Research Article

Prevalence of A1 and A2 Beta-Casein Variants in Bushuev Cattle of Uzbekistan

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Abstract | Milk containing the A1 variant of β-casein is considered to be harmful due to the formation of β-casomorphin-7 peptide. The A1/A2 polymorphism in *CSN2* is therefore of interest for breeding strategies that aim to increase A2 milk frequency. Blood samples were collected from 96 Bushuev cattle in Uzbekistan. Genomic DNA was extracted and genotyped by real-time PCR with SYBR Green detection using allele-specific primers. The A1 allele frequency was 0.35 and A2 - 0.65. Genotype distribution was predominantly A1A2 (54%), followed by A2A2 (38%) and A1A1 (8%). Observed and expected heterozygosity values were 0.54 and 0.45, respectively, with the inbreeding index (Fis) calculated as -0.2. The negative Fis value indicates an excess of heterozygotes in the population. The A2 allele frequency in the Bushuev population is similar to that in other cattle breeds, including Kangayem x Holstein Friesian and Holstein-Zebu crossbred cattle, as well as Frieswal, Vrindvani and Girolando breeds. While the Bushuev cattle population shows a high degree of actual heterozygosity, the lower expected heterozygosity suggests that underlying population processes - such as inbreeding, outbreeding, assortative mating, balancing selection, and other molecular genetics and evolutionary mechanisms-might be influencing the population's genetic structure and genetic diversity. Since A1 variant is potentially harmful for human health, it is essential to conduct screening for A1/A2 alleles among cattle, particularly in breeding bulls and heifers. The genotypic data obtained in this study could be used for the development of breeding programs aimed at increasing the frequency of the desirable A2 allele in future generation of Bushuev breed. In this way, it will be possible to purposefully increase the production of A2 milk, which is considered safer for human health.

Keywords | β-casein (*CSN2*) gene, A1/A2 polymorphism, Bushuev cattle breed, Allele frequency, Genotype frequency, Allele-specific primers, Real-time PCR

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INTRODUCTION

The A2 milk brand, derived from the milk of cows that produce only the A2 type of β-casein protein, also known as original milk, is gaining increasing popularity worldwide. Initially, only the A2 variant, possessing the A2A2 genotype of the β-casein gene, existed. The A1 variant arose as a result of a mutation that occurred during the selective breeding of high milk-yielding cows. These mutations have led to the formation of 13 variants of the β-casein gene, namely A1, A2, A3, A4, B, C, D, E, F, H1, H2, I, and G (Roginsky, 2003). The A1 and A2 variants

differ structurally, with the A1 variant contains histidine at position 67, whereas the A2 variant contains proline at the same position (Sharma *et al*., 2013). The 67th codon of the β-casein gene is polymorphic; the CCT codon encoding proline in the A2 variant is replaced by the CAT codon encoding histidine in the A1 variant (Ganguly *et al*., 2013). This structural difference between the A1 and A2 β-casein proteins results in differential digestion by the intestinal mucosa. During the digestion of A1 β-casein (involving pepsin, pancreatic elastase, and other enzymes), the bond between histidine and the adjacent amino acid is cleaved, producing a bioactive peptide known as beta-casomorphin-7 (BCM-7) (Lien *et al*., 1992). BCM-7 exhibits opioid-like properties and can bind to opioid receptors and nerve cells. The A1 β-casein variant is considered potentially harmful to human health. Health issues associated with the A1 milk variant include atherosclerosis, type 1 diabetes (Kamiński *et al*., 2007), coronary heart disease (McLachlan, 2001; Laugesen and Elliott, 2003), arteriosclerosis (Tailford *et al*., 2003), and sudden infant death syndrome (Sun *et al*., 2003). Additionally, it may also be linked to neurological disorders (Woodford, 2006). It should be noted that A1 beta-casein has been associated in some studies with adverse health outcomes; however, its impact remains controversial and is a topic of ongoing scientific discussion (Parashar and Saini, 2015).

The A1 variant of the β-casein gene is predominantly found in the milk of European, American, Australian, and New Zealand cattle breeds. Holstein and Ayrshire breeds produce a higher proportion of A1 type milk compared to other breeds. In India, local cattle breeds are crossbred with foreign breeds possessing higher milk yield to increase milk production.

Extensive crossbreeding with Holstein-Friesian (HF) and Jersey breeds has significantly increased the proportion of crossbred cattle in Uzbekistan, potentially leading to a rise in the frequency of the A1 allele in Uzbekistan cattle populations. This shift is of particular concern due to the potential health risks associated with the A1 variant of β-casein, underscoring the importance of genotyping breeding bulls to safeguard public health.

Moreover, excessive crossbreeding with Holsteins can lead to recombination loss, where a favorable combination of linked genes is broken apart by meiosis, reducing the benefits of heterosis in advanced generations. These factors highlight the critical need to manage existing genetic variability, particularly the A1/A2 β-casein alleles, both to preserve desirable milk protein profiles and to design future breeding strategies.

The Bushuev breed is a synthetic dairy breed developed in Uzbekistan through crossbreeding local Zebu-type cattle with European taurine breeds.

According to Food and Agriculture Organization of the United Nations, the Bushuev is indigenous dairy cattle breed of Uzbekistan, named after Mikhail Mikhailovich Bushuev (1876-1936), who initiated its selection at the "Golodnostepnaya" experimental station between 1905 and 1918. The breed was developed through crossbreeding local Zebu cows (Adilov *et al*., 2025) with Dutch and Brown Swiss sires, with additional genetic input from Simmental stock; superior hybrids were subsequently bred inter se to stabilize desired traits. Bushuev dairy cattle demonstrate strong adaptation to hot, arid environments and exhibit resistance to haemoparasitic infections. Their milk has a high fat content (4% and above) and protein (3.7–4.2%).

This crossbreeding created a breed with remarkable resistance to hot climates and blood parasite diseases, a strong constitution, and a distinctive ermine-colored coat. The breed's genetic uniqueness also lies in its ability to reflect solar radiation with its white coat while its pigmented skin aids heat emission, alongside its inherent resistance to haemoparasitic infections from its zebu ancestry.

The primary objective of this study was to determine the distribution of A1 and A2 alleles in the population of Bushuev cattle breed in Uzbekistan. This research aimed at the detailed genetic characterization of the A1/A2 β-casein gene polymorphism in the Bushuev cattle breed of Uzbekistan, a unique indigenous breed with mixed ancestry from local zebu and European taurine cattle. By investigating the A1/A2 allele and genotype frequencies of the *CSN2* gene in Bushuev breed, the research could reveal valuable insights into the current genetic structure of the breed, and will help to provide a genetic foundation for developing sustainable breeding strategies and conservation efforts for this endangered local breed.

MATERIALS AND METHODS

Sample collection

This study was conducted in 2024–2025 at the Laboratory of Animal Genetics, Institute of Genetics, Academy of Sciences of the Republic of Uzbekistan. A total of 96 blood samples from Bushuev breed cattle were collected from a farm in Orol Nematlari village, Syrdarya district, Sirdaryo region. Using sterile needles, 10 ml of blood samples were obtained by venepuncture of medial coccygeal vein (*Vena coccygea mediana*, synonym: *Vena caudalis mediana*) and placed in tubes with anticoagulant (EDTA tube).

DNA extraction

DNA isolation from collected blood samples was carried out using GeneJET Genomic DNA Purification Kit (Thermo Fisher Scientific) according to manufacturer's

protocol. Measurement of quantity and quality of genomic DNA were performed using a spectrophotometer NanoDrop Eight (Thermo Fisher Scientific, USA), then the DNA samples were stored at -20 °C until further use.

Single nucleotide polymorphism (SNP) genotyping by real-time PCR

Genotyping of SNPs was performed using SNP Pol DNA Polymerase (Genaxxon Bioscience GmbH, Germany) which is a highly selective DNA polymerase variant, that has been specially developed for single nucleotide discrimination. Primers with a 3'-end mismatch are effectively discriminated by SNP Pol DNA Polymerase. The polymerase amplifies only if the primers fit 100% at the 3'-end. Even a single base alteration (SNP) at the 3' end of primers completely prevents amplification. Primer design was performed by Web-based Allele Specific Primer design tool (WASP) for detecting SNPs and mutations (Wangkumhang *et al*., 2007). The sequences of the designed primers are listed in Table 1.

Figure 1: Design of primers for SNP genotyping.
Nucleotide sequence with black color is intron 6, sequence with green color is exon 7. The allele-specific primers are designed so that their 3′ termini are complementary to the nucleotide variant unique to each allele. The SNP polymorphism rs43703011 is indicated by the arrow. Sequence of universal (common) bk-com-F primer is marked with purple color, whereas sequences of allele-specific bk-A1-R and bk-A2-R primers designed to selectively amplify either the A1 or A2 allele are marked with red and blue color respectively.

Table 1: List of primers used for SNP genotyping.

Primer name	Sequence	Amplicon size
bk-A1-R	5` - ATGTTTTGTGGGAGGCTGTTAT – 3`	564 bp
bk-com-F	5` - TAGTGCTAGAAGTTGGCCATTG – 3`	
bk-A2-R	5` - ATGTTTTGTGGGAGGCTGTTAG – 3`	

SNP rs43703011 in the beta-casein gene (*CSN2*) was determined by allele-specific real-time PCR using designed primers. The allele-specific primer matches at its 3′ end either of the two alleles. The allele-specific PCR reactions were performed in separate wells, with SYBR Green fluorescent labeling and detection. Therefore, this includes two reactions for each sample. Amplification was performed using QuantStudio™ 5 Real-Time PCR System (ThermoFisher, USA) in total volume 25 µl contaning: 17.25 µl of sterile distlled water, 0.25 µl of SNP Pol DNA Polymerase, 0.5 µl of dNTP, 2.5 µl of 10X amplification buffer, 0.5 µl of 10 µM bk-com-F , 0.5 µl of 10 µM bk-A1-R (in the one well) primers and 0.5 µl of 10 µM bk-com-F , 0.5 µl of 10 µM bk-A2-R primers (in the second well), 0.5 µl of SYBR Green (0.2X in the final PCR reaction) and 3 µl of DNA. Amplification of 564 bp fragment of *CSN2* gene consisted of three steps: hold stage: denaturation at 95°C for 2 minutes, PCR stage: followed by 50 cycles of denaturation at 95°C for 15 seconds, primer annealing at 56°C for 10 seconds, extension at 72°C for 90 seconds (with reading fluorescence). Melt Curve stage: rapid heating to 95°C to denature the DNA for 15 seconds, followed by cooling to 60°C for 10 seconds with reading fluorescence at 95°C for 1 second. Data were analyzed using QuantStudio™ Design and Analysis Software v1.5.2 Real-Time PCR Software.

Statistical analyses

To assess variation in the genetic structure of the Bushuev cattle population at the *CSN2* gene, statistical analyses were performed using POPGENE v.1.32. The following metrics were estimated: Allele and genotype frequencies at each locus; the observed (Na) and effective (Ne) numbers of alleles; observed (Ho) and expected (He) heterozygosity; observed (Homo) and expected (Home) homozygosity; Nei's genetic diversity index (H); the fixation index (FIS); Shannon's information index (I); and Nei's genetic distance. The Hardy-Weinberg equilibrium in the Bushuev cattle population was estimated using the chi square test (χ^2).

RESULTS AND DISCUSSION

CSN2 gene rs43703011 polymorphism genotyping of DNA samples of Bushuev breed cattle was conducted by real-time allele-specific real-time PCR with either the bk-com-F + bk-A1-R and bk-com-F + bk-A2-R primers. A total of 96 individuals were analysed to determine variants of the *CSN2* gene. Figure 2 clearly shows that each specific amplification plot can distinguish between A1/A1 homozygous, A1/A2 heterozygous, and A2/A2 homozygous genotypes (Figure 2C). For the individual with two A1 alleles the bk-A2-R primer showed no reaction whereas the bk-A1-R primer showed amplification curve with Ct value 29 (Figure 2 Sample-1, C). In heterozygous DNA samples, both amplification reactions proceed, reflecting the presence of one copy of the A1 allele and one copy of the A2 allele (Figure 2, Sample-2, C). In the individuals homozygous for the A2 allele, no amplification was observed with the bk-A1-R primer, whereas the bk-A2-R primer yielded a clear amplification curve with a threshold cycle (Ct) value of 29 (Figure 2, Sample-3, C). To confirm whether the bk-com-F and bk-A1-R primers amplify only *CSN2* A1 allele, whereas bk-com-F and bk-A2-R primers amplify only *CSN2* A2 allele, corresponding PCR products were directly sequenced by Sanger sequencing using a BigDye™ Terminator v3.1 Cycle Sequencing Kit. Genotypes determined by real-time PCR and Sanger sequencing methods were consistent. In our study, beyond the final Sanger sequencing used for genotype confirmation, we implemented several measures to validate the assay's specificity under actual experimental conditions: (1) Positive controls on each PCR plate: We included well-characterized genomic DNA samples representing the A1A1 and A2A2 genotypes on every PCR plate. These controls were previously confirmed by independent Sanger sequencing and served as internal standards to assess the enzyme's performance in each run. (2) No-template controls (NTCs) were also included to monitor for potential contamination or non-specific amplification. (3) Consistency across replicates: We tested a subset of samples in duplicate and across different runs to assess reproducibility. The genotype calls were 100% concordant. (4) Enzyme validation in preliminary experiments: prior to the main sample analysis, we performed optimization experiments using plasmid constructs containing the known SNP alleles to confirm the allele-specific activity of the SNP Pol DNA Polymerase under our PCR conditions.

The analysis of rs43703011 polymorphism in the *CSN2* gene among the studied animals showed that the predominant genotype was A1/A2 (54%), followed by A2/A2 (38%) and A1/A1 (8%) (Table 2). Allele frequencies for A1 and A2 were 0.35 and 0.65, respectively.

Based on the obtained data, the genetic structure of the studied Bushuev breed cattle population was assessed.

Observed heterozygosity (Ho), defined as the proportion of heterozygous individuals in the sample, was 0.54, while expected heterozygosity (He), calculated from allele frequencies, was 0.45. Comparison of Ho and He revealed a significant excess of heterozygotes. The inbreeding coefficient (Fis) was calculated as -0.2, the negative Fis value indicates an excess of heterozygotes in the population.

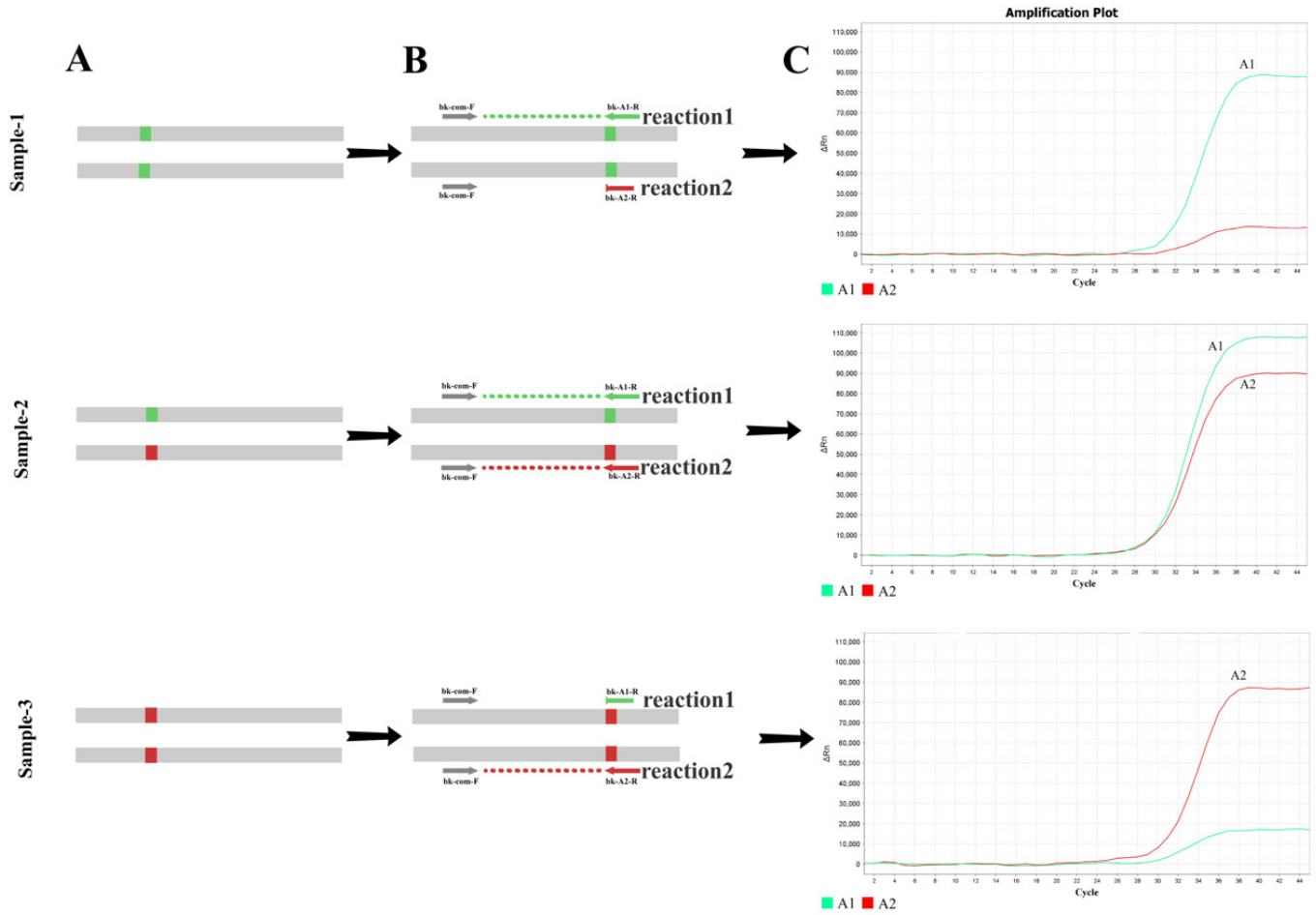

Figure 2: SNP Genotyping of rs43703011 (A1/A2) polymorphism of the CSN2 Gene by real-time PCR using SNP Pol DNA Polymerase (Genaxxon Bioscience GmbH, Germany).
Each real-time PCR assay comprises a universal (common) primer (grey) and allele-specific primers designed to selectively amplify either the A1 (green) or A2 (red) allele. The allele-specific primer matches at its 3′ end either of the two alleles. Separate PCR reactions are conducted for each allele-specific primer, each paired with the common primer and run in parallel. The amplification outcome is dependent on the genotype of the sample: both allele-specific reactions yield a signal in heterozygous samples, whereas only one reaction generates a signal in homozygous samples (B). The presence or absence of amplification in the two reactions enables the determination of the sample's genotype (C).

Table 2: Genotype and allele frequencies of *CSN2* gene in Bushuev cattle population and different crossbred cattle populations.

	Genotype frequency			Allele frequency		Ho	He	Number of samples	P value*	Reference
Breed/type	**A1A1**	**A1A2**	**A2A2**	**A1**	**A2**					
Bushuev	0.08 (8)	0.54 (52)	0.38 (36)	0.35	0.65	0.54	0.45	96	0.0713	this study
Kangayem x Holstein Friesian	0.17 (11)	0.46 (29)	0.37 (23)	0.405	0.595	0.46	0.48	63	0.7227	Malamathi *et al.*, 2014
Holstein-zebu crossbred	0.14 (19)	0.50 (66)	0.36 (48)	0.39	0.61	0.50	0.48	133	0.6279	Pabitra *et al.*, 2022
Frieswal	0.12 (15)	0.40 (50)	0.48 (59)	0.32	0.68	0.40	0.43	124	0.3889	Ganguly *et al.*, 2013
Vrindvani	0.12 (29)	0.48 (113)	0.40 (93)	0.36	0.64	0.48	0.46	235	0.5525	Kumar *et al.*, 2018
Girolando	0.05 (9)	0.35 (63)	0.60 (108)	0.23	0.77	0.35	0.35	180	0.9616	Pereira *et al.* 2016

Note: *P-value for deviation from Hardy-weinberg equilibrium

An A2 allele frequency in Bushuev breed cattle population close to that obtained in our study was reported by Malamathi *et al*. (2014) in Kangayem (Indian zebu cattle breed) X Holstein Friesian; Pabitra *et al*. (2022) in Bangladesh Holstein-zebu crossbred cattle population; Ganguly *et al*. (2013) in Frieswal; Kumar *et al*. (2022) in Vrindvani; and by Pereira *et al*. (2016) in Girolando (Gir (Indian zebu cattle breed) × Holstein).

It should be noted that the Frieswal is Indian crossbreed cattle developed in India (Figure 3). This breed was created by crossing the Holstein Friesian with the indigenous Sahiwal breed to enhance milk production while retaining adaptability to India's diverse climatic conditions. The development of Frieswal cattle represents a strategic effort to improve dairy productivity without compromising the breed's resilience to the environmental challenges posed by the region.

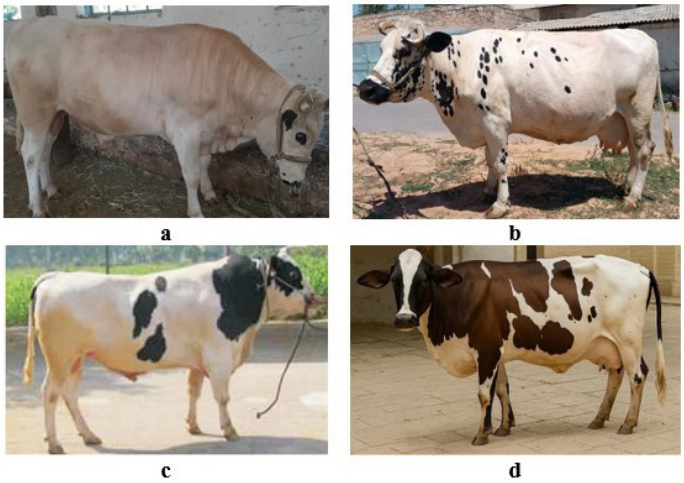

Figure 3: Representatives of Bushuev breed (Uzbekistan) and Frieswal breed (India):
a,Bushuev bull; b, Bushuev cow; c, Frieswal bull; d, Frieswal cow.

In a related context, our previous study involving phylogenetic analysis of the beta-lactoglobulin (BLG) gene in Bushuev dairy cattle (Adilov *et al*., 2025) revealed a close genetic relationship between this breed and Chinese as well as Indian zebu cattle. Notably, the Bushuev breed retains the complete BLG gene from its founder local zebu cattle, which likely originated from Chinese zebu populations derived from Indian zebu cattle. This finding aligns with several prior studies suggesting that zebu cattle were domesticated approximately 8,000–9,000 years before present (B.P.) in India and subsequently dispersed across northwestern South Asia, reaching regions such as China, Afghanistan, Southern Central Asia, and modern-day Iran by around 6,000 B.P. (Pérez-Pardal *et al*., 2018; Semenenko, 2019; Chen *et al*., 2010).

Furthermore, the genetic founder effect from zebu cattle has been associated with beneficial traits such as enhanced adaptability to hot climates and increased resistance to diseases, which are critical for sustaining livestock productivity in challenging environments. These attributes underscore the importance of zebu genetics in the development of crossbreeds like Frieswal, Girolando, Bushuev and others. contributing to their success in hot, humid and arid regions.

The observed heterozygosity (Ho) of 0.54 in the Bushuev cattle population indicates a elevated level of actual genetic diversity compare to expected heterozygosity (He) of 0.45, as it represents the proportion of individuals with two different alleles at a given genetic locus.

Therefore, the mixed ancestry (local Zebu × European breeds) makes the expected frequency of the A1 allele difficult to predict. It is important to note that the initial hybridization of local Zebu with European breeds such as Dutch, Swiss, and Simmental was subsequently followed by breeding inter se of the best obtained crosses and rigorous selection for resistance and productive traits under harsh environmental conditions, including wild pastures and hot climates.

These selection pressure include strong selection for heat resistance, disease resistance, milk composition (high fat, high protein content, high milk productivity) and also daily weight gains and heavier weaning weights of calves. In this regard there is an advantage of the A2 allele over A1 regarding its association with the growth of calves. Hohmann *et al*. (2020) observed that the composite BB|A2A2|AA|AB casein genotype (order of genes on bovine chromosome 6: α s1-|β-|α s2-|κ-CN) was associated with greater average daily weight gains and heavier age-adjusted weaning weights of calves. Therefore, we expect that after selection pressure Bushuev breed had a lower A1 allele and higher A2 allele.

It should be noted that several unrelated elite breeding bulls of the Bushuev breed at the State Breeding Enterprise "Uznaslichilik" were used as semen donors for artificial insemination (AI) in many farms in Syrdarya region and we expect that it also could contribute to higher A2 allele frequency and increased heterozygosity. Artificial insemination in Uzbekistan primarily involves livestock breeding to improve milk production and productivity, with 30% of breedable bovines of Bushuev breed covered by AI, and significant government initiatives supporting these practices. Unrelated breeding bulls are selected through a strategy called outcrossing, which involves breeding individuals with minimal genetic relationship to each other within the same breed to increase genetic diversity, performance, and heterosis. This method combats inbreeding depression, which occurs when closely related

animals are bred and can lead to decreased performance, increased disease susceptibility, and a narrowing of the genetic base of a herd.

There is also additional very important point regarding mixed ancestry of Bushuev breed (local Zebu x European breeds). The comparison between Bos indicus and Bos taurus milk reveals substantial compositional differences that have significant implications for dairy production and nutritional value. Zebu milk is notably richer in total solids, including fat and protein, compared to conventional cow milk. Specifically, it contains 1.8% more dry matter, 1.06% more fat, and 0.41% more protein, with a remarkable increase in casein content (0.76%) (Shahida *et al*., 2024). These compositional advantages make zebu milk particularly desirable for the production of high-value dairy products such as cheese and milk powder, where higher solid content is directly correlated with yield and quality.

Moreover, zebu milk exhibits a significantly higher concentration of trace elements, including cobalt, copper, iron, and zinc - up to 2-2.5 times more than cow milk, which is probably due to specific transporter proteins (Shahida et al., 2024; Collard and McCormick, 2021). These micronutrients play essential roles in both human nutrition and dairy product functionality, further enhancing the value of zebu milk in commercial and health-related contexts.

At the genomic level, the introgression of the A2 allele from zebu into taurine breeds carries broader implications than a single-point gene substitution. This process is akin to introgressing an entire genomic 'neighborhood' rather than a single 'house'. Consequently, not only the desired A2 allele is transferred but also the surrounding casein gene cluster, which spans approximately 250–350 kb in chromosome 6. This genomic region includes multiple genes involved in milk composition and quality, and also other interesting linked genes suggesting that such introgressions could bring a combination of favorable traits beyond just the A2 allele.

In addition, the bovine transferrin gene, located on chromosome 6, is part of a region rich in loci associated with physiological resilience, particularly in response to heat stress. Several studies have identified significant SNPs within this chromosomal region-particularly between markers BTA-76120 and ARS-BFGL-NGS-21182 - that correlate with key heat tolerance traits such as respiratory frequency and cellular protection mechanisms (Nuñez *et al*., 2023). These markers are of particular relevance in hot and arid environments where zebu breeds typically thrive, highlighting the potential adaptive advantages that could be co-introgressed with milk production traits.

The development of the Bushuev breed further exemplifies the complex interplay between genetics, environment, breeding and selection. Initial hybridization of local zebu with European breeds such as Dutch, Swiss, and Simmental was followed by breeding per se and rigorous selection pressure under harsh environmental conditions, including wild pastures and hot climates. This dual-phase process - hybridization followed by breeding inter se and the application of natural plus artificial selection - likely contributed to the retention of adaptive traits from zebu ancestors, including heat resistance and enhanced milk composition, while incorporating desirable production traits from taurine breeds.

It shold be noted that the broader genomic context reveals that simple population genetic models sometimes fail to capture the true complexity of genomes. Advances in technologies such as SNP microarray chips, next-generation sequencing (NGS), and comparative genomics have unveiled dynamic and intricate genomic architectures, indicating that genotypes are more complex than previously thought. Within this framework, heterozygosity-rich regions (HRRs) have emerged as critical genomic features (Smaragdov, 2025).

Runs of heterozygosity (ROHet), also referred to as heterozygosity-enriched regions, emerged as a more recent but very important and relevant concept that have been used to identify genomic regions that are under gene introgression or admixture (Marras *et al*., 2015, 2018). ROHet may mitigate the negative effects associated with harmful homozygous genotype aggregation by preserving heterozygote advantage, particularly at loci involved in immune response, stress tolerance, and reproductive performance. In the meantime, runs of homozygosity (ROH) have been applied to detect genomic regions under selection pressure (Peripolli *et al*., 2017).

Our findings align with these emerging perspectives. It has been shown that intense selection for the A2 allele appears to have increased inbreeding within the Holstein population, as reflected by increased runs of homozygosity (ROH) both genome-wide and specifically on chromosome 6, where the β-casein locus resides (Scott *et al*., 2023). Notably, cows homozygous for the A2 allele were twice as likely to exhibit a run of homozygosity of at least 35 SNP or 1000 kb long across the β-casein locus compared to animals that were homozygous for A1 (Scott *et al*., 2023). This observation suggests that selection for the A2 allele may inadvertently reduce genetic diversity by increasing homozygosity in targeted regions.

Such patterns have important implications for breeding programs. While selecting for desirable alleles like A2 can improve specific traits, it may simultaneously elevate

inbreeding and reduce overall genetic variation, potentially impacting animal health and resilience. Therefore it is logical to suggest that for selecting desirable genotype A2A2 it would be better to combine A2 allele originated from Zebu (this allele is present in Bushuev population) with A2 alleles originated from European breeds (that also present in Bushuev population).

Collectively, these findings support the view that zebu genetics, particularly in the context of milk composition and environmental adaptation, offer valuable opportunities for improving dairy production systems. Strategic introgression and targeted selection can harness the genetic potential of zebu cattle while addressing emerging challenges such as climate change and consumer demand for higher-quality, health-promoting milk products.

High Observed Heterozygosity (0.54) suggests that the Bushuev cattle population is genetically diverse, with many individuals carrying two different alleles at various genetic loci. The lower Expected Heterozygosity (He= 0.45) suggests factors that limit the theoretical diversity, such as a small population size or selection. The fact that Ho is higher than He is an important finding. This difference may be also attributed to the population bottlenecks - a drastic reduction in population size and may explain the difference between Ho and He.

The observation of Ho > He can seem contradictory under the classical understanding of bottlenecks. However, this pattern is actually a well-recognized signature of a recent bottleneck. It reflects a short-term heterozygote excess caused by the loss of rare alleles reducing He, while Ho remains temporarily elevated due to surviving heterozygotes. So, although bottlenecks reduce overall genetic diversity in the long term, they can transiently result in Ho > He, a pattern that is consistent with a recent population bottleneck.

This genetic phenomenon is particularly relevant in the context of the Bushuev cattle breed in Uzbekistan, whose population has recently declined. The reduction is primarily due to the introduction of more productive commercial breeds and a decrease in the cultivation of forage crops, which has led to diminished support for traditional livestock systems. This demographic decline not only poses a risk to the genetic integrity of the Bushuev breed but also emphasizes the urgency of conservation efforts.

To safeguard the unique genetic heritage of this indigenous breed, it is essential to implement molecular genetic studies aimed at characterizing its genetic structure and variability. Additionally, establishing electronic genetic databases will facilitate more efficient breeding and selection strategies, contributing to both the short-term management and long-term preservation of the breed's valuable gene pool.

CONCLUSION

In conclusion, we have successfully genotyped 96 Bushuev cattle for A1/A2 variants of the beta-casein gene using an allele-specific real-time PCR technique.

Due to the fact that the A1 variant of the beta-casein gene is considered potentially harmful to human health, it is essential to conduct screening (genetic selection) for A1/A2 alleles among cattle, particularly in breeding bulls and heifers. The genotypic data obtained in this study could be used in the development of breeding programs aimed at increasing the frequency of the desirable A2 allele in future generation of Bushuev breed. Since culling all A1 allele carriers immediately risks reducing genetic diversity, especially with the high A1A2 genotype frequency (54%), we propose a gradual and sustainable breeding strategy centered on selective use of A2A2 sires. By prioritizing A2A2 bulls in artificial insemination programs while still allowing controlled use of A1A2 females, the frequency of the A2 allele can be increased over successive generations without drastically reducing the effective population size. This method allows for the progressive dilution of the A1 allele while preserving valuable genetic traits unrelated to the A1/A2 status, ensuring genetic continuity and sustainability of the breeding population. This approach aligns with marker-assisted selection principles, ensuring that selection against the A1 allele does not compromise traits like milk yield, fertility and disease resistance. In this way, it will be possible to purposefully increase the production of A2 milk, which is considered safer for human health.

This study provides the first detailed genetic characterization of the A1/A2 β-casein gene polymorphism in the Bushuev cattle breed of Uzbekistan, a unique indigenous breed with mixed ancestry from local zebu and European taurine cattle. By establishing the allele and genotype frequencies of the CSN2 gene (rs43703011), this research offers critical insights into the current genetic structure of the breed, revealing a high prevalence of the health-favorable A2 allele (65%) and significant genetic diversity (Ho= 0.54). The identification of a high A2 allele frequency provides a genetic foundation for developing sustainable breeding strategies aimed at increasing A2 milk production, which is considered to be safer for human consumption. The developed real-time allele-specific PCR genotyping method enables efficient and accurate selection of A2A2 animals, particularly sires for artificial insemination, facilitating the gradual replacement of the A1 allele in the population without compromising genetic diversity. By documenting the genetic variability

and heterozygosity within the Bushuev population, this study supports conservation efforts for an endangered local breed, emphasizing its value as a reservoir of adaptive traits such as heat tolerance, disease resistance, and superior milk composition. This makes the Bushuev breed a strategic asset for climate-resilient dairy production. The results also offer practical guidance for policymakers and breeders in Uzbekistan to refine artificial insemination programs using genetically diverse, A2A2 sires. This will help meet consumer demand for A2 milk while supporting genetic sustainability and local breed conservation. This research contributes to the global understanding of β-casein polymorphism in lesser-studied breeds, expanding the scientific knowledge and providing a valuable perspectives for future comparative, breeding and evolutionary studies on dairy cattle genetics.

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NOVELTY STATEMENT

The Bushuev cattle population was found to exhibit genotypic diversity for the A1/A2 beta-casein variants of the *CSN2* gene.

AUTHOR'S CONTRIBUTION

All authors contributed equally to the manuscript.

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Ethical statement

Approval (reference number 12/TAS/0474) was taken from the Institutional Animal Ethics Committee (IAEC) of Institute of Genetics and Plant Experimental Biology before commencement of the research study and IAEC guidelines were followed during experimentation.

Generative AI and AI-assisted technology statement

The authors declare that they have not used any type of generative artificial intelligence and AI-assisted technologies for the writing of this manuscript, nor for the creation of figures, graphics, and tables.

Conflict of interest

The authors have declared no conflict of interest.

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