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Effect of κ-Casein Polymorphism on Milk Composition in the Orobica Goat

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ABSTRACT

The aim of this work was to study the effects of isoelectrofocusing (IEF) milk protein variants on milk composition in the Italian Orobica goat breed, which is characterized by a rather high frequency of the κ-casein (*CSN3*) B^{IEF} allele. Significant associations were found between the IEF phenotype and protein and casein percentages. A favorable effect of the *CSN3* B^{IEF} variant was found for both protein and casein percentages, with a codominance trend for the 3 phenotypes: BB > AB > AA. Depending on the selection purpose, emphasis could be given to different κ-casein variants in breeding. The high frequency of B^{IEF} could be exploited in breeding strategies to improve the protein and casein percentages when cheese making is a selection objective.
Key words: goat, κ-casein, genetic polymorphism, milk composition

INTRODUCTION

The analysis of CN variation in the domesticated goat (*Capra hircus*) is quite complex because a large number of mutations involve the 4 coding genes (Rando et al., 2000; Caroli et al., 2006), which are tightly linked in the CN cluster (Ferretti et al., 1990; Threadgill and Womack, 1990; Rijnkels, 2002). The 3 calcium-sensitive CN, α_{s1}-CN, β-CN, and α_{s2}-CN, are coded by the *CSN1S1*, *CSN2*, and *CSN1S2* genes, respectively, whereas κ-CN is coded by the *CSN3* gene.

Deep relationships between the large genetic variation and the functional and biological properties affecting milk quality, composition, and technological characteristics have been found mainly in goat *CSN1S1* (Martin, 1993; Grosclaude et al., 1994; Clark and Sherbon, 2000a,b; Serradilla, 2003), which is characterized by high quantitative and qualitative variation. In addition, the *CSN2* and *CSN1S2* genes of the CN cluster have been associated with differences in the level expression of the specific protein, as summarized by Caroli et al. (2006).

For goat *CSN3*, 2 variants were described by Di Luccia et al. (1990) and successively confirmed both at the protein and DNA level (Caroli et al., 2001). Recently, the number of goat *CSN3* variants has increased dramatically. To date, 16 variants have been identified, involving a total of 15 polymorphic sites in *CSN3* exon 4 (Yahyaoui et al., 2001; Angiolillo et al., 2002; Yahyaoui et al., 2003; Jann et al., 2004; Prinzenberg et al., 2005). Of the 16 variants, 13 are protein variants and 3 are silent mutations and thus detectable only at the DNA level (Prinzenberg et al., 2005).

By isoelectric focusing (**IEF**) of milk samples, all *CSN3* variants found in the domesticated goat so far cluster into 2 groups on the basis of the isoelectric point (**IP**): *A*, *B*, *B′*, *B″*, *C*, *C′*, *F*, *G*, *H*, *I*, *J*, *L* (IP = 5.29) and *D*, *E*, *K*, *M* (IP = 5.66). In fact, only 2 IEF patterns are visible, corresponding to these 2 IP groups. The nomenclature of the protein level typing can be thus classified in 2 patterns corresponding to the IP groups: A^{IEF} (IP = 5.29) and B^{IEF} (IP = 5.66) (Prinzenberg et al., 2005).

An interesting difference between the 2 κ-CN IEF variants was suggested by Chianese et al. (2000); namely, that B^{IEF} seems to be associated with a higher milk CN percentage than A^{IEF}. Therefore, the objective of the current study was to analyze the effects of these IEF variants on milk composition in the Italian Orobica goat breed, which is characterized by a rather high frequency of the B^{IEF} allele as well as by low variation in the other CN genes (Caroli et al., 2006). Therefore, it was possible to further investigate the relationship between *CSN3* polymorphism and milk composition by focusing attention on the *CSN3* variation.

MATERIALS AND METHODS

Goat Breed

The Orobica or Valgerola goat is a local population reared in alpine valleys of the Lombardy region in

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Northern Italy. Unlike other goat populations reared in the Lombardy Alps, the Orobica is present mainly in its original geographic area, consisting of 3 valleys characterized by a similar breeding and production system (Associazionerare, 2006). The coat is long-haired and extremely variable both in color (ranging from light gray to brown) and pigment distribution (uniform or differently spotted). Horns are very long in both sexes. The mean weight is 80 kg for the males and 65 kg for the females. Milk production is 205 L for the primiparous goat at 150 d, and 338 L for the pluriparous goat at 210 d (Ministero delle Politiche Agricole, 2006).

A stable consistency of approximately 4,000 head was reported recently (Associazionerare, 2006). The number of animals registered in the herd book was 2,643 in 2005 (Asso.Na.Pa, 2006). In the same year, 973 goats were under official milk recording, with an average milk yield of 291 L, 3.08% fat, and 2.91% protein (AIA, 2006).

Some typical cheeses have been linked to the breed, namely, the Bitto "Valli del Bitto" cheese, made from cow's milk and 15 to 20% Orobica goat milk, and the Maschèrpa de l'aalp, which is produced by adding Orobica goat milk to the whey obtained from Bitto "Valli del Bitto" cheese making (Associazione R.A.R.E., 2006).

Milk Protein Typing

A total of 767 individual milk samples were collected from Orobica goats reared in 54 flocks of Lombardy and enrolled in the recording scheme of the Regional Association of Breeders (**ARAL**) milk quality program. Milk samples were typed by IEF (Caroli et al., 2001).

Association Analyses

For each goat, data were provided by ARAL on the test-day protein, CN, fat, and lactose percentages, evaluated by an automatic infrared apparatus (MilkoScan; Foss Italia, Padova, Italy). The test-day analysis was carried out in the same season (spring). The following linear model was fitted to 520 observations by the GLM procedure in SAS software (SAS Institute, Inc., Cary, NC):

$$y_{ikl} = m + flock_i + \kappa\text{-}CN_j + lactation_k + b \times DIM_{ikl} + e_{ikl}$$

where y_{ikl} is the lth observation of the dependent variable (percentages of protein, fat, CN, and lactose); m is the overall mean; $flock_i$ is the ith flock (54 levels); κ-CN_j is the jth κ-CN IEF phenotype (3 levels: AA, AB, BB); $lactation_k$ is the kth lactation number (5 levels); b is the linear regression coefficient of DIM (DIM_{ikl}) on the dependent variable; and e_{ikl} is the residual error.

Only the *CSN1S1* 0 level and *CSN1S2* AA phenotypes were considered in the statistical analysis because of the rather low frequency of the other phenotypes in the breed, as well as to concentrate attention on the effects of *CSN3*. Moreover, a similar model was fitted on the same data subset, considering the effect of κ-CN as a covariate and estimating the regression effect of the number of B^{IEF} variants in the phenotype on the dependent variables, coded as follows: κ-CN AA = 0 B^{IEF}; κ-CN AB = 1 B^{IEF}; κ-CN BB = 2 B^{IEF}.

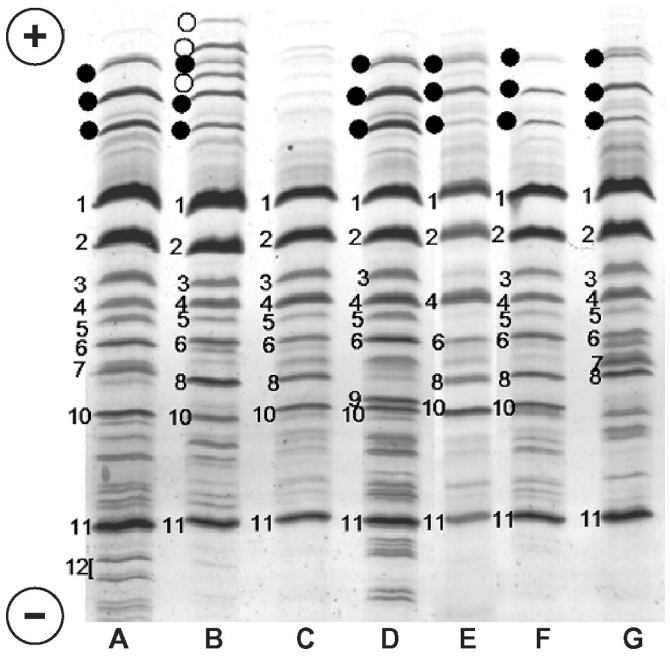

Figure 1. Isoelectrofocusing (IEF) patterns of 7 milk samples (from A to G). Samples belonging to a Maltese goat breed were used as a reference sample source for goat CN typing because of its high phenotype variability. Black dots: *CSN1S1*A* bands; white dots: *CSN1S1*B* bands. A number in the ascending order of isoelectric points (IP) indicates the main bands of the other milk proteins. β-Casein bands: 1, 2, 3, 5; $CSN3*A^{IEF}$ = 4; α-LA = 6; $CSN3*B^{IEF}$ = 7; *CSN1S2*C* = 8; *CSN1S2*E* = 9; *CSN1S2*A* + *F* = 10; β-LG = 11; *CSN1S2*B* = 12 (2 bands). The samples were classified for *CSN1S1* level as follows: samples A, B, D = level 2; samples E, F, G = level 1; sample C = level 0. Bands 3 and 5 are missing in sample E, which is heterozygous for the $CSN2*0_1$ allele.

RESULTS AND DISCUSSION

Milk Protein Typing

Figure 1 shows different IEF patterns obtained from milk of the Maltese goat breed, which was used as the reference sample source for goat CN typing because of its high phenotype variability. The main milk protein bands are indicated. α_{s1}-Casein bands are positioned in the more anodic area of the gel, and are indicated in the figure by black and white dots. The other bands on the gel are indicated by a number in ascending order

Table 1. Frequencies for the isoelectrofocusing (IEF) phenotypes at *CSN1S1*, *CSN1S2*, and *CSN3* in the sample (n = 767)

Locus	IEF phenotype	Frequency (n)	%
CSN1S1	0	656	85.5
	1	111	14.5
CSN1S2[1]	AA	638	83.2
	AB	84	10.9
	AC	29	3.8
	AE	1	0.1
	BB	11	1.4
	BC	3	0.4
	CC	1	0.1
CSN3	AA	393	51.2
	AB	289	37.7
	BB	85	11.1

[1]*CSN1S2* A = *CSN1S2*A* + *CSN1S2*F*.

of IP. The 2 IEF κ-CN variants are respectively identified by the numbers 4 (A) and 7 (B).

Isoelectrofocusing is a rapid and inexpensive milk protein typing tool. In addition to *CSN3* A and B, it allowed us to discriminate *CSN1S1* expression, which can be classified in 3 levels: 0 (null and *F* alleles); 2 (genotypes homozygous or heterozygous for the strong alleles: *A*, *B*, *C*, *H*); and 1 (other genotypes, resulting in an intermediate *CSN1S1* expression). For further genotype details, DNA typing is necessary, as described by Caroli et al. (2006).

For α_{s2}-CN, IEF allowed us to identify 4 patterns, in ascending order of IP: C (number 8), E (9), *A (10), and B (12). Two *CSN1S2* variants, *A* and *F*, comigrate at the A pattern level, whereas the C, E, and B patterns correspond to the *CSN1S2*C*, *E*, and *B* variants, respectively.

The results of IEF screening are given in Table 1. For *CSN1S1*, only levels 0 and 1 were found in the Orobica. A rather low frequency of *CSN1S1* phenotypes carrying strong and intermediate alleles (level 1) was found (14.47%). The *CSN1S2*E* variant occurred in only one sample in the heterozygous condition. The predominant *CSN1S2* pattern was the A (alleles *A* + *F*). The homozygous goats for the A variant made up more than 83%.

Association Analyses

Means and standard deviations of the dependent variables in the samples considered for the association are shown in Table 2. Significant associations were found between the IEF phenotype and protein and CN percentages. Least squares means and standard errors of protein and CN percentages for the IEF phenotypes at *CSN3* are shown in Table 3. A favorable effect of the *CSN3* B^{IEF} variant was found both for the protein and CN percentages ($P < 0.0012$ and $P < 0.0026$, respectively), with a codominance trend for the 3 phenotypes of BB > AB > AA, as confirmed by the regression analysis. In fact, the number of *CSN3* B^{IEF} variants in the phenotype was found to be associated with a highly significant effect on protein ($P < 0.0004$) and CN ($P < 0.0006$) percentages, with an increase of +0.06% (SE = 0.017) protein and +0.05% (SE = 0.014) CN for each B^{IEF} variant added in the phenotype. The B variant additive effect accounted for more than 15% of the phenotypic standard deviation of both traits.

Table 2. Mean and standard deviation of the dependent variables in the samples considered for the association analysis (n = 520)

Variable	Mean	SD
Fat percentage	3.07	0.90
Protein percentage	2.84	0.39
Lactose percentage	4.29	0.46
CN percentage	1.99	0.33

The findings of Chianese et al. (2000) were confirmed by the present work. It should be noted that Chianese et al. (2000) suggested this effect in a Southern Italian breed reared in Sicily, the Girgentana. The occurrence of the same results in another breed from Northern Italy hints of a pleiotropic effect of the gene coding for the *CSN3* B^{IEF} variant on milk protein and CN expression.

For the allele coding for the *CSN3* B^{IEF} variant in the Orobica, Caroli and coauthors (2006) found only *CSN3*D* (frequency = 0.348) by analyzing 66 DNA samples, whereas they found 3 alleles coding for the *CSN3* A^{IEF} variant: *A* (0.083), *B* (0.553), and *C* (0.015). The frequencies of the *CSN3* IEF variants in the present work were 0.663 (A^{IEF}) and 0.337 (B^{IEF}), very close to 0.651 (*CSN3*A* + *B* + *C*) and 0.348 (*D*), respectively. It is most probable that in the 520 milk samples analyzed in the present work, *CSN3*D* is also the unique or, in all cases, the predominant allele coding for *CSN3* B^{IEF}. As summarized in Table 4, the *CSN3*D* protein variant differed from *CSN3* A^{IEF} coded by the most common *CSN3*B* allele in the Orobica for 3 AA substitutions $Gln_{44} \rightarrow Arg_{44}$, $Val_{65} \rightarrow Ile_{65}$, and $Ser_{159} \rightarrow Pro_{159}$. A further AA exchange ($Val_{119} \rightarrow Ile_{119}$) occurred between *CSN3*A*

Table 3. Least squares means ± standard errors of protein and CN percentages for the isoelectrofocusing (IEF) phenotypes at *CSN3* in the data subset used for the association analysis (n = 520)[1]

Locus	IEF phenotype	n	Protein percentage	CN percentage
CSN3	AA	244	2.83 ± 0.02[a]	1.98 ± 0.02[a]
	AB	201	2.91 ± 0.02[b]	2.04 ± 0.02[b]
	BB	75	2.94 ± 0.03[b]	2.07 ± 0.03[b]

[a,b]Means with different superscript letters differ ($P < 0.01$).

Table 4. Amino acid differences among the 16 *CSN3* alleles grouped on the basis of isoelectric point (IP) in 2 isoelectrofocusing (IEF) phenotypes: A^{IEF} (IP = 5.29) and B^{IEF} (IP = 5.66)[1]

CSN3 allele	IEF pattern	AA position (mature protein)								
		44	53	61	65	90	119	145	156	159
		Para-κ-CN					CN-macropeptide			
A	A^{IEF}	Gln	Asn	Tyr	Val	Asp	Val	Val	Ala	Ser
B, *B′*, *B″*	A^{IEF}						Ile			
C, *C′*	A^{IEF}				Ile		Ile		Val	Pro
F	A^{IEF}						Ile			Pro
G	A^{IEF}				Ile		Ile			Pro
H	A^{IEF}		Ser				Ile			
I	A^{IEF}				Ile		Ile			
J	A^{IEF}			Cys			Ile			
L	A^{IEF}				Ile		Ile			Pro
D	B^{IEF}	**Arg**			Ile		Ile			Pro
E	B^{IEF}					**Gly**	Ile			
K	B^{IEF}	**Arg**					Ile			
M	B^{IEF}					**Asn**	Ile	Ala		Pro

[1]See Prinzenberg et al. (2005) for the allele nomenclature, references, and nucleotide differences. Amino acid exchanges modifying IP are bolded.

and *CSN3*D*. The substitution modifying the IP of the *CSN3*D* variant, compared with *CSN3*A* and *CSN3*B*, is $Gln_{44} \rightarrow Arg_{44}$.

Explanations for the favorable effect of the *CSN3* B^{IEF} variant on milk protein and CN expression could be linked to these amino acid differences in the mature protein, possibly affecting both the biological properties of κ-CN (Meisel, 2005) and its biochemical interactions with the other CN fractions in the CN micelle (Lucey et al., 2003). In addition, the genetic variation of κ-CN involving the mature protein could be associated with other polymorphisms in the noncoding sequences (promoter, introns), which might be causative mutations for the expression differences between some alleles, as has already been found for the 2 main bovine *CSN3* alleles (reviewed by Martin et al., 2002). In all cases, quantitative differences at the κ-CN level must be closely considered because of the essential role of this CN in the process of reproduction in mammals; as recently demonstrated in mice, when a null mutation was introduced in κ-CN, it resulted in destabilization of the CN micelles and lactation failure (Shekar et al., 2006).

CONCLUSIONS

The high frequency of B^{IEF} could be exploited in breeding strategies to improve the protein and CN percentages, if cheese making is a selection objective. Simultaneously, the maintenance of strong or intermediate alleles of *CSN1S* (which are rare in the Orobica) should be encouraged. Otherwise, milk nutritional quality could be an interesting breeding objective to enhance the economic value of the Orobica goat. In this case, the selection of CN genotypes or haplotypes should involve consideration of new aspects (i.e., hypoallergenic milk, bioactive peptides), and specific breeding strategies should be applied.

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