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Goat -casein C allele: characterization and detection at the DNA level

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Introduction

Casein genes are organized as a cluster including in the order $_{s1}$-casein (*CSN1S1*), -casein (*CSN2*), $_{s2}$-casein (*CSN1S2*), and k-casein (*CSN3*) (Ferretti *et al.*, 1990; Threadgill and Womack, 1990). In goats the entire casein gene cluster region spans about 250 kb on chromosome 6 (Hayes *et al.*, 1993). Furthermore *CSN1S1* and *CSN2* are only 12 kb apart and convergently transcribed (Leroux and Martin, 1996). Goat caseins show a complex qualitative and quantitative variability, characterised by several genetic polymorphisms as well as by multiple post-translation modifications. Different transcriptional and post-transcriptional mechanisms control casein gene expression, dramatically affecting the technological properties of milk (Martin *et al.*, 2002).
As far as *CNS2* is concerned, three protein variants were found to be associated with a normal -casein content: A, B (Mahé and Grosclaude, 1993) and C (Neveu *et al*., 2002). The last variant was identified by peptide mass fingerprinting and tandem mass spectrophotometry. The variant differed in a mono amino acid substitution, Ala_{177} to Val_{177} of the mature protein, from the *CSN2*A*. Since both amino acids are neutral, the mutation is not detectable by screening protein techniques such as isoelectric focusing. At DNA level the protein polymorphism is justified by a nucleotide transition GCA (Ala_{177}) GTA (Val_{177}), occurring in GenBank accession number AF409096 (Wang *et al.*, 2001, direct submission) if compared to AH001195 sequence (Roberts *et al.,* 1992).
Furthermore, two null *CSN2* alleles were identified, both characterized by mutations responsible for premature stop codons in exon 7 (Ramunno *et al.*, 1995: GenBank Accession number AJ011019; Persuy *et al.*, 1999: GenBank Accession number AF172260). The first null allele was detected in Southern Italian breeds, while the second one was found in Créole and Pyrenean breeds.
In order to get further information on goat *CSN2* variability, a Polimerase Chain Reaction – Single Strand Conformation Polymorphism (PCR-SSCP) method was developed for typing *CSN2*0* as an alternative to Allele Specific PCR (Ramunno *et al.*, 1995). An unknown SSCP pattern was detected and sequenced. Different Italian goat breeds were analysed.

Material and Methods

PCR: A 374 bp fragment containing part of goat *CSN2* exon 7 was amplified by a PCR performed in a 25 µl reaction mixture with 2 µl of DNA solution (100-150 ng), 10 pmol of each primer and 1X PCR Master Mix (Fermentas). *SSCP analysis*: 6 µl PCR product, 8 µl of denaturating solution (0.05% of xylene-cyanol, 0.05% of bromophenol blue, 0.02M EDTA in deionised formamide); heat denaturation (95°C for 8 min); run (16 hours, 280 V, 12°C) on 10% acrylamide : bisacrylamide gels (29:1) with 1.5% glycerol in 0.5X TBE buffer. Sequencing of the unknown SSCP pattern was performed randomly choosing five DNA samples presenting the polymorphism both at the heterozygous and homozygous condition. Typing was performed in seven Italian goat breeds (Camosciata, Cilentana, Garganica, Jonica, Orobica, Maltese, Saanen). A total of 473 DNA samples

were analysed. Samples carrying the *CSN2*0* allele identified by Ramunno *et al.* (1995) were used as reference.

Results and discussion

The PCR-SSCP analysis allowed to identify the null allele and to detect a third polymorphism. The correspondence between the C protein variant and the SSCP polymorphism was demonstrated by sequencing results. The nucleotide transition C T responsible for the amino acid substitution Ala_{177} to Val_{177} was found in all the sequenced samples presenting the polymorphism. The 374 bp fragment was submitted to GenBank (accession number AY563136).
The typing results of the seven Italian goat breeds indicated that *CSN2*C* variant was the most common allele in all breeds except in Saanen, where *CSN2*A* and *CSN2*C* showed similar frequencies (0.51 and 0.49 respectively). In Orobica, a local breed of Lombardy, the highest *CSN2*C* frequency was found (0.975). The null allele was present only in the Southern Italy breeds (Cilentana, Jonica, Garganica, Maltese) with frequencies ranging from 0.05 to 0.09. In these breeds *CSN2*C* occurred with high frequencies, ranging from 0.70 to 0.89.
In conclusion, the PCR-SSCP method here developed allows to identify simultaneously *CSN2*A, CSN2*C*, and *CSN2*0* alleles. Studies on caprine casein variability could usefully include this PCR-SSCP test to identify *CSN2*C* allele at the DNA level. The high frequency of this allele in the breeds typed indicates the importance of recognizing this nucleotide exchange within the caprine casein cluster, and of taking into account the resulting amino acid substitution in further investigations on goat casein variability.

References

Ferretti, L., P. Leone, and V. Sgaramella. 1990. Long range restriction analysis of the bovine casein genes. Nucleic Acids Res. 18:6829-6833.
Hayes, H., E. Petit, C. Bouniol, and P. Popescu.1993. Localisation of the alpha-S2- casein gene (CASAS2) to the homologous cattle, sheep and goat chromosome 4 by in situ hybridisation. Cytogenet. Cell Genet. 64:282-285.
Leroux, C., and P. Martin. 1996. The caprine alphas1 and beta –casein genes are 12-kb apart and convergently transcribed. Anim. Genet. 27:93.
Mahé, M.F., and F. Grosclaude. 1993. Polymorphism of β-casein in the Creole goat of Guadeloupe, evidence for a null allele. Génét. Sél. Evol. 25:403-408.
Martin, P., M. Szymanowska, L. Zwierzchowski, and C. Leroux. 2002. The impact of genetic polymorphisms on the protein composition of ruminants milks. Reprod. Nutr. Dev. 42:433-459.
Neveu, C., D. Mollé, J. Moreno, P. Martin, and J. Léonid. 2002. Heterogeneity of caprine beta-casein elucidated by RP-HPLC/MS: genetic variants and phoshorylation. J. Protein Chem. 21:557-567.
Persuy, M.A., C. Printz, J.F. Medrano, and J.C. Mercier. 1999. A single nucleotide deletion resulting in a premature stop codon is associated with marked reduction of transcripts from a goat β-casein null allele. Anim. Genet. 30:444.
Ramunno, L., P. Mariani, M. Pappalardo, A. Rando, M. Capuano, P. Di Gregorio, and G. Cosenza. 1995. Un gene ad effetto maggiore sul contenuto di caseina β nel latte di capra. XI Conv. ASPA, 185-186, Grado (GO), 19-22 June 1995.
Roberts, B., DiTullio, P., Vitale, J., Hehir, K. and K. Gordon. 1992. Cloning of the goat beta-casein-encoding gene and expression in transgenic mice. Gene 121:255-262
Threadgill, D.W., and J.E. Womack. 1990. Genomic analysis of the major bovine milk proteins genes. Nucleic Acids Res. 18:6935-6942.

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