

## Casein polymorphism in goat's milk

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**Abstract** – In this review we summarize the current knowledge on the extensive goat casein polymorphism. Gene structure, and allelic and non-allelic protein variants, characterized by genomic and proteomic analysis, have been described. The effect of genetic casein polymorphism on technological and nutritional properties of goat's milk has been briefly reported. Goat casein genes show a range of different post-translational modifications and complex patterns of splicing, leading to a great heterogeneity of this fraction. In the light of some recent findings, a genomic and proteomic combined approach appears to be the tool of choice in order to gain an exhaustive characterization of these milk proteins.

goat's milk / casein fraction / heterogeneity / genetics / proteomics

**摘要** – 山羊奶酪蛋白的多态性。本文概述了山羊奶酪蛋白的多态性。从山羊奶不同酪蛋白的基因结构、等位基因和非等位基因蛋白变体以及通过基因组学和蛋白质组学分析获得的酪蛋白相关特性等多个方面,对山羊奶酪蛋白的多态性进行了讨论。并且简要概述了酪蛋白的遗传多态性对山羊奶的加工特性和营养特性的影响。山羊奶中不同的酪蛋白基因之间,显示出较大范围的翻译后修饰差异和序列剪接形式的复杂性,进而导致了这一组分存在着很大的差别。将基因组学与蛋白质组学两种方法结合起来,可以从根本上了解这些乳蛋白的性质。

山羊奶 / 酪蛋白组分 / 异源性 / 遗传学 / 蛋白质组学

**Résumé** – **Polymorphisme de la caséine du lait de chèvre.** Cette revue résume les connaissances actuelles sur le polymorphisme de la caséine de chèvre. La structure des gènes, les variants protéiques alléliques ou non-alléliques caractérisés par analyse génomique et protéomique ont été décrits. L'effet du polymorphisme génétique de la caséine sur les propriétés technologiques et nutritionnelles du lait de chèvre a été brièvement présenté. Les gènes de la caséine de chèvre montrent une large gamme de modifications post-traductionnelles différentes et des motifs complexes d'épissage conduisant à une grande hétérogénéité de cette fraction. À la lumière de certains résultats récents, l'approche génomique et protéomique apparaît comme un outil de choix pour obtenir une caractérisation exhaustive de ces protéines laitières.

lait de chèvre / caséine / hétérogénéité / génétique / protéomique

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## 1. INTRODUCTION

Caseins, the most abundant milk proteins, are a family of acidic, proline-rich phosphoproteins designed to form large, spherical, micellar structures in colloidal suspension with calcium phosphate.

In all species studied so far, casein genes form a cluster of between 250 and 350 kb (Fig. 1), in which the “calcium-sensitive” (Ca-sensitive) caseins,  $\alpha_{s1}$  (*CSN1S1*),  $\beta$  (*CSN2*) and  $\alpha_{s2}$  (*CSN1S2*), are tightly evolutionarily related, whereas  $\kappa$ -casein (*CSN3*) is physically and functionally linked [92]. The organization and orientation of the genes in the cluster is highly conserved, mainly in the 5' and 3'UTRs, in the signal peptide and in the major phosphorylation sites [63]. In all casein genes the first exon encodes the 5'UTR. In the Ca-sensitive caseins the second exon harbors the remaining 12 nt of the 5'UTR and encodes the signal peptide, 15 residues long, and 2 AA of the mature protein. In *CSN3* the signal sequence is encoded by exon 2 and part of exon 3 [92].

In goats, as in cattle, the casein genes cluster region, as first reported by Grosclaude et al. [38], spans 250 kb on chromosome 6 [32, 101].

Caprine casein genes exhibit an unusual and extensive polymorphism that affects milk quality and composition. In milk each casein consists of two or more forms genetically determined by codominant alleles: the primary structures vary considerably due to point mutations (SNP, Single Nucleotide Polymorphism), insertion/deletion and differential splicing patterns. Differences in primary structures can strongly modify electric charge, hydrophobic properties, and the size and shape of the molecule; moreover, large insertions/deletions in the transcription unit as well as mutations in the promoter or in the regulatory sequences can affect the gene transcription rate.

Premature stop codons associated with a decrease in the level of the relevant transcripts have also been reported for each Ca-sensitive casein gene [72, 73, 81, 83, 84].

Lastly, casein heterogeneity in milk can also be caused by post-translational modifications, such as different levels of phosphorylation and glycosylation.

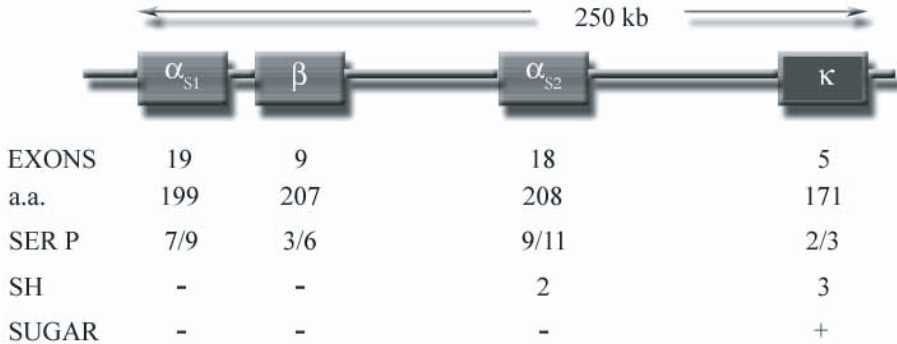
To analyze these phenotypes various electrophoretic techniques have been used, but none of them appears to be fully satisfactory, either for the resolution or the identification of the relevant variants. In recent years, mass spectrometry coupled with appropriate separation techniques, such as high-performance liquid chromatography (HPLC) or two-dimensional electrophoresis (2DE), has become increasingly important as a powerful tool for protein characterization.

The aim of this review is to give an overview of the current knowledge on the polymorphism of the casein fraction in goat's milk as revealed by genomic and proteomic studies, and its potential impact on goat's milk characteristics.

## 2. GOAT CASEIN POLYMORPHISM

### 2.1. $\alpha_{s1}$ -Casein

The *CSN1S1* gene spreads over a quite large transcriptional unit of 16.7 kb and consists of 19 exons varying in length from 24 bp to 358 bp [82]. In these last two decades several studies have reported that the remarkable genetic polymorphism at the  $\alpha_{s1}$ -casein locus is greatly responsible for the individual variations observed in the casein content of the caprine milk (for a review see [59]). At least 17 variants have been observed so far (Tab. I), most of them associated with four quantitative expression levels, ranging from 0 g·L<sup>-1</sup> to 3.5 g·L<sup>-1</sup> per allele. The alleles A, B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub>, B<sub>4</sub>, C, H and L are associated with a



**Figure 1.** Main structural information on goat caseins.

high amount of  $\alpha_{s1}$ -casein ( $\sim 3.5 \text{ g}\cdot\text{L}^{-1}$  per allele), the alleles E and I with an intermediate amount ( $\sim 1.1 \text{ g}\cdot\text{L}^{-1}$  per allele), and the alleles D, F and G with a low amount ( $\sim 0.45 \text{ g}\cdot\text{L}^{-1}$  per allele) [59]. The alleles  $0_1$ ,  $0_2$  and N are responsible for the null phenotype, corresponding to the apparent absence of this fraction in milk [22, 59, 83]. Therefore, milk produced by goats with different CSN1S1 genotypes shows a variable amount of  $\alpha_{s1}$ -casein, ranging from  $7 \text{ g}\cdot\text{L}^{-1}$  in strong allele homozygous goats, to  $0.9 \text{ g}\cdot\text{L}^{-1}$  and  $0 \text{ g}\cdot\text{L}^{-1}$  in weak and null homozygotes, respectively.

The primary structure of the A, B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub>, B<sub>4</sub>, C, E, H, I and L protein variants contains 199 residues and differs in single amino acid substitutions caused by single point mutations; D and F variants are characterized by the internal deletion of 11 and 37 amino acid residues, respectively. Both deletions arise from the 59th residue of the polypeptide chain and lead to the loss of a multiple phosphorylation site [11, 12]. In the F variant the deletion is due to the out-splicing of three exons (9-10-11) during pre-mRNA processing, and the transcription efficiency of the mRNA of allele F is 6 times lower than that of the allele A. The deletion of a single nucleotide occurring within the exon 9 may be the cause of this altered transcription process [52]. The F allele is also characterized by two short

insertions of 11 bp and 3 bp in the 9th intron and by a deletion of 7 bp in the 14th intron [52, 83].

The weak G allele shows the same expression level as the F allele; however, it is characterized by the loss of 13 amino acid residues starting from the 14th position (14→26) and corresponding to the entire exon 4 [58].

Reduced protein synthesis regarding the intermediate allele E is due to the insertion of a 458 bp truncated LINE sequence in position 124 within exon 19, which leads to instability and a threefold reduction of the related messenger amount [45].

The CSN1S1<sup>M</sup> allele, observed for the first time by Chianese et al. [17], shows a C→T transition which leads to the Ser<sup>66</sup>→Leu<sup>66</sup> substitution with regard to variant A and, as a consequence, to the loss of two phosphate groups within the major phosphorylation site [8]. It is a strong variant observed in heterozygous form, probably resulting from interallelic combination.

The null allele  $0_1$  is characterized by a large deletion of about 8.5 kb starting from the 181th nucleotide of intron 12, which involves the last 7 exons of the gene [22]. On the other hand, allele  $0_2$  should present a large and uncharacterized insertion according to Martin et al. [59].

Recently a new “null” allele named N, associated with an apparent lack of

**Table I.** Goat  $\alpha_{s1}$ -casein variants/alleles.

Variants/alleles	Variations	References
<b>A</b>	Glu(77)→Gln	[9, 11]
<b>B<sub>1</sub></b>	Reference 199 aa residues	[37]
<b>B<sub>2</sub></b>	Leu(16)→Pro	[9, 11, 37]
<b>B<sub>3</sub></b>	Leu(16)→Pro; Arg(100)→Lys	[37]
<b>B<sub>4</sub></b>	Leu(16)→Pro; Arg(100)→Lys; Thr(195)→Ala	[37]
<b>C</b>	Leu(16)→Pro; Arg(100)→Lys; Thr(195)→Ala; His(8)→Ile	[9, 11]
<b>D</b>	Leu(16)→Pro Deletion aa. 59→69	[53]
<b>E</b>	Leu(16)→Pro; Arg(100)→Lys; Thr(195)→Ala <i>Insertion of a LINE of 457-458 bp at the 124th n. of 19th exon</i>	[39, 45]
<b>F</b>	Leu(16)→Pro Deletion of aa. 59→95 <i>Deletion of the 23rd nt of the 9th exon and insertion of 11 bp and 3 bp in the downstream intron</i>	[12, 39, 52]
<b>G</b>	Glu(77)→Gln Deletion aa. 14→26	[58]
<b>H</b>	Glu(77)→Gln; Arg(1)→Lys	[16]
<b>I</b>	Uncharacterized	[16]
<b>L</b>	Leu(16)→Pro; Arg(90)→His	[16]
<b>M</b>	Ser(66)→Leu <i>C→T transition at 23rd nt of the 9th exon</i>	[8, 17]
<b>0<sub>1</sub></b>	Deletion ~8.5 kb starting from 181 nt of the 12th intron	[22]
<b>0<sub>2</sub></b>	Large uncharacterized insertion	[59]
<b>N</b>	<i>Deletion of a C at 23rd nt of the 9th exon without insertion of 11 bp and 3 bp in the downstream intron</i>	[83]

$\alpha_{s1}$ -casein, has been found in an Italian population. It is chiefly characterized by the deletion of a cytosine at the 23th nucleotide of exon 9, resulting in a frame-shift that determines a premature stop codon in the 12th exon and, at the same time, by the absence of the 11 bp insertion within the downstream intron, as observed in the F allele [83].

Moreover, proteomic analyses have shown that each  $\alpha_{s1}$ -casein variant can be present as a mixture of up to eight molecular species differing in polypeptide chain length. The full-length protein is the main component, whereas other defective forms, characterized by some internal deletions (residue Gln<sup>78</sup> and/or peptides Glu<sup>110</sup>-Glu<sup>117</sup> and/or Glu<sup>141</sup>-Gln<sup>148</sup>

or combinations of them), derive from skipping events or alternative splicing during primary transcript processing [30, 31].

## 2.2. $\beta$ -Casein

The CSN2 gene is smaller than the other two Ca-sensitive casein genes, consisting of 9 exons ranging from 492 bp and 24 bp.  $\beta$ -Casein, which is the major goat's casein fraction in goat's milk, has long been considered to be monomorphic. The heterogeneity of PAGE and Immunoblotting patterns was thought to be originated only by a discrete phosphorylation rate [15]: in fact, two main phosphorylation levels (5 and 6P) occur with comparable relative abundance [90], but other forms (3 and 4P) have

**Table II.** Goat  $\beta$ -casein variants/*alleles*.

Variants/ <i>alleles</i>	Variations	References
<b>A</b>	Reference 207 aa residues	[9, 93]
<b>A1</b>	C→T at 180th nt of the 9th exon	[23]
<b>O'</b>	166 aa residues	[24, 26, 79, 84]
	C→T at position 373 of the 7th exon	
<b>O</b>	Deletion of an A at position 16 of the 7th exon	[54, 73]
<b>B</b>	Uncharacterized	[54]
<b>C</b>	Ala(177)→Val	[69, 105]
<b>D</b>	Ala(177)→Val	[34]
	Val(207)→Asn	
<b>E</b>	Ser(166)→Tyr	[14]
	C→A at position 370 of the 7th exon	

also been reported [15]. The six phosphorylation sites have been located by homology with bovine  $\beta$ -casein and confirmed by direct characterization [69].

In the 90s the amino acid sequence of the  $\beta$ -casein variant A was determined from the corresponding cDNA precursor [93] (accession number P33048). The occurrence of another allele, named B, was subsequently reported [54], but protein and DNA sequences are still undetermined (Tab. II). In the meantime, goats producing reduced amounts of  $\beta$ -casein were identified: milk samples devoid of or with a reduced amount of  $\beta$ -casein (50%) were detected at a phenotypic level through electrophoretic techniques in some breeds of Southern Italy [15, 26, 79], in Creole goats of Guadalupe [54] and in Pyrenean goats [37]. Two different null alleles, named CSN2<sup>0</sup> and CSN2<sup>0'</sup>, are responsible for this phenotype. The CSN2<sup>0</sup> allele, described by Persuy et al. [72, 73] in Creole goats, originates from a single deletion of an adenine in a row of four adenines in the 7th exon spanning codons 48 ± 49 of the CSN2<sup>A</sup> sequence (GenBank accession number: AF172260) that results in one-nucleotide frame-shift, leading to a premature stop codon at position 73. A weak level of expression was reported for this allele: the amount of CSN2<sup>00</sup> mRNA was roughly 5% of the amount of mRNA

obtained at the same age and stage of lactation from CSN2<sup>AA</sup> goats [72, 73].

The null allele named O', observed in some Italian breeds, presents a C→T transition at the 373rd nucleotide within the 7th exon that produces a premature nonsense codon at position 182 [84]. Northern blot analysis demonstrated that the amount of CSN2<sup>0'</sup> mRNA is 10 times lower than that of CSN2<sup>A</sup> [79]. In spite of the reduced amount and stability of the related mRNA transcript, the premature stop codon in position 182 should be consistent with the expression of a mature protein of 166 residues. Such a protein was recently isolated and fully characterized by coupling RP-HPLC/ESI-MS, MALDI/TOF-MS and tandem mass spectrometry by Cunsolo et al. [24] in a milk sample produced by a homozygous CSN2<sup>0'0'</sup> Sicilian goat.

The C variant was characterized at the protein level, by combined use of peptide mass fingerprinting and tandem mass spectrometry (MS/MS) by Neveu et al. [69]. It had previously been described at the DNA level by Wang et al. (GenBank accession number: AF409096 direct submission) [105]; gene sequencing showed a C→T transition, leading to the substitution Ala<sup>177</sup>→Val<sup>177</sup>.

Another variant, named D, that differs from C only in the last residue

**Table III.** Goat  $\alpha_{s2}$ -casein variants/*alleles*.

Variants/ <i>alleles</i>	Variations	References
<b>A</b>	Reference 208 aa residues	[9]
<b>B</b>	Glu(64)→Lys <i>G→A at the 10th nt of the 9th exon</i>	[9, 10]
<b>C</b>	Lys(167)→Ile <i>A→T at the 15th nt of the 16th exon</i>	[10]
<b>D</b>	205 aa residues Deletion of 122–124 aa residues Thr (121)→Asn <i>Deletion of 106-bp involving the last 11 bp of the 11th exon and the first 95 bp of the following intron</i>	[80]
<b>E</b>	Lys(167)→Ile; Pro(193)→Arg <i>C→G at the 28th codon of the 16th exon</i>	[48, 103]
<b>F</b>	Val(7)→Ile <i>G→A at the 13th nt in the 3rd exon</i>	[80]
<b>0</b>	109 aa residues <i>G→A at the 80th nt in 11th exon</i>	[81]
<b>Sub A</b>	Deletion of the C-terminal tetrapeptide	[25]
<b>Sub E</b>	Deletion of the C-terminal tetrapeptide	[25]

Val<sup>207</sup>→Asn<sup>207</sup>, was identified in a very low amount in a milk sample of a goat from Southern Italy carrying the CSN2<sup>C</sup> allele. This non-allelic variant is a minor component of the casein fraction derived from the C allele, because of a “mistake” in the translation of the CSN2<sup>C</sup> mRNA at the level of the last amino acid residue [34].

A silent allele (A1), characterized by a SNP in the 9th and last exon, was recently sequenced [23]. Lastly, a new allele was identified and characterized: it is named E and seems to be specific to the Frisa goat, a local breed of Northern Italy [14].

### 2.3. $\alpha_{s2}$ -Casein

The CSN1S2 gene is 18.5 kb long and consists of 18 exons which vary from 21 to 266 bp [36]. Seven alleles, showing three different synthesis levels, have been identified so far (Tab. III). The A, B, C, E and F alleles are characterized by point mutations related to single amino acid substitutions [10, 48, 80, 103] and associated with a normal amount of  $\alpha_{s2}$ -casein

(~2.5 g·L<sup>-1</sup>). CSN1S2<sup>D</sup> is a rare defective allele characterized by a deletion, involving part of the exon 11 and of the following intron, that should result in the loss of the codons Pro (CCC<sup>122</sup>), Thr (ACC<sup>123</sup>) and Val (GTG<sup>124</sup>) and the substitution Thr<sup>121</sup>→Asn<sup>121</sup> (EMBL accession no. AJ238684) [80]. Erhardt et al. [29] report the  $\alpha_{s2}$ -casein variant G separated by IEF with an IP between that of variants A and C, that occurs at very low frequencies and is not characterized.

The null allele CSN1S2<sup>0</sup> associated with a non-detectable amount of this casein fraction shows a G→A transition at the 80th nucleotide in exon 11, creating a premature stop codon at position 110 [81]. The total absence of this casein fraction in a CSN1S2<sup>00</sup> homozygous goat from Southern Italy was confirmed by RP-HPLC/ESI-MS analysis [56].

Mass spectrometry studies revealed that in goat's milk each  $\alpha_{s2}$ -casein variant can exist in two forms; the main component corresponds to the full length of 207 residues, while the minor component,

originated by a skipping event, lacks the internal sequence Glu<sup>34</sup>-Glu<sup>42</sup> or Lys<sup>35</sup>-Glu<sup>43</sup> [31].

Recently, two new truncated sub-variants of goat  $\alpha_{s2}$ -casein variants A and E have been identified and fully characterized by RP-HPLC/ESI-MS [25]. These two sub-variants differ from the corresponding full-length proteins (A and E) by the absence of the C-terminal tetrapeptide and seem to be originated by the differential splicing of their correspondent mRNA. These proteins, having an experimentally determined  $M_r$  23 183 and  $M_r$  24 227 Da, respectively, corresponding to AA 1–204 of the full-length variants, were detected as minor components in the casein fraction obtained from milk of a goat belonging to an autochthonous breed from Southern Italy.

The deleted forms of variants A and E, characterized by the internal deletion of Glu<sup>34</sup>-Glu<sup>42</sup>, have also been reported by the same team [25] and by Ferranti et al. [31], attributed to alternative skipping as happens in  $\alpha_{s1}$ -casein.

## 2.4. $\kappa$ -Casein

Caprine  $\kappa$ -casein was first isolated by Zittle and Custer [110] and then characterized at protein level by Mercier et al. [64,65]. Compared with the Ca-sensitive caseins, CSN3 exhibits distinctive properties: it is the only glycosylated and hydrophilic casein, so it is soluble in a broad range of calcium ions and presents a lower phosphorylation level. Its signal peptide consists of 21 residues, while in Ca-sensitive caseins it is 15 residues long [68]. Mercier et al. [64] first reported the occurrence of polymorphism of the goat  $\kappa$ -casein: a neutral substitution Val<sup>119</sup>/Ile<sup>119</sup> was found in an Alpine-Saanen goat, then confirmed by Addeo et al. [1], together with the existence of heterogeneity at the glycosylation rate.

In the 90s different polymorphic patterns were described through different methods: protein electrophoresis [27, 94], chromatographic techniques [46, 51] and capillary electrophoresis [87]. The two main genetic variants, described in an Italian population, were named A and B like their cattle counterpart [27].

cDNA and the promoter region of the caprine CSN3 were first sequenced by Coll et al. [20, 21] and then confirmed by Ward et al. [106]. The  $\kappa$ -Casein gene includes 5 exons, 4 of them carrying more than 90% of the information to encode for the mature protein.

More recently, the presence of the two variants, A and B, previously detected only by gel electrophoresis, was confirmed both at protein and DNA level [13, 18].

Only in the last few years has the CSN3 gene been shown to have many allelic forms [3, 44, 78, 108, 109]. According to the new nomenclature proposed by Prinzenberg et al. [78], at the 4th exon of the CSN3 gene 16 alleles have been characterized, resulting in 13 protein variants (Tab. IV).

All these  $\kappa$ -casein variants found in *Capra hircus* can be divided into two groups on the basis of their isoelectric point (IP): A<sup>IEF</sup> (A, B, B', B'', C, C', F, G, H, I, J, L - IP = 5.29) and B<sup>IEF</sup> (D, E, K, M - IP = 5.66) [78]. Chianese et al. [18] observed that the B<sup>IEF</sup> group of  $\kappa$ -casein variants seems to be associated with higher casein content in milk than the A<sup>IEF</sup> group.

## 3. CURRENT PERSPECTIVE ON THE IMPACT OF GOAT CASEIN POLYMORPHISM

In the last two decades research has continued to reveal the extensive casein polymorphism occurring in goat, but in spite of a large number of papers dealing with the wide heterogeneity of the casein fraction, the studies on the related impact are not so numerous.



**Table IV.** Goat  $\kappa$ -casein variants/*alleles*.

Variants/ <i>alleles</i>	IP group	Variations	References
<b>A</b>	A <sup>IEF</sup>	Reference 171 residues	[20]
<b>B</b>	A <sup>IEF</sup>	Val(119)→Ile G(471)→A	[108]
<b>B'</b>	A <sup>IEF</sup>	Val(119)→Ile	[44]
<b>B''</b>	A <sup>IEF</sup>	C(170)→T, G(471)→A Val(119)→Ile C(290)→T, G(471)→A	[44]
<b>C</b>	A <sup>IEF</sup>	Val(65)→Ile; Val(119)→Ile; Ala(156)→Val; Ser(159)→Pro T(245)→C, G(284)→A, G(309)→A, G(471)→A, C(583)→T, T(591)→C	[78]
<b>C'</b>	A <sup>IEF</sup>	Val(65)→Ile; Val(119)→Ile; Ala(156)→Val; Ser(159)→Pro T(245)→C, G(284)→A, G(309)→A, G(471)→A, C(583)→T, A(509)→G, T(591)→C	[108]
<b>D</b>	B <sup>IEF</sup>	Gln(44)→Arg; Val(65)→Ile; Val(119)→Ile; Ser(159)→Pro T(245)→C, A(247)→G, G(309)→A, G(471)→A, T(591)→C	[13]
<b>E</b>	B <sup>IEF</sup>	Asp(90)→Gly; Val(119)→Ile A(385)→G, G(471)→A	[3]
<b>F</b>	A <sup>IEF</sup>	Val(119)→Ile; Ser(159)→Pro T(245)→C, G(471)→A, T(591)→C	[109]
<b>G</b>	A <sup>IEF</sup>	Val(65)→Ile; Val(119)→Ile; Ser(159)→Pro T(245)→C, G(309)→A, G(471)→A, T(591)→C	[109]
<b>H</b>	A <sup>IEF</sup>	Asn(53)→Ser; Val(119)→Ile A(274)→G, G(471)→A	[44]
<b>I</b>	A <sup>IEF</sup>	Val(65)→Ile; Val(119)→Ile G(309)→A, G(471)→A	[44]
<b>J</b>	A <sup>IEF</sup>	Tyr(61)→Cys; Val(119)→Ile A(298)→G, G(471)→A	[44]
<b>K</b>	B <sup>IEF</sup>	Gln(44)→Arg; Val(119)→Ile A(247)→G, G(471)→A	[44]
<b>L</b>	A <sup>IEF</sup>	Val(65)→Ile; Val(119)→Ile; Ser(159)→Pro G(309)→A, G(471)→A, T(591)→C	[44]
<b>M</b>	B <sup>IEF</sup>	Asp(90)→Asn; Val(119)→Ile; Val(145)→Ala; Ser(159)→Pro T(245)→C, G(384)→A, G(471)→A, T(450)→C, T(591)→C	[78]

Several studies investigated Ca-sensitive caseins ( $\alpha_{s1}$ ,  $\alpha_{s2}$  and  $\beta$ ), located within the micelles, establishing that they affect the technological properties of milk: strong alleles improve cheese-making and fat percentage: on the contrary, defective and null alleles make these features worse [15, 40, 60, 66, 67, 75, 76, 85, 89, 91]. According to these findings genotypes at  $\alpha_{s1}$  and  $\beta$  casein loci have been used for selection in breeding programs in some French and Spanish breeds [4, 55, 74, 91, 97, 99].

More recently, the interest of researchers has been focused on  $\kappa$ -casein, which is located on the surface of the micelles and has been revealed to affect the technological properties of milk: B<sup>IEF</sup> variants being associated with higher casein content in milk than A<sup>IEF</sup> [18, 19]. This association could be due to the direct effect of the different alleles as well as to the haplotype combinations, in which B<sup>IEF</sup> variants are associated with strong alleles at CSN1S1 and CSN1S2 loci [96]. The inclusion of  $\kappa$ -casein genotypes, as an



**Table V.** Main bioactive peptides derived from caseins.

Bioactive peptide	Casein precursor	Bioactivity
Casomorphins	$\alpha$ -, $\beta$ -Casein	Opioid agonist
Casoxins	$\kappa$ -Casein	Opioid antagonist
Casokinins	$\alpha$ -, $\beta$ -Casein	ACE-inhibitory
Immunopeptides	$\alpha$ -, $\beta$ -Casein	Immunomodulatory
Casoplatelins	$\kappa$ -Casein	Antithrombotic
Phosphopeptides	$\alpha$ -, $\beta$ -Casein	Mineral binding

additional criterion, is expected to be beneficial for selection of cheese-making properties.

Because of 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 [96].

In relation to the nutritional aspect, goat's milk has many indisputable health benefits related to a number of medical problems, foremost being malnourishment, gastro-intestinal disorders and cow's milk protein allergies (CMPA) [2, 5, 42, 43, 50, 70, 86, 95]. Unfortunately, most of these studies use bulk, uncharacterized goat's milk instead of individual, characterized milk: this strategy cannot highlight the specific role of casein polymorphism on these nutritional traits.

The overall judgement on goat's milk as a substitute for cow's milk in CMPA is still conflicting: several clinical trials report a high percentage of patients with CMPA that tolerate goat's milk [41, 71, 88, 100], other studies report cases of cross-reactions between cow's and goat's milk [6, 35] or even rare cases of goat's milk allergy [107], sometimes also in the absence of cow's milk sensitivity [49, 102].

Among the several milk allergens that could elicit allergic reactions in sensitized individuals [47, 98],  $\beta$ -lactoglobulin and  $\alpha$ <sub>s</sub>-caseins, absent in human milk, seem to be the most relevant [28, 104]. Genetic casein polymorphism associated with the absence of  $\alpha$ <sub>s</sub>-caseins in milk of some,

rare goats may actually help identify which protein is mainly responsible for the allergic reaction.

To date the allergenic potency of goat's milk with different contents of  $\alpha$ <sub>s</sub>-casein has been investigated in only two trials. A clinical test carried out on guinea pigs revealed that goat's milk lacking  $\alpha$ <sub>s1</sub>-casein is less allergenic than other goat's milk, probably because of a modified  $\beta$ -lactoglobulin/ $\alpha$ <sub>s</sub>-caseins ratio [7]. In an in vitro immunological test the absence or a reduced amount of  $\alpha$ <sub>s2</sub>-casein in goat's milk caused only a small decrease in the allergenic potency of the casein fraction, suggesting that first and foremost whey proteins play a crucial role in the allergic reaction [57].

Caseins represent a reservoir for a wide variety of bioactive peptides (Tab. V), minor regulatory compounds with hormone-like activity, that could affect milk nutritional value [61, 62]. Bioactive peptides are inactive within the sequence of the protein and can be released by enzymatic proteolysis during gastrointestinal digestion or food processing [33, 61]; therefore, mutations in protein genes might abolish or spring biological properties, by generating new sequences. Potential health-enhancing components could remain to be found in milk proteins and it is probably in this area that research may provide the basis for the inclusion of goat caseins as ingredients in functional foods [77].

In conclusion, we believe that the direct impact of this extensive heterogeneity needs to be investigated better in order to

gain new insights into the biological functions of goat caseins, just barely known and little exploited. The evidence of the complex splicing patterns of the casein genes lead to considering the genomic and proteomic combined approach as the only tool of choice for an exhaustive characterization of the goat casein fraction.

## REFERENCES

- [1] Addeo F., Soulier S., Pelissier J.P., Chobert J.M., Mercier J.C., Ribadeau-Dumas B., Preparation and fractionation of goat  $\kappa$ -casein: analysis of the glycan and peptide components, *J. Dairy Res.* 45 (1978) 191–196.
- [2] Alferez M.J.M., Barrionuevo M., Lopez Aliaga I., Sanz Sampelayo M.R., Lisbona F., Robles J.C., Campos M.S., Digestive utilization of goat and cow milk fat in malabsorption syndrome, *J. Dairy Res.* 68 (2001) 451–461.
- [3] Angiolillo A., Yahyaoui M.H., Sanchez A., Pilla F., Folch J.M., Short communication: Characterization of a new genetic variant in the caprine  $\kappa$ -casein gene, *J. Dairy Sci.* 85 (2002) 2679–2680.
- [4] Barbieri M.E., Manfredi E., Elsen J.M., Ricordeau G., Bouillon J., Grosclaude F., Mahé M.F., Bibé B., Influence du locus de la caséine  $\alpha$ s1 sur les performances laitières et les paramètres génétiques des chèvres en race Alpine, *Génét. Sél. Evol.* 27 (1995) 437–450.
- [5] Barrionuevo M., Alferez M.J., Lopez A.I., Sanz S.M., Campos M.S., Beneficial effect of goat milk on nutritive utilization of iron and copper in malabsorption syndrome, *J. Dairy Sci.* 85 (2002) 657–664.
- [6] Bellioni-Businco B., Paganelli R., Lucenti P., Giampietro P.G., Perborn H., Businco L., Allergenicity of goat's milk in children with cow's milk allergy, *J. Allergy Clin. Immunol.* 103 (1999) 1191–1194.
- [7] Bevilacqua C., Martin P., Candalh C., Fauquant J., Piot M., Roucayrol A.M., Pilla F., Heyman M., Goats' milk of defective  $\alpha$ s1-casein genotype decreases intestinal and systemic sensitization to  $\beta$ -lactoglobulin in guinea pigs, *J. Dairy Res.* 68 (2001) 217–227.
- [8] Bevilacqua C., Ferranti P., Garro G., Veltri C., Lagonigro R., Leroux C., Pietrolà E., Addeo F., Pilla F., Chianese L., Martin P., Interallelic recombination is probably responsible for the occurrence of a new  $\alpha$ s1-casein variant found in the goat species, *Eur. J. Biochem.* 269 (2002) 1293–1303.
- [9] Boulanger A., Grosclaude F., Mahé M.F., Polymorphisme des caséines  $\alpha$ s1 et  $\alpha$ s2 de la chèvre (*Capra hircus*), *Génét. Sél. Evol.* 16 (1984) 157–176.
- [10] Bouniol C., Brignon G., Mahé M.F., Printz C., Biochemical and genetic analysis of variant C of caprine  $\alpha$ s2-casein (*Capra hircus*), *Anim. Genet.* 25 (1994) 173–177.
- [11] Brignon G., Mahé M.F., Grosclaude F., Ribadeau Dumas B., Sequence of caprine  $\alpha$ s1-casein and characterization of those of its genetics variants which are synthesized at high level  $\alpha$ s1-CnA, B et C, *Protein Seq. Data Anal.* 2 (1989) 181–188.
- [12] Brignon G., Mahé M.F., Ribadeau Dumas B., Mercier J.C., Grosclaude F., Two of the three genetic variants of goat  $\alpha$ s1-casein which synthesized at a reduced level have an internal deletion possibly due to altered RNA splicing, *Eur. J. Biochem.* 193 (1990) 237–241.
- [13] Caroli A., Jann O., Budelli E., Bolla P., Jäger S., Erhardt G., Genetic polymorphism of goat  $\kappa$ -casein (CSN3) in different breeds and characterization at DNA level, *Anim. Genet.* 32 (2001) 226–230.
- [14] Caroli A., Chiatti F., Chessa S., Rignanese D., Bolla P., Pagnacco G., Focusing on the goat casein complex, *J. Dairy Sci.* 89 (2006) 3178–3187.
- [15] Chianese L., Garro G., Nicola M.A., Mauriello R., Ferranti P., Pizzano R., Cappuccio U., Laezza P., Addeo F., Ramunno L., Rando A., Rubino R., The nature of  $\beta$ -casein heterogeneity in caprine, *Lait* 73 (1993) 533–547.
- [16] Chianese L., Ferranti P., Garro G., Mauriello R., Addeo F., Occurrence of three novel  $\alpha$ s1-casein variants in goat milk, in: Proceedings IDF Milk Protein Polymorphism Seminar, Palmerstone North, New Zealand, 1997, pp. 259–267.
- [17] Chianese L., Garro G., Ferranti P., Speranza L.M., Pilla F., Bevilacqua C., Pietrolà E., Occurrence of new variant of goat  $\alpha$ s1- and  $\alpha$ s2-casein in a breed of Southern Italy, in: Proceedings 25th International Dairy Congress, Aarhus, Denmark, 21–24 September 1998.
- [18] Chianese L., Portolano B., Troncone E., Pizzolongo F., Ferranti P., Addeo F., Alicata M.L., Pilla F., Calcagna G., The quality of Girgentana goat milk, in: Proceedings 7th

- International Conference on Goats, Tour, France, 15–18 May 2000, pp. 946–949.
- [19] Chiatti F., Caroli A., Chessa S., Bolla P., Pagnacco G., Relationships between goat  $\kappa$ -casein (CSN3) polymorphism and milk composition, in: Proceedings of "The role of biotechnology", Villa Gualino, Torino, Italy, 2–5 March 2005, pp. 163–164.
- [20] Coll A., Folch J.M., Sanchez A., Nucleotide sequence of the goat  $\kappa$ -casein cDNA, *J. Anim. Sci.* 71 (1993) 2833.
- [21] Coll A., Folch J.M., Sanchez A., Structural features of the 5' flanking region of the caprine  $\kappa$ -casein gene, *J. Dairy Sci.* 78 (1995) 973–977.
- [22] Cosenza G., Illario R., Rando A., Di Gregorio P., Masina P., Ramunno L., Molecular characterization of the goat CSN1S101 allele, *J. Dairy Res.* 70 (2003) 237–240.
- [23] Cosenza G., Pauciuolo A., Gallo D., Di Berardino D., Ramunno L., A SspI PCR-RFLP detecting a silent allele at the goat CSN2 locus, *J. Dairy Res.* 72 (2005) 1–4.
- [24] Cunsolo V., Galliano F., Muccilli V., Saletti R., Marletta D., Bordonaro S., Foti S., Detection and characterization by high-performance liquid chromatography and mass spectrometry of a goat  $\beta$ -casein associated with a CSN2 null allele, *Rapid Commun. Mass Spectrom.* 19 (2005) 2943–2949.
- [25] Cunsolo V., Muccilli V., Saletti R., Marletta D., Foti S., Detection and characterization by high-performance liquid chromatography and mass spectrometry of two truncated goat  $\alpha_{s2}$ -caseins, *Rapid Commun. Mass Spectrom.* 20 (2006) 1061–1070.
- [26] Dall'Olio S., Davoli R., Russo V., A new goat  $\beta$ -casein variant, *Sci. Tec. Latt. Casearia* 40 (1989) 24–28.
- [27] Di Luccia A., Mauriello R., Chianese L., Moio L., Addeo F.,  $\kappa$ -Casein polymorphism in caprine milk, *Sci. Tec. Latt. Casearia* 41 (1990) 305–314.
- [28] Docena G.H., Fernandez R., Chirido F.G., Fossati C.A., Identification of casein as the major allergenic and antigenic protein of cow's milk, *Allergy* 51 (1996) 412–416.
- [29] Erhardt G., Jäger S., Budelli E., Caroli A., Genetic polymorphism of goat  $\alpha_{s2}$ -casein (CSN1S2) and evidence for a further allele, *Milchwissenschaft* 57 (2002) 137–140.
- [30] Ferranti P., Addeo F., Malorni A., Chianese L., Leroux C., Martin P., Differential splicing of pre-messenger RNA produces multiple forms of goat  $\alpha_{s1}$ -casein, *Eur. J. Biochem.* 249 (1997) 1–7.
- [31] Ferranti P., Lilla S., Chianese L., Addeo F., Qualitative and quantitative variation of deleted protein forms in goat  $\alpha_{s1}$  and  $\alpha_{s2}$  casein phenotypes, in: Proceedings 7th International Conference on Goats, Tour, France, 15–18 May 2000, pp. 573–575.
- [32] Ferretti L., Leone P., Sgaramella V., Long range restriction analysis of the bovine casein genes, *Nucleic Acids Res.* 18 (1990) 6829–6833.
- [33] Fitzgerald J.R., Murray B.A., Bioactive peptides and lactic fermentations, *Int. J. Dairy Technol.* 59 (2006) 118–125.
- [34] Galliano F., Saletti R., Cunsolo V., Foti S., Marletta D., Bordonaro S., D'Urso G., Identification and characterization of a new  $\beta$ -casein variant in goat milk by high-performance liquid chromatography with electrospray ionization mass spectrometry and matrix assisted laser desorption/ionization mass spectrometry, *Rapid Commun. Mass Spectrom.* 18 (2004) 1–11.
- [35] Gjesing B., Osterballe O., Schwartz S., Wahn V., Lowenstein H., Allergen specific IgE antibodies against antigenic components in cow milk and milk substitutes, *Allergy* 41 (1986) 51–56.
- [36] Groenen M.A., Dijkhof R.J.M., Verstege A.J.M., Van der Poel J.J., The complete sequence of the gene encoding bovine  $\alpha_{s2}$ -casein, *Gene* 123 (1993) 187–193.
- [37] Grosclaude F., Martin P., Casein polymorphism in the goat, in: Milk Protein Polymorphism Seminar (II), Proceedings IDF Int. Dairy Fed., Bruxelles, Belgium, 1997, pp. 241–253.
- [38] Grosclaude F., Joudrier P., Mahé M.F., Polymorphisme de la caséine  $\alpha_{s2}$  bovine : étroite liaison du locus  $\alpha_{s2}$ -Cn avec les loci  $\alpha_{s1}$ -Cn,  $\beta$ -Cn et  $\kappa$ -Cn, mise en évidence d'une délétion dans le variant  $\alpha_{s2}$  Cn, *Ann. Génét. Sél. Anim.* 10 (1978) 313–327.
- [39] Grosclaude F., Mahé M.F., Brignon G., Di Stasio L., Jeunet R., A Mendelian polymorphism underlying quantitative variations of goat  $\alpha_{s1}$ -casein, *Génét. Sél. Evol.* 19 (1987) 399–412.
- [40] Grosclaude F., Ricordeau G., Martin P., Remeuf F., Vassal L., Bouillon J., Du gène au fromage : le polymorphisme de la caséine  $\alpha_{s1}$  caprine, ses effets, son évolution, *INRA Prod. Anim.* 7 (1994) 3–19.
- [41] Grzesiak T., Goat milk, milk of the future for infants, in: Freund G. (Ed.), Intérêts nutritionnel et diététique du lait de chèvre,

- Les Colloques n° 81, INRA Éditions, Paris, France, 1997, pp. 127–147.
- [42] Hacelaf W., Boukhrelda M., Benbouabdellah M., Coquin P., Desjeux J.F., Boudraa G., Touhami M., Comparative digestibility of goat's versus cow's milk fats in children with digestive malnutrition, *Lait* 73 (1993) 593–599.
- [43] Haenlein G.F.W., Goat milk in human nutrition, *Small Rumin. Res.* 51 (2004) 155–163.
- [44] Jann O.C., Prinzenberg E.M., Luikart G., Caroli A., Erhardt G., High polymorphism in the  $\kappa$ -casein (CSN3) gene from wild and domestic caprine species revealed by DNA sequencing, *J. Dairy Res.* 71 (2004) 188–195.
- [45] Jansà-Perez M.J., Leroux C., Bonastre A.S., Martin P., Occurrence of a LINE sequence in the 3' UTR of the goat alpha s1-casein E-encoding allele associated with reduced protein synthesis level, *Gene* 147 (1994) 179–187.
- [46] Jaubert A., Martin P., Reverse phase HPLC analysis of goat caseins. Identification of  $\alpha_{s1}$  and  $\alpha_{s2}$  genetic variants, *Lait* 72 (1992) 235–247.
- [47] Kitagawa S., Zhang S., Harari Y., Castro G.A., Relative allergenicity of cow's milk and cow's milk-based formulas in an animal model, *Am. J. Med. Sci.* 310 (1995) 183–187.
- [48] Lagonigro R., Pietrosa E., D'Andrea M., Veltri C., Pilla F., Molecular genetic characterization of the goat  $\alpha_{s2}$ -casein E allele, *Anim. Genet.* 32 (2001) 391–393.
- [49] Lamblin C., Bourrier T., Orlando J.P., Sauvage C., Wallaert B., Allergy to goat's or sheep's milk without allergy to cow's milk, *Rev. Fr. Allergol. Immunol. Clin.* 41 (2001) 165–168.
- [50] Lara-Villoslada F., Olivares M., Jimenez J., Boza J., Xaus J., Goat milk is less immunogenic than cow milk in a murine model of atopy, *J. Pediatr. Gastroenterol. Nutr.* 39 (2004) 354–360.
- [51] Law A.J.R., Tziboula A., Fractionation of caprine  $\kappa$ -casein and examination of polymorphism by FPLC, *Milchwissenschaft* 48 (1993) 68–71.
- [52] Leroux C., Mazure N., Martin P., Mutation away from splice site recognition sequences might cis-modulate alternative splicing of goat  $\alpha_{s1}$ -casein transcripts. Structural organization of the relevant gene, *J. Biol. Chem.* 267 (1992) 6147–6157.
- [53] Mahé M.F., Grosclaude F.,  $\alpha_{s1}$ -Cn D, another allele associated with a decreased synthesis rate at the caprine  $\alpha_{s1}$ -casein locus, *Génét. Sél. Evol.* 21 (1989) 127–129.
- [54] Mahé M.F., Grosclaude F., Polymorphism of  $\beta$ -casein in the Creole goat of Guadeloupe: evidence for a null allele, *Génét. Sél. Evol.* 25 (1993) 403–408.
- [55] Manfredi E., Serradilla J.M., Leroux C., Martin P., Sánchez A., Genetics for milk production, in: *Proceedings 7th International Conference on Goats*, Tour, France, 15–18 May 2000, pp. 191–196.
- [56] Marletta D., Bordonaro S., Galliano F., Cunsolo V., Saletti R., Pastore N., D'Urso G., Identification of CSN1S2<sup>0</sup> allele in a Sicilian goat breed and characterization of  $\alpha_{s2}$  casein fraction by HPLC/ESI-MS, in: *Proceedings of the 7th World Congress on Genetics Applied to Livestock Production*, Montpellier, France, 19–23 August 2002, comm. 09–32.
- [57] Marletta D., Bordonaro S., Guastella A.M., Falagiani P., Crimi N., D'Urso G., Goat milk with different  $\alpha_{s2}$ -casein content: analysis of allergenic potency by REAST inhibition assay, *Small Rumin. Res.* 52 (2004) 19–24.
- [58] Martin P., Leroux C., Characterization of a further  $\alpha_{s1}$ -casein variant generated by exon skipping, in: *Proceedings XXIV International Conference on Animal Genetics*, Prague, Cz., 23–29 July 1994, Abs E. 43, p. 88.
- [59] Martin P., Ollivier-Bousquet M., Grosclaude F., Genetic polymorphism of caseins: a tool to investigate casein micelle organization, *Int. Dairy J.* 9 (1999) 163–171.
- [60] Martin P., Szymanowska M., Zwierzchowski L., Leroux C., The impact of genetic polymorphisms on the protein composition of ruminant milks, *Reprod. Nutr. Dev.* 42 (2002) 433–459.
- [61] Meisel H., Overview on milk protein-derived peptides, *Int. Dairy J.* 8 (1998) 363–373.
- [62] Meisel H., Fitzgerald R.J., Biofunctional peptides from milk protein: mineral binding and cytomodulatory effects, *Curr. Pharm. Des.* 9 (2003) 1289–1295.
- [63] Mercier J.C., Vilotte J.L., Structure and function of milk protein genes, *J. Dairy Sci.* 76 (1993) 3079–3098.
- [64] Mercier J.C., Addeo F., Pelissier J.P., Structure primaire du caséinomacropéptide

- de la caséine kappa caprine, *Biochimie* 58 (1976) 1303–1310.
- [65] Mercier J.C., Chobert J.M., Addeo F., Comparative analysis of the amino acid sequences of caseinomacropeptides from seven species, *FEBS Lett.* 72 (1976) 208–214.
- [66] Moatsou G., Vamvakaki A.N., Mollé D., Anifantakis E., Léonil J., Protein composition and polymorphism in the milk of Skopelos goats, *Lait* 86 (2006) 345–357.
- [67] Moioli B., Pilla F., Tripaldi C., Detection of milk protein genetic polymorphisms in order to improve dairy traits in sheep and goats: a review, *Small Rumin. Res.* 27 (1998) 185–195.
- [68] Moreno F.J., Recio I., Olano A., López-Fandiño R., Heterogeneity of caprine  $\kappa$ -casein macropeptide, *J. Dairy Res.* 68 (2001) 197–208.
- [69] Neveu C., Mollé D., Moreno F.J., Martin P., Léonil J., Heterogeneity of caprine beta-casein elucidated by RP-HPLC/MS: genetic variants and phosphorylations, *J. Protein Chem.* 21 (2002) 557–567.
- [70] Park Y.W., Comparison of buffering components in goat and cow milk, *Small Rumin. Res.* 8 (1992) 75–81.
- [71] Park Y.W., Hypo-allergenic and therapeutic significance of goat milk, *Small Rumin. Res.* 14 (1994) 151–159.
- [72] Persuy M.A., Printz C., Medrano J.F., Mercier J.C., One mutation might be responsible for the absence of beta-casein in two breeds of goats, *Anim. Genet.* 27 (1996) 96.
- [73] Persuy M.A., Printz C., Medrano J.F., Mercier J.C., A single nucleotide deletion resulting in a premature stop codon is associated with marked reduction of transcripts from a goat beta-casein null allele, *Anim. Genet.* 30 (1999) 444–451.
- [74] Piacere A., Ricordeau G., Manfredi E., Sigwald J.P., Lahaye P., Bibe B., Bouillon J., From cooperative genetical research work to technological transfer into the selection schemes: the french example on genetic improvement of protein content in goat milk, in: *Proceedings 7th International Conference on Goats*, Tour, France, 15–18 May 2000, pp. 18–22.
- [75] Pierre A., Michel F., Le Graet Y., Variation in size of goat milk casein micelles related to casein genotype, *Lait* 75 (1995) 489–502.
- [76] Pierre A., Michel F., Le Graet Y., Zahoute L., Casein micelle size in relation with casein composition and  $\alpha_{s1}$ ,  $\alpha_{s2}$ ,  $\beta$  and  $\kappa$  casein contents in goat milk, *Lait* 78 (1998) 591–605.
- [77] Pilla F., Valentini A., Lenstra J.A., Martin P., Animal genetics and functional food, in: *Livestock farming system Product quality based on local resources leading to improved sustainability*, EAAP publication N° 118, Wageningen Academic Publisher, The Netherlands, 2003, pp. 49–59.
- [78] Prinzenberg E.M., Gutscher K., Chessa S., Caroli A., Erhardt G., Caprine  $\kappa$ -casein (CSN3) polymorphism: new developments of the molecular knowledge, *J. Dairy Sci.* 88 (2005) 1490–1498.
- [79] Ramunno L., Mariani P., Pappalardo M., Rando A., Capuano M., Di Gregorio P., Cosenza G., A major-effect gene for  $\beta$ -casein content in goat milk, in: *Atti XI Congresso Nazionale ASPA*, Grado, Italy, 19–22 June 1995, pp. 185–186.
- [80] Ramunno L., Cosenza G., Pappalardo M., Longobardi E., Gallo D., Pastore N., Di Gregorio P., Rando A., Characterization of two new alleles at the goat CSN1S2 locus, *Anim. Genet.* 32 (2001) 264–268.
- [81] Ramunno L., Longobardi E., Pappalardo M., Rando A., Di Gregorio P., Cosenza G., Mariani P., Pastore N., Masina P., An allele associated with a non detectable amount of  $\alpha_{s2}$  casein in goat milk, *Anim. Genet.* 32 (2001) 19–26.
- [82] Ramunno L., Cosenza G., Rando A., Illario R., Gallo D., Di Bernardino D., Masina P., The goat  $\alpha_{s1}$  casein gene: gene structure and promoter analysis, *Gene* 334 (2004) 105–111.
- [83] Ramunno L., Cosenza G., Rando A., Paucillo A., Illario R., Gallo D., Di Bernardino D., Masina P., Comparative analysis of gene sequence of goat CSN1S1 F and N alleles and characterization of CSN1S1 transcript variants in mammary gland, *Gene* 345 (2005) 289–299.
- [84] Rando A., Pappalardo M., Capuano M., Di Gregorio P., Ramunno L., Two mutations might be responsible for the absence of  $\beta$ -casein in goat milk, *Anim. Genet.* 27 (Suppl. 2) (1996) 31.
- [85] Rando A., Ramunno L., Masina P., Mutations in casein genes, *Zootec. Nutr. Anim.* 26 (2000) 105–114.
- [86] Razafindrakoto O., Ravelomanana N., Rasolofo A., Rakotoarimanana R.D., Gourgue P., Coquin P., Briend A., Desjeux J.F., Goat's milk as a substitute for cow's



- milk in undernourished children: a randomised double-blind clinical trial, *Lait* 73 (1993) 601–611.
- [87] Recio I., Perez-Rodriguez M.L., Amigo L., Ramos M., Study of the polymorphism of caprine milk caseins by capillary electrophoresis, *J. Dairy Res.* 64 (1997) 515–523.
- [88] Reinert P., Fabre A., Use of goat's milk for infant feeding. Experience at Creteil (France), in: Freund G. (Ed.), *Intérêts nutritionnel et diététique du lait de chèvre*, Les Colloques N° 81, INRA Éditions, Paris, France, 1997, pp. 119–121.
- [89] Remeuf F., Influence du polymorphisme génétique de la caséine  $\alpha_{s1}$  caprine sur les caractéristiques physico-chimiques et technologiques du lait, *Lait* 73 (1993) 549–557.
- [90] Richardson B.C., Creamer L.K., Comparative micelle structure III. The isolation and chemical characterization of caprine  $\beta_1$ -casein and  $\beta_2$ -casein, *Biochim. Biophys. Acta* 365 (1974) 133–137.
- [91] Ricordeau G., Mahé M.F., Persuy M.A., Leroux C., François V., Amigues Y., Fréquences alléliques des caséines chez les chèvres des Pyrénées. Cas particulier de la caséine  $\beta$  nulle, *INRA Prod. Anim.* 12 (1999) 29–38.
- [92] Rijnkels M., Multispecies comparison of the casein gene loci and evolution of casein gene family, *J. Mammary Gland Biol. Neoplasia* 7 (2002) 327–345.
- [93] Roberts B.T., Di Tullio P., Vitale J., Hehir K., Gordon K., Cloning of the goat  $\beta$ -casein coding gene and expression in transgenic mice, *Gene* 121 (1992) 255–262.
- [94] Russo V., Davoli R., Dall'Olio S., Tedeschi M., Research on goat milk polymorphism, *Zoot. Nutr. Anim.* 12 (1986) 55–62.
- [95] Sabbah A., Hassoun S., Drouet M., Cow's milk allergy and its substitution by goat's milk, in: Freund G. (Ed.), *Intérêts nutritionnel et diététique du lait de chèvre*, Les Colloques n° 81, INRA Éditions, Paris, France, 1997, pp. 111–118.
- [96] Sacchi P., Chessa S., Budelli E., Bolla P., Cerotti G., Soglia D., Rasero R., Cauvin E., Caroli A., Casein haplotype structure in five Italian goat breeds, *J. Dairy Sci.* 88 (2005) 1561–1568.
- [97] Sánchez A., Ilahi H., Manfredi E., Serradilla J.M., Potential benefit from using the  $\alpha_{s1}$ -casein genotype information in a selection scheme for dairy goats, *J. Anim. Breed. Genet.* 122 (2005) 21–29.
- [98] Savilahti E., Kuitunen M., Allergenicity of cow milk proteins, *J. Pediatr.* 121 (1992) 12–20.
- [99] Serradilla J.M., The goat  $\alpha_{s1}$ -casein gene: a paradigm of the use of a major gene to improve milk quality?, in: Gabiña D., Sanna S. (Eds.), *Breeding programmes for improving the quality and safety of products. New traits, tools, rules and organization*, CIHEAM-IAMZ, Zaragoza, 2003, pp. 99–106.
- [100] Taitz L.S., Armitage B.L., Goat's milk for infants and children, *Brit. Med. J.* 288 (1984) 428–429.
- [101] Threadgill D.W., Womack J.E., Genomic analysis of the major bovine casein genes, *Nucleic Acids Res.* 18 (1990) 6935–6942.
- [102] Umpierrez A., Quirce S., Maranon F., Cuesta J., Garcia-Villamuzza Y., Lahoz C., Sastre J., Allergy to goat and sheep cheese with good tolerance to cow cheese, *Clin. Exp. Allergy* 29 (1999) 1064–1068.
- [103] Veltri C., Lagonigro R., Pietrolà E., D'Andrea M., Pilla F., Chianese L., Molecular characterization of the goat  $\alpha_{s2}$ -casein E allele and its detection in goat breeds of Italy, in: *Proceedings 7th International Conference on Goats*, Tour, France, 15–18 May 2000, p. 727.
- [104] Wal J.M., Cow's milk proteins allergens, *Ann. Allergy Asthma Immunol.* 89 (2002) 3–10.
- [105] Wang Q., Huang Z., Chen M.J., Wang S.Z., Zeng Y.T., 2001 available on <http://www.ncbi.nlm.nih.gov/>
- [106] Ward T.J., Honeycutt R.L., Derr J.N., Nucleotide sequence evolution at the  $\kappa$ -casein locus: evidence for positive selection within the family Bovidae, *Genetics* 147 (1997) 1863–1872.
- [107] Wuthrich B., Allergy to cheese produced from sheep's and goat's milk but not to cheese produced from cow's milk, *J. Allergy Clin. Immunol.* 96 (1995) 270–273.
- [108] Yahyaoui M.H., Coll A., Sanchez A., Folch J.M., Genetic polymorphism of the caprine  $\kappa$ -casein gene, *J. Dairy Res.* 68 (2001) 209–216.
- [109] Yahyaoui M.H., Angiolillo A., Pilla F., Sanchez A., Folch J.M., Characterization and genotyping of the caprine  $\kappa$ -casein variants, *J. Dairy Sci.* 86 (2003) 2715–2720.
- [110] Zittle C.A., Custer J.H., Identification of the  $\kappa$ -casein among the components of whole goat casein, *J. Dairy Sci.* 49 (1966) 788–791.