

Characterization of goat α_{s2} -CN D, E and G by electrophoretic technique and mass spectrometry analysis

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The goat α_{s2} -CN D was characterised by the highest anodic mobility respect to goat α_{s1} -CN by PAGE pH=8.6 (1). Since the same behaviour was exhibited by PAGE analysis at pH=3.0 it was concluded that this goat α_{s2} -CN consisted of a molecular size lower than the normal phenotypes α_{s2} -CN A, B and C. On the other hand, studies carried out by Ramunno *et al.* (2) by PCR analysis, showed that goat α_{s2} -CN D were lacking of the tryptptides Pro¹²²Thr¹²³Val¹²⁴. These results weren't enough to justify the above electrophoretic experiments. In this work the MW of the new α_{s2} -CN variant proteins was calculated by using LC/MS/TOF analysis. It was 19832Da despite to 24719Da of the reference goat α_{s2} -CN A with a DMW of 4900Da. By an extensive use of ES/MS and peptide mapping, combining HPLC with ESI/TOF-MS, the primary structure of α_{s2} -CN D was achieved. The results indicated that this protein was 159 aa long due the lacking of the sequence 84-124 expressed by the exon 11 in α_{s2} -CN 207 aa long. This incorrect splicing could explain also the minor quantitative level of this phenotype in the milk if compared to the one of α_{s2} -CN A. This study was carried out on milk goat sample containing the heterozygous phenotype α_{s2} -CN DA classified on the base of relative negative net charge and pI. The analytical procedure, instead, showed for the phenotype α_{s2} -CN A a MW of 24733Da having more than 14 D respect to MW of reference α_{s2} -CN A (MW=24719). The variant tryptic peptide α_{s2} -(f4-22) was consistent with the substitution Ser⁸(A)–Thr⁸(G) which in turn explain the same mobility at alkaline pH and the pI value very similar between α_{s2} -CN A and the new variant. This last, considering the available nomenclature of the occurred alleles named in the letters order from A to F was called α_{s2} -CN G.

References

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