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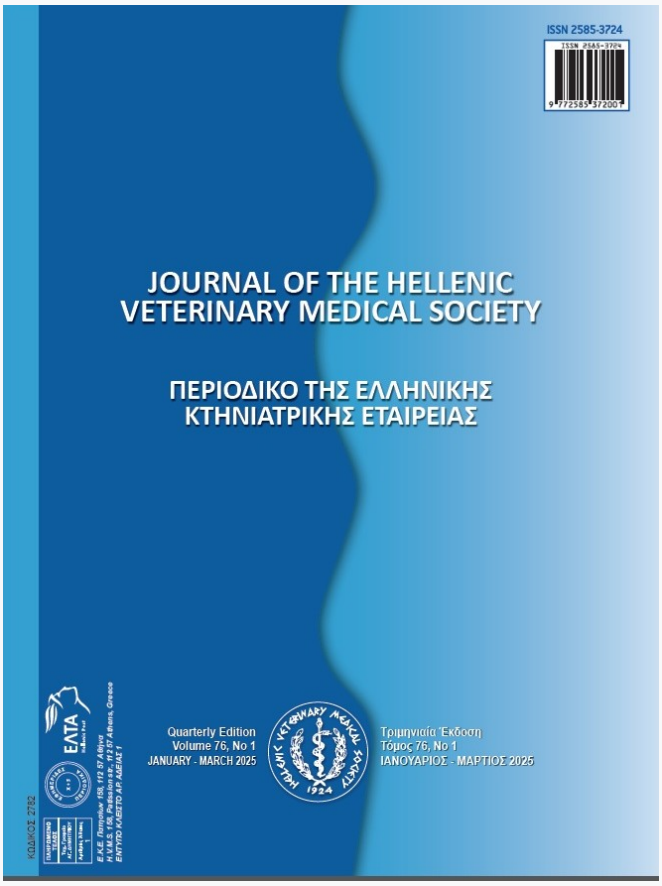

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Genetic variability of alpha-casein, beta-casein, and kappa-casein genes in Holstein-Friesian, Simmental and Brown Swiss cattle

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ABSTRACT: This study aims to determine the genotypes and allele frequencies of polymorphisms of bovine αS1-casein (*CSN1S1*), αS2-casein (*CSN1S2*), β-casein (*CSN2*) and κ-casein (*CSN3*) genes in Holstein-Friesian, Simmental, and Brown Swiss breeds. DNA was isolated from milk samples and gene regions were amplified using optimized PCR protocols. Sanger sequencing was performed to genotyping PCR products. *CSN1S1* and *CSN1S2* genes in three cattle breeds were observed to be monomorphic. Three different genotypes were observed for the *CSN2* gene in Holstein-Friesian and two genotypes in Brown Swiss, while the Simmental breed was monomorphic. For the *CSN3* gene was observed three different genotypes in Holstein-Friesian and Brown Swiss, two different genotypes in Simmental. Also, a novel single nucleotide polymorphism (SNP) was identified in the sequence region in the *CSN2* gene exon 7 of three cattle breeds in this study. This SNP, c.249C>G, were non-synonymous, which leads to the change of p.N68K in amino acid residue of bovine β-casein. This SNP is thought to be detected for the first time in *Bos taurus* genus. However, it was concluded that it would be appropriate to carry out more studies on this new SNP point in larger populations.

***Key words*:** Alpha-casein; Beta-casein; Cattle milk; Kappa-casein; Sequence.

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INTRODUCTION

Milk is produced in mammary glands of mammals after birth, and it is the primary source of nutrition for their infants. It is composed of several solid components including minerals, lactose, fat, and protein (Bodnár et al., 2018). Cattle milk proteins can be grouped into 2 categories: caseins and whey proteins. Caseins are organized in micelles and account for about 80% of milk proteins. Casein micelles have evolved to concentrate, stabilize, and deliver vital nutrients, namely calcium and protein, to the newborn (De Kruif and Holt, 2003; Zhang et al., 2016).

Casein refers to a set of milk-specific proteins that are distinguished by ester-bound phosphate, high proline concentration, and limited solubility at pH levels ranging from 4.0 to 5.0 (Lawrence and Lawrence, 2011). There are four types of caseins: αS1-, αS2-, β- and κ-caseins. These are phosphoproteins with a high proline content and low levels of sequence identity (Mills et al., 2004). αS1-casein, αS2-casein, β-casein, and κ-casein are coded by the *CSN1S1*, *CSN1S2*, *CSN2* and *CSN3* genes, respectively (Caroli et al., 2009; Peñagaricano and Khatib, 2012). The four casein genes are connected and structured in a casein region of approximately 250 kb on bovine chromosome 6 (Threadgill and Womack, 1990).

Up to 40% of the casein in the milk of cows is made up of the αS1-casein (*CSN1S1)*. It has one main component and one minor component. It has 199 residues of amino acids. The αS1-casein signal peptide is composed of 15 amino acid residues, making the pre-form of αS1-casein 214 amino acids in length (Farrell et al., 2004). 28 variants have been discovered according to the Uniprot database (https://www.uniprot.org/). B and C are the two most prevalent variations, and the difference between the two variants arises from amino acid position 192, Glu replaces Gly (Caroli et al., 2009; Lühken et al., 2009). Čítek et al. (2023) reported that cows with the CSN1S1 BB genotype had significantly higher milk, protein, and fat yields than BC, but the effect of CSN1S1 genotypes on the milk technological quality was non-significant. Also, Ozdemir et al. (2018) reported on the significant influence of CSN1S1 gene polymorphism on protein, fat yield, and content.

The αS2-casein (*CSN1S2*), which is a member of the casein family constitutes about 10% of the bovine casein fraction. *CSN1S2* consists of two major forms and several minor components. *CSN1S2**A, which is the reference protein form of *CSN1S2* is a single polypeptide of 207 amino acids. The αS2-CN signal peptide is composed of 15 amino acid residues, making the pre-form 222 amino acid residues in length (Farrell et al., 2004; Ibeagha-Awemu et al., 2007). 33 variants have been discovered according to the Uniprot database (https://www.uniprot.org/). Variants B and C are specific to zebu and yaks, respectively, whereas the D variant has been found at low frequencies in some European breeds and the African Namchi taurine breed (Ibeagha-Awemu et al., 2007).

The *CSN2* gene is located on chromosome 6 and has 9 exons and 8 introns in its structure. It consists of 209 amino acids and accounts for approximately 37% of the total casein in milk. The β-casein signal peptide is composed of 15 amino acid residues, making the pre-form 224 amino acids in length (Farrell et al., 2004; Doval et al., 2021). The second-largest protein fraction in cow milk among casein is β-casein (Shashank et al., 2018). 14 variants have been identified in the Uniprot database (https://www.uniprot.org/). A1, A2, A3, B and C variants are the five most common variants (Doval et al., 2021). A1 and A2 variants of beta casein are frequently detected in dairy cattle (Kamiński et al., 2007). Exon VII (498 bp), the CSN2 gene's longest exon, contains the nucleotide modifications that encode the amino acid variations between the A1, A2, A3, and B variants (Kumar et al., 2020). The difference between A1 and A2 variants of beta-casein is nucleotide sequence change from CCT (A2) to CAT (A1) in 67th amino acid position. This situation causes the substitution of proline (A2) by histidine (A1, B) in the amino-acid sequence (Jaiswal et al., 2014). Jinsmaa and Yoshikawa (1999) reported that the gastrointestinal digestive of the A1 variant leads to the release of a seven-amino acids (Tyr-Pro-Phe-Pro-Gly-Pro-Ile) peptide named β-casomorphine 7 (BCM-7), with a strong opioid activity. Several studies have reported that consumption of 'A1-milk' (due to the presence of BCM-7) may be a risk factor for human health (Elliott et al., 1999; Wasilewska et al., 2011; Demirel and Çak, 2018; Kaplan et al., 2022).

The κ-casein gene consists of a polypeptide chain of 169 amino acids and encodes the milk protein that is important for the balance and structure of casein micelles. The κ-casein signal peptide is composed of 21 amino acid residues, making the pre-form 190 amino acids in length (Farrell et al., 2004). Kruchinin et al. (2023) reported 14 polymorphic variants of the *CSN3* gene, whereas Uniprot database (http://www.uniprot.org/) has been identified 7 variants. The two common

genetic variants are designated A and B (Caroli et al., 2009). These alleles differ by substitutions in 2 amino acids at positions 136 and 148. The A allele includes the threonine and asparagine amino acids, whereas the B allele has isoleucine and alanine in the same positions, respectively (Mahmoudi et al., 2020).

This study aims to determine the genotypes and allele frequencies of *CSN1S1*, *CSN1S2*, *CSN2* and, *CSN3* genes in Holstein-Friesian, Simmental and Brown Swiss breeds.

MATERIAL AND METHODS

Animals

All animal procedures in this study were approved by the Van Yuzuncu Yil University Animal Researches Local Ethic Committee Approval Certificate (Türkiye; permission number: 2017/12). Van province, which conducted the present study is in eastern Türkiye and is a border city with the largest lake in the country. Since the number of lactating animals in the dairy cattle farm of Van Yuzuncu Yil University Livestock Application and Research Center was 20 head and DNA isolations were obtained from milk, 20 cows constituted the material of the study. Screening for polymorphisms was performed using genomic DNA obtained from three cattle breeds: Holstein-Friesian (n = 6), Simmental (n = 7), and Brown Swiss (n = 7). The cows included in the study were fed with a total mixed ration. The shelter structure where the animals were kept was a free-stall shelter. Cows were milked twice a day in the milking parlor (herringbone, 2×4).

DNA isolation and PCR amplifications

Before starting DNA isolation, 50 mL of raw milk (in 10 mL centrifuge tubes) was centrifuged at 2000 g for 10 minutes. The milk fat and most of the supernatant from above the somatic cell and milk protein pellet were removed, leaving 10 mL of milk. The remaining 10 mL of milk was centrifuged at 2000 g for 10 minutes. The supernatant was removed, and the remaining portion (approximately 1 mL) was transferred to a 2 mL centrifuge tube. DNA was isolated by Milk DNA Preservation and Isolation Kit (Cat. 44800, Norgen, Canada), according to the manufacturer's instructions. DNA quality was assessed using spectrophotometric analysis (BioDrop, UK), ensuring high purity for subsequent PCR amplification.

PCR amplifications were conducted in a Rotor-Gene Q thermocycler (Qiagen, Germany). Primers used in PCR amplification of *CSN1S1*, *CSN1S2*, *CSN2*, and *CSN3* are presented in Table 1.

We tested the PCR conditions reported in the references (Koczan et al., 1993; Mclachlan, 2006; Ibeagha-Awemu et al., 2007; Patel et al., 2008; Miluchova et al., 2009a; Miluchova et al., 2009b) to reach the optimum annealing temperature. We determined the most adequate amplification conditions evaluating preliminary PCR studies. The amplification conditions of *CSN1S1* were 94°C for 5 min followed by 35 cycles at 94°C for 30 s, 59°C for 50 s, and 72°C for 30 s, and then an extra extension step of 72°C for 5 min. The same amplification conditions were used for other genes except for annealing temperatures and times. Annealing temperatures and times for *CSN1S2*, *CSN2* and *CSN3* genes are 63°C for 50 s, 65°C for 50 s and 65°C for 1 min, respectively.

Electrophoresis

To achieve reliable sequencing outcomes, purity of PCR products was checked by electrophoresis (90 V, 45 min) in 2% agarose gel stained with SafeView™ Classic (Cat. No: G108, ABM, Canada). Additionally, the purity of a PCR amplicon was determined by the single, integrity, and brightness of electrophoretic bands. The size of PCR products in Table 1 should be clearly visible on the agarose gel electrophoresis.

Table 1. Primers used in PCR amplification of *CSN1S1*, *CSN1S2*, *CSN2*, and *CSN3*

Gene	Primer (5′→ 3′)	PCR product (bp)	References
CSN1S1	F: TGCATGTTCTCATAATAACC R: GAAGAAGCAGCAAGCTGG	310	Koczan et al., (1993) and Miluchova et al., (2009a)
CSN1S2	F: AAAACAAGCAGCCAAGAAGC R: TTCCCAGTCTCCCCAGTATG	356	Ibeagha-Awemu et al., (2007)
CSN2	F: CCTTCTTTCCAGGATGAACTCCAGG R: GAGTAAGAGGAGGGATGTTTTGTGGGAGGCTCT	121	Mclachlan, (2006) and Miluchova et al., (2009b)
CSN3	F: ATCATTTATGGCCATTCCACCAAAG R: GGCCATTTCGCCTTCTCTGTAACAGA	350	Patel et al., (2008)

Genotyping and Data Analysis

The screening for polymorphisms and genotyping were carried out using the Sanger sequencing method. The purification process of PCR products was performed with Agencourt® AMPure® XP PCR purification. After purification, the Applied Biosystems 3130 Genetic Analyzer (Thermo Fisher, UK) device was used for forward and reverse primers and bidirectional sequence readings. Peak readings for each nucleotide were evaluated in the results.

The cows' CSN1S1, CSN1S2, *CSN2*, and *CSN3* genes were genotyped in SnapGene (Ver. 6.2) using the sequences obtained from Sanger sequencing. These four genes were aligned with the BLAST tool in the NCBI database to find similar and different regions in their sequences. For the *CSN2* gene, reference sequences of *Bos taurus* and *Bubalus bubalis* species from the NCBI database and sequences from different genotypes the *CSN2* in the present study were analyzed with Mega 11 and BioEdit (Ver. 7.7.1) programs. The single nucleotide variation in the *CSN2* gene was checked and output by BioEdit software.

Statistical analysis

Allele and genotype frequencies for the genetic variants of *CSN1S1*, *CSN1S2*, *CSN2*, and *CSN3* genes were determined by the direct counting method. The chi-square ($\chi 2$) test was used to check whether the population was in Hardy-Weinberg equilibrium (HWE). Additionally, current study calculated Polymorphic information content (PIC) and Heterozygosity (He) values in this study. PIC of a marker corresponds to its ability to detect polymorphism among individuals of a population, and the higher that capacity, the greater its value. It is one of the indicators of marker quality in genetic studies. The heterozygosity of a marker is the likelihood of an individual being heterozygous at the marker site and depends on the number of alleles and their frequency in the population. The heterozygosity value ranges from zero (without heterozygosity) to 1.0 (high number of alleles with equal frequency) (Serrote et al., 2020). In brief, PIC measures the informativeness of a marker in detecting polymorphism, and heterozygosity refers to the likelihood that two alleles at a locus are different. POPGENE Version 1.32 (Yeh et al., 1997) was used for PIC, He, HWE and $\chi 2$ analyses. Post hoc power analysis was carried out using the G*Power tool (version 3.1.9.7; Universities of Düsseldorf, Mannheim and Kiel, Germany) to determine the study's sample size. Post hoc power analysis results (effect size=0.30, α=0.05 and 1-β=0.80) revealed that 20 animals would be needed for the sample size.

RESULTS

The *CSN1S1* and *CSN1S2* genes were determined to be monomorphic in this study. For the *CSN1S1* gene, the BB genotype and B allele were detected in three cattle breeds. For the *CSN1S2* gene, the AA genotype and A allele were detected in three cattle breeds. Genotype and allele frequencies, He, PIC, and HWE of the *CSN2* gene are presented in Table 2.

For the *CSN2* gene, three different genotypes (A1A1, A1A2, and A2A2) were observed in the Holstein Friesian cattle breed. Holstein Friesian cattle in the study were in harmony with HWE. One genotype (A2A2) was observed in the Simmental cattle breed. Therefore, HWE could not be calculated for the Simental cattle breed. Two different genotypes (A1A1 and A2A2) were observed in the Brown Swiss cattle breed. The results of this study found that there was a deviation from HWE equilibrium for the *CSN2* polymorphisms in the Brown Swiss cattle breed. Genotype and allele frequencies, He, PIC, and HWE of the *CSN3* are presented in Table 3.

The *CSN3* gene in Holstein Friesian, Simmental, and Brown Swiss cattle was observed as three genotypes (AA, AB, and AE), two genotypes (AB and BB), and three genotypes (AA, AB, and BB), respectively. Three cattle breeds in the study were consistent with the HWE.

Table 2. Genotype and allele frequencies, He, PIC, and HWE of the CSN2 gene

	Genotype						Allele					
	A1A1		A1A2		A2A2		A1	A2				
Breed	n	F	n	F	n	F	F	F	He	PIC	χ^2	P
Holstein Friesian	2	0.33	2	0.33	2	0.33	0.50	0.50	0.50	0.375	0.67	0.414
Brown Swiss	1	0.14	0	0.0	6	0.86	0.14	0.86	0.2449	0.2149	7	0.008**
Simmental	0	0.0	0	0.0	7	1.0	0.0	0.0	0.0	0.0	-	-

F: Frequency; n: number of animals; He: heterozygosity; PIC: polymorphism information content; $\chi 2$: Chi-square; **P<0.01 : not consistent with HWE.

Table 3. Genotype and allele frequencies, He, PIC, and HWE of the *CSN3*

	Genotype								Allele						
	AA		AB		BB		AE		A	B	E				
Breed	n	F	n	F	n	F	n	F	F	F	F	He	PIC	χ^2	P
Holstein Friesian	3	0.50	2	0.33	0	0.0	1	0.17	0.75	0.17	0.08	0.4028	0.3633	0.67	0.414
Brown Swiss	1	0.14	1	0.14	5	0.72	0	0.0	0.21	0.79	0.0	0.3367	0.28	2.32	0.128
Simmental	0	0.0	5	0.71	2	0.29	0	0.0	0.36	0.64	0.0	0.4592	0.3538	2.16	0.142

F: Frequency; n: number of animals; He: heterozygosity; PIC: polymorphism information content; χ2: Chi-square

A part of electropherogram of the Sanger sequencing of A1A1 and A2A2 genotypes in exon 7 of *CSN2* are presented in Figure 1. The nucleotide difference between A1A1 and A2A2 genotypes is seen in substitution C245A (red arrow). In addition, substitution C249G, the novel SNP found in three cattle breeds in this study, is shown by the blue arrow. C249G variation was revealed in all twenty cattle screened in the study.

Alignment of the sequences of the *CSN2* gene are presented in Figure 2. The sequence region obtained because of Sanger sequencing was aligned with the Bioedit program. As a result of alignment, a difference was detected from the reference sequence on the same nucleotide in three cattle breeds. While the Cytosine (C) nucleotide for the *CSN2* gene is located in the reference sequence, Guanine (G) nucleotide was detected in this study. The novel SNP, C249G causes a non-synonymous mutation because the asparagine (N) amino acid found in codon 68 of the *CSN2* gene is converted to the amino acid lysine (K). Additionally, as a result of BLAST performed at NCBI, it was observed that there was a SNP similar to this study in the 68th amino acid localized in Exon 7 of the *CSN2* gene of *Bubalus bubalis* (water buffalo / GQ176287).

DISCUSSION

This study reports genotype and allele frequencies *CSN1S1*, *CSN1S2*, *CSN2* and *CSN3* variants in Holstein Friesian, Simmental, and Brown Swiss breed.

Concerning the *CSN1S1* gene, BB genotype (1.0) and B allele (1.0) were detected in three cattle breeds.

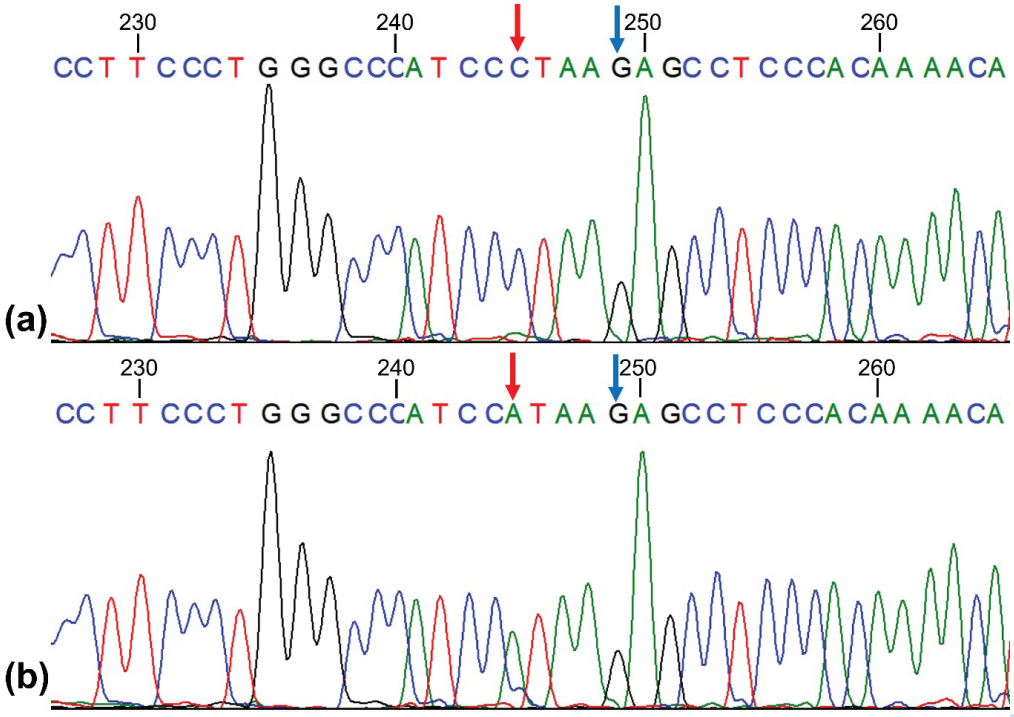

Figure 1. An electropherogram of the Sanger sequencing in exon 7 of *CSN2*

(a) A part of the sequence map of the A2A2 gene in exon 7 of *CSN2*. The red arrow represents the difference nucleotide of the A2A2 gene from the A1A1 gene and the blue arrow indicates the different nucleotide detected in this study.
(b) A part of the sequence map of the A1A1 gene in exon 7 of *CSN2*. The red arrow represents the difference in the nucleotide of the A1A1 gene from the A2A2 gene, and the blue arrow indicates the different nucleotide detected in this study.

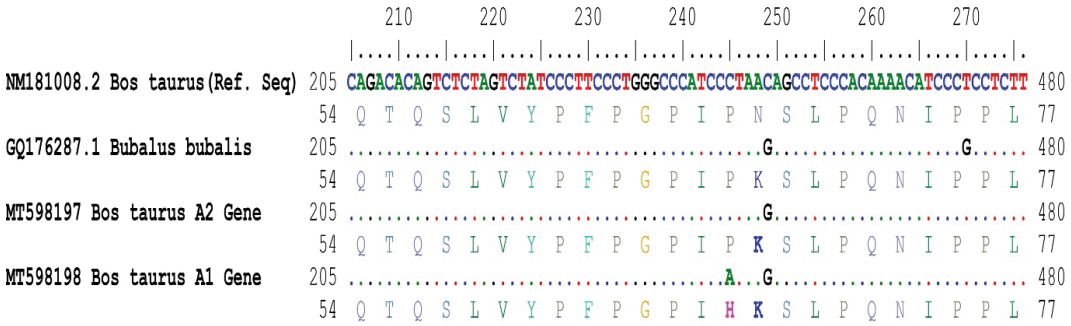

Figure 2. Alignment of the sequences of the *CSN2* gene

Numbers represents the position of coding region. Dots (.) represent the identity with the reference sequence (NM810008.2). Nucleotide substitutions are denoted by different letters. MT598197 and MT598198 are sequences detected in this study. Lines 1, 3, 5, 7 are nucleotide sequences, lines 2, 4, 6, 8 are abbreviations of amino acids.

Kaygısız and Doğan (1999) reported the frequencies of *CSN1S1* gene were 0.958 and 0.042 for B and C allele, respectively. Heck et al. (2009) found the frequencies of *CSN1S1* gene were 0.997 and 0.003 for B and C allele, respectively. Bovenhuis and Van Arendonk (1991) and Ardıçlı et al. (2018) reported that the frequencies of *CSN1S1* gene were 0.95 and 0.05 for B and C allele, respectively. In the current study, B allele was detected as the most frequent allele of the *CSN1S1* gene in three cattle breeds. This result was consistent with the findings of Bovenhuis and Van Arendonk (1991), Kaygısız and Doğan (1999), Heck et al. (2009) and Ardıçlı et al. (2018).

According to findings related to *CSN1S2* gene, AA genotype (1.0) and A allele (1.0) were detected in three cattle breeds. Ibeagha-Awemu et al. (2007) reported A, B and D allele frequencies of *CSN1S2* in German Simmental cattle were 0.987, 0.0 and 0.013, in Anatolian Black cattle were 0.867, 0.133 and 0.0 for A, B and D allele, respectively. Caroli et al. (2010) found that A allele frequency of *CSN1S2* in Original Pinzgauer cattle from Austria and Germany were 0.755 and 0.855, respectively. In the present study, AA allele was observed as the most frequent allele of the *CSN1S2* gene in three cattle. This result was consistent with the findings of Ibeagha-Awemu et al. (2007) and Caroli et al. (2010).

The fact that the CSN1S1 and CSN1S2 genes were determined to be monomorphic in the present study may indicate that only these genotypes were preferred in breeding programs in the past. The fact that both genes are monomorphic can be interpreted as a disadvantage in terms of dairy cattle breeding. Since there is no variation in the genes, the selection application will not be successful. Similarly, Kolenda and Sitkowska (2021) reported that through the years, a selection aiming at improving milk performance might have changed the genotype frequencies of different SNPs, rendering some of them monomorphic. Moreover, they pointed out that if a SNP is no longer found in different variants, perhaps its removal from the microarray and replacement with a new SNP that would be valuable to breeders should be considered.

The frequencies of A1A1, A1A2 and A2A2 genotypes in Holstein Friesian and Brown Swiss cattle breeds were found to be 0.045, 0.045, 0.910 and 0.034, 0.034, 0.932, respectively. Simmental cattle breed was monomorphic in terms of A2A2 genotype. The frequency of A1 and A2 allele in Holstein Friesian and Brown Swiss cattle breeds were found to be 0.50, 0.50 and 0.14, 0.86, respectively. The A1 allele frequency in this study was lower than the finding (0.54) Hanusová et al. (2010) reported in the Holstein Friesian cattle breed. While the A2 allele frequency of Holstein Friesian in this study was found lower than finding (0.692) reported from Heck et al. (2009), it was found consistent with finding (0.498) reported by Bovenhuis and Van Arendonk (1991). In this study, *CSN2* gene was monomorphic in Simmental cattle breed, however, it was polymorphic in Holstein Friesian and Brown Swiss cattle breeds. The high frequency of A2A2 genotype in the three breeds suggested that semen with A2 gene was used in artificial insemination instead of A1 gene which carries health risks.

Genotype frequencies of the *CSN3* gene in Holstein Friesian cattle breed were observed as AA (0.50), AB (0.33) and AE (0.17). Also, A, B, and E allele frequencies were determined as 0.75, 0.17 and 0.08, respectively. In the current study, A allele was the most frequent allele of the *CSN3* gene in Holstein Friesian cattle breed.

This result was consistent with the findings reported by previous studies (Bovenhuis and Van Arendonk, 1991; Kaygısız and Doğan, 1999; Heck et al., 2009; Gurses et al., 2018; Demirel, 2019; Adamov et al., 2020). Genotype frequencies of the *CSN3* gene in Brown Swiss cattle were observed as AA (0.14), AB (0.14) and BB (0.72). Also, A and B allele frequencies were determined as 0.21 and 0.79, respectively. The A allele frequency reported by Kaygısız and Doğan (1999) was higher than the present study, but the B allele finding was lower than the current study. In the current study, B allele was the most frequent allele of the *CSN3* gene in Simmental cattle breed. This result was consistent with findings reported by Gurses et al. (2018).

In this study, HWE values could not be calculated for the *CSN1S1* and *CSN1S2* genes in three cattle breeds and for the *CSN2* gene in only Simmental cattle since these genes are monomorphic. For the *CSN3* gene, the three cattle breed populations in the study were observed to be consistent with HWE, while for the *CSN2* gene, only the Holstein population was consistent. The present results showed there was a deviation from HWE equilibrium for the CSN2 polymorphisms in the Brown Swiss cattle breed. This deviation may occur due to a variety of breeding methods, including selection and inbreeding.

Botstein et al. (1980) reported that a marker with a PIC value of less than 0.25 is considered low or uninformative, while values between 0.25 and 0.5 are mildly informative. In addition, they stated that markers with PIC values of 0.5 or higher are highly informative for genetic studies. In our research, PIC values for *CSN2* and *CSN3* genes ranged from 0.22 to 0.38. This result showed that a marker at a particular locus may be mildly informative in distinguishing the polymorphism rate. This may be because the minor allele frequency for both polymorphisms is very low. In the current study, the HE values of CSN2 and CSN3 genes in three breeds ranged from 0.249 to 0.500. Consistent with our results, Soyudal et al. (2018) reported the HE values of CSN2 and CSN3 genes in Holstein cattle as 0.4925 and 0.2809, respectively. The low heterozygosity values in this study may be due to high levels of inbreeding or high selection pressure.

In the present study, the nucleotide sequences of the CSN1S1, CSN1S2, CSN2, and CSN3 genes were determined by Sanger sequencing. The nucleotide sequences of these four genes were matched with the reference sequences available in GenBank by BLAST. The BLAST result revealed the C249G variation in the exon 7 region of the CSN2 gene. Interestingly, this SNP was reported for the first time in *Bos taurus* with our study. However, a similar variation was reported in a different species, *Bubalus bubalis*. Fan et al. (2021) revealed the c.249C>G SNP in the exon 7 region of the CSN2 gene in River and Swamp Buffaloes using PCR product direct sequencing in China. They reported that this SNP was non-synonymous because it caused the p.Asn68Lys change in the mature peptide of Buffalo *β*-casein.

The sample size in the study was limited due to the small number of lactating animals on the farm where the study was conducted. Therefore, the monomorphism of the CSN1S1 and CSN1S2 genes may be due to the sample size. In addition, polymorphic sites can be detected by scanning different regions of these genes. Moreover, the C249G variation we encountered as a result of Sanger sequencing may have occurred due to a mutation in only twenty cows used in the study. For this reason, it is highly recommended that the sample size be increased for future studies.

CONCLUSION

The casein genes are evaluated as reasonable candidate genes for marker-assisted selection in dairy animals. Casein gene frequencies belonging to Holstein Friesian, Simmental, and Brown Swiss cattle breeds determined in this study are expected to be a literature source for future studies. Also, the present study revealed a novel non-synonymous SNP in the exon 7 region of the CSN2 gene in *Bos taurus*. However, more information is needed to understand whether this variation occurred and why/how it occurred. It was concluded that it would be appropriate to investigate the polymorphic structures of casein genes and the relationships between yield characteristics and casein genes in larger populations.

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CONFLICT OF INTEREST

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