.03	6.67 $\pm$ 0.02	6.68 $\pm$ 0.02	6.64 $\pm$ 0.02
Ratios:				
fat : protein	1.21 : 1	1.22 : 1	1.20 : 1	1.26 : 1
protein : fat	0.83 : 1	0.82 : 1	0.84 : 1	0.79 : 1
fat: dry skimmed residue*	0.41 : 1	0.42 : 1	0.42 : 1	0.45 : 1
protein : dry skimmed residue	0.34 : 1	0.35 : 1	0.34 : 1	0.35 : 1
Energy value of milk, MJ	270.37 $\pm$ 2.28	271.42 $\pm$ 3.40	275.01 $\pm$ 5.29	277.32 $\pm$ 8.98
Kappa-casein AB genotype				
Number of animals, heads	18	6	3	3
Density, kg/m ³	1028.29 $\pm$ 0.07	1028.26 $\pm$ 0.11	1028.16 $\pm$ 0.10	1028.33 $\pm$ 0.18
Titrated acidity, °T	18.06 $\pm$ 0.37	17.80 $\pm$ 0.13	17.98 $\pm$ 0.41	17.65 $\pm$ 0.05
Active acidity, pH	6.74 $\pm$ 0.03	6.68 $\pm$ 0.03	6.71 $\pm$ 0.04	6.66 $\pm$ 0.06
Ratios:				
fat : protein	1.21 : 1	1.26 : 1	1.38 : 1	1.08 : 1
protein : fat	0.83 : 1	0.79 : 1	0.73 : 1	0.93 : 1
fat: dry skimmed residue	0.42 : 1	0.43 : 1	0.44 : 1	0.42 : 1
protein : dry skimmed residue	0.35 : 1	0.32 : 1	0.35 : 1	0.39 : 1
Energy value of milk, MJ	280.04 $\pm$ 3.86 [#]	274.55 $\pm$ 4.44	280.12 $\pm$ 5.66	285.85 $\pm$ 12.1
Kappa-casein BB genotype				
Number of animals, heads	3	–	2	–
Density, kg/m ³	1028.20 $\pm$ 0.06	–	1028.50	–
Titrated acidity, °T	18.10 $\pm$ 0.17	–	17.80	–
Active acidity, pH	6.78 $\pm$ 0.06	–	6.73	–
Ratios:				
fat : protein	1.13 : 1	–	1.12 : 1	–
protein : fat	0.88 : 1	–	0.89 : 1	–
fat: dry skimmed residue	0.41 : 1	–	0.43 : 1	–
protein : dry skimmed residue	0.37 : 1	–	0.38 : 1	–
Energy value of milk, MJ	287.57 $\pm$ 4.26 ^{##}	–	292.17	–

Note: [#]p < 0.05; ^{##}p < 0.01 – the probability of difference was calculated for cows with AA genotype of the corresponding line; * – mass fraction of skimmed milk residue

The ratio of fat and protein mass fractions to skimmed milk residue, regardless of cow genotype and origin, ranged from 0.41–0.45 : 1 and 0.32–0.39 : 1. Against this background, the corresponding ratios are mostly optimal, and the milk of cows of all groups meets the consumer requirements.

The higher content of mass fractions of fat, protein, and lactose in milk led to an increase in its energy value in experimental cows with the BB genotype of Starbuck and Elevation lines by 17.20 MJ, or 6.4 % ($P < 0.01$), and by 7.53 MJ, or 2.7 %, and by 17.16 MJ, or 6.2 %, and by 12.05 MJ, or 4.3 %. The calculations of the energy value of milk show that animals with the AB genotype were inferior to those with the BB genotype, but exceeded those with the AA genotype of these lines by 9.67 MJ, or 3.6 %, and 5.11 MJ, or 1.2 %, respectively. Similarly, the dominant effect of the descendants of the Chifa and Valiant lines on cows with the AB genotype was observed in those with the AA genotype, causing an increase in energy concentration in milk by 3.13 MJ, or 1.2 %, and 8.53 MJ, or 3.1 %.

CONCLUSION

The findings of this study on the polymorphism of the kappa-casein gene in the Ukrainian Black-and-White dairy breed, taking into account its linear origin, may be used to improve breeding programs aimed at enhancing both milk yield and milk quality. First and foremost, this creates a scientifically sound basis for the widespread implementation of marker-assisted selection, more precise evaluation of animals' breeding value, earlier prediction of their productive and technological traits, optimization of breeding programs, and formation of highly productive herds with enhanced technological properties. The results of the study indicate the polymorphism of the experimental population of Ukrainian Black-and-White dairy cows, represented by three genotypes AA (63.1 %), AB (31.6 %), BB (5.3 %), with a predominance of the A allele frequency (0.79). However, when evaluating the correspondence of the actual frequency of their occurrence to the theoretically expected one at the locus that ensures the synthesis of kappa-casein, genetic equilibrium was maintained. The productive traits of cows with different kappa-casein genotypes are determined by the heredity of sires: cows with AA genotype of the Starbuck line have 5.8 and 12.3 % higher milk production than animals with AB and BB genotypes, while cows with BB genotype of the Elevation line have a higher content of protein mass fractions by 0.42 and 0.49 % and fat by 0.18 and 0.09 % compared to animals with AA and AB genotypes.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest: the authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Animal studies: the experiments conducted on animals do not contradict the current legislation of Ukraine (Article 26 of the Law of Ukraine 5456-VI of 16.10.2012 „On the Protection of Animals from Cruelty”) as amended as of 04.08.2017, and the „General Ethical Principles for Animal Experiments” adopted by the First National Congress on Bioethics (Kyiv, 2001) and international bioethical standards (materials of the IV European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Purposes (Strasbourg, 1985).

AUTHOR CONTRIBUTIONS

Conceptualization, [I.K.; A.P.; N.B.; N.P.] methodology, [I.K.; A.P.]; validation, [O.P.; K.R.; O.K.]; formal analysis [N.B.; A.P.]; investigation, [I.K.; I.P.]; data curation, [I.K.; A.P.; I.P.]; writing – original draft preparation, [I.K.; I.P.]; writing – review and editing, [O.P.; O.K.; N.B.]; visualization, [O.K.; K.R.; N.B.; O.P.]; supervision, [N.B.; K.R.]; project administration, [I.K.; A.P.]; funding acquisition, [I.K.; N.B.; A.P.; I.P.; O.K.; K.R.; O.P.].

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ПОЛІМОРФІЗМ ГЕНА КАППА-КАЗЕЇНУ ТА ЙОГО ЗВ'ЯЗОК З МОЛОЧНОЮ ПРОДУКТИВНІСТЮ І ЯКІСТЮ МОЛОКА КОРІВ РІЗНОГО ЛІНІЙНОГО ПОХОДЖЕННЯ

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Вступ. Ефективність молочного скотарства значною мірою зумовлюється підвищенням продуктивності корів і залежить від якості молока, яка значно погіршилася за останні роки. Метою дослідження було визначити поліморфізм гена каппа-казеїну та його зв'язок з молочною продуктивністю і якістю молока у корів різного походження.

Матеріали та методи. Геномну ДНК екстрагували з індивідуальних зразків біологічного матеріалу (зразки волосся з цибулинами), відібраного від корів, за допомогою комерційного набору реагентів "ДНК-сорб В" виробництва фірми

AmpliSens. Полімеразну ланцюгову реакцію проводили на ампліфікаторі Amply-4-2 (фірма Biokom), детекцію – за використання УФ-трансілюмінатора (фірма Biokom). Молочну продуктивність фіксували за результатами індивідуальних щомісячних контрольних доїнь корів.

Результати. Генетичний аналіз довів поліморфність локусу каппа-казеїну в піддослідного масиву корів української чорно-рябої молочної породи, що представлений трьома генотипами: AA (63,1 %), AB (31,6 %), BB (5,3 %) із переважанням частоти алеля A (0,79). Корови з генотипом AA лінії Старбака демонструють максимальну молочну продуктивність, перевершуючи тварин з генотипом AB на 361,6 кг, або 5,8 % на тлі вірогідно суттєвої переваги над представницями з генотипом BB – на 727,6 кг, або 12,3 % ($p < 0,01$). Водночас, особини із генотипом AB лінії Чіфа за рівнем надойв молока за лактацію переважали тварин із генотипом AA лише на 25,8 кг, або 0,4 %, тоді як у групи корів із генотипом AB лінії Валіанта відмінність за відповідним показником, навпаки, виявилася нижчою проти тварин із генотипом AA цієї ж лінії на 559,0 кг, або 9,2 %. У ході лабораторного аналізу з'ясовано, що корови з генотипом BB ліній Старбака і Елевейшна мали вищі значення вмісту в молоці масової частки жиру на 0,01 і 0,20 %, порівняно з тваринами із генотипом AB і AA лінії Старбака та на 0,09 і 0,18 % – лінії Елевейшна. Цим тваринам властиві й вищі значення масової частки білка на 0,39 і 0,25 % та 0,45 і 0,49 %.

Висновок. Результати досліджень вказують на поліморфність піддослідного масиву корів української чорно-рябої молочної породи, що представлений трьома генотипами AA (63,1 %), AB (31,6 %), BB (5,3 %) із переважанням частоти алеля A (0,79). Коровам-нащадкам із генотипом AA лінії Старбака властива вища на 5,8 і 12,3 % молочна продуктивність, ніж у тварин із генотипами AB і BB, тоді як коровам із генотипом BB лінії Елевейшна – більший вміст масових часток білка – на 0,42 і 0,49 % і жиру – на 0,18 і 0,09 % щодо тварин із генотипами AA і AB.

Ключові слова: поліморфізм, каппа-казеїн, корови, молоко, лінія