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A NEW METHOD FOR CHARACTERIZATION OF CATTLE β-CASEIN VARIANTS A^1, A^2, A^3 AND B

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Polymorphism of β-casein is quite complex, due to its high genetic variability and to the presence of a large number of cases of not characterized or not well clarified variants. In cattle eleven variants are known, however only four (A^1, A^2, A^3 and B), are universally distributed in nearly all *Bos taurus* and *Bos indicus* populations. The variant A^1 is particularly important, because, according to some recent studies, it might cause or aggravate one type 1 diabetes (which is the type seen most commonly in children), heart disease and autism. The lenght of β-casein gene is 8,5 kb and it is composed by nine exons, that have a size from 24 to 498 bp. Amino acid differences among the variants A^1, A^2, A^3 and B are located in exon VII, which encoded for 82% of mature protein. Used protocols for the characterization of β-casein variants A^1, A^2, A^3 and B are based on the following methods: AS-PCR, PCR-SSCP and Real Time PCR. We describe a new method based on the analysis of specific single nucleotide polymorphism (SNPs) and on the technique of ddNTPs primer extension. This tecnique is based on the extension, in presence of fluorescence-labeled dideoxy nucleotide (ddNTP, terminators), of an unlabeled oligonucleotide primer that bind to the complementary template immediately adjacent to the mutant nucleotide position. Given that there are no unlabeled dNTPs, a single ddNTP is added to its 3' end, resulting in a fluorescence-labeled primer extension product which is readily separated by capillary electrophoresis**.** In order to verify this new method, we tested one hundred reference samples (fifty Frisian Holstein and fifty Brown Swiss), previously analysed using Real Time PCR method. We used DNA extracted from different biological materials (blood, hair, milk and semen), and in all cases we confirmed the genotypes of reference samples.