SHORT COMMUNICATION

Genetic variability at αs_2-*casein* gene in *Girgentana* dairy goat breed

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Abstract

Casein genes are highly polymorphic and the high degree of variability has qualitative and quantitative effects on milk composition thereby affecting chemical, physical and technological properties of goat milk. The aim of this work was to evaluate the genetic polymorphisms of the *as*$_2$*-casein* (*CSN1S2*) gene in the endangered *Girgentana* dairy goat breed in order to assess the genotypes distribution, as it is known genotype influences technological and nutritional milk properties. The study was performed on 207 sample of *Girgentana* goat breed, analysed with different PCR protocols. The most frequent alleles was A (0.722), followed by F (0.225), C (0.051) and E (0.002) while B, D and 0 alleles were not found. Genotypes detected were AA (0.512), AF (0.338), AC (0.082), FF (0.043), CF (0.020) and EF (0.005). Our results suggested that *Girgentana* goat breed could be used for the production of milk with high fat and protein content and with optimal technological ability, suitable for cheese making.

Introduction

In the milk of ruminants more than 95% of proteins are synthesised by six structural genes, four caseins (αs_1-, β-, αs_2- and κ-caseins) and two whey proteins (a-lactalbumin and b-lactoglobulin). Caseins are the main protein component of milk. In caprine species the entire casein gene cluster region spans about 250 kb on chromosome 6 (Hayes *et al*., 1993). Casein genes are highly polymorphic and the high degree of variability, together with post-translational modifications and differential splicing patterns, has qualitative and quantitative effects on milk composition thereby affecting chemical, physical and technological properties of goat milk (Rando *et al*., 2000; Martin *et al*., 2002; Marletta *et al*., 2007).

So far, eight alleles have been identified, which are associated with different expression levels of *as*$_2$*-casein* (*CSN1S2*) in milk: A, B (Boulanger *et al*., 1984), C (Bouniol *et al*., 1994), E (Lagonigro *et al*., 2001) and F (Ramunno *et al*., 2001a) are *strong* alleles associated with a normal content (about 2.5 g/L per allele) of this protein in milk. Intermediate *CSN1S2* D allele is associated with a reduced level (about 1.5 g/L per allele), while null *CSN1S2* 0 allele is associated with non-detectable amount of *CSN1S2* in milk (Ramunno *et al.,* 2001a, 2001b). Moreover, Erhardt *et al.* (2002) reported the G allele associated with a normal content of *as*$_2$*-casein* typed at protein level by isoelectric focusing, but not characterized at molecular level.

The *Girgentana* goat is an ancient Sicilian goat breed reared in Southern Italy for its good dairy production. Average milk production was 224±66 L in the first lactation, and 320±109 L for later lactations (AIA, 2011). Due to sanitary policies the size of the *Girgentana* goat breed decreased of almost 90% in 20 years. In 1983, the population consisted of 30,000 individuals but, nowadays, only 651 heads are reared in Sicily (ASSONAPA, 2012). Over the last years this breed has become almost extinct, in part as a consequence of the marked decrease in fresh goat milk consumption.

The aim of this work was to evaluate the genetic polymorphisms of the *CSN1S2* gene in the endangered *Girgentana* dairy goat breed in order to assess the genotypes distribution, as it is known genotype influences technological and nutritional milk properties.

Materials and methods

A total of 207 samples of *Girgentana* goat breed, all females enrolled in the herd book were randomly collected in 10 flocks located in different areas of Sicily. The number of animals sampled per flock ranged from 15 to 25 individuals.

From each animal about 10 mL of blood were collected from the jugular vein, using vacuum tubes containing EDTA as anticoagulant. Genomic DNA was extracted from buffy coats of nucleated cells using a salting out method (Miller *et al.,* 1988). After checking the quantity and quality of the DNA using NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA), samples were diluted to a final concentration of 50 ng/µL in ultrapure water and stored at 4°C until use.

The *CSN1S2* B and C alleles were characterized by Allele Specific-PCR (Vacca *et al.,* 2009b). Since primer pair used in Allele Specific-PCR did not discriminate C and E alleles, C allele was assigned after *CSN1S2* E allele identification which was obtained using primer pair by Chessa *et al.* (2008) and restriction enzyme by Lagonigro *et al.* (2001). The D, 0, and F alleles were detected using PCR-restriction fragment length polymorphism protocol by Ramunno *et al.* (2001a). *CSN1S2* A allele was assigned by exclusion after genotyping for all other alleles has been carried out and presence or absence of heterozygous conditions was detected. Primers sequences and annealing temperature are shown in Table 1.

All PCR and digestion products were analysed by electrophoresis on agarose gel stained with ethidium bromide.

The obtained data were used to calculate genotype and allele frequencies and Hardy-Weinberg equilibrium probability test (with default parameters) using GENEPOP version 4.0.11 (Rousset, 2008). Expected (He) and Observed (Ho) heterozygosity were calculated using GENETIX software package version 4.05 (Belkhir *et al.,* 1996).

Results and discussion

The genotype and allele frequencies at *CSN1S2* locus are reported in Table 2. The most frequent allele was A (0.722), followed by F (0.225), C (0.051) and E (0.002). Alleles B, D

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Table 1. Primers sequences, annealing temperature and reference.

Name	Direction	Sequence	Ta, °C	Reference
B1Z	Forward	5'-CTATCAGATCATCTAGTGAG-3'	53	Vacca *et al.* (2009a, 2009b)
B1Y	Reverse	5'-CTCTGGGGCAACTTT-3'	53	Vacca *et al.* (2009a, 2009b)
B1X	Reverse	5'-CTCTGGGGCAACTTC-3'	53	Vacca *et al.* (2009a, 2009b)
C2Z	Forward	5'-CTGAAGAAGAAAAGAATCGCC-3'	53	Vacca *et al.* (2009a, 2009b)
C2X	Reverse	5'-CTGGTAATACTGGCTGATTT-3'	53	Vacca *et al.* (2009a, 2009b)
C2Y	Reverse	5'-CTGGTAATACTGGCTGATTA3'	53	Vacca *et al.* (2009a, 2009b)
CASDf	Forward	5'- GACACATAGAGAAGATTC-3'	51	Ramunno *et al.* (2001a)
CASDr	Reverse	5'- CGTTGGGACATTTTATCT-3'	51	Ramunno *et al.* (2001a)
C16 Fw	Forward	5'- CTGTTTGGTATCATTTAGAATTTAT-3'	56	Chessa *et al.* (2008)
E16 Rv	Reverse	5'- CTCTTTTTATTACAAAAGACAACTT-3'	56	Chessa *et al.* (2008)
CASFf	Forward	5'- TCTCTTGCCATCAAAACA-3'	54	Ramunno *et al.* (2001a)
CASFr	Reverse	5'- TGGTCTTTATTCCTCTCT-3'	54	Ramunno *et al.* (2001a)

Ta, annealing temperature.

and 0 were not found in the analysed *Girgentana* goat individuals. Six genotypes were detected and the only alleles found in homozygous condition were A and F, whereas the others were found in heterozygous condition (Table 2). The most common genotype was AA (0.517) followed by AF (0.335) and AC (0.081).

Genotype influences the rate of *CSN1S2* in goat milk compared to the total casein content, in fact, in presence of *CSN1S2* strong or intermediate genotypes, this protein fraction represent 16% of total casein content. On the other hand, *CSN1S2* genotypes 0/not 0 are associated with a reduction of up to 9% which results in the total absence of this protein in milk with *CSN1S2* 00 genotype (Marletta *et al*., 2002).

Our results are in agreement with those reported for *Girgentana* goat breed by Marletta *et al.* (2004, 2005), who reported the absence of B, D, and 0 alleles in this breed. Alleles D and 0 were also absent in some local goat breeds reared in Italy (Sacchi *et al.*, 2005; Vacca *et al.*, 2005) and in Egyptian goat population (Othman and Ahmed, 2006). Moreover, we detected *CSN1S2* E allele that was not reported in the study of Marletta *et al.* (2004).

According to our results, in a study performed on casein loci in four Sicilian dairy goat breeds, Gigli *et al*. (2008) found that A and F were the most frequent alleles (0.547 and 0.287, respectively). In contrast with our results, they reported the presence of the B and D alleles in *Girgentana* goats breed, and of E allele in all breeds except *Girgentana* one. Moreover, allele frequencies at *CSN1S2* locus in *Girgentana* and *Argentata dell'Etna* Sicilian goat breeds were A>F>C (Marletta *et al.,* 2004) that differ from Tunisian native goats (A>C>F) as reported by Vacca *et al.* (2009a).

Girgentana goat breed was in Hardy-Weinberg equilibrium at this locus (P>0.05).

Table 2. Genotype and allele frequencies at locus in *Girgentana* goat breed.

Genotype	N.	Frequency	Allele	Frequency
AA	106	0.512	A	0.722
AC	17	0.082	C	0.051
AF	70	0.338	E	0.002
CF	4	0.020	F	0.225
EF	1	0.005		
FF	9	0.043		

N, number of individuals.

Considering the heterozygosity values obtained by Marletta *et al.* (2004), it is possible to note that our results for He value are in agreement with those reported for Girgentana goat breed (He 0.403 *vs* 0.423), and that our Ho value is higher (Ho 0.440 *vs* 0.316) than that reported by these authors. Results of our study demonstrate that our samples showed a major genetic variability in terms of number of allele (3 *vs* 4) at this locus compared with results obtained by Marletta *et al.* (2004). However, our results showed lower genetic variability of *Girgentana* goat breed compared with that reported by the same authors for *Argentata dell'Etna* goat breed (He=0.661).

Conclusions

The results of our study showed the absence of intermediate and null alleles in *Girgentana* goat breed as previously reported in other studies (Marletta *et al.,* 2004; Gigli *et al.,* 2008), therefore, these results can be considered as an upgrade of previous ones. This feature indicates that *Girgentana* goat breed could be used for the production of milk with high fat and protein content and with optimal technological ability, suitable for cheese making (Ramunno *et al.,* 2007). Moreover, considering that *CSN1S2* locus is closely linked to *CSN1S1*, *CSN2* and *CSN3* loci and alleles at these loci are inherited together as haplotype (Hayes *et al.,* 1993; Rijnkels, 2002) further studies are required to determine the relationship between alleles at *CSN1S2* locus and at the three other casein loci.

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