

P006**Genetic variability detected at the (c-type) milk lysozyme encoding gene in donkey**

Gianfranco Cosenza¹, Barbara Auzino², Roberta Ciampolini², Daniela Gallo¹, Marco Iannaccone¹, Rosanna Capparelli¹, Alfredo Pauciullo³

¹Dipartimento di Agraria, University of Napoli "Federico II", Italy

²Dipartimento di Scienze Veterinarie, University of Pisa, Italy

³Dipartimento di Scienze Agrarie, Forestali e Alimentari, University of Torino, Italy

Contact: giacosen@unina.it

Lysozyme is known to be a natural antimicrobial agent since it catalyses the hydrolysis of glycosidic bonds of mucopolysaccharides in bacterial cell walls. It inhibites the development of many pathogens bacteria, thus making the milk somewhat selective in regards to the milk bacteria content. Three major distinct types of lysozymes have been identified: chicken-type (c-type), invertebrate-type (i-type), and goose-type (g-type). In particular, there are at least 4 non-stomach lysozyme genes in ruminants (i.e., mammary gland, kidney, trachea, intestinal). Lysozymes in ruminants and equine milk are

considered to be the c-type because of their similarity to chicken egg white lysozyme. The c-type lysozyme content in donkey's milk varies during the different stages of lactation with a mean value of 1.0 mg/ml and proved to be higher than that in bovine, ovine, caprine (traces), whereas it was very close to mare's milk. In the equine species, the c-type lysozyme encoding gene (4 exons) maps on chromosome 6 and transcribes a mRNA of 1329bp, coding for a protein of 148aa. To our knowledge, no information on genetic variability has been reported so far at this *locus* in donkey. Consequently, in order to detected variability, total RNA was extracted from milk somatic cells of 6 unrelated Ragusana donkeys reared in Central Italy. The mRNA fragment comprised between the last 84nt of exon 1 and the first 285nt of exon 4 was amplified by RT-PCR and sequenced. Primers (For GCAAGGTCCTTG-AAAGATGT and Rev ACCAGCATTAGTCTATTTCG) were designed using as template the genomic donkey sequence (EMBL ID: NW_014638180). The obtained sequence (465bp) is relative to the cDNA tract spanning the last 64nt of exon 1 to the 236th nt of exon 4. Stop codon is located at the 65th-67th nt of exon 4. Sequences comparison showed a transition G→A at the 160th nt of exon 2 (NW_014638180:g-1784688C>T) responsible for the aa change Arg⁵⁰→Gln. The presence of the codon CGA at exon 2 of the donkey milk lysozyme encoding gene might represent the ancestral condition of the gene in *equidae*, as it has also been found in other donkey and male sequences. The identification of this SNP could represent the first report of polymorphism at this *locus* in donkey. Next step of the research will be the analysis of a large number of samples in order to establish the frequency of this mutation in donkey species and to evaluate if and how the new genetic variant may influence functional and biological properties of donkey's milk.