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) BIEF allele. Significant associations were found between the IEF phenotype and protein and casein percentages. A favorable effect of the CSN3 BIEF 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 BIEF 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, αs1-CN, β-CN, and αs2-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 Lucia 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: AIEF (IP = 5.29) and BIEF (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 BIEF seems to be associated with a higher milk CN percentage than AIEF. 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 BIEF 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
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 Mascherpa 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):

\[ y_{ikl} = m + \text{flock}_i + \kappa-CN_j + \text{lactation}_k + b \times \text{DIM}_{ikl} + e_{ikl} \]

where \( y_{ikl} \) is the \( l \)th observation of the dependent variable (percentages of protein, fat, CN, and lactose); \( m \) is the overall mean; \( \text{flock}_i \) is the \( i \)th flock (54 levels); \( \kappa-CN_j \) is the \( j \)th \( \kappa-CN \) IEF phenotype (3 levels: AA, AB, BB); \( \text{lactation}_k \) is the \( k \)th 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 \( \kappa-CN \) 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: \( \kappa-CN \) AA = 0 B\( _{\text{IEF}} \); \( \kappa-CN \) AB = 1 B\( _{\text{IEF}} \); \( \kappa-CN \) BB = 2 B\( _{\text{IEF}} \).

**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. \( \alpha_s1-\text{Casein} \) 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.
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, cosegregate at the A pattern level, whereas the C, E, and B patterns correspond to the CSN1S2*E, C, 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 heterogeneous 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 analysis 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.

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 Gln44→Arg44, Val65→Ile65, and Ser159→Pro159. A further AA exchange (Val119→Ile119) occurred between CSN3*A

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

<table>
<thead>
<tr>
<th>Locus</th>
<th>IEF phenotype</th>
<th>Frequency (n)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSN1S1</td>
<td>0</td>
<td>656</td>
<td>85.5</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>111</td>
<td>14.5</td>
</tr>
<tr>
<td>CSN1S2</td>
<td>AA</td>
<td>635</td>
<td>83.2</td>
</tr>
<tr>
<td></td>
<td>AB</td>
<td>84</td>
<td>10.9</td>
</tr>
<tr>
<td></td>
<td>AC</td>
<td>29</td>
<td>3.8</td>
</tr>
<tr>
<td></td>
<td>AE</td>
<td>1</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>BB</td>
<td>11</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>BC</td>
<td>3</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>1</td>
<td>0.1</td>
</tr>
<tr>
<td>CSN3</td>
<td>AA</td>
<td>393</td>
<td>51.2</td>
</tr>
<tr>
<td></td>
<td>AB</td>
<td>289</td>
<td>37.7</td>
</tr>
<tr>
<td></td>
<td>BB</td>
<td>85</td>
<td>11.1</td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat percentage</td>
<td>3.07</td>
<td>0.90</td>
</tr>
<tr>
<td>Protein percentage</td>
<td>2.84</td>
<td>0.39</td>
</tr>
<tr>
<td>Lactose percentage</td>
<td>4.29</td>
<td>0.46</td>
</tr>
<tr>
<td>CN percentage</td>
<td>1.99</td>
<td>0.35</td>
</tr>
</tbody>
</table>

### 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)

<table>
<thead>
<tr>
<th>Locus</th>
<th>IEF phenotype</th>
<th>Protein percentage</th>
<th>CN percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSN3</td>
<td>AA</td>
<td>244</td>
<td>2.83 ± 0.02 $^a$</td>
</tr>
<tr>
<td></td>
<td>AB</td>
<td>201</td>
<td>2.91 ± 0.02 $^b$</td>
</tr>
<tr>
<td></td>
<td>BB</td>
<td>75</td>
<td>2.94 ± 0.03 $^b$</td>
</tr>
</tbody>
</table>

$^a,b$Means with different superscript letters differ ($P < 0.01$).
and CSN3*D. The substitution modifying the IP of the CSN3*D variant, compared with CSN3*A and CSN3*B, is Gln44→Arg44.

Explanations for the favorable effect of the CSN3 BIEF 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 BIEF 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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