



**Official Journal of
the Animal Science
and Production
Association (ASPA)**

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ISSN 1594-4077

eISSN 1828-051X

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**[http://www.
tandfonline.com/tjas](http://www.tandfonline.com/tjas)**

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**Italian Journal of
Animal Science**

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volume 16

supplement 1

2017

italian journal of animal science

ASPA 22nd CONGRESS

Perugia, June 13-16, 2017

Book of Abstracts

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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 detect 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 GCAAGGTCTTTG-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 236thnt of exon 4. Stop codon is located at the 65th-67thnt of exon 4. Sequences comparison showed a transition G→A at the 160thnt of exon 2 (NW_014638180:g-1784688C > T) responsible for the aa change Arg⁹⁰→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.

P007

First SNP discovery in ACACA gene and association study with milk yield in Mediterranean river buffalo

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Table 1 Genotyping data and effects of *ACACA* SNP genotypes on milk yield.

Locus	Trait	Genotype		
<i>ACACA</i>	Genotype distribution	CC (430)	CT (112)	TT (9)
	N. lactations	854	223	19
	N. records	5603	1498	135
	Average milk quantity	8.36	8.18	7.32

The *ACACA* enzyme catalyses the first committed step of fatty acid synthesis in mammalian cytosol, the carboxylation of acetyl-CoA to malonyl-CoA, leading to the biosynthesis of long-chain fatty acids. To our knowledge no information on DNA genetic variability at *ACACA locus* has been reported so far in buffalo species. Consequently, in order to detect polymorphisms at Italian Mediterranean river buffalo *ACACA locus* and test possible associations with milk yield, we analyzed 551 subjects belonging to 14 farms, located in Salerno and Caserta province. A total of 7096 records for milk yield measured monthly with an automatized milk recording system on 1096 lactations were used. The DNA regions of the *ACACA* gene spanning partial exons 1 of 10 individual samples, randomly chosen, were amplified and sequenced using primers (GACAGTTTCTGACCTTTTGGTG and AGACCTCTCTG-CTTCCAA) designed on the genomic buffalo sequence (EMBL acc. no. NW_005785166). Sequence comparison showed a transversion C→T at position 34 of the exon 1 (5' UTR) (NW_005785166:g4381303G > A). The genotyping of DNA samples was performed at the KBiosciences (<http://www.kbiosciences.co.uk>) laboratory. The major allele had a relative frequency of 0.88 and the *locus* was in Hardy-Weinberg equilibrium. A mixed linear model procedure of SAS 9.1 (SAS Institute) was used for the association analysis between genotypes and milk yield. The model included fixed effects of the genotype, farm, calving season, days in milk, parity and the random effect of the animal. None of the three genotypes had significant associations with milk yield (Tab. 1). In conclusion, in this study, we report the first SNP identification at the *ACACA locus* in Mediterranean river buffalo. Although we found no association between the detected polymorphism and milk yield, our work provides a starting point for studies of the future possible association between *ACACA* variation and other milk phenotypic traits in buffalo.

P008

The interleukin-10 polymorphism g.3936 G > A is uncoupled with bovine tuberculosis susceptibility in water buffalo (*Bubalus bubalis*)

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