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Genetic polymorphism of goat *CSN1S1* and *CSN1S2* genes and their impact on milk composition

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As it is well known, six main proteins are present in ruminants milk: four caseins (αs1, αs2, β e k) and two main whey protein, β-lactoglobulin and α-lactoalbumin. In ruminants the four caseins represent about the 80% of milk proteins. Three (αs1, β and αs2) of the four caseins are sensitive to calcium precipitation, show similar molecular weights (around 24 kDa), promoter regions, leader peptide sequences and locations of the major phosphorylation site. These data support the hypothesis of a common evolutionary origin of these genes from the duplications of a unique ancestral gene [1].

At the present, the genomic organization and the nucleotide sequences of these genes are known. These genes have been mapped in the order *CSN1S1*, *CSN2*, *CSN1S2* and *CSN3* in a 250 kb (kilobase) DNA region of chromosome 6 in cattle and goat. In goat the *CSN1S1* and *CSN2* genes are convergently transcribed at only 12 kb apart [1; 2].

The goat *CSN1S1* gene extends over 16.7 kb including 1138 bp of exonic regions and about 15.7 kb of intronic regions. The main feature of the goat *CSN1S1* gene is the extremely split architecture. It contains 19 exons ranging in size from 24 to 385 bp and 18 introns from 90 to 1685 bp [3].

The goat *CSN1S1* gene represents, since many years, an excellent model for demonstrating that the major part of the variability observed in the αs1-casein content in the goat milk is due to the presence of autosomal alleles at a single structural *locus* (*CSN1S1*). So far, at least 17 alleles have been identified, which are associated to different levels of αs1-casein expression in the milk. A first group of alleles are related to a normal content of αs1-casein, whereas I and E alleles are associated to an intermediate content, and D, F and G alleles are related to a low level of αs1-casein in the milk. Alleles *CSN1S1* N, 01 and 02 are 'null' alleles and have been associated with the apparent absence of αs1-casein in the milk [3; 4; 5; 6].

Most of the mutational events responsible for the formation of such alleles have been already identified: particularly, the alleles associate to a normal content of this casein fraction have originated from single nucleotide substitutions [4; 5; 6]. While the molecular event characterizing the I allele is not known [4], the E allele is characterized from an insertion of a DNA segment (LINE, Long Interspersed Nuclear Element, 457 nucleotides long) which took place inside of the 19th exon [7].

Regarding the alleles related to a low level of αs1-casein in the goat milk, for the αs1-Cn D and G alleles, were observed mRNA characterized by the out-splicing of the exons 9 and 4, respectively [8]. For the G allele, cDNA sequencing revealed that exon 4 is skipped during the course of pre-mRNA processing. The mutational event responsible for the outsplicing of exon sequence, is a transition (G → A) occurring in the 5' splice site consensus sequence [5]. At this moment, it is unknown the molecular event responsible of this erroneous splicing in D allele

The F allele is, instead, characterized from a deletion of the 23rd nucleotide of the 9th exon and the insertion of 11 bp and 3 bp in the subsequent intron. By means of Northern blot analysis the amount of αs1 casein mRNA transcribed from F allele was estimated to be at least 6 time lower than that transcribed from A allele [9]. Furthermore, the F allele was shown to yield multiple alternatively spliced transcripts (at least 9), among which the most representative mRNA population is characterized by the alternative skipping of exon 9, 10 and 11 and responsible as consequence, for the synthesis of a form of αs1 casein deprived of 37 aa [3; 9].

The 01 allele, the true null allele, is characterized from the deletion of a DNA segment of nearly 8.5 kb (starting from the 181 nucleotide of the intron 12, and including the last 7 exons of the gene) [10], while a large insertion, so far uncharacteized, is the mutational event responsible of the 02 allele [5].

Recently, in a goat population reared in the Naples province, a new allele at the this *locus* (named N) have been identified, being associated to an apparent lack of synthesis of αs1-casein [3]. Sequence data and typing results show that the *CSN1S1* N allele is characterized, like to the *CSN1S1* F allele by the deletion of cytosine at the nucleotide 23 of the exon 9. The cytosine deletion is resulting in one-nucleotide frameshift and determines a premature stop codon in exon 12 [3]. The presence in the *CSN1S1* N allele of a premature termination codon (PTC) might be responsible for the apparent lack of αs1 casein synthesis. The correlation between the presence of a PTC and the absence of protein synthesis has been also found in other eukaryotic genes [11] and, in particular, at the *loci* of other two Ca-sensitive caseins of goat: αs2 [12] and β [13; 14]. However, N allele is characterized by the absence, contrary to F allele, of the insertion of 11 and 3 bp in the subsequent intron [3]. The molecular data available, in would allow to hypothesize that the *CSN1S1* N allele could be originated by an interallelic recombination event [3].

Analysis by means of Quantitative Real Time PCR shows that the amount of mRNA transcribed by the *CSN1S1* N allele is apparently the 33% of that transcribed by the *CSN1S1* F allele. Comparison of transcripts produced by the N and F alleles shows a remarkable variability in alternative splicing (at least 12 populations of CSN1S1 N mRNA). Particularly, for the F allele a higher ratio was observed compared to the N allele in the amount of transcripts characterized by outsplicing of exons 9, 10 and 11 (nearly 60% *vs* 21%) [3]. It is possible to hypothesize that the observed differences in the expression of the goat *CSN1S1* gene could be the direct consequence of more elaborated systems of gene regulation.

The effects of αs1-casein polymorphism on milk yield and composition, micelle structure, renneting properties and cheese yield have been thoroughly studied in different breeds. Results can be summarised as followed: (1) no differences exist among genotypes with respect to milk yields; (2) it shows a significant effect on the diameter of the micelles and on their calcium content which are lower in milks "AA"; (3) goat milk with high levels of αs1 casein has a better milk composition, including total solids, fat protein, casein phosphorus and lower pH than milks with low levels of αs1-casein; in particular, the intermediate variant E appear to improve milk composition over null variant milk composition (4) goat milk with αs1 casein A/A has a higher total nitrogen and higher fat level than milk with null content of this casein fraction (5) milk from genotypes constituted with "strong" alleles shows better renneting properties (faster coagulation and firmer curd) than "intermediate" and "null" genotypes, and these render better properties than "weak" genotypes; and (6) cheese yields of different genotypes are ranked in the same way as for renneting properties; (7) cheeses made with milk from these genotypes have less typical goat flavour than those from "weak" genotypes, due to different fatty acid profiles [review 15].

Goat milk proteins have many significant differences in their amino acid compositions from the milk of other mammalian species, especially in relative proportions of the various milk proteins and in their genetic polymorphisms The major protein in cow milk is αs1-casein, but goat milk may differ genetically by having either none or much. This in turn indicates and may explain significant differences to cow milk in digestion by infants and patients [16], which traditionally have been explained by the "homogenized" nature of goat milk fat.

Bevilacqua *et al.* [17] noted that "contradictory results have been reported on the use of goat milk in cow milk allergy." It was suggested that this could be due to this "high genetic polymorphism of goat milk proteins". The authors found that guinea pigs fed goat milk with low αs1-casein produced significantly less antibodies to β-lactoglobulin than animals fed with goat milk containing higher αs1-casein. They suggested that the digestion of β -lactoglobulin was enhanced in the relative absence of α-s1-casein.

In recent years a remarkable genetic polymorphism has been revealed also at the *CSN1S2 locus* in goat. The *CSN1S2* gene organization is very similar to that of genes coding for the other two calcium sensitive caseins In bovine this gene is about 18.5 Kb long and is divided in 18 exons ranging in size from 21 to 266 nucleotides [18].

To date, the 7 alleles identified at this *locus* would seem to be associated with three different expression levels: whereas *CSN1S2*A, B, C, E and F alleles are associated with a normal content of αs2-

casein [19; 20; 21; 22; 23] the *CSN1S2*D allele is associated with an intermediate amount of this casein fraction [23]. The *CSN1S20* allele is a "null" allele being associated to the apparent absence of αs2-casein in milk [12].

At molecular level, the *CSN1S2*A, B, C, E and F alleles differ in point mutations and, as a consequence, in amino acid substitutions at the protein level [21; 22; 23]. The *CSN1S2*D allele is characterized by a 106-bp deletion, involving the last 11 bp of the exon 11 and the first 95 bp of the subsequent intron [23]. The mutation that characterizes the null allele is a transition (G→A) at nucleotide 80 of the exon 11. This mutation is responsible of the formation of a premature stop-codon and, of consequence, for the apparent lack of αs2 casein synthesis [12]. Dot blot analyses showed that the level of *CSN1S2* mRNA of mammary gland cells from the *CSN1S2 0/0* goat is about 10% of the normal value [12]. Likewise for the goat *CSN1S1* gene, the analysis of the length of cloned RT-PCR fragments, identified 2 mRNA populations for A allele, 3 for the D allele [23] and at least 6 for 0 allele [24].

Alleles associated with a null amount of protein have been found for the an other goat calcium sensitive casein fraction, β casein [14; 25]. Therefore, at present, *Capra hircus* is the only species for which a null allele is available for each of these proteins.

In conclusion, by means of selection based on parent genotyping at the DNA level it will be possible to obtain goat populations producing milk characterised by the absence of either αs1-casein, or αs2-casein or β-casein. Such milk could be useful for specific technological processes of transformation or for specific nutritional or dietary purposes in order to attenuate the negative consequence of some metabolic deficiencies and allergies and to contribute to the prevention of some diseases

Considering the remarkable quantitative polymorphism characterizing the main protein fractions enooding gene, some goat milk types are more similar to the woman milk and, therefore, could be used, with better results, in the human feeding. In fact, the woman milk possess particular characteristics. It is lacking in the β-lactoglobulin fraction and it is characterized by a general low content in casein, in particular, αs2 casein absence and αs1 casein traces, similar to the milk produced by goats with genotype *CSN1S2* 0/0.

The milk produced by goats homozigotes for *CSN1S2* 0 allele, being characterized by the absence of such protein fraction, is similar in composition to the woman milk and, therefore, it could, perhaps, find profit employment in the feeding of the newborn, limiting intolerance phenomena to this specific milk protein fraction.

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