

# Genetic polymorphisms of kappa-casein (CSN3) gene and its association with bovine milk properties

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## Abstract

Kappa-casein is an important subtype of casein protein which has many beneficial effects on the dairy industry as well as to the consumers' health. This dual importance of kappa-casein underscores the interconnected relationship between the quality of milk and the sustainability of the dairy industry. Among the several factors, genes play a major role in influencing milk properties. In line with that, numerous genetic variants of the kappa-casein gene have been identified and the two most prevalent variants, A and B, have shown significant associations with enhanced milk properties such as constituents, yield and technological properties. Hence, the review aims to provide an updated overview on the genetic polymorphisms of kappa-casein A and B variants in several bovine breeds from various parts of the world and their associations with milk properties particularly milk constituents, milk yield and technological properties such as rennet milk coagulation time and thermal resistance.

**Key words:** Kappa-casein; CSN3 gene; A and B variants; milk yield; milk constituents; rennet milk coagulation time.

## Introduction

The constituents, yield and technological properties of bovine milk play a pivotal role in both the consumers' and industry's perspectives, shaping the dynamics of the dairy sector. Milk is one of the most nutritious food products, which can have a significant impact on the consumers' health (Pathumsha, 2016). Simultaneously, the dairy industry places significant emphasis on milk constituents, yield, and technological properties as key determinants of production efficiency, economic viability, and the development of innovative dairy products (Ariza *et al.*, 2019). This dual importance underscores the interconnected relationship between the quality of milk and the sustainability of the dairy industry. In this context, understanding the factors influencing milk constituents, yield and technological properties is essential for both consumers seeking optimal nutrition and the dairy industry striving for economic success and growth. The milk yield, technological properties and its constituents are influenced by many factors such as breeds, environments, and other managerial factors (Albazi *et al.*, 2023, Yuldashbaev *et al.* 2020). These studies have shown that genetic variants of many genes influence the milk traits in bovine and suggested selecting animals based on genetic markers (Miluchová *et al.*, 2018).

Kappa-casein is the most important protein among the four milk casein proteins. Studies have shown that the A and B variants of kappa-casein gene (CSN3) are associated with enhanced milk traits such as constituents, yield, and technological properties of milk such as rennet coagulation time and thermal resistance. Hence, it was suggested that CSN3 A and B variants can be used as good markers to assist in choosing bovines for selective breeding programs to improve milk traits (Gurses and Yuce, 2012). This also has the potential to contribute to economic growth as bovines' have been described as "living banks" during periods of economic distress and be a promising treatment for malnourished children (which is a predisposing factor that contributes to many diseases). According to the World Health Organization (WHO), (2023) more than 30 million children in 15 worst-affected countries worldwide currently suffer from acute malnutrition and of which 8 million children are severely malnourished (Birthal and Negi, 2012; Pathumsha, 2016; Taylor, 2023). Treating these undernourished children would contribute in achieving the sustainable development goal (SDG) 2 target 2, which is to end all forms of malnourishment by 2025 (Johnston, 2016). Not only improving the milk traits but also assessing the variants will be a good initiative to maintain animal biodiversity.

Therefore, the primary objective of this review is to provide comprehensive insights into the genetic polymorphisms of kappa-casein A and B variants in several bovine breeds and their associations with milk properties particularly milk constituents, milk yield and technological properties such as rennet milk coagulation time and thermal resistance.

## Casien

Casein and whey protein are two important proteins found in bovine milk and 80% of milk protein is made up of casein. This protein contains calcium and phosphate and exists as micelles. Casein can be categorized into four major classes known as alpha s1 ( $\alpha$ s1-Cs), alpha s2 ( $\alpha$ s2-Cs), beta ( $\beta$ -Cs) and kappa-casein ( $\kappa$ -Cs) (Davoodi *et al.*, 2016). Kappa-casein is a single polypeptide chain with a highly hydrophobic N-terminal, and a hydrophilic C-terminal. It is made up of 169 amino acids with a molecular weight of 19.2kDa. Kappa-casein is the only glycosylated casein in Eutherians and consists of sialic acid, galactose and galactosamine. It makes up to 12% of the casein in milk (Deb *et al.*, 2014; Fan *et al.*, 2019; Jaiswal and Worku, 2022). Kappa-casein plays a crucial role in variety of processes, such as in the synthesis, stability, and aggregation of casein micelles (Gurses and Yuce, 2012; Jiménez-Montenegro *et al.*, 2022). Additionally, it acts as a stabilizer to prevent the pressure of calcium on the casein. Thereby ensuring that the micelles retain their colloidal state (Bayraktar, 2022). In addition, it is essential to produce caseino-macropptide, which is necessary for the coagulation of milk, to increase the efficiency of digestion, to prevent neonate hypersensitivity to ingested proteins and to inhibit gastric pathogens (Troch *et al.*, 2017; Khushbakht and Ujan, 2022). Furthermore, kappa-casein is a slow digesting protein, which results in a more sustained release of amino acids (Trommelen *et al.*, 2020). This increases satiety and reduces appetite, which helps with weight management and reduction (Bendtsen *et al.*, 2013). Moreover, it provides an increase supply of bioavailable calcium and bioactive peptides (Fan *et al.*, 2019). Studies have shown a number of positive health effects of kappa-casein, such as preventing dental cavities, anticancer effects, antioxidant properties, antimicrobial activities and hypocholesterolemia effect (Davoodi *et al.*, 2016; Ledesma-Martínez *et al.*, 2019; Halavach *et al.*, 2020).

## Genetic variations or polymorphisms of Kappa-casein gene

Most of the expression of the kappa-casein is determined by the protein coding region exon IV of the kappa-casein gene (CSN3). The CSN3 gene comprises of a thirteen kilobase sequence located on chromosome six and divided into five exons (Bangar *et al.*, 2021). A number of polymorphisms in the kappa-casein gene have been identified to give rise to fourteen variants or haplotypes namely A, B, B2, C, D, E, F1, F2, G1, G2, H, I and J and one synonymous variant A1 (Tanaskovska *et al.*, 2016; Fan *et al.*, 2019). Of them, A and B have been notably higher in frequency, and play a key role in influencing milk traits. These variants are associated with

enhanced milk traits such as constituents, yield, and technological properties of milk such as rennet coagulation time and thermal resistance.

Table 01 shows the allele and genotype frequencies of CSN3 gene in the investigated bovine species. The B variant differs from A by two single nucleotide polymorphisms (SNPs) rs43703015 and rs43703016 and causing the substitutions of amino acids in both 136 and 148 positions. Threonine (ACC) and aspartic acid (GAT) at position 136 and 148 in A variant, are substituted by isoleucine (ATC) and alanine (GCT) respectively, in B variant at the same location (Kovalchuk *et al.*, 2019; Vanvanhossou *et al.*, 2021). These double amino acid substitutions function as a diallelic system due to significant linkage disequilibrium created (Jiménez-Montenegro *et al.*, 2022). Furthermore, CSN3 A variant is the more frequent variant compared to CSN3 B variant (Lisson *et al.*, 2013). Thereby these SNPs lead to the genotypes AA, AB, and BB of CSN3. The B variant resulting due to these SNPs consist of HindIII recognition site while it is absent in A variant. Hence, the addition of HindIII restriction enzyme will provide 521 and 353 base pair (bp) fragments for B variant and no cleavage will be observed at A variant, which appears as a single uncut form (Pinder *et al.*, 1991; El Nahas *et al.*, 2013). Another additional distinguishing feature is the number of HinfI I sites in CSN3 A and B variants. A variant has two restriction sites for HinfI at 350 bp yielding two major fragments of 134 bp and 132 bp and a minor fragment of 84 bp, while B variant had only one restriction site yielding two fragments of 266 bp and 84 bp (Shivashanker *et al.*, 2022). These polymorphisms are controlled by co-dominant autosomal genes that are inherited according to the Mendelian inheritance (Laisin *et al.*, 2021).

## Associations of kappa-casein A and B variants with bovine milk constituents

### Protein content of bovine milk and kappa-casein A and B variants

Bovine milk contains approximately 3.35 g of protein per 100 ml (Antunes *et al.*, 2023). Most of the studies conducted on various bovine breeds such as Holstein, Holstein Friesian, Polish Holstein Friesian, Czech Simmental, Jersey, Red Sindhi, Red and White, White Backed, Brown Swiss and their crosses; reveal that the BB genotype of kappa-casein or the B variant alone is linked to significantly higher protein content compared to other genotypes and variants (Wolanciuk, 2015; Gurses *et al.*, 2016; Yaser and Senkal, 2019; Cítek *et al.*, 2021; Velmatov *et al.*, 2021). These associations were proven by a meta-analysis as well (Ozdemir *et al.*, 2018). Studies suggested that this could be due to the increased expression of CSN3 B variant due to the increased stability of its mRNA, the linkage of different promoter regions to the kappa-casein A and B variants or due to a higher level of mRNA for the CSN3 B variant (Bovenhuis *et al.*, 1992; Debeljak *et al.*, 2000). Some studies conducted on different bovine breeds such as Colombian Holstein, Holstein, Latvia Blue and Brown, Black Motley, Anatolian Black, Jersey, Simmental, Alatau and Tropical milking Criollo also have found positive associations with higher protein content of milk and BB genotype. However, these studies failed to show a significant association due to limited sample size which do not align with the central limit theorem (Gurses and Yuce, 2012; Zambrano-Burbano *et al.*, 2012; Wolanciuk, 2015; Gurses *et al.*, 2016; Petrovska *et al.*, 2017; Becerril-Pérez *et al.*, 2020; Berezkina *et al.*, 2020; Khastayeva *et al.*, 2021). Contradictorily, some studies conducted on Holstein, East Anatolian Red and Crossbred (Friesian x Jenoubi) mentioned that AB and AA genotypes are associated with a higher protein content (Gurses and Yuce, 2012; Awad *et al.*, 2016; Gurses *et al.*, 2016; Albazi *et al.*, 2023). Studies conducted on Bulgarian Rhodope, Crossbreeds of Friesian and Bunaji, Anatolian Black, Holstein Friesian, Holstein Frieswal and Gaolua observed no significant association with the genotypes (Gurses and Yuce, 2012; Ren *et al.*, 2013; Deb *et al.*, 2014; Anggraeni *et al.*, 2017; Bankar *et al.*, 2018; Soyudal *et al.*, 2019; Mehandzhiyski *et al.*, 2019; Angelova *et al.*, 2021). Hence, the association between kappa-casein genetic variants and protein content appears to vary among different bovine breeds, leading to diverse and sometimes contradictory observations in numerous studies.

### Fat content of bovine milk and kappa-casein A and B variants

Bovine milk consists of (3% to 5%) of milk fat (Moneeb *et al.*, 2021). Some studies on different bovine breeds, such as Montbéliarde, Czech Simmental, Holstein, Holstein Friesian, Brown Swiss, Jersey, White-Backed, Polish Black, Polish Red, Jersey, Crossbreed Red Sindhi, Red and White and Latvian Blue bovines suggest a significant association between the BB genotype and B variant and higher fat content and the findings were confirmed with meta-analyses as well (Bugeac *et al.*, 2013; Dođru, 2015; Wolanciuk, 2015; Gurses *et al.*, 2016; Gopi and Venkataramanan, 2017; Petrovska *et al.*, 2017; Ozdemir *et al.*, 2018; Cítek *et al.*, 2021; Velmatov *et al.*, 2021; Dragh *et al.*, 2023). Although, the studies on Brown Swiss, Bulgarian Rhodope, Montbéliarde, Hallikar and Malnad Gidda bovines show no significant association, a positive association with the BB genotype and B variant and higher fat content were noted with limited number of sample size (Gurses *et al.*, 2016; Mehandzhiyski *et al.*, 2019; Khastayeva *et al.*, 2021; Shivashanker *et al.*, 2022). This association was mentioned on a meta-analysis (Mahmoudi *et al.*, 2020). This association could result due to the higher casein content in kappa-casein B variant.

The casein net can trap fat. Hence higher casein content traps more fat leading to a higher fat content in the bovine milk (Djedović et al., 2015).

**Table 1** The allele and genotype frequencies of kappa-casein gene in the investigated bovine species.

Bovine specie	Frequencies of variants		Frequencies of genotypes			References
	A	B	AA	AB	BB	
Black Motley of the Udmurt Republic	-	-	0.56	0.40	0.04	(Berezkina <i>et al.</i> , 2020)
Montbéliarde from France	0.35	0.65	0.09	0.53	0.38	(Bugeac <i>et al.</i> , 2013)
Simmental in Serbia	0.67	0.33	0.43	0.48	0.10	(Djedović <i>et al.</i> , 2015)
Crossbreds of Simmental and Red Holstein in Serbia	0.88	0.13	0.75	0.25	0.00	(Djedović <i>et al.</i> , 2015)
Busha in Serbia	0.67	0.33	0.42	0.50	0.08	(Djedović <i>et al.</i> , 2015)
Crossbreds of Friesian and Bunaji in Nigeria	0.75	0.25	0.53	0.43	0.33	(Laisin <i>et al.</i> , 2021)
Holstein in Turkey	0.74	0.19	0.51	0.31	0.02	(Gurses <i>et al.</i> , 2016)
Brown Swiss in Turkey	0.36	0.64	0.15	0.41	0.44	(Gurses <i>et al.</i> , 2016)
Jersey in Turkey	0.31	0.69	0.12	0.38	0.50	(Gurses <i>et al.</i> , 2016)
Anatolian Black in Turkey	0.50	0.50	0.30	0.40	0.30	(Bayraktar, 2022)
Holstein in Turkey	0.29	0.71	0.50	0.41	0.09	(Bayraktar, 2022)
Anatolian Black in Turkey	0.75	0.25	0.50	0.50	-	(Gurses and Yuce, 2012)
East Anatolian Red in Turkey	0.78	0.22	0.57	0.43	-	(Gurses and Yuce, 2012)
Brown Swiss in Turkey	0.35	0.63	-	-	-	(Dođru, 2015)
Holstein in Turkey	0.169	0.831	0.04	0.26	0.70	(Soyudal <i>et al.</i> , 2019)
Polish Black-and-White Holstein-Friesian	0.48	0.52	0.23	0.50	0.27	(Wolanciuk, 2015)
Jersey in Poland	0.62	0.38	0.38	0.47	0.14	(Wolanciuk, 2015)
Polish Red	0.27	0.73	0.07	0.39	0.53	(Wolanciuk, 2015)
White-backed in Poland	0.55	0.45	0.30	0.50	0.20	(Wolanciuk, 2015)
Chinese Holsteins	0.85	0.15	0.73	0.25	0.02	(Alim <i>et al.</i> , 2015)
Holstein Friesian in Indonesia	0.91	0.09	0.23	0.71	0.06	(Anggraeni <i>et al.</i> , 2017)
Frieswal cross breed of Holstein Friesian and Sahiwal in India	0.79	0.21	0.58	0.42	0.00	(Deb <i>et al.</i> , 2014)
Red Sindhi in India	0.59	0.32	0.09	0.75	0.25	(Gopi and Venkataramanan, 2017)
Jersey Crossbreed in India	0.48	0.34	0.18	0.58	0.42	(Gopi and Venkataramanan, 2017)
Jersey in India	0.16	0.36	0.48	0.34	0.66	(Gopi and Venkataramanan, 2017)
Gaolao in India	0.93	0.07	0.89	0.09	0.02	(Bankar <i>et al.</i> , 2018)
Holstein in Iraq	0.41	0.53	0.38	0.18	0.44	(Yaser and Senkal, 2019)
Holstein Friesian in Iraq	0.40	0.60	-	-	-	(Dragh <i>et al.</i> , 2023)
Crossbred dairy in Crossbred (Friesian x Jenoubi in Iraq	0.53	0.46	0.20	0.67	0.13	(Albazi <i>et al.</i> , 2023)
Latvian Blue	0.64	0.36	0.32	0.64	0.05	(Petrovska <i>et al.</i> , 2017)
Latvian Brown	0.78	0.21	0.59	0.35	0.04	(Petrovska <i>et al.</i> , 2017)
Holstein-Friesian in the Republic of North Macedonia	0.55	0.36	0.93	0.73	0.27	(Tanaskovska <i>et al.</i> , 2016)
Girolando in Brazil	0.86	0.14	0.73	0.25	0.02	(Barbosa <i>et al.</i> , 2019)
Holstein in Slovakia	0.83	0.17	0.70	0.28	0.03	(Miluchová <i>et al.</i> , 2018)
Crossbreed of Simmental and Holstein in Slovakia	0.76	0.24	0.58	0.36	0.06	(Trakovická <i>et al.</i> , 2012)
Sahiwal in Pakistan	0.92	0.08	0.82	0.16	0.02	(Shahlla <i>et al.</i> , 2014)
Achai in Pakistan	0.70	0.18	0.12	0.79	0.21	(Ghafoor <i>et al.</i> , 2015)
Sahiwal in Pakistan	0.92	0.08	0.00	0.96	0.04	(Ghafoor <i>et al.</i> , 2015)
Brown Holstein in Bulgaria	0.00	0.00	0.16	0.59	0.27	(Angelova <i>et al.</i> , 2021)
Bulgarian Rhodope	-	-	0.19	0.56	0.20	(Mehandzhiyski <i>et al.</i> , 2019)
Black-and-White cows in Russia	0.81	0.19	0.67	0.29	0.04	(Shaidullin <i>et al.</i> , 2021)
Kholmogory in Russia	0.80	0.20	0.645	0.32	0.04	(Shaidullin <i>et al.</i> , 2021)
Czech Fleckvieh cattle breed	0.552	0.418	0.296	0.487	0.158	(Bartonova <i>et al.</i> , 2012)
Palestian Holstein Friesian	0.80	0.20	-	-	-	(Zyiad and Fawzi, 2014)
Lithuanian milk cattle	0.721	0.227	0.492	0.386	0.021	(Morkuniene <i>et al.</i> , 2016)
Holstein Friesian in Egypt	0.80	0.20	-	-	-	(Awad <i>et al.</i> , 2016)
Simmental in the Republic of Kazakhstan	0.63	0.37	0.45	0.35	0.20	(Khastayeva <i>et al.</i> , 2021)
Alatau in the Republic of Kazakhstan	0.75	0.25	0.55	0.40	0.05	(Khastayeva <i>et al.</i> , 2021)
Tropical milking Criollo in Mexico	0.39	0.61	0.09	0.60	0.31	(Becerril-Pérez <i>et al.</i> , 2020)

### **Lactose content of bovine milk and kappa-casein A and B variants**

Bovine milk contains approximately 5 g lactose per 100 ml (milliliters) of milk (Misselwitz et al., 2019). Studies on kappa-casein A and B variants and lactose content showed diversified findings. Due to the limited number of samples, studies conducted on Holstein, Holstein-Friesian, Montbéliarde, Freiswal and Goaloa noted a non-significant positive association between A variant or AA genotype and higher lactose content (Bijl et al., 2014; Deb et al., 2014; Awad et al., 2016; Bankar et al., 2018; Soyudal et al., 2019; Dragh et al., 2023). Conversely, studies on Crossbreeds of Friesian and Bunaji, Anatolian Black, East Anatolian, Tropical milking Criollo, Alatau and Simmental bovines, mention non-significant positive association with kappa-casein AB and BB genotypes and higher lactose content, respectively (Gurses and Yuce, 2012; Bijl et al., 2014; Deb et al., 2014; Bankar et al., 2018; Becerril-Pérez et al., 2020; Laisin et al., 2021; Khastayeva et al., 2021; Dragh et al., 2023).

N-acetyl neuraminic acid content of bovine milk and kappa-casein A and B variants

Sialic acid is a diverse group of nine carbon carboxylated monosaccharides found in glycomacropptide which is a milk-derived bioactive peptide released from kappa-casein by the action of chymosin (Nakano and Ozimek, 2014; Córdova-Dávalos et al., 2019; Ling et al., 2022). N-acetyl neuraminic acid is the common sialic acid derivative that links to the peptide threonine residues of kappa-casein during post translational modification. It is crucial for stabilizing glycoprotein structures and plays vital roles in cell interaction, signaling, and various physiological functions including neurodevelopment (Ghosh, 2020; Ling et al., 2022; Liu et al., 2022).

Studies on N-acetyl neuraminic acid content in relation to kappa-casein A and B variants are limited. A study conducted on Holstein bovines indicated that the degree of glycosylation is higher in bovines with the kappa-casein AB genotype compared to the AA genotype. This association is attributed to the increased glycosylation of kappa-casein B variant because it has a different conformation due to the presence of threonine instead of isoleucine, thereby it creates a new binding site for oligosaccharides (Robitaille et al., 1991; Holland and Boland, 2014). This fact was also reported in other studies too (Bonfatti et al., 2014). Similar findings are reported in studies conducted on Friesian and its' crossbreeds and Jersey bovines (Coolbear et al., 1996). Additionally, a significant correlation is noted between milk yield and N-acetyl neuraminic acid content, suggesting that an increase in N-acetyl neuraminic acid content positively influences milk yield (Robitaille et al., 1991a, b).

### **Calcium content of bovine milk and kappa-casein A and B variants**

Bovine milk, containing 0.126% calcium, helps to achieve the daily requirement of the body in one liter (Pravina et al., 2013). Few studies explore the association between kappa-casein variants A and B and calcium content. Studies involving Holstein-Friesian and Montbéliarde bovines found no significant association but suggest higher calcium content with B variant (Bijl et al., 2014). In Holstein and Black Motley bovines, the kappa-casein BB genotype is positively related to higher calcium content, potentially due to the larger casein micelles trapping more calcium phosphate (Berezkina et al., 2020). However, a study on Holstein bovines indicates a positive relation between kappa-casein AB genotype and higher calcium content, presenting conflicting results (Ren et al., 2013).

### **Phosphorous content of milk and kappa-casein A and B variants**

Phosphorus, the second most abundant mineral in the human body, is present in bovine milk at 84.4 mg per 100 ml. Limited studies explore the association between kappa-casein A and B variants and phosphorus content. Two studies on Holstein-Friesian, Holstein and Black Motley bovines reported a non-significant positive association with higher phosphorus content and B variant and BB genotype respectively (Bijl et al., 2014; Berezkina et al., 2020). The observation with BB genotype attributed to the larger casein micelles, which capture more calcium phosphate on their surface, potentially leading to increased phosphorus content (Berezkina et al., 2020).

### **Associations of kappa-casein A and B variants with the milk yield**

The impact of kappa-casein A and B variants on milk yield remains controversial, with studies reporting conflicting findings. Studies conducted on Polish Black-and-White, Polish red, Holstein Friesian, Jersey, White-backed, Czech Simmental and their crosses found significant associations with higher milk yield and AA genotype and A variant (Wolanciuk, 2015; Gurses et al., 2016; Cítek et al., 2021; Albazi et al., 2023; Dragh et al., 2023). However studies conducted on Friesian, Holstein, Holstein Friesian, Simmental, Czech Fleckvieh Frieswal, Sahiwala and their crosses mentioned that AB genotype produced a significantly higher milk yield compared to other genotypes (Bartonova et al., 2012; Deb et al., 2014; Shahlla et al., 2014; Djedović et al., 2015; Awad et al., 2016; Tanaskovska et al., 2016; Laisin et al., 2021). Studies on Red and White bovines reported that BB genotype produced significantly higher milk yield compared to other genotypes (Velmatov et al., 2021). Some studies conducted on Black-Motley, Simmental, Alatau and Tropical milking Criollo bovines have shown a non-significant positive association between the BB genotype and higher milk yield, while others indicate no significant association (Becerril-Pérez et al., 2020; Berezkina et al., 2020; Khastayeva et al., 2021). Meta-analyses further confirm the complexity of these associations, emphasizing the need for a deeper understanding of kappa-

casein variants and their influence on milk yield (Mahmoudi et al., 2020). Different bovine breeds, populations, and methodologies contribute to the variability in results, and further research is essential to unravel the intricate relationship between kappa-casein variants and the milk production trait.

### **Rennet milk coagulation time of bovine milk and kappa-casein A and B variants**

Milk coagulation, a crucial step in cheese production, involves the destabilization of casein micelles. It takes place in two phases, the enzymatic phase, and the non-enzymatic phase (Troch et al., 2017). During the enzymatic phase, renin cleaves the C-terminal of kappa-casein, leading to micelle destabilization. The non-enzymatic phase occurs at pH 6.6, resulting in spontaneous micelle combination to form a gel-like coagulum. Numerous studies investigate the association between kappa-casein A and B variants and rennet milk coagulation time. The studies conducted on Holstein, Holstein-Friesian, Czech Simmental bovines and their crosses, have shown a significant positive association between kappa-casein BB genotype and shorter rennet milk coagulation time (Tanaskovska et al., 2016; Cítek et al., 2021). While some studies on Simmental, Bulgarian Rhodope, Holstein, Brown Holstein, Alatau and Latvian Brown bovines noted non-significant positive association with AB and BB genotypes and shorter rennet milk coagulation time (Bonfatti et al., 2014; Petrovska et al., 2017; Mehandzhyski et al., 2019; Yaser and Senkal, 2019; Angelova et al., 2021; Khastayeva et al., 2021). Evidence suggests that the difference in the primary amino acid sequence of kappa-casein B variant could interact favorably with the enzyme rennin (Cítek et al., 2021). Further, the degree of glycosylation also influences coagulation time, with higher glycosylation associated with shorter coagulation time. The size of casein micelles, fat globules, and their interactive effects further impact coagulation properties, where smaller casein micelles and larger fat globules lead to faster coagulation. (Panthi et al., 2017). Overall, the studies highlight the complex interplay of genetic variants, glycosylation, and micelle characteristics in determining the rennet milk coagulation time property.

### **Thermal resistance of bovine milk and kappa-casein A and B variants**

Thermal resistance of milk has been defined as the technological property of milk to withstand elevated temperatures without the clotting of the proteins. There are few studies that mention about the association between kappa-casein A and B variants and thermal resistance. Some studies conducted on Holstein, Frieswal, Crossbreeds of Black-Motley and Holstein and Kholmogorskaya bovines of the Tatarstan type reported that, no significant association between kappa-casein A and B variants and thermal resistance but showed that the B variant was associated with higher thermal resistance (Zambrano-Burbano et al., 2012; Deb et al., 2014; Miluchová et al., 2018; Tyulkin et al., 2018).

### **Conclusion**

Kappa-casein, a crucial protein in the dairy industry, is influenced by variants such as A and B of the CSN3 gene, impacting milk properties. Despite the varied findings, selecting the B variant as the genetic marker shows promising result for enhancing the milk properties, but further research is needed to explore the associations with other factors such as sample size, environmental conditions, bovine breeds, and management approaches in different countries and the milk properties.

**Conflict of interest** - none.

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