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Short communication: CSN1S1-CSN3 (α_{S1}-κ-casein) composite genotypes affect detailed milk protein composition of Mediterranean water buffalo

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ABSTRACT

The aim of the study was to investigate the effect of composite *CSN1S1-CSN3* [α_{S1}-κ-casein (CN)] genotype on milk protein composition in Mediterranean water buffalo. Content of α_{S1}-CN, α_{S2}-CN, β-CN, γ-CN, κ-CN, glycosylated and unglycosylated κ-CN, α-lactalbumin, and β-lactoglobulin was measured by reversed-phase HPLC using 621 individual milk samples. Genotypes at *CSN1S1* and *CSN3* were also obtained by reversed-phase HPLC. Two alleles were detected at *CSN1S1* (corresponding to the A and B variants, O62823: p.Leu193Ser,) and at *CSN3* (corresponding to the X1 and X2 variants, CAP12622.1: p.Ile156Thr). Increased proportions of α_{S1}-CN in total casein (TCN) were associated with genotypes carrying *CSN1S1* A. Genotypes associated with a marked decrease of the proportion of α_{S1}-CN in TCN (composite genotypes AB-X1X1 and BB-X1X2) were associated with marked increases in the proportion of α_{S2}-CN. In addition, composite genotypes carrying the X1 allele at *CSN3* were associated with a greater proportion of α_{S2}-CN in TCN relative to those carrying *CSN3* X2. Composite genotypes greatly affected also the variability of ratios of κ-CN to TCN, with genotypes carrying the X1 allele at *CSN3* being associated with decreased ratios. The decreased content of glycosylated κ-CN associated with *CSN3* X1 was responsible for the overall lower content of total κ-CN in milk of X1-carrying animals. Increasing the frequency of specific genotypes might be an effective way to alter milk protein composition, namely the proportion of α_{S1}-CN, α_{S2}-CN, and κ-CN in TCN, and the degree of glycosylation of κ-CN.
Key words: *CSN1S1*, *CSN3*, casein, water buffalo

Short Communication

Several studies investigating constituents of bovine and goat milk reported a marked influence of milk protein genotypes on protein composition (Martin et al., 2002), and gene-assisted selection exploiting variation at milk protein loci has been suggested as an effective way to alter protein composition and enhancing technological properties of milk (Caroli et al., 2009).

In Mediterranean water buffalo, only 2 genetic variants of α_{S1}-CN (Chianese et al., 2009), κ-CN (Bonfatti et al., 2012b), and α-LA (Chianese et al., 2004) have been detected, with the latter being virtually monomorphic, whereas, for α_{S2}-CN, β-CN, and β-LG, no polymorphism has been detected (Chianese et al., 2009). Recently, effects of *CSN* composite genotypes on milk coagulation properties and effects of nongenetic factors on the detailed milk protein composition have been investigated by Bonfatti et al. (2012 a,b), evidencing that phenotypic variability of protein composition exists and that coagulation properties of buffalo milk are affected by *CSN* composite genotype. To date, effects exerted by *CSN* genotypes on the detailed buffalo milk protein composition have not been investigated. The aim of this study was to estimate the effects of *CSN1S1* and *CSN3* genotypes on the detailed milk protein composition in Mediterranean water buffalo.

One milk sample, which derived from the blending of consecutive morning and evening milking, was collected from 621 Mediterranean water buffaloes reared in 15 commercial farms. The farms were located in the Campania region, in the south of Italy. Milk was frozen immediately after collection, transferred in dry ice to the Department of Comparative Biomedicine and Food Science at the University of Padova, and preserved at −40°C until reversed-phase (**RP**)-HPLC analysis.

Contents of α_{S1}-CN, α_{S2}-CN, β-CN, γ-CN, glycosylated, and unglycosylated κ-CN, β-LG, and α-LA were measured using the RP-HPLC method of Bonfatti et al. (2008) for bovine milk. Peaks of A and B α_{S1}-CN variants, α_{S2}-CN, β-CN, γ-CN, X1 and X2 κ-CN, β-LG, and α-LA were purified by semipreparative RP-HPLC (Bonfatti et al., 2008), lyophilized, weighted, and used as calibration standards. Hence, a specific calibration equation was obtained for each protein fraction or protein genetic variant. For γ-CN, the same calibration equation of β-CN was used. After preliminary compari-

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sons of chromatograms with results of DNA sequencing, genotypes of buffaloes for *CSN1S1* and *CSN3* were also derived by RP-HPLC (Bonfatti et al., 2012b). Polymorphisms consisted of a C > T transversion at nucleotide 578 of *Bubalus bubalis* exon 17 of *CSN1S1* complete coding sequence AJ005430.1 (O62823: p.Leu193Ser), and a T > C transversion at nucleotide 467 of the *CSN3* complete coding sequence HQ677596 (CAP12622.1: p.Ile156Thr). Total casein (**TCN**) content (g/L) was computed as the sum of α_{S1}-CN, α_{S2}-CN, β-CN, γ-CN, and total κ-CN contents. Total whey protein content (**WH**, g/L) was calculated as the sum of α-LA and β-LG contents. Protein composition was measured as percentage ratios of α_{S1}-CN (**α_{S1}-CN%**), α_{S2}-CN (**α_{S2}-CN%**), β-CN (**β-CN%**), γ-CN (**γ-CN%**), and total κ-CN (**κ-CN%**) to TCN, and of β-LG to WH (**β-LG%**). Because WH was the sum of α-LA and β-LG contents, the percentage of α-LA was not considered, because its correlation with β-LG% was −1. The logarithmic transformation of γ-CN% (**Logγ-CN%**) was used to improve the distributional features of the trait. In addition, the percentage ratio of glycosylated κ-CN to total κ-CN (**glyco-κ-CN%**) was calculated.

Effects of composite *CSN1S1-CSN3* genotypes on protein composition were investigated using Bayesian methodology. The model included the effect of herd test day (15 levels), the effect of parity (first parity, from second to fourth parity, and more than 4 parities), the effect of DIM class (less than 60 d, from 60 to 220 d, from 221 to 260 d, from 261 to 280 d, and more than 280 d) and the composite *CSN1S1-CSN3* genotype effect (7 genotypes). In buffalo, AI is not a common practice, because heat detection is difficult and misleading paternity identification can occur (Rosati and Van Vleck, 2002). Hence, pedigree information was available for a limited number of animals and accounting for additive genetic effects of the animals, as well as reconstruction of *CSN1-CSN3* haplotypes and estimation of their effects, was unfeasible. Marginal posterior densities of parameters of interest were obtained by performing numerical integration through Gibbs sampling, and the mean of the marginal posterior density was used as a point estimate of the parameter. Marginal posterior densities of the differences between effects of each composite genotype relative to the reference genotype were obtained from samples of genotype effects solutions. Genotype BB-X2X2, which was the most frequent, was chosen as reference genotype. Bounded uniform priors were used for all effects considered in the models. A chain of 100,000 iterations with a burn-in of 20,000 iterations was generated in each analysis.

Descriptive statistics for the detailed composition of milk protein are reported in Table 1. The average protein composition was consistent with that reported by Addeo et al. (1977), Addeo et al. (1996), and D'Ambrosio et al. (2008). The most abundant fractions in TCN were α_{S1}-CN and β-CN, whereas γ-CN, a product of β-CN degradation, accounted for only 0.5% of TCN. More than 30% of total κ-CN was in the glycosylated form, and the ratio of glycosylated κ-CN to total κ-CN ranged from 17 to 50%. Minor allele frequency at *CSN1S1* and *CSN3* was 0.33, with A (*CSN1S1*) and X1 (*CSN3*) being the less frequent alleles. Observed and expected (assuming linkage equilibrium) composite genotype frequencies are reported in Table 2. Comparison of such frequencies by a chi-squared test indicates that the investigated loci are in linkage disequilibrium ($P < 0.001$). Allele *CSN1S1* B is more frequently associated with *CSN3* X2 than with *CSN3* X1. Because the AA-X2X2 genotype was observed in 1 animal only, that record was discarded.

The effects of *CSN1S1-CSN3* composite genotypes, measured in standard deviation units of the traits and relative to genotype BB-X2X2, on the detailed milk protein composition are depicted in Figure 1. Composite genotypes affected the amounts of TCN and WH. These effects were larger for TCN than for WH. In general, all genotypes were associated, relative to genotype BB-X2X2, to an increase (ranging from 0.1 to 0.5 SD) in TCN and to a decrease or no important change in WH. Because composite genotypes considered exclusively *CSN* genes, a limited effect on variation of WH was expected. However, relevant negative effects on WH were observed for genotypes AA-X1X2 and AB-X1X1. Genotype AA-X1X2 was also associated with a marked increase in TCN, resulting in a greater casein-to-protein ratio compared with other *CSN1S1-CSN3* genotypes.

Effects exerted by composite genotypes on casein composition were of considerable extent. Overall, increased α_{S1}-CN% was associated with *CSN1S1* homozygous genotypes carrying the A allele. The largest difference between composite genotype effects for α_{S1}-CN% was greater than 1 standard deviation of the trait and was observed between AA-X1X1 and BB-X1X2 genotypes. For bovine breeds, knowledge of the effects of *CSN1S1* variants on milk protein composition is scarce, because in this species the gene is almost monomorphic, but across-*CSN1S1* genotype variation in α_{S1}-CN milk content has been reported (Graml and Pirchner, 2003). Genotypes negatively affecting α_{S1}CN% were also associated with an important increase in α_{S2}-CN%. Besides the effects exerted by *CSN1S1*, variation of α_{S2}-CN% was influenced by alleles at *CSN3*. In particular, composite genotypes carrying allele *CSN3* X1 were associated with a greater proportion of α_{S2}-CN% relative to those carrying *CSN3* X2. The effect exerted by the *CSN3* gene was easily detectable when aver-