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Kappa Casein in Farm Animals: A Review

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ABSTRACT

A review of the results reported by different research workers has been compiled; with the aim to focus on various aspects encompassing the application of the important areas of research work on milk kappa casein. The major whey proteins in cow milk are alpha-lactalbumin and beta-lactoglobulin. Casein is a slow-digesting protein in contrast to whey protein that is rapid digesting. Milk Casein is of 3 or 4 types and each type has a similar structure but distinct molecules with different compositions, genetic variations, and functional properties. The main components of the cow milk casein complex are αs1 (CSN_1S_1), αs_2 (CSN_1S_2), β (CSN_2), and κ-caseins (CSN_3) with each one of them occurring in two or more variants. Transgenic technologies could be used to alter milk composition in cows. Kappa-casein has been associated with differences in milk yield, composition and processing. Research work on casein has been attempted in different species (cow, buffalo, sheep, goat, horse, camel), yet it needs be extended to the remaining species and breeds of animals. The accurate and early identification of milk protein genotypes can have a direct impact on reviewing and developing dairy cattle breeding programs, to achieve the target of increasing production of milk and quality of its constituents on an economic footing. The review details the applied aspects of casein, classification and structure, polymorphism of kappa casein gene in different species of animals.

KEYWORDS: Evolution, genes, kappa casein, polymorphism, structure

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1. INTRODUCTION

Liquid milk is considered to be a wholesome food and is also used in industrial processes for many dairy products. Good milk quality is essential for commercial dairy products. The production of cheese depends on the quality of milk Caseins. It has been reported that the genotype of animals has a relation with the production of cheese but the effect of genotype is reported to be inconsistent. The milk casein which is very important concerning cheese production is kappa casein (KCN). The inconsistent findings of an association between production traits and protein polymorphism emphasized the need to confirm under different climatic conditions. The focus on the study of KCN gene manipulation is also an important area of research for the benefit of the patients suffering from metabolic diseases through creating low-phenylalanine containing milk.

Improvements in livestock and dairy technologies are the keys to boost up milk production. Milk being a wholesome food is not only contributing nutrients but is also providing food security as well as in reducing poverty & malnutrition at the global level. (Hemme and Otte, 2010). Milk protein is one of the important constituents of milk of all species. Ganguli et al. (1964) reported that the composition of cow and buffalo milk was identical with respect to the types and quantity of amino acids. Barlowska et al. (2011) opined that the most universal raw material for the processing of dairy products is cow milk. Due to their high protein content, including casein, and fat, sheep and buffalo milk are very good raw material, especially for cheese making. Camel milk owes high nutritional value as its milk contains a high proportion of antibacterial substances and a thirty times higher concentration of vitamin C in comparison to cow milk. The milk from goat is a good raw material for dairy processing and also to some extent as a therapeutic product.

The comparative milk protein percentage was noted to be 4.71–4.79 in Gir Cows and 5.31–5.94 in Jaffarabadi buffaloes, both at 1 – 3 months lactation (Ninan et al., 2014). Eckles et al. (1997) reported milk protein to be 6.52, 3.82 and 2.00 percentage in Ewe, Doe and Mare, respectively. The milk protein percentage in Camels is around 2.50–4.50 (Anonymous, 2002). Medrano and Aguilar-Cordova (1990) reported the milk protein percentage to be 3.29, 3.64, 3.48, 3.75, 3.98 and 3.32 in Holstein, Brown Swiss, Ayrshire, Gurnsey, Jersey and Short Horn cattle breeds, respectively.

The total protein component of milk is partitioned into two major categories of milk proteins namely 1. Caseins and 2. Whey proteins. Both caseins proteins and whey proteins are good sources for essential amino acids. The milk proteins are heterologous groups with varied structures and properties (Barlowska et al., 2011). Thirteen major milk proteins have been identified along with their genetic variants (Lucey et al., 2017). The present review encompasses various species like cattle, buffaloes, sheep and goat to highlight the Research done, the applied aspects of kappa casein of milk and to emphasize the area of future research so as to enable the livestock entrepreneur for better commercial and economic benefits.

2. CASEIN V/S WHEY PROTEINS

The major whey proteins in cow milk are alpha-lactalbumin and beta-lactoglobulin. Casein is a slow-digesting protein and whey is a fast-digesting protein. The casein family contains phosphorus but the serum (whey) proteins do not contain phosphorus. The casein will coagulate or precipitate at pH 4.6., whereas whey proteins remain in solution in milk at pH 4.6. Casein in milk is of 3 or 4 types and each type is having a similar structure but is distinct molecules with different compositions, genetic variations, and functional properties. Caseins make up between 20–45% of human milk proteins as against 80% in bovine milk (Kunz and Lonnerdal, 1990). The main components of the cow milk casein complex are αs1 (CSN_1S_1), αs_2 (CSN_1S_2), β (CSN_2), and κ-caseins (CSN_3), and each one occurs in two or more variants. The κ-casein (CSN_3) molecule is a single-chain polypeptide of 169 amino acids with a molecular weight of 19.2 KDa (Rachagani and Gupta, 2008, Galila et al., 2008) and is the only protein fraction that contains the sulphur amino acids cysteine and methionine, constituting approximately 13% of milk casein (Farrell et al., 2004). The amino acids of casein help in the growth and development of the young. Milk proteins include enzymes, proteins involved in transporting nutrients, antibodies and growth factors (Haenlein 1996). Kappa-caseins (κ-CN) exist as A or B variants and are of particular importance for the milk quality (Medrano and Aguilar-Cordova, 1990); Pizarro et al. (2020) stated that Goat milk casein proteins (αs_1, αs_2, ß, and κ) are encoded by 4 loci (CSN_1S_1, CSN_2S_2, CSN_2, and CSN_3) clustered within 250 kb on chromosome 6.

Table 1: Old and new symbols for bovine casein gene nomenclature (Bawden and Nicholas, 1999)

Gene	Old symbol	New symbol
αs_1–casein	CASA 1	CSN_1S_1
αs_2–casein	CASA 2	CSN_1S_2
β–casein	CASB	CSN_2
κ–casein	CASK	CSN_3

3. APPLIED ASPECTS OF CASEIN (Kunz and Lonnerdal, 1990)

Casein being a major component of cheese–supplies amino acids, carbohydrates, calcium and phosphorus. It is a food additive and can be used as binder for safety matches. It forms soluble aggregates called 'casein micelle' in which k-casein molecules stabilize the structure. B gene is associated with an increase in milk, protein, and cheese yield. Kappa–casein is split into insoluble para casein and soluble hydrophilic glycopeptide–caseinomacropeptide, which is responsible for increased efficiency of digestion, prevention of neonate hypersensitivity to ingested proteins and inhibition of gastric pathogens. Casein has anti-oxidant and radical scavenging properties. The proteolytic fragments of α-s 2 casein exhibit anti-bacterial activity viz. Casocidin-1 inhibits *E. coli* and *Staph. carnosis* growth, Casoparan peptide activate macrophage phagocytosis and peroxidase release and Casohypotensin and casoparan may be involved in bradykinin regulation

4. CASEIN STRUCTURE

The major protein component of ruminant milk is the caseins (αs_1, αs_2, β, and κ) secreted in the form of stable calcium phosphate micelles. The micelles contribute towards the low viscosity of the milk despite the high protein concentration. The casein micelles are stabilized by stearic and electrostatic repulsion of the polar C-terminal domain of the kappa casein protein (Horne, 1992) and functional importance for protein and mineral nutrition of the offspring and in demonstrating the physical properties of milk.

The various models proposed for the conformation of casein in the micelle include a) Several sub micelles form the micelle molecule with kappa casein at the periphery (Dalgleish, 1998 and Drohse and Foltmann, 1989), b) Casein inter-linked fibres form the nucleus (Esteves et al., 2003) and c) Model that suggests a double link among casein molecules for gelling to take place (Holt, 1992).

5. KAPPA CASEIN CLASSIFICATION AND STRUCTURE

Kappa casein can be classified into different groups based on the comparison of hydrophobicity, carbohydrate, and amino acid content and composition and site of proteolytic cleavage (Table 2).

Kappa-casein needs a definite structure to fulfill its function as the interface between calcium-sensitive casein and milk serum. Other milk caseins do not seem to have a role that requires well defined structures. This divergence may reflect differences in the mechanism of milk clotting between mammalian species. Kappa-casein is cleaved into five chains viz. Casoxin-C, Casoxin-6, Casoxin-A, Casoxin-B and Casoplatelin (Ginger and Grigor, 1999).

Glycosylation degree of Kappa casein is higher in colostrum as compared to milk and is more in milk during mastitis infection (Dziuba and Minkiewicz, 1996). Goat milk contains larger micelles, including more calcium and other minerals compared to cow's milk. These larger micelles are attributed to improve the cheese making properties of goat milk (Rahmatalla et al., 2021). As a result of post-translational modifications, caprine kappa casein appears heterogeneous in milk protein electrophoresis assays with at least five forms of which the main one being non-glycosylated form (Addeo et al., 1978; Recio et al., 1997).

Table 2: Groups of kappa Casein

Group	Site	Species	References
Group No 1	Chymosin sensitive band is Phe–Met	Cattle, sheep, goat, water buffalo	Mercier et al., 1976a
Group No 2	Cleavage site is Phe–Ile or Phe – Leu	Human, camel, mouse, rat, pig	
Separate Group	Chymosin cleavage site is Phe – Ala	Marsupials	Stasiuk et al., 2000

6. KAPPA CASEIN GENES

Caseins are encoded by four tightly linked and clustered genes, approximately covering an area of 250 Kb genomic DNA fragments. Kappa casein cDNA has been characterized in several species, including cattle (Gorodetskii and Kaledin, 1987), sheep (Furet et al., 1990) and goat (Coll et al., 1993). The casein gene locus has been mapped on chromosome no. 6 in bovine (Threadgill and Womack, 1990) and caprine species (Hayes et al., 1993). The order of the casein genes in the cluster is αs_1, β, αs_2 and κ with the kappa casein located in the region 95–120 Kb downstream of the αs_2–gene and about 200 Kb from the αs_1–gene in the bovine genome (Rijnkels et al., 1997).

Kappa casein mRNA in goats contain an open reading frame of 579 bp coding for 21 amino acids of signal peptide and 171 amino acids of the mature protein. The signal peptide of kappa casein is different in both length and amino acid sequence from the consensus sequence of the calcium-sensitive genes. The structure of the gene is also quite different and has been described in bovine (Alexander et al., 1988), goat (Coll et al., 1995), and rabbit (Baranyi et al., 1993). The coding sequence for mature protein is contained in exon three (9 amino acids) and four (162 amino acids). The sole milk protein gene whose signal peptide is encoded by two exons (2 and 3), is that of kappa casein. The 3'–untranslated region is contained within exons 4 and 5. Repetitive elements have been identified in the 5' flanking region of the kappa casein gene from different species (Coll et al., 1995 and Gerencser et al., 2002). Similar repetitive elements have also been found in introns 2 and 3

(Coll et al., 1995). The bovine kappa casein gene contains a microsatellite repeat in introns 3 with 6 alleles (Lien and Rogne, 1993; Leveziel et al., 1994). The caprine kappa casein promoter contains two types of repetitive elements: a 206–bp SINE (short interspersed nuclear element) and a 114–bp fragment of a LINE (Long interspersed nuclear element).

6.1. Evolution of kappa casein gene

The polymorphism present in milk proteins is due to genetic variation and respective variants are transmitted with no dominance. However, other processes like phosphorylation and glycosylation can cause certain heterogeneity in caseins (Mclean, 1987).

Kappa casein is structurally related to the γ-fibrinogen and kappa casein genes have been shown to possess higher degree of conservations (Patel et al., 2011). The kappa casein gene was postulated to be evolutionarily related to the fibrinogen (g-chain) gene family whose cleavage by thrombin results in blood clotting (Jolles et al., 1978). This hypothesis is sustained by the structural and functional similarities between the proteins and by the nucleotide sequence similarities between κ-casein and fibrinogen cDNA (Thompson et al., 1985).

Although the kappa casein gene is not evolutionarily related to the genes encoding the calcium-sensitive caseins, it is physically and functionally linked to them and the four genes are co-ordinately expressed at high levels in tissue and stage-specific fashion. Thus, the expression pattern of the kappa casein seems to be familiar to that of other caseins despite the different organization of its 5' flanking region. Nevertheless, kappa casein genomic clones (from goat, cow, and rabbit) were either non-functional (Ninomiya et al., 1994; Rijnkels et al., 1995) or were poorly expressed (Persuy et al., 1996; Baranyi et al., 1993) in transgenic mouse lines under their own regulatory sequences. In contrast, the kappa casein gene has been shown to be expressed in the mammary gland of transgenic mice (Persuy et al., 1996; Gutierrez et al., 1996) and transgenic cattle (Brophy et al., 2003) when linked to the β-casein regulatory sequences. These observations suggest that regulatory elements might be involved in the expression of the entire casein gene locus (may be located in the 5' proximal region of the cluster) analogous to the Locus Control Region (LCR) described for the β-globin gene cluster (Grosveld et al., 1987; Li et al., 2002).

6.2. Polymorphism of the kappa casein gene

The caprine kappa casein was first isolated by Zittle and Custer (1966) and its amino acid composition was determined by Richardson et al. (1973). Subsequently, the complete amino acid sequence of 171 residues was established (Mercier et al., 1976a, b). The main differences in the amino acids between caprine and bovine kappa caseins are located in the C-terminal portion of the protein. Compared to their bovine counterpart, ovine and caprine k-caseins have in common the insertion of two amino acid residues Val–His (Valine–Histamine) between positions 131 and 132 (Mercier et al., 1976b).

6.2.1. Cattle

The association of milk casein gene polymorphism has been shown with differences in milk composition, processing and quality (McLean, 1987) and yield characteristics (Lin et al., 1986). Kappa casein in bovines is widely polymorphic with nine genetic variants characterized to date. In cattle, the kappa-casein gene (CSN_3) presents two common genetic variants, A and B, and these alleles differ by substitutions in 2 amino acids, at positions 136 (Thr–Ile) and 148 (Asp–Ala) and both occurring in the CMP region of the protein (Grosclaude et al., 1973, Mercier et al., 1973). The six kappa casein variants (A, B, C, D, E, F, and G) can be genotyped by PCR-RFLP using *Hind* III (or *Hinf* I), *Hae* III, *Hha* I, and *Mae* II endonucleases (Schlieben et al., 1991; Prinzenberg et al., 1996). The genetic analysis of the promoter regions of the kappa-casein gene (CSN_3) was done by Keating et al. (2007) in 42 cattle representing 9 different breeds. They found that 2 distinct haplotypes (A and B) exist at this locus, differing from each other by single base changes at positions -514 (T/G), -426 (T/C) and -384 (T/C) where haplotype A has T, T and T and haplotype B has G, C and C. The AA and AB haplotypes occurred at a higher frequency of 69.0 % and 21.4 % being homozygous and heterozygous, respectively. Molee et al. (2011) in a study on two groups of Holstein cows (< 87.5% and > 87.5%) found five genotypes of beta-casein (A1A2 and A1B being most and least frequent) and five genotypes of kappa casein AA being most frequent and BB and BE being least frequent), in both the groups. Galila et al. (2008) through their work reported that among the examined 20 Holstein cattle, 17 were found to be of the genotype AA and 3 were of the AB genotype. The BB genotype could not be detected among the studied animals. Higher protein percentage in milk is associated with kappa-casein genotype AB compared to AA, but there was no difference in the fat percentage. Mir et al. (2014) determined the allele and genotype frequencies of genetic variants in five milk protein genes (αs_1 casein, ß-casein, κ-casein, α-lactalbumin, and ß-lactoglobulin) using the SNaPshot genotyping method and concluded that higher milk production in the population of Sahiwal in Pakistan is associated with the AB genotype identified in the kappa casein gene. Thus, incorporation of genotypes AB and BB may help to improve the milk yield in the Sahiwal cattle. A similar observation was reported by Akter et al. (2020) in native cattle of Bangladesh. They reported that within the case of κ-casein gene, AA genotype

(0.73) had higher frequency followed by AB (0.23) and BB (0.04) genotype. Ladyka (2022) evaluated the Ukrainian domestic dairy breeds and reported that the distribution of genotype frequency for kappa casein gene varies significantly in different breeds viz. Lebedyn–19% A/A, 50% A/B and 31% B/B; Ukrainian Brown dairy–30% A/A, 41% A/B and 30% B/B; Simmental–44% A/A, 46% A/B and 10% B/B; Ukrainian Black-and-White dairy–58% A/A, 27% A/B and 15% B/B. The association of genetic polymorphism with milk production and composition has stimulated interest in using genetic polymorphism of casein genes in molecular marker assisted selection (MAS) to improve milk productivity in farm animals (Kumar et al., 2007). Genetic polymorphism in kappa casein gene serve as informative biomarkers of milk yield and composition (Albazi et al., 2023). Chiatti et al. (2007) found a favourable effect of the CSN_3 variant for both protein and casein percentages, and the codominance trend for the 3 phenotypes was BB>AB > AA. The level of kappa casein in the milk was reported to be doubled in the transgenic cows and the milk had a slightly yellowish tinge when compared with normal milk due to the light scattering properties of smaller casein micelles present in the milk. The future use of transgenic technologies could be to a) Benefit animal health, for example, by improving the growth and survival of calves, b) Prevent animal diseases, such as mastitis, c) Make milk with human health benefits and d) Assist milk processing into dairy products.

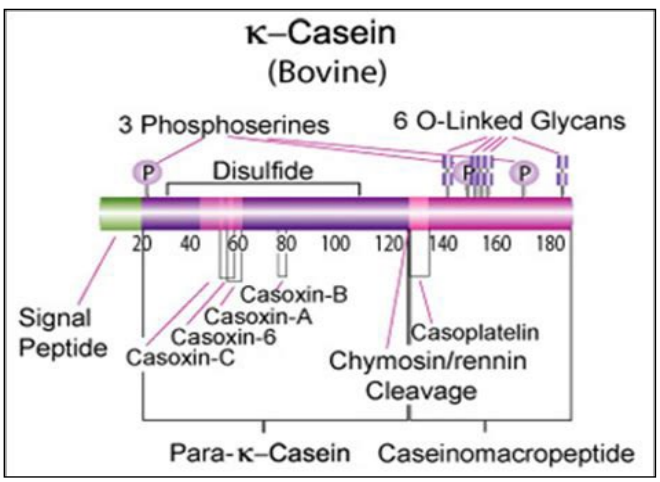

Figure 1: Structure of Bovine K-casein; Source: http://2014.igem.org/Team:SF_Bay_Area_DIYbio/Parts

6.2.2. Buffalo

The literature reviewed here includes work on Italian water buffalo, Murrah buffalo, Nili-Ravi buffalo and Egyptian buffalo in connection to kappa-casein variants.

Addeo and Mercier (1977) determined the complete amino acid sequence of caseinomacropeptide (C terminal fragment released by kappa casein) in Italian water buffalo (Bubalus arnee), and found that it contains 64 amino acid residues together with one phosphoserine which differs from its bovine (Bos taurus) B counterpart by 10 amino acid substitutions. Water buffalo kappa-casein is homologous to the bovine variant B. They felt that a variant Thr136-Ala148 might be the wild type of the Bos genus.

However, Mitra et al. (1998) detected polymorphism in alleles A and B of kappa-casein (*CSN3*) in Murrah, Nili-Ravi, and Egyptian buffaloes. They did not detect the genotype BB among the animals studied (Otaviano et al., 2005). However, DNA sequencing of the amplified fragment (GenBank Acc. No. U96662) revealed one polymorphism at codon 135 (ThrACC->I1eATC) in buffalo; the frequencies of 135 Thr/Ile alleles were estimated as 0.88 and 0.12, respectively. Similarly, Al- Shawa (2020) found polymorphism at the level of κ-CN gene with two different alleles 'A' and 'B' in Egyptian buffaloes, by the method of PCR-RFLP. Further Al-Shawa (2019) opined that polymorphism in the κ-CN gene should be involved within modern selection programs as a potential candidate associated with dairy performance traits i.e. gene assisted selection (GAS) that will permit the selection of animals at an early age for breeding programs. In a study on 115 female Murrah breed and its crossbred buffaloes, Otaviano et al. (2005) using PCR-RFLP and SSCP techniques demonstrated that the animals were monomorphic (only allele B) for the kappa-casein gene. They opined that this makes it difficult to quantify the gene action on milk yield and its constituents. Abdel Dayem et al. (2009) using the PCR-RFLP technique concluded that bulls were monomorphic for the kappa-casein gene and they possess only allele B in homozygosis form.

Sharma et al. (2011) conducted studies on the evolutionary relatedness of mammary-derived kappa casein gene of riverine buffalo (Bubalus bubalis). They reported that the kappa-casein gene which is involved in milk clotting with fibrinogen gamma-chain, leucine-rich repeat interacting protein 1 gene, and chymosin gene involved in blood clotting are inter-related. Kappa casein and chymosin are involved in milk clotting while fibrinogen γ chain and leucine-rich repeat interacting protein 1 gene are involved in blood clotting. Ozsensoy (2020) observed in Anatolian water buffaloes the presence of Hinf I/BB genotype, associated with higher milk protein amount and milk fat.

6.2.3. Sheep

By protein electrophoresis, the kappa casein seems to be monomorphic in sheep and no variants were detected in protein electrophoresis (Alais and Jolles, 1967; Soulier et al., 1974). However, Addeo et al. (1992) using chromatographic techniques suggested possible polymorphism. At the DNA level, restriction analysis showed a two-allele polymorphism with *Hind* III and *Pvu* II enzymes (Di Gregorio et al., 1991) and three allele polymorphisms with *Pst* I endonuclease (Leveziel et al., 1991). In sheep two κ-CN variants were found (Ceriotti et al., 2004) compared to bovine κ-CN which has 11 variants with A and B being the most common (Farrell et al., 2004) and goat which has 16 κ-CN variants (Prinzenberg et al., 2005).

6.2.4. Goat

Variants in kappa casein using different techniques documented in the literature have been reviewed here for different goat breeds. Kappa casein variants have been characterized in different goat breeds (Yahyaoui et al., 2001; Caroli et al., 2001; Angiolillo et al., 2002). The first evidence of genetic polymorphism was reported by Mercier et al., (1976a, b) while sequencing the CMP from the milk of an Alpine Saanen goat. They found either Valine or Isoleucine at position 119 and considered that the animal sequenced was heterozygous at this position. Isoleucine residue was postulated to be predominant since it was found in the sequence of bovine and ovine species.

Quantitative differences at the Kappa casein (CSN_3) level must be closely considered because of their essential role in the reproduction process of mammals. Gupta et al. (2009) found Kappa casein (CSN_3) gene polymorphism in exon 4 and also identified different variants A, B, C, D, E, G and confirmed the DNA levels in Jakhrana goat breeds. The polymorphism seems to be associated with effects on milk production traits (Angulo et al., 1994). The genetic variation of kappa casein involving the mature protein could be associated with other polymorphisms in the non-coding sequences (promoter, introns) which might be causative mutations for the expression differences between some alleles, as has already been found for the two main bovine kappa casein (CSN_3) alleles (Martin et al., 2002). The favourable effect of the kappa casein (CSN_3) B variant on milk protein and kappa casein (CSN_3) expression could be linked to these amino acid differences in the mature protein, possibly affecting both the biological properties of kappa casein and its biochemical interactions with the other casein fractions in the casein micelle (Lucey et al., 2003; Meisel, 2005). Kappa casein protein (only casein fraction that contains the sulphur amino acids cysteine and methionine), constitutes approximately 13% of milk casein. Among the eleven alleles identified the *A, B* and *E* were the most common (Farrell et al., 2004). Great differences have been found among goat breeds and populations in the polymorphism distribution within casein genes and haplotypes. The haplotypes coding for CSN_1S*B, CSN_2*A, CSN_1S_2*A and CSN_3*B protein variants was postulated as ancestral among the haplotypes considered by Sacchi et al. (2005) and Caroli et al. (2006). Kiplagat et al. (2010) found in East African goats that the prevalent casein variant was CSN_3*B with frequencies which ranged from 0.750 to 0.953. The CSN_3*A was the second most common allele. In Egyptian breeds, allele B is the most common allele and appeared at a frequency of 68.2%, whereas allele C appeared with 22.7%. Allele A was displayed in different frequencies ranging from 10 to 45.5%. The appearance of each allele was in homozygous or heterozygous genotypes (Othman and Ahmed, 2007). There is a strong influence of the kappa casein (CSN_3) gene in combination with the β-lactoglobulin (LGB) gene on milk protein composition and milk coagulation properties (Matejicek et al., 2008).

The monomorphic patterns of the Kappa casein (CSN_3) gene (exon 4) were studied with *Hae* III restriction endonucleases and genotype frequency in Barbari, Marwari, Beetal, Surti and local breeds of the region of Madhya Pradesh (Kumar et al., 2009). Patel et al. (2011a) noted that in the Zalawadi goat breed, PCR–RFLP analysis with *Hae* III restriction enzyme confirmed AA genotype in CSN_3 gene. CSN_3 gene was found to be polymorphic in Zalawadi goat and restriction endonucleases BSeN1 and Alw441 revealed GG, GA, and AA genotype and CC, Ct, and TT genotype, respectively (Patel et al., 2011b). Goat breeds of Lithuania exhibited genetic diversity in milk proteins which were found to be polymorphic for αs_1–casein, αs_2–casein, kappa casein, and β–lactoglobulin genes (Baltrenaite et al., 2009). In South Africa, in 5 goat populations, limited genetic variation was found for kappa casein, with the Saanen goat population exhibiting no variation at all for the kappa casein gene (Scheepers et al., 2010). Veress et al. (2004) in their studies on Hungarian milk goat breeds concluded that the direct relationship between the allelic variants CSN_3S_1, CSN_1S_2, and CSN_2 genes and casein content, which further influence the physico-chemical properties of milk could be utilized in breeding schemes aiming at the improvement of milk processing quality and cheese yields. Rashayedeh et al. (2020) investigated the genetic polymorphism and association of milk protein genes and yield in Awassi sheep strains of Palestine and reported significant effects of *β-LG* genotypes on fat content and density of milk in both strains, whereas certain *κ-CN* genotype showed effects on solid non-fat milk content in local Awassi only.

Milk of goat has low levels of αs_1 casein. It is difficult to digest but lower curd tension makes it easier to digest than that present in cow milk. Goat milk has A_2 beta-casein and is recommended to those who try to avoid A_1 beta-casein. In Germany, Caprine (isoform–A) and ovine (isoform–B) dairy products are differentiated by the isoelectric focusing (IEF) of para-k-casein peptide (Tsartsianidou et al., 2016). However, they found that the nucleotide polymorphism within the goat k-casein gene in indigenous breeds of Greece altered the isoelectric point of para-k-casein peptide and led to false positive results.

7. CONCLUSION

With the abundance of casein proteins, kappa-casein has been associated with differences in milk yield, composition and processing. Research work on casein has been attempted in different species, yet it is insufficient and has to be extended to the remaining species and breeds of animals. The precise and timely identification of milk protein genotypes are likely to assist in reviewing and developing dairy cattle breeding programs, to achieve the target of increasing production of milk and quality of its constituents on economic footing.

8. REFERENCES

Abdel Dayem, A.M.H., Karima Gh, M.M., Nawito, M.F., Ayoub, M.M., Darwish, S.F., 2009. Genotyping of kappa-casein gene in Egyptian buffalo bulls. Livestock Science 122(2–3), 286–289.

Addeo, F., Mauriello, R., Moio, L., Laezza, P., Chianese, L., Di Luccia, A., 1992. Ovine casein variant identification using electrophoretic immunochemical and chromatographic techniques. Milchwissenschaft 47, 283–287.

Addeo, F., Mercier, J.C., 1977. Primary structure of the casein macropeptide of kappa casein of buffalo. Biochimie 59(4), 375–379.

Addeo, F., Soulier, S., Pelissier, J.P., Chobert, J.M., Mercier, J.C., Ribadeau-Dumas, B., 1978. Preparation and fractionation of goat k-casein: analysis of the glycan and peptide components. Journal of Dairy Research 45(2), 191–196.

Akter, S., Anower, A.K.M.M., Hossain, M.M.K., Alin, M.A., Jahan, N., Bhuyan, A.A., Salimullah, M., Alam, J., 2020. Genotyping of κ-casein and ß-lactoglobulin genes in native cattle from Barishal region of Bangladesh. Animal Biotechnolgy 33(1), 110–115. https://doi.org/10.1080/10495398.2020.1781145.

Alais, C., Jolles, P., 1967. Isolation, purification, and analysis of two κ-casein like fractions from sheep casein 1, 2. Journal of Dairy Science 50, 1555–1561.

Albazi, W.J., Al-Jhuwaini, T.M., Alamely, M., Jeddoa, Z.M., Mousa, R., Al-Dawmy, F., Atallah, A.H., Altaee, R., Jabber, E., Al-Shimmary, F., Salin, S., Al-Himaery, N., 2023. Association of kappa casein gene polymorphism with milk production traits in crossbred dairy cows. Ciencia Animal Brasileria 24, e-74079E. https://doi.org/10.1590/1809-6891v24e-74079E.

Alexander, L.J., Stewart, A.F., Mackinlay, A.G., Kapelinskaya, T.V., Tkach, T.M., Gorodetsky, S.I., 1988. Isolation and characterization of the bovine Kappa casein gene. European Journal of Biochemistry 178(2), 395–401.

Al Shawa, Z.M., El Zarei, M.F., Ghazy, A.A., Amin, M.S., Ayoub, M.A., Merdan, S.M., Mokhtar, S.A., 2019. Genetic profile of κ–casein gene based on RFLP technique in association with milk traits in Egyptian buffaloes. Journal of Animal, Poultry and Fish Production; Suez Canal University 8(1), 9–20.

Al-Shawa, Z.M., El Zarei, M.F., Ghazy, A.A., Ayoub, M.A., Merdan, S.M., Mokhtar, S.A., 2020. Polymorphic, allelic and genotypic frequencies of κ-casein and ß-LG genes in Egyptian buffaloes. Journal of Agricultural Science 12(4), 283. doi.10-5539/jas.v12n4p283.

Angiolillo, A., Yahyaoui, M.H., Sanchez, A., Pilla, F., Folch, J.M., 2002. Characterization of new genetic variants in the caprine Kappa casein gene. Journal of Dairy Science 85(10), 2679–2680.

Angulo, C., Amills, M., Ares, J.L., Jimenez, I., Jordana, J., Sanchez, A., Serradilla, J.M., 1994. Genetics of caseins and its relation with yield, composition and cheese making of milk in Spanish breeds of Goats. In: Proceedings of the 74th Meeting of EAAP, August 25–29, 1994, Lillehammer, Norway, pp: 209–209.

Anonymous, 2002. Handbook of Animal Husbandry (3rd Edn.), pub, DIPA, ICAR, New Delhi.

Baltrenaite, L., Kerziene, S., Morkuniene, K., Miceikiene, I., 2009. Genetic diversity in milk proteins among goats breed in Lithuania. Acta Universitatis Latviensis 753, 117–124.

Baranyi, M., Bozse, Z.S., Buchberger, J., Zrause, I., 1993. Genetic polymorphism of milk proteins in Hungarian spotted and Hungarian grey Cattle: A possible new genetic variants of β-lactoglobulin. Journal of Dairy Science 76(2), 630–636.

Barlowska, J., Szwajkowska, M., Litwinczuk, Z., Krol, J., 2011. Nutritional value and technological suitability of milk from various animal species used for dairy production. Comprehensive Reviews in Food Science and Food Safety 10(6), 291–302.

Bawden, W.S., Nicholas, K.R., 1999. Molecular genetics of milk production. In: Fries, R., Ruvinsky, A. (Eds.), The genetics of cattle. CABI Publishing, New York, NY. Pages 539–576 CABI Record Number: 19990105991, CABI Publishing, Wallingford, UK. ISBN (Hardback): 978-0-85199-258-7.

Brophy, P., Smolenski, G., Wheeler, T., Wells, D., L'Huileer, P., Laible, G., 2003. Cloned transgenic cattle produce milk with higher levels of β-casein and κ-casein. Nature Biotechnology 21(2), 157–162.

Caroli, A., Chiatti, F., Chessa, S., Rignanese, D., Bolla, P., Pagnacco, G., 2006. Focusing on the goat casein gene complex. Journal of Dairy Science 89(8), 3178–3187.

Caroli, A., Jann, O., Budelli, E., Bolla, P., Jager, S., Erhardt, G., 2001. Genetic polymorphism of goat Kappa casein (CSN3) in different breeds and characterization at DNA levels. Animal Genetics 32(4), 226–230.

Ceriotti, G., Chessa, S., Bolla, P., Budelli, E., Bianchi, L., Duranti, E., Caroli, A., 2004. Single nucleotide polymorphisms in the ovine casein genes detected by polymerase chain reaction-single strand conformation polymorphism. Journal of Dairy Science 87(8), 2606–2613.

Chiatti, F., Chessa, S., Bolla, P., Cigalino, G., Caroli, A., Pagnacco, G., 2007. Effect of κ-casein polymorphism on milk composition in the orobica goat. Journal of Dairy Science 90(4), 1962–1966

Coll, A., Folch, J.M., Sanchez, A., 1993. Nucleotide sequence of the goat kappa-casein cDNA. Journal of Animal Science 71(10), 2833.

Coll, A., Folch, J.M., Sanchez, A., 1995. Structural features of the 5′ flanking region of the caprine κ-casein gene. Journal of Dairy Science 78, 973–977.

Creamer, L.K., 1991. Some aspects of casein micelle structure. P 148–163 in Interactions in Food Proteins. Parris, N., Barford, R. (Eds.), ACS Symp. Ser. 454. Am. Chem. Soc., Washington, DC. (c.f. Creamer et al. (1998). Journal of Dairy Science 81, 3004–3012)

Dalgleish, D.G., 1998. Casein micelles as colloids: Surface structures and stabilities. Journal of Dairy Science 86(11), 3013–3018.

Di Gregorio, P., Rando, A., Pieragostini, E., Masina, P., 1991. DNA polymorphism at the casein loci in sheep. Animal Genetics 22(1), 21–30.

Di Gregorio, P., Rando, A., Ramunno, L., Masina, P., Pieragostini, E., 1989. Polymorphism of the DNA region containing casein genes in sheep and goat. XXIV Simposio Internazionale Zootecnica, 275–282.

Drohse, H.B., Foltmann, B., 1989. Specificity of milk clotting enzymes towards bovine κ-casein. Biochimica et Biophysica Acta 995(3), 221–224.

Dziuba, J., Minkiewicz, P., 1996. Influence of glycosylation on micelle-stabilizing ability and biological properties of C-terminal fragments of cow's κ-casein. International Dairy Journal 6(11-12), 1017–1044.

Eckles, C.H., Combs, W.B., Macy, H., 1997. Milk and milk products. (4th Edn). Tata McGraw Hill Publishing Co., New Delhi.

Esteves, C.L.C., Lucey, J.A., Wang, T., Pires, E.M.V., 2003. Effect of pH on the gelation properties of skim milk gels made from plant coagulants and chymosin. Journal of Dairy Science 86(8), 2558–2567.

Farrell, H.M.J., Jimenez-Flores, R., Bleck, G.T., Brown, E.M., Butler, J.E., Creamer, L.K., Hicks, C.L., Hollar, C.M., Ng-Kwai-Hang, K.F., Swaisgood, H.E., 2004. Nomenclature of the proteins of cows' milk-sixth revision. Journal of Dairy Science 87(6), 1641–1674.

Furet, J.P., Mercier, J.C., Soulier, S., Gaye, P., Hue-Delahaie, D., Vilotte, J.L., 1990. Nucleotide sequence of ovine k-casein cDNA. Nucleic Acids Research 18(17), 5286.

Galila, A., Darwish, F., 2008. A PCR-RFLP assay to detect genetic variants of kappa-casein gene in cattle and buffalo. Arab Journal of Biotechnology 11(1), 11–18.

Ganguli, N.C., Prabhakaran, R.J.V., Iya, K.K., 1964. Composition of the caseins of buffalo and cow milk. Journal of Dairy Science 47(1), 13–18.

Gerencser, A., Barta, E., Boa, S., Kastanis, P., Bosze, Z., Whitelow, C.B., 2002. Comparative analysis on the structural features of 5' flanking regions of κ-casein genes from six different species. Genetics Selection Evolution 34(1), 117–128.

Ginger, M.R., Grigor, M.R., 1999. Comparative aspects of milk caseins. Comparative Biochemistry and Physiology Part B 124(2), 133–145.

Gorodetskii, S.I., Kaledin, A.S., 1987. Nucleotide sequence analysis of cow kappa casein cDNA. Genetika 23(4), 596–604.

Grosclaude, F., Mercier, J.C., Ribadeau-Dumas, B., 1973. Genetic aspects of cattle casein research. Netherlands Milk Dairy Journal 27, 328. Annales de Genetique et de Selection Animale 4(4), 515–521. www.gse-journal.org/articles/gse/pdf/1972/04/AGSE_0003-4002_1972_4_4_ART0003.pdf.

Grosveld, F., van Assendelft, G.B., Greaves, D.R., Kollias, G., 1987. Cell 51, 975-985 (c.f. Rijnkels et al, 1995).

Gupta, S.C., Kumar, D., Pandey, A., Malik, G., Gupta, N., 2009. New k-casein alleles in Jakhrana goat affecting milk processing properties. Food Biotechnology 23(1), 83–96.

Gutierrez, A., Mega, E.A., Meade, H., Shoemaker, C.F., Medrano, J.F., Andersson, G.B., Murray, J.D., 1996. Alternations of the physical characteristics of milk from transgenic mice producing bovine k–casein. Journal of Dairy Science 79(5), 791–799.

Haenlein, G.F.W., 1996. Nutritional value of dairy products of Ewe and goat milk. In: Proceedings of the IDF/CIRVAL Seminar Production and Utilization of Ewe and Goat Milk, vol. 9603. Crete, Greece, Internat. Dairy Fed. Publ., Brussels, Belgium, Pp. 159–178. (c.f. Haenlein, G.F.W. 2004. Goat milk in human nutrition. Small Ruminant Research 51, 155–163.

Hayes, H., Petit, E., Bouniol, C., Popescu, P., 1993. Localisation of the alpha-S2- casein gene (CASAS2) to the homologous cattle, sheep and goat chromosome 4 by in situ hybridisation. Cytogenetics and Cell

Genetics 64(3–4), 281–285.

Hemme, T., Otte, J., 2010. Status and prospects for smallholder milk production: a global perspective. Food and Agriculture Organization of the United Nations.

Hobor, S., Kunej, T., Lenasi, T., Dovc, P., 2006. Kappa casein gen (CSN_3) in horse: Genetic variability in exon 1 and 4. Acta Agriculturae Slovenica 88(2), 83–89.

Holt, C., 1992. Structure and stability of bovine casein micelles. Advances in Protein Chemistry 43, 63–151.

Horne, D.S., 1992. Ethanol stability. In: Fox, P.F. (Ed.), Advanced dairy Chemistry Vol. I. Proteins. London, Elsevier Applied Science, 657–690. ISBN: 978-1-85166-761-1, CABI Record Number: 19930457654, Elsevier Applied Science, London, UK.

Iametti, B.S., Tedeschi, G., Oungre, E., Bonomi, F., 2001. Primary structure of kappa-casein isolated from mares' milk. The Journal of Dairy Research 68(1), 53–61.

Jolles, P., Loucheux-Lefebvre, M.H., Henschen, A., 1978. Structure relatedness of kappa casein and fibrinogen γ-chain. Journal of Molecular Evolution 11(4), 271–277.

Keating, A.F., Davoren, P, Smith, T.J., Ross, R.P., Cairns, M.T., 2007. Bovine kappa-casein gene promoter haplotypes with potential implications for milk protein expression. Journal of Dairy Science 90(9), 4092–4099.

Kiplagat, S.K., Agaba, M., Kosgey, I.S., Okeyo, M., Indetie, D., Hanotte, O., Limo. M.K., 2010. Genetic polymorphism of kappa-casein gene in indigenous Eastern Africa goat populations. International Journal of Genetics and Molecular Biology 2(1), 1–5.

Kumar, A., Rout, P.K., Mandal, A., Roy, R., 2007. Identification of the CSN1S1 allele in Indian goats by the PCR-RFLP method. Animal 1(8), 1099–1104.

Kumar, A., Rout, P.K., Mandal, A., Roy, R., 2009. Kappa casein gene polymorphism in Indian goats. Indian Journal of Biotechnology 8, 214–217. http://nopr.niscpr.res.in/handle/123456789/3893.

Kunz, C., Lonnerdal, B., 1990. Human-milk proteins: analysis of casein and casein subunits by anion-exchange chromatography, gel electrophoresis and specific staining method. The American Journal of Clinical Nutrition 51(1), 37–46.

Ladyka, V., 2022. Evaluation of cow genotypes by kappa-casein of dairy breeds. Acta Fytotechnica et Zootechnica 25(1). ISSN 1336-9245.

Leveziel, H., Metenier, L., Guerin, G., Cullen, P., Provot, C., Bertaud, M., Mercier, J.C., 1991. Restriction fragment length of ovine casein genes: close linkage between the αs_1, αs_2, β and κ-casein loci. Animal Genetics 22(1), 1–10.

Leveziel, H., Rodellar, C., Leroux, C., Pepin, L., Grohs, C., Vaiman, D., Mahe, M.F., Martin, P., Grosclaude, F., 1994. A microsatellite within the bovine κ-casein gene reveals a polymorphism correlating strongly with polymorphisms previously described at the protein as well as the DNA level. Animal Genetics 25(4), 223–228.

Li, M.H., Zhao, S.H., Bian, C., Wang, H.S., Weid, H., Liu, B., Yu, M., Fan, B., Chen, S.L., Zhu, M.J., Lia, S.J., Xiong, T.A., Li, K., 2002. Genetic relationships among twelve Chinese indigenous goat populations based on microsatellite analysis. Genetics Selection Evolution 34(6), 729–744.

Lien, S., Rogne, S., 1993. Bovine casein haplotypes number, frequencies and applicability as genetic marker. Animal Genetics 24(5), 373–376.

Lin, C.Y., McAllister, A.J., Ng-Kwai-Hang, K.F., Hayes, J.F., 1986. Effects of milk protein loci on first lactation production in dairy cattle. Journal Dairy Science 69(3), 704–712.

Lucey, J.A., Johnson, M.E., Horne, D.S., 2003. Perspectives on the basis of the rheology and texture properties of cheese. Journal of Dairy Science 86(9), 2725–2743.

Lucey, J.A., Otter, D., Horne, D.S., 2017. A 100-year review: Progress in the chemistry of milk and its compounds. Journal of Dairy Science 100(12), 9916–9932.

Martin, P., Szymanowska, M., Zwierzcowski, L., Leroux, C., 2002. The impact of genetic polymorphisms on the protein composition of ruminant milks. Reproduction Nutrition Development 42(5), 433–459.

Matejicek, A., Matejickova, J., Stipkova, M., Hanus, O., Gencurova, V., Kyselova, J., Nemcova, E., Kott, T., Sefrova, J., Krejcova, M., Melcova, S., Holzelova, I., Bouska, J., Frelich, J., 2008. Joint effects of CSN3 and LGB genes on milk quality and coagulation properties in Czech Fleckvieh. Czech Journal of Animal Science 6, 246-252. 03.pdf (agriculturejournals.cz).

McLean, D.M., 1987. Influence of milk protein variants on milk composition, yield and cheese making properties. Animal Genetics 18, 100–102.

Medrano, J.F., Aguilar-Cordova, E., 1990. Genotyping of bovine kappa-casein loci following DNA sequence amplification. Biotechnology 8(2), 144–146.

Meisel, H., 2005. Biochemical properties of peptides encrypted in bovine milk proteins. Current Medical Chemistry 12(16), 1905–1919.

Mercier, J.C., Addeo, F., Pelissier, J.P., 1976a. Structure primaire de la caseinomacropeptide caprine. Biochimie 58(11-12), 1303–1310. PMID: 1016651.

Mercier, J.C., Brignon, G., Ribadeau-Dumas, B., 1973. Structure primaire de la caséine k-B bovine Séquence ulpecul. Europe Journal of Biochemistry 35(2), 222–235.

Mercier, J.C., Chobert, J.M., Addeo, F., 1976b. Comparative analysis of the amino acid sequence of the casinomacropeptide from seven species. FEBS Letters 72(2), 208–214.

Mir, S.N., Ullah, O., Sheikh, R., 2014. Genetic polymorphism of milk protein variants and their association studies with milk yield in Sahiwal cattle. African Journal of Biotechnology 13(4), 555–565.

Miranda, G., Anglade, P., Mahe, M.F., Erhardt, G., 1993. Biochemical characterization of the bovine genetic kappa-casein C and E variants. Animal Genetics 24(1), 27–31.

Mitra, A., Schlee, P., Krause, I., Blusch, J., Werner, T., Balakrishnan, C.R., Pirchner, F., 1998. Kappa-casein polymorphisms in Indian dairy cattle and buffalo: a new genetic variant in buffalo. Animal Biotechnology 9(2), 81–87.

Molee, A., Boonek, L., Rungasakinin, N., 2011. The effect of kappa casein genes on milk yield and milk composition in different percentages of Holstein in crossbred dairy cattle. Animal Science Journal 82(4), 512–516.

Ninan, J., Arya, J.S., Gajbhiye, P.U., 2014. Correlation between milk and plasma components in Gir cattle and Jaffarabadi buffaloes during different lactation stages. Indian Journal of Dairy Science 67(1), 57–61.

Ninomiya, T., Hirabayashi, M., Sagara, J., Yuki, A., 1994. Functions of milk protein gene 5′ flanking regions on human growth hormone gene. Molecular Reproduction and Development 37(3), 276–283. https://doi.org/10.1002/mrd.1080370306.

Otaviano A.R., Tonhati, H., Sena, J.A.D., Muñoz, M.F.C., 2005. Kappa-casein gene study with molecular variations in female buffaloes, bubalus bubalis. Genetics and Molecular Biology 28(2), 237–241.

Othman, E., Ahmed, S., 2007. Genotyping of the caprine kappa casein variants in Egyptian Breeds. International Journal of Dairy Science 2(1), 90–94.

Ozsensoy, Y., 2020. Assessment of polymorphism on kappa casein gene of Anatolian water buffalo breed using PCR-RFLP method. Turkish Journal of Veterinary and Animal Sciences 44(4), 904–909.

Patel, S.B., Pande, A.M., Rank, D.N., Arya, J.S., Ninan, J., 2011a. Kappa casein gene polymorphism in Zalawadi goats. Indian Journal of Biotechnology 10(2), 235–237.

Patel, S.B., Rank, D.N., Ninan, J., Pande, A.M., 2011b. Polymorphic characteristics of kappa casein in Zalawadi goat. The Indian Journal of Field Veterinarians, 7(1), 9–12.

Persuy M.A., Printz, C., Medrano, J.F., Mercier, J.C., 1996. One mutation might be responsible for the absence of beta-casein in two breeds of goats. Animal Genetics 27, 96. https://hal.inrae.fr/hal-02686110.

Pizarro, M.G., Landi, V., Navas, F.J., Martínez, A., Fernández, I., Delgado, J.V., Leon, J.M., 2020. Nonparametric analysis of casein complex genes epistasis and their effects on phenotypic expression of milk yield and composition in Murciano-Granadina goats. Journal of Dairy Science 103(9), 8274–8291. https://doi.org/10.3168/jds.2019-17833.

Prinzenberg, E.M., Gutscher, K., Chessa, S., Caroli, A., Erhardt, G., 2005. Caprine kappa-casein (CSN3) polymorphism: new developments in molecular knowledge. Journal of Dairy Science 88(4), 1490–1498.

Prinzenberg, E.M., Hiendleder, S., Ikonen, T., Erhardt, G., Prinzenberg, E.M., Hiendleder, S., Erhardt, G., Ikonen, T., 1996. Molecular genetic characterization of new bovine κ-casein alleles CSN3-F and CSN3-G and genotyping by PCR.-RFLP. Animal Genetics 27(5), 347–349. https://doi.org/10.1111/j.1365-2052.1996.tb00976.x.

Rachagani, S., Gupta, I.D., 2008. Bovine kappa-casein gene polymorphism and its association with milk production traits. Genetics and Molecular Biology 31(4), 893–897.

Rahmatalla, S.A., Arends, D., Ahmed, A.S., Hassan L.M.A., Krebs, S., Reissmann, M., Brockmann, G.A., 2021. Capture sequencing to explore and map rare casein variants in goats. Frontiers in Genetics 12, 620253. https://doi.org/10.3389/fgene.2021.620253.

Rashayedeh, F.S., Sholi, N., Al-Atiyat, R.H., 2020. Genetic polymorphism of milk genes (β-lactoglobulin and κ-casein) in indigenous awassi and improved awassi sheep of Palestine. Livestock Research for Rural Development 32(5), 75.

Richardson, B.C., Creamer, L.K., Munford, R.E., 1973. Comparative micelle structure. I. The isolation and chemical characterisation of caprine κ-casein. Biochimica et Biophysica Acta 310(1), 111–117. https://doi.org/10.1016/0005-2795(73)90013-5.

Rijnkels, M., Kooiman, P.M., de Boer, H.A., Pieper, F.R., 1997. Organisation of the bovine casein gene locus. Mamm. Genome 8(2), 148–152.

Rijnkels, M., Kooiman, P.M., Krimpenfort, P.J.A., de Boer, H.A., Pieper, F.R., 1995. Expression analysis of the individual bovine β-; αS_2- and K-casein genes in transgenic mice. Biochemical Journal 311(3), 929–937

Sacchi, P., Chessa, S., Budelli, E., Bolla, P., Ceriotti, G., Soglia, D., Rasero, R., Cauvin, E., Caroli, A., 2005. Casein haplotype structure in five Italian goat breeds. Journal of Dairy Science 88(4), 1561–1568.

Scheepers, R.C., van Marle-Koster, E., Visser, C., 2010. Genetic variation in the kappa-casein gene of South African goats. Small Ruminant Research 93(1), 53–56. doi:10.1016/j.smallrumres. 2010.03.007.

Schlieben, S., Erhardt, G., Senft, B., 1991. Genotyping of bovine k-casein (k- CN A, k-CN B, k-CN C, k-CN E) following DNA sequence amplification and direct sequencing of k-CN E PCR product. Animal Genetics 22(4), 333–342.

Sharma, A., Kanwar, S.S., Tantia, M.S., Vijh, R.K., 2011. Evolutionary relatedness of mammary derived kappa casein gene of riverine buffalo (*Bubalus bubalis*). Wayamba Journal of Animal Science 3, (1305537960) 116–117.

Soulier, S., Ribadeu-Dumas, B., Deanmur, R., 1974. Purification des caséines χ de brebis. European Journal of Biochemistry 50(2), 445–452.

Stasiuk, S.X., Summers, E.L., Demmer, J., 2000. Cloning of marsupial kappa-casein cDNA from brushtail possum (*Trichosurus ulpecula*). Reproduction fertility and Development 12(3–4), 215–222.

Thompson, M.D., Dave, R.J., Nakhashi, H.L., 1985. Molecular cloning of mouse mammary gland Kappa casein: comparison with rat Kappa casein and rat and human γ-fibrinogen. DNA 4(4), 263–271.

Threadgill, D.W., Womack, J.E., 1990. Genomic analysis of the major bovine milk proteins genes. Nucleic Acids Research 18(23), 6935–6942.

Tsartsianidou, V., Triantafillidou, D., Karaiskou, N., Tarantili, P., Triantafillidis, G., Georgakis, E., Triantafyllidis, A., 2016. Caprine and ovine greek dairy products. The official German method generates false-positive results due to κ-casein gene polymorphism. Journal of Dairy Science 100(5), 3539–3547.

Veress, G., Kuzsa, S., Bosze, Z., Kukovivs, S., Javor, A., 2004. Polymorphism of the αs_1-casein, κ-casein and ß-lactoglobulin genes in the Hungarian milk goat. South African Journal of Animal Science 34(5), 20–23.

Yahyaoui, M.H., Coll, A., Sanchez, A., Folch, J.M., 2001. Genetic polymorphism of the caprine kappa casein gene. Journal of Dairy Research 68(2), 209–216.

Zittle, C.A., Custer, J.H., 1966. Identification of the k-casein among the components of whole goat casein. Journal of Dairy Science 49(7), 788–791.