

Water Buffalo a_{s1} -CN polymorphism determination by immunoelectroforetic technique and LC/ESI/MS analysis

Chianese L.¹, Quarto M.¹, Pizzolongo F.¹, Calabrese M.G.¹, Caira S.², Mauriello R.¹ and Addeo F.¹

¹ Dipartimento di Scienza degli Alimenti, Università degli Studi di Napoli Federico II, Portici, Italy

² Istituto di Scienze dell'Alimentazione del CNR, Avellino, Italy

The Mediterranean Water Buffalo breed reared in Italy has historically subsisted without cross-breeding for more than sixteen centuries with other foreign animals. Therefore, the Italian water buffalo can be defined a pure autochthon breed having evident morphological differences with other European and Asian breeds. In previous studies (1, 2, 3) we demonstrated that some specific genetic variants, occurred in Venezuelan Water Buffalo milk, were lacking in the Italian milk. Recently has been detected a novel a -La variant in Mediterranean Water Buffalo breed (4). In this research a comprehensive study on the genetic polymorphism of milk proteins was performed to correctly trace milk from the water buffalo breed reared in Campania in order to adopt a standard as a sufficient basis for traceability of the PDO Mozzarella cheese. To this aim electrophoretic technique as well as high-resolution ESI/MS analysis were carried out on individual milk samples of Mediterranean race

UTLIEF and PAGE at alkaline pH profiles of whole Water Buffalo casein showed just one phenotype for a_{s1} -CN, b-CN and k-CN respectively and three phenotypes for a_{s2} -CN as reported previously by Chianese *et al.* (1). These results confirmed a substantial invariance of water buffalo caseins, while by RP-HPLC analysis two a_{s1} -CN variants, with different retention times were observed. LC/ESI-QTOF/MS analysis of the novel phenotype consisted of three components with molecular mass of 23252.86, 23332.86 and 23412.86 Da respectively. After dephosphorylation, the a_{s1} -CN showed a molecular mass of 22772.86 Da; it is characterized by a difference of 26 Da in the molecular mass value compared with the most common a_{s1} -CN variant. The tryptic peptides determined for the novel variant was identical with the yielded by the common variant except for the molecular mass 2950,42 Da. This peptide gave by tandem MS sequence 166YVPLGTQYPDAPSFSDIPNPIGSENSGK¹⁹³. The novel a_{s1} -CN variant (B) differ from the common variant (A) by single silent amino acid substitution Leu¹⁷⁸(A)→Ser¹⁷⁸(B). The presence of those "silent" variants will add a new perspective in studies involving genetic polymorphism of Mediterranean Water Buffalo milk proteins.

References

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