SHORT COMMUNICATION

Genetic variability at αs2-casein gene in Girgentana dairy goat breed

Marisa Palmeri, Salvatore Mastrangelo, Maria T. Sardina, Baldassare Portolano
Dipartimento di Scienze Agrarie e Forestali, Università di Palermo, Italy

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 αs2-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.

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 GENEPOP version 4.0.11 (Rousset, 2008). Expected (He) and Observed (Ho) heterozygosity were calculated using GENEPOP version 4.0.11 (Rousset, 2008). Expected (He) and Observed (Ho) heterozygosity were calculated using GENEPOP version 4.0.11 (Rousset, 2008). Expected (He) and Observed (Ho) heterozygosity were calculated using GENEPOP version 4.0.11 (Rousset, 2008). 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

Results and discussion

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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 CSNIS2 in goat milk compared to the total casein content, in fact, in presence of CSNIS2 strong or intermediate genotypes, this protein fraction represent 16% of total casein content. On the other hand, CSNIS2 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 CSNIS2 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 CSNIS2 E allele that was not reported in the study of Marletta et al. (2004).

According to our results, 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 goat breeds, and of E allele in all breeds except Girgentana one. Moreover, allele frequencies at CSNIS2 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. (2009).

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

| Name | Direction | Sequence | Ta, °C | Reference |
|------|-----------|----------|--------|-----------|
| BIZ  | Forward   | 5’-CTATCACGATCTAGTAC-3’ | 53     | Vacca et al. (2009b) |
| B1Y  | Reverse   | 5’-CTCTGGGCAACTTT-3’ | 53     | Vacca et al. (2009a) |
| B1X  | Reverse   | 5’-CTCTGGGCAACTTT-3’ | 53     | Vacca et al. (2009a) |
| C2Z  | Forward   | 5’-CTGGAAGAAAAGATCATC-3’ | 53     | Vacca et al. (2009b) |
| C2X  | Reverse   | 5’-CTGGAAGAAAAGATCATC-3’ | 53     | Vacca et al. (2009b) |
| C2Y  | Reverse   | 5’-CTGGAAGAAAAGATCATC-3’ | 53     | Vacca et al. (2009b) |
| CASDf | Forward | 5’-CTGGTAATCTGCTGATT-3’ | 51     | Ramunno et al. (2001a) |
| CASDr | Reverse | 5’-CTGGTAATCTGCTGATT-3’ | 51     | Ramunno et al. (2001a) |
| C16 Fw | Forward | 5’-CTGTGGTGATACATGTTAT-3’ | 56     | Chessa et al. (2008) |
| E16 Fv | Reverse | 5’-CTCTTTTAATACAAAAAGACATTT-3’ | 56     | Chessa et al. (2008) |
| CASFf | Forward | 5’-CTCTGGGCAACTTT-3’ | 53     | Ramunno et al. (2001a) |
| CASFr | Reverse | 5’-CTGTTTGGTATCATTTAGAATTTAT-3’ | 56     | Ramunno et al. (2001a) |

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 | 0.335     | 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 CSNIS2 locus is closely linked to CSNIS1, 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 CSNIS2 locus and at the three other casein loci.

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