

Major proteins in goat milk: an updated overview on genetic variability

Maria Selvaggi · Vito Laudadio · Cataldo Dario ·
Vincenzo Tufarelli

Received: 7 February 2013 / Accepted: 20 December 2013 / Published online: 1 January 2014
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Abstract Milk and dairy products are very important in Mediterranean diet because of their health promoting and organoleptic properties. In many developing countries, goat rearing has a key role in livestock production. What makes goats so popular is their ability to provide high quality food under diverse climatic conditions and resilience to extreme and capricious environments. In the last years, the interest concerning caprine milk has been increasing also to find a new exploitation for local breeds. To promote the goat dairy products there is a clear need to know the quality and the technological aspects of milk produced. That being so, the purpose of this study was to review the available literature on the major goat milk proteins with a particular attention to recent findings on their genetic variability. Moreover, the main effects of different protein variants on milk yield and composition were also discussed.

Keywords Milk · Casein · Whey protein · Dairy goats · Polymorphism · Genetic variants

Introduction

Milk and dairy products are very important in Mediterranean diet because of their health promoting and organoleptic properties. In many developing countries, goat rearing plays a key role in livestock production, in fact it is estimated that over 80 % of the world's goat population is

located in Asia and Africa. The domestication of goat is well known since ancient times, as it is thought to have arisen about 10,000 years ago in the mountains of Iran [1]. In the past goats were considered as marginal species for subsistence farming of poor populations underestimating their economic role and potentialities. To date, goats are no more only synonymous of underdevelopment and poverty in fact goat milk plays a vital role in human nutrition in the area acknowledged as the cradle of modern civilization. What makes goats so popular is their ability to provide high quality food under diverse climatic conditions, as well as their resilience to extreme and capricious environments [2]. Although it may not be important in certain parts of the world, the contribution of goat milk production to the economic and nutritional wellbeing of humanity is undeniable in many developing countries, especially in the Mediterranean, Middle East, Eastern Europe and South American countries. In the last years, interest concerning caprine milk has been increasing also to find a new exploitation for local breeds. In developed countries belonging to Europe, Oceania and North and South America, goat milk production is assuming an increasingly economic relevance, especially due to the production of goat cheeses which are suitably selected as a gourmet food, and receive the highest prices among cheeses on the market [3]. Consumption of goat milk and dairy products is also associated with beneficial health effects. New potential uses and marketing exploitation strategies for goat milk are also being investigated, for example, as a basis for nutraceutical and infant foods. Dairy products are used as vehicles for other functional ingredients such as phytosterols, fish fatty acids and many probiotic bacteria. Another aspect of demand for goat milk derives from the increasing number of people with cow milk allergies and other gastrointestinal diseases. Cow milk allergy is the most frequent

M. Selvaggi · V. Laudadio · C. Dario (✉) · V. Tufarelli
Department of DETO—Section of Veterinary Science and
Animal Production, University of Bari 'Aldo Moro',
70010 Valenzano, BA, Italy
e-mail: cataldo.dario@uniba.it

allergy in the first years of life. In the absence of maternal milk, allergic subjects need an alternative protein source, which is usually based on hydrolyzed cow milk proteins (caseins or whey proteins) or soybean-based formula. Milk from various mammalian species (goat, donkey and horse) has been suggested as a possible alternative to cow milk also for infant-food formulae. That being so, quality and safety of goat milk must be necessarily improved to enhance the growing interest in goat dairy products all over the world.

In order to promote the goat dairy products, there is a clear need to know the quality and technological aspects of milk produced. There are many qualitative differences between goat milk and milks produced by other domestic species not only in terms of composition. As a consequence, goat milk have peculiar properties and specific technological destinations; the milk protein composition in toto and the unique micellar structure determine particular differences in the rheological proprieties of the curds produced after coagulation.

Milk is a secretion of the mammalian gland, whose composition and physical characteristics of vary from species to species. It is a complex oil-in-water emulsion containing fat, protein, lactose, minerals as well as enzymes, cells, hormones and immunoglobulins. The two major categories of milk proteins are: insoluble proteins (the casein family) and soluble proteins (whey proteins) found in lactoserum. Hence, caseins include α s1, α s2, β , and κ -caseins, while the whey proteins are α -lactalbumin and β -lactoglobulin (Table 1). Milk also comprises important minor proteins, such as serum albumin, immunoglobulins, lactoferrin, transferrin, calcium-binding protein, prolactin, folate-binding protein and proteoseptone. The content of nitrogen components in goat milk varies according to breed, genetics, season, stage of lactation and feed. Goat milk has a natural whey protein to casein ratio of about 20:80. The non-protein nitrogen

content is in overall 5.8 % of total nitrogen. The main components of the non-protein nitrogen fraction are urea, free amino acids, nucleosides, nucleotides and polyamines [3, 4].

More than 95 % of the proteins contained in ruminant milk are synthesized from six structural genes that encode well characterized proteins, the whey proteins (β -lactoglobulin and α -lactalbumin) and the caseins (α s1, α s2, β , and κ -caseins).

The caseins are a family of phosphoproteins synthesized in the mammary gland in response to lactogenic hormones and other stimuli and secreted as large colloidal aggregates termed micelles, which are responsible for most of the unique physical properties of milk. Their relevance for the dairy industry worldwide have made them an important target for study by many scientists leading to them becoming perhaps the most widely studied food proteins. Initially, the caseins were considered as a homogenous substance. Subsequently, Linderstrom-Lang and Kodama [5] demonstrated that they were composed of two fractions: one which precipitated in the presence of calcium (calcium-sensitive caseins) and the other which was responsible for stabilizing the former against precipitation (calcium-insensitive caseins). Later, Mellander [6] demonstrated the presence of three different components designated as α -, β -, and γ -casein. Further studies revealed that an even greater number of components was present in the casein fraction [7] and much efforts were dedicated to clarify these minor components. However, with the expansion of molecular biology, it is now possible to affirm that only four types of caseins exist and the earlier heterogeneity that was recognized by electrophoresis is due to effects of post-translational processing, alternative splicing of the gene product or genetic polymorphisms [8]. In 1984, the American Dairy Science Association Committee on Nomenclature and Classification proposed that the nomenclature developed for the bovine caseins, namely the α s1, α s2, β and κ -caseins, be adopted for investigations of milk proteins in other species.

Caseins are the major protein fraction of milk in many species and their function is to transport calcium phosphate in milk and hence to provide the newborns with a source of calcium and phosphorus for bone formation as well as to contribute to the requirement for amino acids [9]. The caseins, being secreted proteins, possess N-terminal signal peptides that direct the passage of the newly synthesized polypeptides into the lumen of the endoplasmic reticulum, whereupon the signal peptides are removed from the primary translation products to yield the mature caseins. The calcium-sensitive caseins show similar molecular masses (around 24 kDa), promoter regions, leader peptide sequences and locations of the major phosphorylation site. These data support the hypothesis of a common

Table 1 Concentrations of protein fractions in goat

Total protein (g/kg)	37.20
Total casein (g/kg)	24.00
α s1-Casein (% of total casein)	5.60
α s2-Casein (% of total casein)	19.20
β -Casein (% of total casein)	54.80
κ -Casein (% of total casein)	20.40
Whey proteins (g/kg)	7.40
α -Lactalbumin (% of total whey protein)	24.00
β -Lactoglobulin (% of total whey protein)	53.70
Minor whey proteins (% of total whey protein)	22.30
NPN $\times$ 6.38 (g/kg)	5.80

Adapted from Park et al. [3], Barth and Behnke [11], Jenness [37], Mora-Gutierrez et al. [40]

evolutionary origin of these genes from the duplications and modifications of a unique ancestral gene [10]. The remainder of the sequences appear to be subject to few functional constraints and have accumulated many nucleotide substitutions, as revealed by comparative sequence analysis [9]. In goat, a high polymorphism has been found at the four casein genes with several alleles associated with null or reduced expression of the respective protein. Besides caseins nitrogen (76 % of total nitrogen content of milk), the nitrogen content of milk is also distributed among whey proteins (18 %) and non-protein nitrogen (6 %). As previously reported, the term whey proteins has been used to describe the group of milk proteins that remain soluble in milk serum or whey after precipitation of caseins at pH 4.6 and 20 °C and after rennet casein precipitation [11, 12]. The former whey protein source is known as acid whey, the latter is referred to as sweet or rennet whey [13]. Unlike casein, whey proteins contain no phosphorus and are characterized by a high content of sulfur-containing amino acids, mainly methionine and cystine. This aspect is very important for the requirement of newborns since they need 4–6 % of sulfur-containing amino acids (on total amino acids content) in order to well support growth [14, 15].

Whey proteins are globular molecules with a substantial content of α -helix motifs, in which the acidic/basic and hydrophobic/hydrophilic amino acids are distributed in a fairly balanced way along their polypeptide chains [16]. About 75 % of all milk whey proteins are albumins (α -lactalbumins and β -lactoglobulins) a valuable source of bioactive peptides with extra physiological activity. From a nutritional point of view, milk whey proteins have been considered superior to casein in various aspects. They have a better amino acid profile, being similar to those of human milk. Therefore, whey proteins are recommended for the formulation of milk products for replacement of bovine milk in infant nutrition [17]. It was observed that the caseins undergo much lower digestion and absorption than the whey proteins [18]. Whey proteins are used as food ingredients due to their important functional properties such as solubility, viscosity, water-holding capacity, gelation, adhesion, emulsification and foaming [19].

Due to the current and growing interest on this topic and considering the large number of new information, the purpose of this study was to review the available literature on goat milk proteins with a particular attention to recent findings on milk protein genetic variants to support scientists in the acquirement of the new advances on this matter. Moreover, the effects of different protein variants on milk yield and composition were discussed.

Caseins

α s1- and α s2-Caseins

Among calcium-sensitive caseins, α s1-casein has the greater solubility in the presence of calcium. It is a highly phosphorylated protein and it is a structural component of the casein micelle playing a key role in cheese curd formation [12, 20]. The gene encoding for the α s1-casein is known as CSN1S1 gene. In many eutherian species, the nucleotide sequence of this gene shows the presence of a highly conserved signal sequence of 15 residues even if quite divergent sequences for the mature proteins suggest that they are encoded by a rapidly evolving gene family. The α s1-casein consists of 214 amino acids in each species [21]. Bovine α s1-casein exists in two phosphorylated forms containing 8 and 9 phosphates/mol [22]. Different variants of the α s1-caseins have been described within species. In cattle, eight protein variants of the α s1-casein are known and classified from A to H. The two most common variants (B and C) differ in the amino acid exchange Glu→Gly at amino acid position 192 of the mature protein [12].

The α s2-caseins are a more disparate group than the α s1-caseins and have been identified from a wide number of species such as ovine, caprine and bovine [9, 23, 24]. The α s2-casein is the least investigated protein probably due to the difficult in isolation and purification. However, it is found throughout mammalian milks and, in some rare instances, contributes up to 29 % of the composition of the whole casein content. Furthermore, it is found in higher concentration in larger caprine casein micelles [25]. The α s2-caseins are highly phosphorylated peptides. The complete amino acid sequence of α s2-casein consists 207 residues with a large number of positively charged side chains, especially in the C-terminal segment [9]. Like κ -casein, it contains two Cys residues. However, it has a low Pro content. No reports on the presence of α s2-casein in human milk are found [26]. The gene encoding for α s2-casein (CSN1S2) has a signal sequence highly conserved but the mature proteins are quite divergent as for α s1-caseins. The role of α s2-casein in casein micelles has not been yet studied in depth. The α s2-casein comprises up to 10 % of the casein fraction in bovine milk; it consists of two major and several minor components exhibiting varying levels of post-translational phosphorylation as well as minor degrees of intermolecular disulfide bonding [12, 27]. Many protein variants of the α s2-casein have been reported in bovine milk (A–D). The protein variant A was initially determined chemically [28] and later by cDNA and genomic DNA sequencing [9, 29]. Variants B and C are specific for zebus and yaks, respectively [30–32],

whereas the D variant has been found at low frequencies in some European bovine breeds and in the African Namchi taurine breed [33–36]. The original variant A is the most frequent in all breeds so far investigated and also almost fixed in most western breeds. The different forms of the gene and protein are caused by single nucleotide polymorphisms that result in amino acid changes.

Until the late 1980s, it was believed that goat milk lacked the α s1-casein [37]. Subsequently, many α s1-casein variants have been found in goat milk with marked difference in their level of expression [38–40]. In fact, the polymorphism of α s1-casein is one of the most interesting and extensively studied in goat and cow milk [41]. The types of mutations are single nucleotide substitutions, deletions or large insertions. The α s1-casein shows great individual quantitative variations due to the occurrence of genetic polymorphisms [41, 42] (see also Table 2). Nine of the currently identified alleles in goat milk (A, B₁, B₂, B₃, B₄, C, H, L and M), identified as strong alleles, are associated with a high level of α s1-casein in milk (~3.5 g/l), two (E and I) with medium levels (1.1–1.7 g/l), and three (D, F and G; weak alleles) with low levels (~0.45 g/l). The O₁, O₂ and N are “null” alleles and produce no α s1-casein in goat milk [41, 43–50]. Due to the important role in cheese-making ability of α s1-casein, the impact of low α s1-casein content in milk can be significant [51–54]. Optimization of curd firmness is crucial to cheese-makers to optimize cheese yield. In addition, shorter coagulation time (the point at which coagulation appears firstly or the time when casein micelles have aggregated sufficiently to form visible flocs) and higher coagulation rate (the measure of how quickly the curd firms once coagulation has begun) would shorten the processing time required for cheese production. The potential to improve milk coagulation properties by selecting for high α s1-casein milk may be significant. Research on milk from Saanen and Alpine goat breeds showed that α s1-casein content is related with other milk components and coagulation properties [53]. In fact, goat milks containing higher or intermediate α s1-casein had significantly higher levels of all milk components than milks without α s1-casein. Milks with higher solid not fat and protein content began coagulating later than milk with low solids, suggesting that protein delays the onset of coagulation. Milks with high levels of α s1-casein had long coagulation time and formed firm curds probably due to the presence of higher amounts of α s1-casein and α s2-casein in the milk [55]. These two protein fractions may delay curd formation by binding Ca²⁺ ions, making fewer available for binding after proteolysis of κ -casein by rennet [53]. Moreover, average coagulation rate values of milks containing high amounts of α s1-casein were significantly higher than for milks lacking this protein [55]. To exploit the genetic variability could be a good opportunity for

Table 2 Milk protein genetic polymorphisms in goat

	Variant	References
α s1-Casein	A, B ₁ , B ₂ , B ₃ , B ₄ , C, D, E, F, G, H, I, L, M, N, O ₁ , O ₂	Grosclaude et al. [41], Moiola et al. [43], Rando et al. [44], Bevilacqua et al. [45], Ramunno et al. [46], Sacchi et al. [47], Chianese et al. [49], Grosclaude and Martin [50]
α s2-Casein	A, B, C, D, E, F, G, O	Boulanger et al. [62], Bouniol et al. [63], Lagonigro et al. [65], Ramunno et al. [66], Ramunno et al. [68], Erhardt et al. [67], Marletta et al. [69]
β -Casein	A, A1, B, C, D, E, O, O'	Mahé and Grosclaude [84], Neveu et al. [85], Ramunno et al. [87], Persuy et al. [88], Galliano et al. [89], Cosenza et al. [90], Caroli et al. [91]
κ -Casein	A, B, B', B'', C, C', D, E, F, G, H, I, J, K, L, M	Caroli et al. [140], Yahyaoui et al. [121], Jann et al. [141], Prinzenberg et al. [122], Caravaca et al. [142]
α -Lactalbumin	Three nucleotide substitutions	Cosenza et al. [172], Lan et al. [173]
β -Lactoglobulin	Many nucleotide substitutions and one indel	Chianese et al. [188], Yahyaoui et al. [187], Pena et al. [190], Graziano et al. [189], Ballester et al. [191], Kumar et al. [192]

dairy farmers, in fact milk from animals possessing strong alleles may be used for the dairy industry; while animals with mild or null alleles can be reared to obtain milk for allergic subjects [56–58]. Goat milk lacking α s1-casein is less allergenic than other goat milk. For goat breeding programs, this new knowledge could be a challenge and rewarding, especially since selection for or against α s1-casein is now practiced in some countries.

It has been assessed that the variance in α s2-casein content account for the unique physicochemical characteristics of goat caseins explaining a large part of differences in the technological properties between goat and cow milks [53, 59–61]. Concerning the polymorphism of α s2-casein in goat, at least eight alleles have been identified, associated with three synthesis levels [47] (see Table 2). Boulanger et al. [62] and Bouniol et al. [63] found three variants (A–C) differing by amino acid substitutions [64]. Variants A, B, C, E [65], F [66] and G [67] are related to normal synthesis levels; whereas D and O are associated with lower and null synthesis

level respectively [68]. The content of α s2-casein was associated with allergenic properties of milk: variants A, B, C, E and F displayed a higher allergenic potency than did D and O [69]. D variant is a rare defective allele characterized by a deletion, involving part of the exon 11 and of the following intron, that should results in the loss of the codons Pro (CCC₁₂₂), Thr (ACC₁₂₃) and Val (GTG₁₂₄) and the substitution Thr→Asn at position 121 [66]. The null allele shows a G→A transition at the 80th nucleotide in exon 11, creating a premature stop codon at position 110 [68]. Mass spectrometry studies revealed that in goat milk each α s2-casein variant can exist in two forms; the main component corresponds to the full length of 207 residues, while the minor component, caused by a skipping event, lacks the internal sequence Glu₃₄-Glu₄₂ or Lys₃₅-Glu₄₃ [70]. Recently, two new truncated subvariants of goat α s2-casein variants A and E have been identified [70, 71]. An evolutionary pathway has been proposed [47] starting from the A variant and leading independently to the B, C, F alleles, each characterized by a different amino acid substitution with respect both to goat variant A and to the bovine and ovine sequences. Thus, A allele may be considered the ancestral variant.

β -Caseins

β -Caseins are described as “calcium-sensitive” because they precipitate in the presence of low concentrations of this cation. They widely contribute to determine the surface properties of casein micelles being essential for curd formation when milk is clotted by the enzyme chymosin [72]. The β -casein family includes several eutherian and two marsupial proteins for which the amino acid and nucleotide sequence is known [9, 73–77]. Eutherian β -caseins gene sequences are characterized by a highly conserved 15 residue signal sequence although the mature proteins show a degree of divergence among species in terms of amino acid identity similar to that observed for the α s1-caseins [78]. The β -caseins are particularly rich in glutamines; the presence of a single major phosphorylation site near the N-terminus is well identified. These proteins have a lower number of phosphorylation sites and a lesser phosphorylation degree compared to α s1- and α s2-caseins. At least 12 bovine variants for β -casein, A1, A2, A3, B, C, D, E, F, G, H1, H2, I have been reported [12]. The variant A2 is the most common variant used as reference. Lien et al. [79] studied the most common genetic variants in many Nordic breeds (A2, A1, B, A3). Recently, Jensen et al. [80] reported the variant I in Danish dairy breeds indicating that it might be a relatively common variant.

In goat milk, the β -casein is the most abundant protein [37]. The heterogeneity of polyacrylamide gel electrophoresis and immunoblotting patterns was thought to be originated only by a discrete phosphorylation rate [81].

Two main phosphorylation levels (6 and 5P) corresponding to β 1 and β 2 proteins occur with not negligible abundance [82] but other forms have also been reported (3 and 4P) by Chianese et al. [81]. Caprine β -casein was initially considered as monomorphic; later, several studies showed the presence of a null phenotype in different goat breeds [83, 84]. Subsequently, three variants A, B and C [84, 85], differing by a single amino acid substitution, were associated with normal β -casein content. The C variant was found to be most frequent in Italian goat breeds [86]. Moreover, two null alleles were characterized [87, 88]. To date, genetic polymorphism of the goat β -casein gene (CSN2) is characterized by extensive variability (Table 2), which is under the control of at least 8 autosomal alleles: A, A1, B, C, D, E, and 2 null alleles (O and O') [84, 85, 89–91]; the genetic variants A, A1, B, C, D, and E being related to a normal β -casein content. Two null alleles (O and O') of the CSN2 gene are both characterized by mutations responsible for premature stop codons in exon 7 [87, 88]. CSN2 variant O originates from a single deletion of an A at position 16 of the 7th exon of the gene creating a premature stop codon at position 73; whereas O' allele is characterized by a transition (C→T) at position 373 of the 7th exon of the gene, leading to a premature stop codon at position 182. These phenomena may determine non-functional messengers and the absence of β -casein in milk [64]. Both the null alleles are associated with a non-detectable amount of this protein in milk. The mRNA analysis revealed that the transcript product amounts were almost 10 [87] and 100 [88] times smaller for the null alleles compared to the A variant. The alignment among the mature β -casein sequence of different species suggests that A is the ancestral allele in comparison with C [86]. In fact, the presence of Ala at position 177 of the mature protein coded by goat A allele has been found also in other ruminant species, whereas the C allele codes for Val at the same amino acid position. Studies about the coagulation ability of individual milks with “null” β -casein showed that longer rennet coagulation times were needed in comparison with normal milk and weaker curd firmness occurred [3]. The analysis of milk coagulation properties with and without β -casein demonstrates that milk without β -casein shows longer coagulation times and low curd firmness. Moreover, it was found that cheese yield from milk without β -casein is about 80 % of that obtained from milk with a normal content of this casein [81].

κ -Caseins

The κ -casein represents about 15 % of the total caseins. The amino acid and nucleotide sequence of κ -casein from many eutherian species is already known [92–97]. The κ -casein consists of 169 amino acids residues (19,023 Da)

and occurs in polymeric form via disulfide bonds ranging in sizes from 60 to 600 kDa. Compared to other caseins, κ -casein is the only one soluble in the presence of calcium ions and has a much smaller phosphate component. The signal peptide of κ -casein is 21 residues in length (compared to 15 in calcium-sensitive caseins). This casein is the only eutherian casein that has been shown to contain carbohydrate moieties leading to the conclusion that this protein is not related to the calcium-sensitive caseins [95, 98, 99]. κ -Casein is susceptible to cleavage by the chymosin (rennin). Jollés et al. [98] have shown that cleavage occurs at a specific Phe-Met bond in the C-terminal portion of bovine κ -casein. Nakhasi et al. [100] proposed that κ -casein be classified into two separate groups depending on the species of origin. Group I κ -caseins (cow, sheep, goat, water buffalo) differ from group II κ -caseins (rat, mouse, pig, human) for hydrophobicity, carbohydrate content, amino acid composition and site of proteolytic cleavage. Group I κ -caseins contain the archetypal Phe-Met bond. In group II κ -caseins however, the cleavage site is specified by Phe-Ile or Phe-Leu. This divergence might reflect differences in the mechanism of clotting in ruminant and non-ruminant mammals [101]. The κ -caseins are the only glycosylated caseins from eutherian milk. The carbohydrate groups are attached to κ -casein via *O*-glycosidic linkages to Ser and Thr residues within the C-terminal portion of the molecule. Glycosylation occurs post-translationally and is catalysed by membrane bound *O*-glycosyl-transferases within the Golgi apparatus of mammary epithelial cells [102, 103]. Glycosylation increases during the colostral period and as a consequence of infections, but decreases with successive periods of lactation [104]. Human κ -casein is more highly glycosylated than that of the sheep or cow and carbohydrate residues may account for up to 55 % of the molecular weight [105]. The carbohydrate portion of bovine κ -casein consists of galactose, *N*-acetyl galactosamine and *N*-acetyl neuraminic acid. The κ -casein from sheep milk contains *N*-glycolyl-neuraminic acid in addition to these three sugars [106]. Human κ -casein is yet more complex and contains fucose, galactose, *N*-acetyl glucosamine, *N*-acetyl galactosamine and *N*-acetyl neuraminic acid [105, 107, 108]. Hydrolytic action by chymosin at Phe-Met releases a hydrophobic N-terminal segment (1–105) and a hydrophilic soluble C-terminal portion (106–169) known as para- κ -casein and macropeptide, respectively [109]. The macropeptide is glycosylated, with the carbohydrate moieties mostly linked to Thr, containing *N*-acetylneuraminic acid, galactose and galactosamine. Para- κ -casein possesses no carbohydrates. Macropeptides from cow and sheep contain only one glycosylated Thr, whereas the human one has ten sites of glycosylation [107]. The uneven distribution of carbohydrates, in addition to the high content of Asp and Glu in the C-terminal sequence

results in high net negative charges (3.5 at pH 6.8) that are involved in the stabilization of the κ -casein. The overall acidic character of the macropeptide is conserved in κ -caseins for several species, including cow, zebu, buffalo, goat, ewe, sow and human [110]. κ -Casein is mainly sited on the surface of the casein micelles being responsible for their stability [111]. The presence of a glycan moiety in the C-terminal region of κ -casein stabilizes the micelle [104, 112]. The level of glycosylation does not influence the micelle structure but it does affect the susceptibility of κ -casein to hydrolysis by chymosin, with a decreasing susceptibility as the level of glycosylation increases [113, 114].

The genetic polymorphism of the κ -casein gene (CSN3) in several species: cattle [115, 116], buffalo [117–119], goat [120–123], sheep [124], yak [125] and *equidae* species [126–128]. Eleven variant alleles of the κ -casein gene have been identified in cattle, namely A, B, C, E, F¹, F², G¹, G², H, I and J [12]. Initially, a variant called D was detected by polyacrylamide gel electrophoresis analysis, however this variant was later found to be identical to κ -casein C [129]. Nevertheless, only two alleles, namely A and B, are commonly identified in *Bos taurus* dairy cattle [130]. These two variants differ in the amino acids 136 and 148 [131]. Several studies on κ -casein variants resulted in its significant association with lactation performance, milk composition, milk protein content and rheological properties [132–135].

Goat κ -casein was isolated for the first time by Zittle and Custer [136]. The complete amino acid sequences of 171 residues was determined by Mercier et al. [93]. Coll et al. [137, 138] reported the nucleotide sequence of cDNA and the promoter region of the gene. The gene comprises five exons which more than 90 % of the coding region for mature protein (141 amino acids out of 171) is part of exon 4 [121, 139]. Initially, the structural analysis of the goat κ -casein *locus* indicated the presence of a biallelic polymorphism (A and B variants) [140]. To date, based on the published sequences, the CSN3 gene is considered highly polymorphic with up to 16 different alleles: A, B, B', B'', C, C', D, E, F, G, H, I, J, K, L and M that yield 13 protein variants [121, 122, 141, 142] (Table 2). Allele frequencies studies indicate that A and B are the most frequent CSN3 variants in Spanish, French, Italian and Egyptian goat breeds [121, 122, 143, 144]. The F allele was described and found only in wild goat (*Capra pyrenaica*) while the G have been found at relatively high frequencies only in Italian goats [121, 139]. The variants C, D, H, K, I, L and M have been observed only in few breeds at low or intermediate frequencies [122, 141]. Relevant surveys were carried out also by Angiolillo et al. [120] on the following breeds: Spanish (Murciano-Granadina and Canaria), French (Alpine and Saanen), Italian (Teramana, Garganica,

Girgentana, Sarda, Cilentana Nera, Montefalcone). Prinzenberg et al. [122] analyzed the following populations: GermanWeisse Edelziege, Saanen, Nigeria Borno goat, Cameroon Red Sokoto, West African Dwarf, Anatolian hair goat and Angora at the same *locus*. These researches demonstrated that the differences in the occurrence and frequency of the alleles in these goat breeds reflect their geographic origin. The highest number of alleles was found in the goat breeds from the Near East. In particular, Yahyaoui et al. [121] suggested that the distribution of such alleles has been influenced either by selection pressure for milk production or, more likely, by genetic drift. Moreover, the same authors affirmed that A allele appear later in the evolutionary pathway. In many breeds, the B allele was the most common suggesting that this variant may be the ancestral one [144]. The increasing interest on κ -casein is mainly due to its role on technological properties of milk because of κ -casein is located on the surface of the micelles. Goat CSN3 alleles A and B had different effects on milk casein and protein contents. These two milk components, together with fat content, determine the yield as well as the organoleptic properties of cheese. In particular, the amount of casein associated with the BB and AB genotypes was greater than that found in AA goats; in the same way, the total protein content was higher for the BB and AB goats compared with their AA counterparts. Therefore, genotyping the CSN3 *locus* should be considered when performing marker-assisted selection in dairy goats [142]. More recently, it was reported that CSN3 genotype has a significant effect on the rennet coagulation time being BB genotype better than AB without any influence on cheese yield [145]. Together with the investigations on variants associated with strong alleles at CSN1S1 and CSN1S2 *loci*, the inclusion of κ -casein genotypes, as an additional criterion, may supports the selection for cheese-making properties. In fact, due to the tight association among casein genes, the estimation of the relationship between casein variants and milk production traits can be improved by considering the entire casein haplotype instead of single gene typing [47].

Whey proteins

α -Lactalbumin

The α -lactalbumin is a calcium metalloprotein with a single strong Ca^{2+} binding site, representing one of the major whey proteins in ruminant milks [146, 147]. It stimulates lactose synthesis in the mammary gland by interacting with the enzyme UDP-galactosyl-transferase, giving rise to the heterodimer enzyme lactose synthase [148, 149]. Being this protein necessary for the production of lactose and for

the water movement into mammary secretory vesicles and then into the alveolar lumen, the role of α -lactalbumin is crucial for milk secretion [150]. This protein is rich in essential and conditionally essential amino acids and is a dominant protein in human milk [151]. The α -lactalbumin has a high content of lysine and cysteine and a particularly high content of tryptophan [152].

The structure of the gene (LALBA) encoding this protein has been reported in rat [153], human [154], bovine [155], caprine [156] and guinea pig [157] species, it has an organization similar to that of human lysozyme-encoding gene [158] suggesting a common ancestral origin. The bovine α -lactalbumin has been localized on chromosome 5 [159, 160]. The gene spans approximately 3,090 bp being composed of four exons and three introns [155, 161]. Two genetic variants of α -lactalbumin (A and B) have been most commonly found in cattle [12]. The B variant is present in the milk of most *Bos taurus* cattle, and both the A and B variants are found in the milk of *Bos indicus* cattle and in the droughtmaster (*B. indicus* $\times$ *B. taurus*) [162–165]. Osterhoff and Pretorius [166] have been reported the presence of the A variant in South African cows of European descent. The A variant is present at a low frequency in the milk of some Italian and Eastern European *B. taurus* breeds [167]; it contains a Glu at position 10 of the mature protein, while the B variant has an Arg substitution at that position [168]. A lot of investigations show that the SNP in α -lactalbumin change the gene expression and deal with differences in milk yield and composition [161, 169]. A third genetic variant, named C, has also been reported in Bali cattle (*B. javanicus*) [170]. Until now, there is no evidence of this third allele neither in *B. taurus* nor in *B. indicus*. Recently, a new bovine milk protein variant was discovered (D variant); the SNP causing the amino acid change 65Gln>His is located in exon 2 of α -lactalbumin gene. This amino acid change is not expected to affect protein function [171].

The goat α -lactalbumin gene, located on chromosome 5, is organized in four exons coding for a 123 amino acid polypeptide chain. Vilotte et al. [156] isolated and characterized a genomic clone encoding goat α -lactalbumin. The sequence was aligned with that of its bovine counterpart, finding that the 5'-flanking regions are highly homologous, but two small insertions were detected in the caprine sequence. One of these insertions fills the gap found in the so-called 'milk-box' consensus sequence of the bovine gene. No further studies on the variability of this gene in goats are known. In contrast to numerous studies that had been carried out on goat casein genes and their polymorphisms, studies on goat α -lactalbumin gene and its polymorphisms were not so frequent. It was only reported by Cosenza et al. [172] that this gene showed three different mutations in Italian goat populations: one in the first intron; one in the third exon and one in the 3' untranslated

region. The third exon mutation, a C→T transition identifies a silent allele at position 80 of the mature protein. The intronic mutation consists of a T→C transition in the 13th nucleotide of the first intron and in a C→G transversion located 187 nucleotides beyond the fourth exon. More recently, Lan et al. [173] found that a mutation in exon 3 (M63868: g.1897T>C) identified an amino acid substitution Leu→Pro (p.L100P) of the α -lactalbumin. Here, the allele characterized by the presence of C was designated as A2, while that carrying a T was called A1. Phylogenetic analysis of LALBA gene in different species revealed that this gene is highly conserved. The comparison of the gene sequence has also highlighted the conservation of amino acids with a functional roles [174]. A possible reason to justify the highly conserved sequence may be the in α -lactalbumin physiological functions, i.e. lactogenesis in the animal; however the mutations located in exonic region may be linked to the adaptation of animals in different natural or conditioned environments [175]. Due to the strong correlation between α -lactalbumin and the nutritional value and the functional properties (i.e. gelling, film-forming, foaming and emulsifying) of whey and whey products, to deeply investigate the eventual presence of protein variants and genetic polymorphisms could be an interesting perspective.

β -Lactoglobulin

The β -lactoglobulin is a globular protein member of the lipocalin family, small proteins with many properties, such as the ability to bind small hydrophobic molecules. Although no clear physiological function has been yet defined for the β -lactoglobulin, beyond providing amino acids to the offspring, a role in the transport of retinol and fatty acids has been suggested [176, 177]. The β -lactoglobulin is present in the milk of many species, principally in ruminants. It is absent in milk from rodents, rabbits and camels in which instead another major whey protein (whey acidic protein) is found [176]. Human milk is free of β -lactoglobulin, while this protein is the major whey protein of sheep, goat and mare milk. Businco and Bellanti [178] reported that β -lactoglobulin is responsible for the onset of allergic forms to milk proteins that affect a considerable percentage of infants nourished with maternal milk replacements produced with cow milk. β -lactoglobulin was the first protein in which polymorphism was found. It is well known that β -lactoglobulin gene (BLG) is located on chromosome 3 in ovine and chromosome 11 in goat and cattle [179]. Its polymorphisms may be helpful as informative molecular markers for milk yield and composition as well as for rheological properties of milk. To date, twelve polymorphic variants of this protein are known in cattle, but the two most frequent, A and B, were shown to

be linked with differences in milk protein yield and quality [8, 180, 181]. These two variants differ by two amino acid substitutions in the polypeptide chain arising from two single nucleotide substitutions in the BLG: Asp 64→Gly and Val 118→Ala [182, 183].

The complete sequence of the goat BLG was described. Similarities of this gene with ovine and bovine counterparts were 96.9 and 94.7 %, respectively [184, 185]. The β -lactoglobulin transcription unit spans 4.7 kb arranged in seven small exons and six introns [186]. In goat, no variants producing amino acid change have been characterized at the DNA level. Genetic polymorphisms in the 5'-flanking region were also observed by Yahyaoui et al. [187] in Murciano-Granadina, Canaria, Payoya, Malaguena and Saanen breeds without any effect either on transcription level and on the protein. However, Chianese et al. [188] found an individual variability in β -lactoglobulin content in Girgentana goat breed; subsequently, the gene promoter region in individuals of this breed with reduced β -lactoglobulin content was characterized and a new polymorphism located in the promoter region, not related to the β -lactoglobulin content was detected [189]. Moreover, different variants have been described by Pena et al. [190] and Yahyaoui et al. [187] in the 3' untranslated region (exon 7) and in the proximal promoter region of the gene in Spanish and French goats. Ballester et al. [191] observed 15 β -lactoglobulin polymorphisms in different goat breeds. Nine polymorphisms are located in the promoter region and six in the exons of the gene. All polymorphisms are single nucleotide substitutions with the exception of one deletion/insertion in the promoter region. Kumar et al. [192] observed two alleles having an effect on 90 days milk production in Jamunapari and Barbari goat breeds: animals carrying AA genotype yielded more milk than AB ones.

Minor proteins

Milk protein fraction also includes other minor proteins such as immunoglobulins, lactoferrin, ferritin, transferrin, proteose-peptone, glycomacropeptides, calmodulin, lactoperoxidase, lysozyme, prolactin and folate-binding protein and various growth factors. The content of minor proteins is similar in goat and cow milk but the content of folate-binding protein is higher in goat than in cow milk (12 vs. 8 μ g/ml) [3]. Folate-binding protein is a glycoprotein with about 22 % carbohydrates [37]. Lactoferrin is a glycoprotein present in milk of all vertebrate species. This protein is the major iron binding protein in human and mare milk, whereas transferrin is abundant in milk from rats and rabbits [193]. Immunoglobulins classes (IgG, IgA and IgM) are also present in goat milk. Among minor whey proteins, goat milk also has proteose-peptones like bovine

and other species milks. Proteose-peptones are characterized as a mixture of heat-stable, acid-soluble, phosphoglycol proteins [3]. Although present at low concentrations, minor components of milk can influence profound metabolic, immunological and physiological processes and thus contribute to some of the key advantages of goat milk in developing nutritional products [194].

Conclusions

Goat milk is a product with interesting nutritional and technological characteristics as well as a potential use in dietetics and therapeutics. Milk production traits, which are under control of several genes, are very important in goat due to their economic relevance. As a consequence, the improvement of milk yield and composition is of great significance for breeders. The use of molecular technologies potentially offer a way to select a breeding animal for a wide range of traits and to enhance reliability in predicting the mature phenotype of the individual. Therefore, molecular markers that are significantly related to livestock productions can play an important role for the genetic improvement of domestic animals through conventional breeding strategies [195, 196]. With the advent of genomic DNA analysis techniques it is possible to study the milk proteins gene polymorphisms to identify the favorable genotypes related to the highest milk yield or to the better protein profile for the peculiar destinations of milk. The direct relationship between the allelic variants of genes and milk protein content, which influence the physico-chemical properties of milk, may be utilized in breeding schemes aiming to improve the milk quality. Some authors suggested an influence of goat milk polymorphisms on allergenic effects of milk, but these aspects need to be more intensely investigated. However, the existence of a high genetic diversity in goat milk protein genes suggests their use for human nutrition. Nevertheless, further studies must be carried out in order to enhance the utilization of DNA polymorphisms to improve dairy traits in goat species.

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