Short communication: Effect of α_{S1}-casein (*CSN1S1*) and κ-casein (*CSN3*) genotypes on milk composition in Murciano-Granadina goats

F. Caravaca,* J. Carrizosa,† B. Urrutia,† F. Baena,‡ J. Jordana,§ M. Amills,§ B. Badaoui,§ A. Sánchez,§ A. Angiolillo,# and J. M. Serradilla‡[1]

*Departamento de Ciencias Agroforestales, Escuela Universitaría de Ingeniería Técnica Agrícola, Universidad de Sevilla, 41013 Sevilla, Spain
†Instituto Murciano de Investigación y Desarrollo Agrario y Alimentario (IMIDA), Estación Sericícola, La Alberca, Murcia, Spain
‡Departamento de Producción Animal, Campus de Rabanales, Universidad de Córdoba, 14071 Córdoba, Spain
§Departament de Ciència Animal i dels Aliments, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain
#Dipartimento di Scienze e Tecnologie per l'Ambiente e il Territorio–Università del Molise, 86090 Pesche (IS), Italy

ABSTRACT

The effects of the caprine α_{S1}-casein (*CSN1S1*) polymorphisms on milk quality have been widely demonstrated. However, much less is known about the consequences of the κ-casein (*CSN3*) genotype on milk composition in goats. Moreover, the occurrence of interactions between *CSN3* and *CSN1S1* genotypes has not been investigated. In this study, an association analysis between *CSN1S1* and *CSN3* genotypes and milk quality traits was performed in 89 Murciano-Granadina goats. Total milk yield as well as total protein, fat, solids-not-fat, lactose, α_{S1}-casein (CSN1S1), and α_{S2}-casein (CSN1S2) contents were recorded every other month during a whole lactation (316 observations). Data analysis using a linear mixed model for repeated observations revealed no interaction between the *CSN1S1* and *CSN3* genotypes. With regard to the effect of the *CSN3* locus, AB and BB genotypes were significantly associated with higher levels of total casein and protein content compared with the AA *CSN3* genotype. In strong contrast with French breeds, the *CSN1S1* genotype did not affect protein, casein, and fat concentrations in Murciano-Granadina goats. These results highlight the importance of taking into consideration the *CSN3* genotype when performing selection for milk composition in dairy goats.

Key words: goat, α_{S1}-casein, κ-casein, milk composition

Caseins are the main nutritional source for newborns in placental mammals (Brawand et al., 2008). Genes encoding these milk proteins are organized as a cluster in the following sequence (Grosclaude et al., 1978; Ferretti et al., 1990; Rijnkels et al., 1997): α_{S1}-casein (*CSN1S1*), β-casein (*CSN2*), α_{S2}-casein (*CSN1S2*), and

κ-casein (*CSN3*). In goats and cattle, this gene cluster has been mapped to a 250-kb region on chromosome 6 (Martin et al., 2002). At the functional level, CSN1S1 plays a major role in the efficient transportation of caseins from the endoplasmic reticulum to the Golgi compartment (Chanat et al., 1999), whereas CSN3 is essential for regulating micelle formation and stabilization (Alexander et al., 1988).

The *CSN1S1* and *CSN3* genes are highly polymorphic in goats, whereas *CSN1S2* and *CSN2* are much less variable (Martin et al., 2002; Moioli et al., 2007). The caprine *CSN1S1* locus has 18 allelic variants associated with high, medium, low, and no CSN1S1 content in milk (Ramunno et al., 2004; Caroli et al., 2007; Moioli et al., 2007). In French and Italian breeds, genetic variation at this locus has been shown to affect protein, casein, and fat levels in milk, as well as milk rheology and organoleptic properties of cheese (see reviews by Grosclaude and Martin, 1997; Martin et al., 2002; Moioli et al., 2007). The *CSN3* gene is also extremely polymorphic, with up to 16 different alleles, *A*, *B*, *B′*, *B″*, *C*, *C′*, *D*, *E*, *F*, *G*, *H*, *I*, *J*, *K*, *L*, and *M* (Yahyaoui et al., 2003; Jann et al., 2004; Prinzenberg et al., 2005) that yield 13 protein variants. Allele frequency studies indicate that *A* and *B* are the most frequent *CSN3* variants in Spanish, French, and Italian goat breeds (Yahyaoui et al., 2003; Prinzenberg et al., 2005). Alleles *F* and *G* have been found at relatively high frequencies only in Italian goats (Yahyaoui et al., 2003). The remaining variants (*D*, *H*, *K*, *I*, *L*, *C*, and *M*) have been detected in only a few breeds at low or intermediate frequencies (Jann et al., 2004; Prinzenberg et al., 2005). The effects of these largely nonsynonymous *CSN3* polymorphisms on milk yield and composition are mostly unknown, although associations between CSN3 isoelectrofocusing (**IEF**) variants and protein and casein contents have been reported for the Orobica goat breed (Chiatti et al., 2007). However, because IEF only distinguishes between 2 variants, A^{IEF} (*A*, *B*, *B′*, *B″*, *C*, *C′*, *F*, *G*, *H*, *I*, *J*, and *L*) and B^{IEF} (*D*, *E*, *K*, and *M*), there is still a

need for fine mapping of the amino acid substitutions responsible for these effects.

The objectives of the current study were first to investigate the association between the variants of *CSN1S1* and *CSN3* genes and milk yield and composition traits in the Murciano-Granadina breed; and second, to test for the existence of interactions between both loci.

Five hundred and nine Murciano-Granadina goats were selected at 3 farms located in Murcia (southeastern Spain). Herds were reproductively unconnected and full siblings were avoided in the experimental design. Blood samples were taken from these 509 goats and genomic DNA was extracted according to Vidal et al. (2005). Subsequently, goats were genotyped for the *CSN1S1* gene following the protocol reported by Caravaca et al. (2008). It should be noted that data from 138 of these 509 goats had been previously analyzed to investigate the effect of the *CSN1S1* genotype on the synthesis rate of this protein (Caravaca et al., 2008). In the current experiment, the *CSN3* gene was genotyped by primer extension analysis (Yahyaoui et al., 2003) in 242 out of the 509 goats. The nomenclature proposed by Prinzenberg et al. (2005) was used to denote *CSN3* alleles. Based on *CSN1S1* and *CSN3* genotype information, 89 goats were selected using the following criteria: at the *CSN1S1* locus, only BB and EE genotypes were chosen, because they could be ascertained unambiguously with the genotyping methods employed (see above), they were found at sufficient frequencies (Table 3), and they represented genotypes associated with high and medium CSN1S1 synthesis rates. With regard to *CSN3*, only AA, AB, and BB goats were selected because these 3 genotypes were the most abundant ones. A potential limitation of this experiment was that the *CSN1S2* and *CSN2* loci were not genotyped in the population under study. In fact, null alleles have been reported for the *CSN1S2* and *CSN2* loci, being associated with null amounts of either of these proteins, whereas the *D* allele of the *CSN1S2* gene causes a reduction in the concentration of this casein (Persuy et al., 1999; Ramunno et al., 2001). However, a preliminary analysis (results not shown) performed at the protein and DNA levels suggested that these alleles are very rare or even absent in the Murciano-Granadina breed.

The total volume of milk collected at one milking was determined every other month for each of the 89 goats through an entire lactation period. Total protein, fat, SNF, and lactose contents and SCC were determined in milk samples at a professional laboratory (Milk Interprofessional Laboratory, Cantabria, Spain) using a spectrophotometer (C9 CombiFoss FT 6000, Foss Electric, Hillerød, Denmark) for routine analyses. In addition, a near infrared spectrophotometer, previously calibrated (Agüera et al., 2004), was used to measure

Table 1. Total number of goats and milk samples (within parentheses) analyzed for each α_{S1}-casein (*CSN1S1*) and κ-casein (*CSN3*) genotypic class

| *CSN3* genotype | *CSN1S1* genotype | | |
	BB	EE	Total
AA	10 (34)	6 (20)	16 (54)
BB	15 (55)	17 (64)	32 (119)
AB	15 (48)	26 (95)	41 (143)
Total	40 (137)	49 (179)	89 (316)

total casein, CSN1S1, and CSN1S2 contents using the method described by Caravaca et al. (2008). After purging data, 316 records from 89 goats were used for the statistical analyses. Table 1 shows the number of goats and observations of each *CSN1S1* and *CSN3* genotype used in this study.

The MIXED procedure (SAS Institute, 2007) was used for the association analysis, as suggested by Littell et al. (1998) for repeated observations analysis. The mixed model used in the first round of analysis was Y = Xβ + Zu + e, where β was the vector of fixed parameters or values of levels of fixed factors: *CSN1S1* and *CSN3* genotypes (2 and 3 levels, respectively) and their interaction (6 levels); herd-year-season (5 levels); parity number (5 levels); number of kids born (3 levels), and number of months elapsed from kidding to sampling day (10 levels); X was the incidence matrix for these effects; u was the vector of random animal effects; Z was the incidence matrix for random effects, and e was a vector of the residual random errors, assumed to be normally distributed with mean E (e) = 0 and variance V (e) = R. In this case, repeated measures of the variable within goat existed that were assumed to be correlated. Therefore, the REPEATED instruction of the mixed linear model procedure of SAS was implemented with the CS option, which defines a compound symmetric structure of R. This structure assumes that all repeated measures have the same variance and that all pairs of measures on the same animal have the same correlation. Thus, the covariance between repeated measures was only due to the individual component and not to the fact that they were taken from the same individuals at different time points. In a second round of analysis, the aforementioned statistical model, including only those factors being significant (see Table 2), was used to reanalyze each variable. Linear combinations of parameter values L′β (L being a vector of coefficients) and their corresponding variance V (L′β), estimated with this model, allowed for testing differences between genotype means using a *t* distribution. The Bonferroni correction for multiple comparisons was applied.

The frequencies of genotypes observed for *CSN1S1* (n = 509) and *CSN3* (n = 242) loci in the Murciano-

Table 2. P-values obtained for each fixed factor considered in the mixed model used to analyze the traits under study

	Trait							
Factor[1]	Daily milk yield, kg	Milk fat content, %	Milk protein content, %	Milk lactose, %	Casein content, %	CSN1S1 content, g/L	CSN1S2[2] content, g/L	SNF, %
CSN3 genotype	0.34	0.25	0.03	0.83	0.02	0.95	0.05	0.11
CSN1S1 genotype	0.35	0.60	0.23	0.1	0.07	<0.0001	0.73	0.36
CSN3 × *CSN1S1*	0.47	0.95	0.84	0.15	0.89	0.94	0.87	0.74
Herd-year-season	0.07	0.009	0.90	0.001	0.10	0.35	0.0003	0.46
Number of kids born	0.07	0.34	0.55	0.96	0.93	0.95	0.79	0.69
Lactation ordinal	0.83	0.17	0.82	0.48	0.85	0.65	0.51	0.86
Month of lactation	<0.0001	<0.0001	<0.0001	<0.0001	0.0001	<0.0001	<0.0001	<0.0001

[1]*CSN3* = κ-casein; *CSN1S1* = α_{S1}-casein.
[2]CSN1S2 = α_{S2}-casein.

Granadina breed are shown in Table 3. Allelic frequencies computed from these genotypic frequencies were in general agreement with previously published values for Murciano-Granadina goats, indicating that the *E* allele is the most frequent *CSN1S1* allele in peninsular Spanish breeds (Jordana et al., 1996). With regard to *CSN3*, the *A* and *B* alleles were in the majority, with a combined genotypic frequency (AA + AB + BB) of 0.997 (Table 3). Other authors have obtained similar results for *CSN3* in an independent Murciano-Granadina population (Yahyaoui et al., 2003) as well as in several European, Asian, and African breeds (Yahyaoui et al., 2003; Prinzenberg et al., 2005; Sacchi et al., 2005). The most frequent combination of *CSN1S1-CSN3* genotypes in the experimental sample was EE-AB (0.26), followed by EE-BB (0.17) and BB-BB and BB-AB (both 0.15). The remaining combinations were present at frequencies <0.10. Sacchi et al. (2005) genotyped 430 goats at the *CSN1S1*, *CSN1S2*, and *CSN3* loci in 5 Italian breeds. These authors found that the most frequent *CSN1S1-CSN3* haplotypes observed were *E-D* (at the time of this study, the *B* allele was referred to as *D*) in Vallesana breed (0.27), and *F-D* in Jonica (0.21) and Garganica (0.21) breeds. The haplotype frequencies in the Vallesana breed were similar to those reported in the current work.

The most relevant finding of the association analysis was that *CSN3* polymorphisms displayed significant effects on goat milk quality (Table 4). In contrast to Chiatti et al. (2007), specific *CSN3* alleles (*A* vs. *B*) were compared rather than IEF variants (which represent a pool of alleles). A marked influence of *CSN3* polymorphisms on total casein content (expressed as mean percentage values) was found. The amount of casein associated with the BB (2.94% ± 0.10) and AB (2.95% ± 0.09) genotypes was significantly greater ($P < 0.05$) than that found in AA goats (2.74% ± 0.11). Similarly, the total protein content (expressed as percentage) was significantly greater ($P < 0.05$) for the BB

(3.70% ± 0.06) and AB (3.70% ± 0.05) goats compared with their AA (3.47% ± 0.08) counterparts.

This association is difficult to interpret from a biological point of view because the *CSN3 A* and *B* variants only differ by a single amino acid substitution at position 119: the *A* allele has a valine and the *B* allele an isoleucine at this position (Yahyaoui et al., 2001). Valine and isoleucine are both aliphatic amino acids with similar biochemical properties. Analysis with the Polyphen software (Ramensky et al., 2002) showed that in most mammalian species, Ile119 is a highly conserved residue, suggesting that it might have an important functional role. In the absence of a causal effect, the Ile119Val polymorphism might be linked to one or several regulatory mutations located in the noncoding regions of the *CSN3* gene (e.g., promoter, introns, 3′-untranslated region). It is notable that associations between *CSN3* genotype and milk quality have been reported in cattle, although the polymorphisms involved are completely different. In cattle, the *B* variant, which differs from the *A* variant at 2 positions (*A*: Thr136 and Asn148, *B*: Ile136 and Ala148), has been associated with milk with increased protein content and a shorter renneting time (reviewed by Martin et al., 2002).

In a previous study (Caravaca et al., 2008) a significant effect of the *CSN1S1* genotype on the synthesis rate of this protein was reported, although associations with

Table 3. Genotype frequencies (within parentheses) for the α_{S1}-casein (*CSN1S1*; n = 509) and κ-casein (*CSN3*; n = 242) loci in the Murciano-Granadina goat breed

CSN1S1 genotypes[1]		*CSN3* genotypes	
BE (0.3)	A/0-A/0 (0.033)	AB (0.442)	AG (0.004)
EE (0.277)	BF (0.035)	BB (0.413)	BG (0.004)
BB (0.139)	EF (0.025)	AA (0.124)	
A/0-E (0.096)	A/0-F (0.008)	AF (0.008)	
A/0-B (0.056)	FF (0.001)	BF (0.008)	

[1]Genotyping protocols for the goat *CSN1S1* gene did not allow us to distinguish between *A* and *0* alleles so they are shown as *A/0*.

Table 4. Least squares means and standard errors of milk composition traits in Murciano-Granadina goats (n = 89; 316 records) with different α_{S1}-casein (*CSN1S1*) and κ-casein (*CSN3*) genotypes

	Trait							
Genotype	Daily milk yield, kg	Fat, %	Protein, %	Casein, %	CSN1S1, g/L	CSN1S2,[1] g/L	Lactose, %	SNF, %
CSN3								
AA	2.08 ± 0.25	4.83 ± 0.29	3.47 ± 0.08[a]	2.74 ± 0.11[a]	7.51 ± 0.58	3.04 ± 0.13	4.47 ± 0.06	8.72 ± 0.18
AB	1.97 ± 0.20	5.09 ± 0.23	3.70 ± 0.05[b]	2.95 ± 0.09[b]	7.46 ± 0.46	3.25 ± 0.11	4.49 ± 0.05	8.96 ± 0.14
BB	2.17 ± 0.22	5.18 ± 0.26	3.70 ± 0.06[b]	2.94 ± 0.10[b]	7.56 ± 0.51	3.24 ± 0.12	4.50 ± 0.05	8.97 ± 0.16
CSN1S1								
BB	2.01 ± 0.21	5.07 ± 0.25	3.57 ± 0.13	2.82 ± 0.10	8.61 ± 0.49[a,2]	3.19 ± 0.11	4.53 ± 0.05	8.93 ± 0.15
EE	2.14 ± 0.21	4.99 ± 0.25	3.67 ± 0.13	2.94 ± 0.10	6.41 ± 0.49[b,2]	3.16 ± 0.12	4.45 ± 0.05	8.84 ± 0.15

[a,b]Different letters indicate significant differences between means for $P < 0.05$ with Bonferroni's correction.

[1]CSN1S2 = α_{S2}-casein.

[2]These results were obtained from a subset of goat data (n = 89) previously used in an experiment (n = 138) published by Caravaca et al. (2008).

other milk traits were not explored. The Murciano-Granadina population employed in this previous experiment (n = 138) was similar to that used in the current work (data from 89 of these 138 goats have been analyzed again). No significant associations between *CSN1S1* genotype and total protein, fat, and casein contents have been found (Table 4). This is an unexpected result because Mahé et al. (1993) and Manfredi et al. (1995) reported associations between *CSN1S1* genotypes and these 3 traits in French breeds. However, results obtained in French breeds were not completely conclusive and notable differences among goat breeds and individuals can be found. For instance, in Saanen goats, AF individuals had a lower milk protein and fat contents than FF individuals, whereas in the Alpine breed, the opposite was true (Manfredi et al., 1995). Similarly, a within-family association analysis encompassing 5 sires and their offspring showed significant differences for protein and fat contents between *A* and *F* alleles just in 1 of 2 families sired by AF bucks (Mahé et al., 1993). These inconsistencies reflect the complex interactions between the *CSN1S1* genotype and other loci and environmental factors that modulate the amounts of fat and proteins in milk. It is reasonable to conclude that mutations in the *E* allele (i.e., the long interspersed nucleotide element insertion at exon 19) and the *F* allele (polymorphisms leading to skipping of exons 9 to 11 during mRNA processing) of *CSN1S1* result in a dramatic decrease of the rate of CSN1S1 synthesis. At the same time, other components of milk such as total protein, casein, and fat concentrations may be more or less affected by *CSN1S1* polymorphisms, depending on the population under consideration.

Finally, we investigated whether there was any interaction between the *CSN1S1* and *CSN3* genotypes. This issue was raised by the observations of Chanat et al. (1999), who demonstrated that caseins tended to be retained in the endoplasmic reticulum of mammary epithelial cells from *CSN1S1*-00 goats. This suggested that the interaction of CSN1S1 with caseins forming the micelle was necessary for the efficient transportation of these lactoproteins from the endoplasmic reticulum to the Golgi compartment. The current analysis did not show evidence of the existence of a significant interaction between the *CSN1S1* and *CSN3* genotypes, suggesting that there may be other undefined biochemical mechanisms that can compensate for the deficient casein transportation associated with alleles that result in milk with low CSN1S1 content.

In summary, the caprine *CSN3* alleles *A* and *B* had differential effects on milk casein and protein contents. These 2 milk components, together with fat content, determine the yield of cheese as well as its organoleptic properties. Therefore, genotyping the *CSN3* locus should be considered when performing marker-assisted selection in dairy goats. Moreover, the current work confirmed that in the Murciano-Granadina breed, the *CSN1S1* genotype affects the CSN1S1 synthesis rate but not milk protein or casein concentrations. Further studies are needed to replicate and validate these results in other goat breeds and to investigate the effect of *CSN1S1* and *CSN3* polymorphisms on the rheological properties of milk.

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