Сәкен Сейфуллин атындағы Қазақ агротехникалық зерттеу университетінің Ғылым жаршысы: пәнаралық = Вестник науки Казахского агротехнического исследовательского университета имени Сакена Сейфуллина: междисциплинарный. – Астана: С. Сейфуллин атындағы Қазақ агротехникалық зерттеу университеті, 2025. -№ 1 (124). - P. 124-132. - ISSN 2710-3757, ISSN 2079-939X

doi.org/ 10.51452/kazatu.2025.1(124).1856
UDC 636.082.12 **Research article**

Genetic structure of Auliekol cattle breed using 50K SNP BeadChip

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Received: 31-01-2025 **Accepted:** 27-03-2025 **Published:** 31-03-2025

Abstract

Background and Aim. The Auliekol cattle breed is a meat-producing breed, predominantly found in Kazakhstan. The breed was developed from 1960 to 1990 by crossing the Kazakh Whiteheaded breed with Charolais and Aberdeen Angus bulls. The aim of this study was to analyze the genetic and population structure using a genotyping assay, and examining the relationship between the ancestral breeds.

Materials and Methods. DNA was extracted from 48 blood samples of the Auliekol breed and genotyped using Bovine SNP 50K BeadChip. Genetic distances were calculated using Nei's method based on SNP data in R.Principal Component Analysis (PCA) was performed to explore the population structure and genetic stratification using PLINK.

Results. According to Nei's genetic distance, Charolais (0.0046) and Kazakh Whiteheaded (0.00693) are genetically closest to the Auliekol breed, whereas the Kazakh breed from Russia (0.0113) shows greater genetic differentiation, and Angus (0.024) is the most distant. The principal component analysis (PCA) demonstrated that Auliekol breed has a distinct genetic structure separate from European cattle breeds. The analysis also showed the hybrid origin of the breed and the influence of the Kazakh Whiteheaded and European breeds on the formation of the traits of the Auliekol breed.

Conclusion. The findings from the structural analysis contribute to the development of a system of breeding records and preservation of the Auliekol breed of meat productivity in Kazakhstan.

Keywords: Auliekol cattle breed; genotyping; 50K SNP; population structure; genetic diversity.

Introduction

Cattle breeding for meat production is a significant sector in animal husbandry, aimed at providing humanity with meat and meat products. Cattle domestication began around 10,000 years ago, marking a significant achievement in agriculture. With technological advancements, various cattle breeds have been selectively bred for specific productivity traits(meat and dairy) and adaptability to diverse environmental conditions. The Angus beef breed is known for its high meat quality, excellent marbling score and calving ease [1]. According to *McTavish* et al. [2], the genetic background of the Angus breed, along with other European breeds, has played a crucial role in shaping the development of cattle breeds such as the Auliekol in Kazakhstan. The contribution of the Angus breed to Auliekol cattle was particularly important for meat quality, ensuring a tender texture and marbled composition [2]. The Charolais breed, originating from France, is another important contributor to the Auliekol breed. Charolais cattle are known for their excellent muscle mass, large frame, and fast growth rate. They are often used in crossbreeding programs to enhance the size and meat yield of other cattle breeds. Charolais bulls are highly regarded for producing offspring with good muscle development, which translates into higher-quality meat with greater yields. The Charolais breed is primarily white or cream-coloured and has significantly influenced the development of beef cattle worldwide [2]. The Kazakh Whiteheaded breed is known for its adaptability to the harsh climate conditions of Kazakhstan, making it an ideal foundation breed for developing cattle that can thrive in the country's diverse environments [3 ,4].

The Auliekol breed has thus inherited valuable traits from all three parent breeds: adaptability to Kazakhstan's climate from the Kazakh Whiteheaded, muscle mass and body structure from the Charolais, and meat quality and tenderness from the Angus [3]. The Auliekol breed is particularly well adapted to the steppe and semi-steppe regions of Kazakhstan. It is known for its high meat productivity and strong adaptability to the local environment (Figure 1).

Figure 1 – Auliekol breed typical animals (by Ussenbekov E.S)

The Auliekol breed is one of the leading meat-producing cattle breeds in Kazakhstan. In the 1960s, breeders initiated crossbreeding of Charolais and Aberdeen Angus bulls with Kazakh Whiteheaded cows, which ultimately led to the establishment of the Auliekol breed in the 1990s [5]. All three breeds contributed to the Auliekol characteristics, combining their strongest qualities. Over 70% of Auliekol cattle are polled, which is one of the breed's defining characteristics. Bulls and cows are light grey. The breed has distinctive external characteristics: a strong skeleton, large head, short muscular neck, height at the withers (1.3 m for cows and 1.4 m for bulls), chest circumference (2.45 m), thick short hair, skin with five layers (compared to only three in other breeds), and significant body weight (males weighing 950-1.200 kg, females 550-700 kg). It is known for its resilience and strong immunity [6,7]. Animals swiftly and readily adapt to the local environment, showing minimal sensitivity to temperature and weather variations [7].The main advantages of the Auliekol breed include its adaptability to the climate of Kazakhstan, ability to consume hay made from grasses that other breeds usually cannot, ease of grazing cows and bulls, strong immunity, rapid weight gain in, and high-quality meat. The Auliekol breed develops and matures quickly. The meat from the Auliekol breed is similar to that of Angus due its marbled composition. There are no fat accumulations around the muscular tissue; however, thin layers of fat are present within the meat fibers [8].

Genetic analysis of three cattle breeds in Kazakhstan (Alatau, Kazakh Whiteheaded, and Auliekol) using DNA fingerprinting revealed that the genetic distance between the Kazakh Whiteheaded and Auliekol breeds was the smallest (D =0.025). The Kazakh Whiteheaded breed exhibited the highest heterozygosity (H =0.54) compared to the Auliekol breed (H =0.38), confirming its greater genetic variability [9]. Microsatellite locus analysis confirmed the relationship between the Kazakh Whiteheaded breed with Hereford cattle, attributed to the use of Hereford bulls in its development [10,11]. Whole-genome genotyping data using single nucleotide polymorphisms (SNPs) showed that the Kazakh Whiteheaded breed clustered with Herefords in principal component analysis (PCA), as well as in structural, and phylogenetic analyses [12, 13, 14]. The Kazakh Whiteheaded breed retained a proportion of Turano-Mongolian genetic components from local cattle and the Kalmyk breed [13]. The Auliekol breed exhibited heterogeneity and formed its own distinct cluster in PCA and ADMIXTURE analysis [14]. Genetic analysis identified selection signatures in the Kazakh Whiteheaded breed in genes of coat color (*KIT, KITLG*, and *EDN3*), growth (*LCORL-NCAPG*), milk fat content 0(*DGAT1*), and protein content (FKBP2) [12,13]. The Kazakh Whiteheaded breed, homozygous for *CAPN1* (CC) and *GH* (VV), exhibited superior productive traits, including higher slaughter weight and increased milk yield [15].

This study aims to describe the genetic structure using 50K SNP BeadChip of the Auliekol breed and the breeds involved in its formation, such as Angus, Charolais, and Kazakh Whiteheaded cattle.

Materials and Methods

The research material consisted of blood samples (48 samples) from the Auliekol breed. Of these: 15 samples (14 from cows and 1 from a bull), were obtained from the Parasat Agrofarm in the Kostanay region. 11 blood samples (10 cows and 1 bull) were taken from the Krasny Kordon farm in Kostanay region. 13 blood samples (4 cows and 9 bulls) were obtained from the InsPEK LLP in Kostanay region. 9 blood samples (bulls) were obtained at the *Zhunusov A.B.* farm in the Almaty region.

Genomic DNA was isolated using the traditional phenol-chloroform extraction method [16]. For DNA extraction, 400 μL of buffer (100 mM Tris, 20 mM EDTA, 10 mM NaCl, pH 8.0) was added to 100 μL of blood. Then, 5 μL of proteinase K (20 mg/mL) and 25 μl of 10% SDS were added. The mixture was incubated for 3 hours at 56°C. DNA was purified twice with ethanol and then eluted in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.4). The Auliekol breed DNA was genotyped using the BovineSNP50 v.3 SNP array (Illumina, USA) at Miratorg-Genetika LLC.

Genetic distances were calculated using Nei's method based on SNP data in R [17] to assess genetic similarity among breeds. Principal Component Analysis (PCA) was performed in PLINK [18] to investigate population structure and genetic stratification. The process involved preparing the input data in binary format (.bed, .bim, .fam) and performing quality control to filter SNPs and samples with high genotype drop rate, low minor allele frequency (MAF), or Hardy-Weinberg disequilibrium. PCA was performed on the pruned dataset using the --pca command, which outputs eigenvalues (.eigenval) and eigenvectors (.eigenvec). The results were visualized by plotting the first two principal components (PC1 and PC2) using the ggplot2 package in R [17] to identify clustering patterns and assess population structure.

Results and Discussion

DNA was extracted from 48 blood samples was quantified using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA). The average DNA concentration obtained from the samples was approximately 448.93 ng/μL, demonstrating the success of the isolation process and yielding sufficient DNA for downstream molecular applications such as genotyping. DNA concentrations ranged from a minimum of 73.9 ng/μL to a maximum of 1528.4 ng/μL, indicating variability among samples (Figure 2).

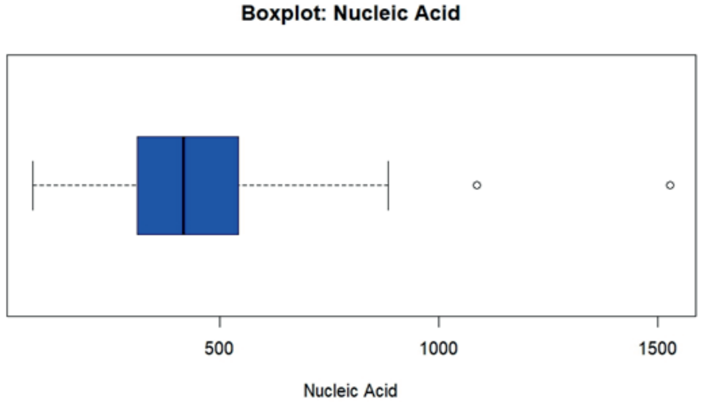

Figure 2– Boxplot of DNA Concentration Distribution from Extraction Samples

This range suggests effective extraction for most samples, although a few may require optimization to improve consistency. Despite this variability, the overall yield confirms the effectiveness of the extraction protocol for high-throughput and precision genetic analyses. Such a wide range of DNA concentrations could be attributed to differences in sample quality, source material, or handling during extraction. Further standardization may help reduce this variability and ensure uniform results across samples.

SNP genotyping data quality control was performed using PLINK v1.9 (13 February 2023). The filtering criteria included the removal of SNPs with more than 5% missing genotypes (--geno 0.05), ensuring that only markers with reliable genotyping quality were retained. To eliminate low-informative variants, SNPs with a minor allele frequency (MAF) below 5% (--maf 0.05) were excluded. Additionally, markers showing significant deviations from Hardy-Weinberg equilibrium (p-value < 1e-6, --hwe 1e-6 midp) were filtered out to avoid potential technical artifacts or population structure effects. After applying these quality control measures, 25,295 variants and 186 samples passed the filters.

Based on the calculated Nei's genetic distance values, the following conclusions can be drawn:

Charolais has the smallest genetic distance to the Auliekol breed (0.0046), indicating a high genetic similarity. Kazakh Whiteheaded (KWH_KZ) is also relatively close (0.00693), which is expected since both breeds are bred in Kazakhstan and may share common ancestors. Kazakh (Kazakhskaya) breed from Russia has a greater distance (0.0113), suggesting some genetic differentiation between cattle populations in Kazakhstan and Russia. Angus exhibits the largest genetic distance (0.024), from the Auliekol breed, highlighting significant genetic divergence.

The Principal Component Analysis (PCA) plot illustrates the genetic distribution of the Auliekol breed (ALEKZ), the Kazakh Whiteheaded breed (KWHKZ), and multiple European breeds. The data reflect the unique genetic profiles of these breeds and their contributions to cattle breeding in Kazakhstan (Figure 3).

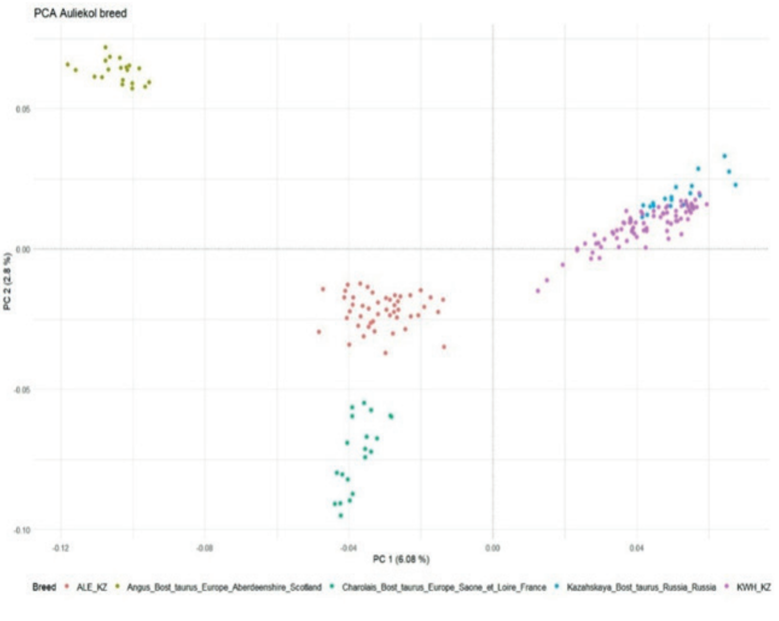

Figure 3 – Principal Component Analysis (PCA) of Cattle Genetic Data: ALEKZ – Auliekol breed; KHWKZ – Kazakh Whiteheaded breed; Kazakhskaya Bostaurus Russia – Kazakh cattle (unnamed breed) from the Russian Federation; Angus Bos taurus Europe Aberdeenshire - Angus breed from Scotland (UK); Charolais Bos Taurus Europe Saone-et-Loire – Charolais breed from France

The Auliekol breed (ALEKZ), represented in red on the PCA plot, is one of Kazakhstan's most significant beef cattle breeds. Its development began in the 1960s through crossbreeding Charolais and Aberdeen Angus bulls with the Kazakh Whiteheaded breed (KWHKZ). This selective breeding aimed to create animals with superior meat quality, high growth rates, and adaptability to Kazakhstan's steppe and semi-desert climates (Figure 3).

By the 1990s, the Auliekol breed was fully established, becoming a cornerstone of the country's beef cattle industry. The Kazakh Whiteheaded breed (KWHKZ), marked in blue on the PCA plot, is one of Kazakhstan's oldest and most widespread cattle breeds. It was developed to meet the demand for meat and dairy products under challenging climatic conditions. The genetic position of KWHKZ on the PCA plot highlights its distinct characteristics and structure, which is also observed in previous studies [11, 12, 14]. Its proximity to European breeds, such as Charolais and Aberdeen Angus, reflects its foundational role in the creation of the Auliekol breed.

The KWHKZ provided traits such as efficient weight gain on pastures and adaptability to local conditions, making it a critical component in forming a productive and resilient hybrid breed. The PCA analysis reveals clear genetic differentiation among the breeds. The Auliekol breed forms a distinct cluster, underscoring its genetic uniqueness due to targeted breeding efforts. Similar results were obtained in a study conducted in 2019 and 2022 using SNP markers [12, 14]. Using Bovine 50K BeadChip and GGP Bovine 150K SNP markers, studies were conducted and analyzed that the Kazakh Whiteheaded and Auliekol cattle breeds have their own genetic structure and are different from European and Russian breeds [14, 19, 20]. This is also shown by our analysis and further confirms the unique genetic composition. According to the PCA results, the Kazakh White-Headed breed is genetically closer to European breeds compared to the Auliekol breed, which indicates a greater role in selection and crossing programs. It is also evident that the Charolais and Angus breeds, presented as separate clusters, are genetically distant from the local Kazakh breeds. However, they have contributed to the Auliekol breed, particularly in terms of good meat quality, high productivity and large breed size [21, 22]. The Kazakh Whiteheaded is located closer to the Auliekol breed in the PCA plot, reflecting to its role as a maternal lineage and its contribution to adaptability traits which are crucial in the development of this breed [23, 24]. In contrast, the Kazakh breed from Russia forms a genetically distinct cluster, suggesting a limited influence on the genetic composition of the Auliekol breed.

Conclusion

The close relationship between the Auliekol and Kazakh Whiteheaded breeds is clearly visible in the PCA plot and genetic distance calculations, demonstrating their fundamental role in the development of the Auliekol breed. This highlights the significant contribution of KWHKZ to the adaptability and growth characteristics of the Auliekol breed. The contribution of the Charolais and Aberdeen Angus breeds is also evident, as they form separate genetic clusters while still having a notable impact on the genetic composition of the Auliekol breed.

In conclusion, the observed genetic differentiation between Auliekol, Kazakh Whiteheaded and European breeds confirms the effectiveness of targeted crossbreeding and selection programs in Kazakhstan. The unique genetic structure of the Auliekol breed reflects Kazakhstan's strategic efforts to enhance livestock production through selective breeding programs. The findings of this study provide valuable insights for future breeding programs aimed at enhancing productivity, adaptability, and meat quality in Kazakhstan's cattle industry. Further research into the genetic diversity and population structure of other local breeds will be instrumental in optimizing breeding strategies and ensuring sustainable livestock production.

Authors' contributions

AK, TB, BR and DK: conceptualized and designed the study, conducted a comprehensive literature search, analyzed the collected data, and prepared the manuscript. DL and YU: performed final editing and proofreading of the manuscript. All authors read, reviewed, and approved the final version of the manuscript.

Funding information

The work was performed within the framework of a grant-funded project for scientific and (or) scientific and technical research for 2023–2025 from the Ministry of Science and Higher Education of the Republic of Kazakhstan (Grant No. AP19674808: “Creation of genetic passports and study of genetics of local Kazakhstani cattle breeds using genome resequencing”).

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50K SNP BeadChip көмегімен Әулиекөл мал тұқымының генетикалық құрылымы

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Түйін

Алғышарттар мен мақсат. Ірі қараның Әулиекөл тұқымы – Қазақстанда ең көп таралған етті мал тұқымы. Тұқым 1960-1990 жылдары аралығында қазақтың ақбас тұқымын Шароле және Абердин-ангус бұқаларымен шағылыстыру арқылы жасалған. Зерттеудің мақсаты генотиптік талдауды қолдана отырып, генетикалық және популяциялық құрылым, сондай-ақ тұқымдар арасындағы байланыстар талданды.

Материалдар мен әдістер. Әулиекөл тұқымының 48 қан үлгісінен ДНҚ бөлініп алынып, Bovine SNP 50K BeadChip микрочипі арқылы генотиптелді. Генетикалық қашықтық R. SNP деректеріне негізделген Nei әдісімен есептелді. Негізгі компоненттер талдауы (PCA) популяция құрылымын және генетикалық стратификацияны зерттеу үшін PLINK бағдарламасы арқылы жүргізілді.

Нәтижелер. Нейдің генетикалық қашықтығы бойынша Әулиекөл тұқымына ең жақын Шароле (0,0046) және Қазақ Ақбас (0,00693) болса, Ресейден келген Қазақ тұқымы (0,0113) біршама генетикалық дифференциацияны көрсетеді, ал Ангус (0,024) ең алыс. Негізгі компоненттік талдау (PCA) Әулиекөл тұқымының Европалық ірі қара тұқымдарынан ерекше, бөлек генетикалық құрылымы бар екенін көрсетті. Талдау нәтижелері тұқымның буданды шығу тегін және Әулиекөл тұқымының белгілерінің қалыптасуына қазақтың Ақбас және Европалық тұқымдарының әсерін көрсетті.

Қорытынды. Құрылымдық талдаудан кейін алынған нәтижелер асыл тұқымды есепке алу жүйесін дамытуға және Әулиекөл тұқымын Қазақстандағы ет өнімділігін сақтапқалуға ықпал етеді.

Кілт сөздер: Әулиекөл ірі қара мал тұқымы; генотиптеу; 50К SNP; популяция құрылымы; генетикалық әртүрлілік.

Генетическая структура аулиекольской породы крупного рогатого скота с использованием 50K SNP BeadChip

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Аннотация

Предпосылки и цель. Аулиекольская порода крупного рогатого скота – наиболее распространённая в Казахстане мясная порода крупного рогатого скота. Порода была выведена в 1960-1990 годах путем скрещивания Казахской белоголовой породы с быками Шаролезской и Абердин-ангусской пород. Целью данного исследования является анализ генетической и популяционной структуры с использованием панели генотипирования, а также изучение взаимосвязи между предковыми породами.

Материалы и методы. ДНК была выделена из 48 образцов крови Аулиекольской породы и прогенотипированы с использованием микрочипа Bovine SNP 50K BeadChip. Генетические расстояния рассчитывались с использованием метода Nei на основе данных SNP в R. Анализ главных компонент (PCA) был использован для изучения популяционной структуры и генетической стратификации с помощью программы PLINK.

Результаты. На основании генетической дистанции Nei наиболее близки к Аулиекольской породе породы Шароле (0,0046) и Казахская белоголовая (0,00693), в то время как Казахская порода из России (0,0113) демонстрирует некоторую генетическую дифференциацию, а наиболее далека от нее - Ангус (0,024). Изучение главного компонентного анализа (PCA) показало, что Аулиекольская порода имеет отчётливую отдельную генетическую структуру от Европейских пород крупного рогатого скота. Анализ также показал гибридное происхождение породы и влияние Казахской белоголовой и Европейской пород на формирование признаков Аулиекольской породы.

Заключение. Полученные результаты структурного анализа способствуют развитию системы племенного учета и сохранению Аулиекольской породы мясной продуктивности в Казахстане.

Ключевые слова: Аулиекольская порода крупного рогатого скота; генотипирование; 50K SNP; структура популяции; генетическое разнообразие.