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RESEARCH ARTICLE
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Analysis and Characterization of the Amino Acid Composition of Milk Protein in First-Calf Cows of the Black-And-White and Holstein Breeds

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ABSTRACT

This study was conducted at the Akas Agricultural Company LLP in West Kazakhstan to evaluate the amino acid composition and technological properties of milk proteins in first-calf cows of the Black-and-White breed and Holstein cattle of German and Dutch origin, as well as their crossbreeds, in order to identify the most promising genotype and establish optimal directions for further breeding. Five groups of animals were kept under identical conditions, and milk samples were analyzed using thin-layer chromatography and microscopic methods. Significant genotype-related differences were revealed: milk of crossbreeds contained the highest levels of essential amino acids, particularly leucine, arginine, lysine, and phenylalanine, compared with pure Black-and-White cows. Holstein cows and their crossbreeds demonstrated improved protein profiles, with higher casein content and more favorable casein micelle structures. These characteristics enhanced the technological properties of milk, including coagulation time and suitability for dairy processing. The findings indicate that crossbreeding Black-and-White cows with Holsteins increases the nutritional and biological value of milk, making it more suitable for high-quality dairy production.

Keywords: Amino Acid Composition, Casein, First-Calf Cows; Holsteins of Dutch and German Breeding; Milk

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INTRODUCTION

Milk composition exhibits a high degree of variability and is affected by a group of different genetics, environment, and management factors such as breed, feeding system, herd management practices, lactation stage and seasonality (Belkhemas et al., 2021; Stepanova et al., 2021; Ryazanov et al., 2022; Nokusheva et al., 2023). The major components, such as protein, fat, and lactose, have a very important role in characterizing the nutritional and technological characteristics of milk (Butar-Butar et al., 2024; Myktybayeva et al., 2024), with protein from 2.8 to 3.6%, fat from 2.8 to 6%, and lactose from 4.5 to 4.8% in commercial milk (Esposito et al., 2014; Martini et al., 2016; Kulpiisova et al., 2024).

The amino acid composition of milk proteins is of particular significance, as it varies based on breed, diet, and management practices, thereby directly influencing the nutritional and technological quality of both milk and dairy products (Halavach, 2024). Genetic variation substantially contributes to the variability of milk protein fractions and amino acid composition, emphasizing the need for breed-specific evaluation to improve milk protein quality (Visentin et al., 2022; Maheshwari et al., 2024). Enhancing amino acid profiles not only increases the nutritional value and biological functionality of dairy products but also improves farm profitability (Sanjulián et al., 2025). Comparative studies of purebred and crossbred cows indicate that crossbreeding may improve the balance of essential amino acids and the overall biological value of milk proteins (Levina et al., 2021; An et al., 2025). In addition, Fourier-transform mid-infrared spectroscopy (FT-MIR) has proven effective for detecting phenotypic and

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regional differences in milk composition, allowing for high-throughput evaluation of amino acid and protein traits (Tiplady et al., 2022; Li et al., 2024). Furthermore, regional assessments demonstrate that environmental and management factors influence milk protein profiles, highlighting the importance of context-specific evaluations when selecting animals for breeding programs (Landi et al., 2021; Thum et al., 2023).

Despite the development of highly specialized breeding of beef cattle in Kazakhstan, challenges continue regarding the access of the population to high-quality dairy products (Kuandykova et al., 2024; Zhaksalykov et al., 2024). The improvement of dairy cattle breeding remains insufficient, and many breeds cannot meet the current standards for efficacy (Uskenov et al., 2023). Highly effective dairy animals with desirable adaptability, reproductive potential, and longevity are in short supply (Bauer et al., 2023). Due to the high import volume of foreign animals, especially Holstein cattle, it is also important to estimate their acclimatization, productivity, and gene potential in the framework of the production system in the Republic of Kazakhstan (Noori ALajwadi & Owaid, 2024). These studies should also include the determination of the best practices for their use (Buienbayeva et al., 2024; Kenzhebaev et al., 2024). In recent years, German and Dutch line Holstein cattle have been widely introduced (Karymsakov et al., 2025), for pure breeding and for crossbreeding programs with the indigenous Black-and-White cattle (Papusha et al., 2023; Traore et al., 2023). The employment of this gene pool source, which is deemed world-leading in dairy farming (Silvi et al., 2024; Utami et al., 2025), should enhance the production of high-quality and competitive dairy products in the Republic of Kazakhstan.

In this context, the goal of the current study is to characterize the profile of the amino acids as a significant pointer for the assessment of the quality of dairy products derived from the first-calf Black-and-White and Holstein animals of various lineages in West Kazakhstan and define the best genotypes for the development of prospective breeding activities. One of the current and prominent tasks for the agro-industrial complex of the Republic of Kazakhstan is giving the general public access to high-quality food products, which requires effective implementation of the entire set of existing resources (Karymsakov & Strekozov, 2021). In this context, the development of animal production, in part specifically in terms of milk and dairy products, plays a determining role (Shaikenova et al., 2021).

MATERIALS & METHODS

Location

The study was conducted at Akas Agricultural Company LLP in the West Kazakhstan region of Kazakhstan, and laboratory studies were conducted at the laboratories of Orenburg State Agrarian University and Zhangir Khan West Kazakhstan Agrarian and Technical University.

Experimental Design

To conduct the study, five groups of 12 first-calf cows each were formed according to the principle of analog groups, taking into account their origin, live weight, and physiological condition. Group 1 included purebred Black-and-White cows; Group 2 consisted of purebred Holsteins of German breeding; and Group 3 comprised purebred Holsteins of Dutch breeding. Group 4 consisted of half Holstein (German origin) × half Black-and-White cows, while Group 5 included half Holstein (Dutch origin)×half Black-and-White cows. Cows from different groups were kept in the same feeding and housing conditions throughout the study period, consistent with the conditions adopted by the farm and meeting the zootechnical requirements.

Amino Acid Analysis

The Amino Acid Analysis of milk proteins was analyzed using thin-layer chromatography (TLC), enabling the separation, identification, and semi-quantitative determination of individual amino acids based on their mobility and retention factors. Morphometric parameters of milk fat globules, including number, size distribution, and mean diameter, were assessed through high-resolution microscopic analysis with an 8-megapixel (MP) digital imaging system integrated with a 10.5-inch liquid crystal display (LCD) touchscreen (OPTO-EDU, Beijing, model A59.3521). Technological properties of milk were evaluated by examining its coagulation dynamics, curd formation, and protein stability using standardized milk fractionation and coagulation assays under controlled laboratory conditions. All analyses were conducted under consistent environmental conditions to ensure reproducibility and comparability of the data across different genotypes.

Experimental Stages

In the first stage, a comparative analysis of the levels of essential amino acids was performed, followed by an analysis of the levels of non-essential amino acids. Next, the biological value of milk proteins was assessed based on the amino acid index. Special attention was paid to studying the components of the milk protein of first-calf cows in different experimental groups. Then, the ratio of casein fractions was determined, which allowed for clarification of the protein complex's structure. The size and mass of casein micelles were measured, reflecting the physicochemical properties of milk. The final stages included an assessment of rennet coagulability and an analysis of the duration of the coagulation phase, which was of practical importance for determining the suitability of milk for cheese production.

Data Analysis

The data were processed using the method of variation statistics, aided by digital technology. The difference was considered significant at $P<0.05$, $P<0.01$, and $P<0.001$.

RESULTS AND DISCUSSION

The analysis of amino acid profiles in milk protein from first-calf animals of different genotypes showed a

substantial effect of genotype upon milk quality. Crossbred animals (groups 4 and 5) always had a larger concentration of essential amino acids, whereas Black-and-White animals (group 1) had the minimum levels; purebred Dutch and German Holsteins (groups 2 and 3) fell between these limits (P<0.05) (Table 1, Fig. 1). The essential amino acids, such as leucine, lysine, phenylalanine, and arginine, were found predominantly in higher amounts in Holsteins' and crossbreds' milk compared to Black-and-White animals, and crossbreds showed maximum superiority. Conversely, histidine, threonine, and valine were comparatively in lower quantities in all groups. This trend demonstrates the heterosis effect in crossbred animals, which was in accordance with prior work concerning protein composition in milk (Mode et al., 2023).

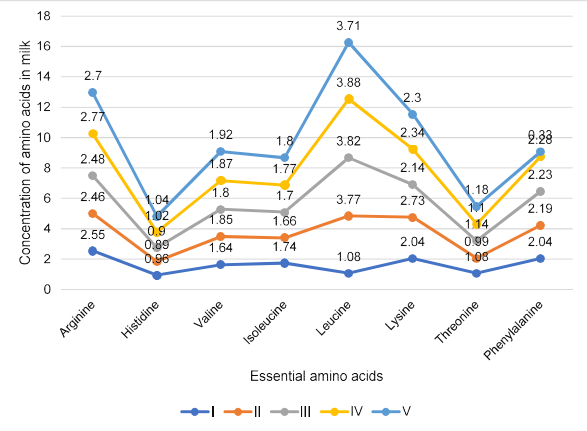

Fig. 1: Comparative analysis of essential amino acid levels.

In a similar manner, the total concentration of non-essential amino acids exhibited a comparable pattern: the lowest concentrations were observed in Black-and-White cows, intermediate concentrations in Holsteins, and the highest concentrations in crossbreeds (P<0.05) (Fig. 2). Among the non-essential amino acids, glutamic acid, proline, aspartic acid and serine were present in the highest quantities, whereas glycine, alanine, and tyrosine were found in lower amounts. Black-and-White cows demonstrated reduced levels of alanine, glycine, serine, and tyrosine when compared to crossbreeds (P<0.05), although they had elevated levels of glutamic acid and proline. The comprehensive amino acid index, which serves as an indicator of the biological value of milk proteins, was determined to be highest in crossbreeds and lowest in black-and-white cows (P<0.05), thereby corroborating the effect of heterosis. These findings align with earlier research suggesting that genetic variability plays a significant role in the disparities observed in milk protein fractions and amino acid composition, underscoring the necessity for breed-specific assessments aimed at enhancing milk protein quality (Visentin et al., 2022; Halavach, 2024).

The protein content, in particular casein, is a prime determining factor of the nutritional as well as technological properties of milk. Crossbred animals had maximum combined α- and β-casein fractions, whereas Holsteins and black-and-white animals had lower casein content (P<0.05) (Table 2, Fig. 3). Conversely, γ-casein was most concentrated in Holsteins, whereas crossbreed animals showed lower levels, reflecting casein composition variability based on genotype. Further, casein micelle dimensions as well as its mass were found to be affected by genotype: crossbreed animals had significantly higher micelle diameter and higher micelle mass in comparison to purebred Holsteins as well as Black-and-White animals, indicating an enhancement in technological characteristics of the milk (P<0.05) (Fig. 3). This excellence of crossbred animals in terms of micelle size (0.45–1.71%) and micelle mass (1.23–2.94%) is in tune with previous work relating to genotypes and breeds affecting casein micelle characteristics (Visentin et al., 2022; Saha et al., 2024; Solarczyk et al., 2024). Furthermore, associate research work has shown that crossbred animals, in general, produce milk endowed with better amino acid composition, hence better nutritional value as well as enhanced technological characteristics, in conformity with our results (Levina et al., 2021; Li et al., 2024).

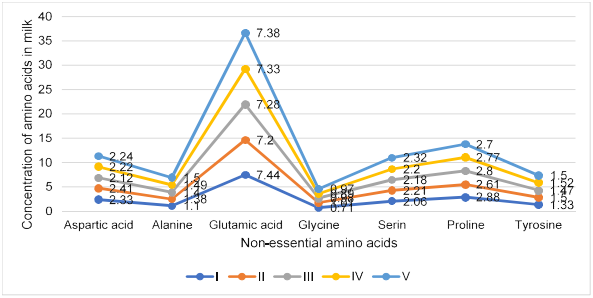

Fig. 2: Comparative analysis of the levels of non-essential amino acids.

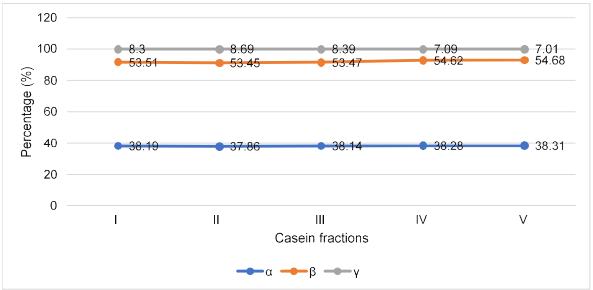

Fig. 3: Ratio of casein fractions in milk (%).

Coagulation characteristics of milk, essential in cheese and cottage cheese production, differed in accordance with genotype. The greatest coagulation time in total, as well as gelation phase, was exhibited in Holstein cows of German and Dutch breeding lines, whereas crossbred animals exhibited minimum time, and black-and-white cows were between these two categories (P<0.05) (Fig. 4). The coagulation time in Holsteins was greater relative to black-and-white as well as crossbred animals, being 0.48% to 3.53% greater, in accordance with prior research that indicates significant roles of casein genotypes in rennet coagulation time (RCT), as well as in curd formation (Gai et al., 2023; Lavon et al., 2023; Sanjayaranj et al., 2023). These results are in accordance with prior inquiries that

Table 1: Components of the milk protein of the first-calf cows of the experimental groups

Indicator	Group									
	1		2		3		4		5	
	X±Sx	Cv	X±Sx	Cv	X±Sx	Cv	X±Sx	Cv	X±Sx	Cv
Mass content of protein	3.19±0.027^{b}	2.61	3.14±0.016^{c}	1.75	3.17±0.034^{b}	3.17	3.26±0.018^{a}	2.15	3.27±0.026^{a}	2.12
including casein	2.63±0.012^{b}	1.55	2.58±0.008^{c}	1.03	2.61±0.019^{b}	2.38	2.69±0.008^{a}	0.92	2.69±0.004^{a}	0.57
α	1.00±0.007^{b}	2.24	0.98±0.001^{c}	0.40	1.00±0.008^{b}	2.68	1.03±0.005^{a}	1.85	1.03±0.006^{a}	2.26
β	1.41±0.020^{b}	4.75	1.38±0.008^{c}	2.20	1.40±0.020^{b}	4.84	1.47±0.007^{a}	1.84	1.47±0.003^{a}	0.71
γ	0.22±0.014^{a}	26.32	0.22±0.010^{a}	16.80	0.22±0.011^{a}	19.37	0.19±0.007^{b}	15.99	0.19±0.004^{b}	6.59
whey proteins (albumin and globulin)	0.57±0.019^{b}	12.53	0.54±0.011^{c}	7.63	0.56±0.014^{b}	9.56	0.60±0.008^{a}	4.74	0.61±0.007^{a}	4.05

Note: Values are presented as mean ± standard error (X±Sx); Cv — coefficient of variation (%); Different superscript letters in the same row (a, b, c) indicate statistically significant differences between groups at P<0.05; Comparison is made within rows (between groups for the same indicator); Values sharing the same letter are not significantly different; different letters indicate significant differences.

Table 2: Casein micelle size and weight in the milk of the first-calf cows of the experimental groups

Indicator	Group									
	1		2		3		4		5	
	X±Sx	Cv	X±Sx	Cv	X±Sx	Cv	X±Sx	Cv	X±Sx	Cv
Casein micelle size, °A	625.40±3.174^{c}	1.96	618.80±4.099d	2.37	622.60±1.351c	0.83	628.20±1.194^{a}	0.74	629.40±1.304^{a}	0.76
Micelle weight, mln units of molecular weight (MW)	129.60±2.387^{b}	6.96	127.80±1.084c	2.79	128.10±0.942c	2.83	131.20±1.519^{a}	4.60	131.56±1.378^{a}	4.22

Note: Values are presented as mean ± standard error (X±Sx); Cv — coefficient of variation (%); Different superscript letters in the same row (a, b, c) indicate statistically significant differences between groups at P<0.05; Comparison is made within rows (between groups for the same indicator); Values sharing the same letter are not significantly different; different letters indicate significant differences.

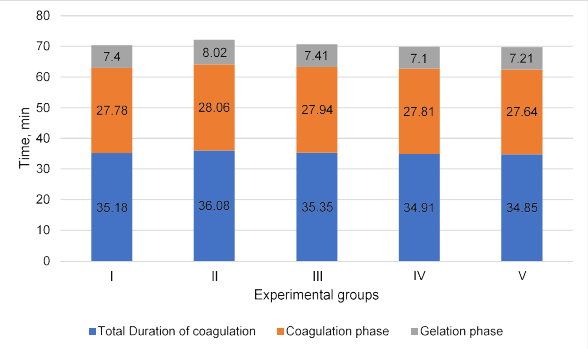

Fig. 4: Rennet coagulability of milk from first-calf cows in the experimental groups.

presumed Holsteins would have greater coagulation times in view of greater levels of γ-casein, whereas crossbred animals are frequently found to possess desirable technological attributes in favoring cheese production (Panchal et al., 2021; Żbik et al., 2024).

Compared to global data, our results are in line with prior studies demonstrating that crossbreeding improves essential amino acid balance as well as general biological value in milk proteins (Levina et al., 2021; Nayik et al., 2024). Additionally, several environmental as well as management aspects, such as feeding regimens as well as herd keeping, are known to affect milk protein composition (Belkhemas et al., 2021; Stepanova et al., 2021; Ryazanov et al., 2022). Nevertheless, irrespective of said aspects, genetic effect remained a main factor in our research, showing that crossbreeding local Black-and-White varieties of domestic livestock with imported Holsteins is a potential method for improving quality in Kazakhstan's milk.

Integrated results of amino acid composition, casein fraction composition, micelle size and weight, and coagulation of milk confirm the better nutritional and technological properties of crossbred cow's milk. Accordingly, crossbred first-calf animals contained most essential and non-essential amino acids (16.98–17.03g/kg), and their amino acid index was 3.37–5.75% greater than in purebred animals (P<0.05), evidencing heterosis expression. The minimum content of amino acids was found in Black-and-White animals (15.60g/kg), and in purebred Holsteins, it was in an intermediary position (16.04–16.21g/kg). The results are in agreement with data describing the superiority of crossbred animals for milk production in terms of greater amino acid composition and higher casein content (Halavach, 2024; Myktybayeva et al., 2024).

The technological properties of milk, as defined by casein micelle characteristics and coagulation properties, once again validate crossbreed superiority in dairy farming. This supports the importance of adopting crossbreeding measures to improve quality in milk and processing performance in Kazakhstan, thus addressing national objectives in expanding dairy output (Kozhanov et al., 2024; Mukhametzhanova et al., 2024; Belgibayeva, 2025).

Overall, crossbreeding Black-and-White breeding animals with Holsteins significantly improves the biological value and technological quality of milk, mainly by augmenting essential and non-essential amino acids, achieving an optimal casein fraction composition, producing larger, higher-density micelles, and exhibiting desirable coagulation properties. Purebred Holsteins retain a few Holstein advantages in γ-casein content and longer coagulation times, whereas Black-and-White animals have generally inferior quality milk protein. These results confirm the relevance of genotype as well as heterosis in dictating milk composition and quality for high-grade dairy farming.

Conclusion

The results of the analysis of the amino acid composition of milk of Black-and-White cattle compared to Holsteins of different breeding allow us to conclude that the content of dry matter in the milk of crossbreeds in comparison with animals of the maternal breed differed by 0.12-0.19%, compared to the animals of the paternal breed by 0.17-0.34%, and the mass fraction of fat differed, respectively, by 0.05-0.08% and 0.07-0.14%, density by 0.07-0.12Å and by 0.14-0.30Å, and energy value by 3.48-

5.24kJ (1.12-1.69%) and 4.73-9.26kJ (1.53-3.02%). Additionally, the amino acid composition of crossbreed cow milk improved, as indicated by an increase in the content of essential amino acids in milk protein.

DECLARATIONS

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Conflict of Interest: The authors declare no conflict of interest.

Data Availability: Data available from the corresponding authors by request.

Ethics Statement: All animal procedures were carried out in accordance with the Guidelines for Working with Laboratory (Experimental) Animals in Preclinical (Non-Clinical) Studies of the Republic of Kazakhstan by Recommendation No. 33 of the Board of the Eurasian Economic Commission dated November 14, 2023.

Author's Contribution: BK and YS designed the study. BS (Berik Satybaev) and IZh organized the experimental work and collected samples. BY and BS (Bekzhassar Sidikhov) performed laboratory analyses and data processing. KM contributed to statistical analysis and interpretation of results. DB prepared the initial draft of the manuscript. NY and FZ supervised the project and critically revised the manuscript. All authors read and approved the final version.

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