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SHORT REPORT

Survey of single strand conformation polymorphism of kappa-casein gene in Alpine and Saanen goats in Iran

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To study the genetic polymorphism of κ-casein (*CSN3*) gene as candidate gene for marker-assisted selection in different domestic livestock species, blood samples were randomly withdrawn from 34 Alpine and 42 Saanen goats. DNA was extracted from blood samples and a 416 bp fragment from exon 4 of the CSN3 gene was amplified by polymerase chain reaction (PCR). PCR products were analysed using single strand conformation polymorphism (SSCP) method. Results indicated that there were two (K_1 and K_2) conformational patterns with frequencies 0.91 and 0.09 for Alpine and 0.76 and 0.24 for Saanen goats, respectively. A Chi-square test confirmed Hardy-Weinberg equilibrium for the gene in both breeds.

KEY WORDS: *CSN3* / goat / genetic polymorphism / SSCP

κ-casein (*CSN3*) plays an important role in the formation, stabilization and aggregation of the casein micelles thus altering the manufacturing properties and digestibility of milk [Jann 2004]. Genes coding for this protein cover an area of

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approximately 250 kb of the genomic DNA, all located on chromosome 6 [Rijnkels *et al*. 1997]. Of a goat *CSN3*, two variants were described by Di Luccia *et al*. [1990], successively confirmed both at the protein and DNA level [Caroli *et al*. 2001]. Recently, the number of goat *CSN3* variants identified has increased dramatically. To date, 16 variants have been identified, involving a total of 15 polymorphic sites in *CSN3* exon 4 [Yahyaoui *et al*. 2003, Prinzenberg *et al*. 2005]. Of the 16 variants, 13 are protein variants and 3 are silent mutations detectable only at the DNA level [Prinzenberg *et al*. 2005]. Allelic 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 identified in only few breeds at low or intermediate frequencies [Jann *et al*. 2004, Prinzenberg *et al*. 2005].

Alpine is a goat known for its high production of milk. The breed originates from French Alps. Mature weight is around 57 kg. Alpine goats can range in colour from white or gray to brown and black. Saanens derive their name from the Saanen valley in the south of Canton Berne, Switzerland. It is a white or cream-coloured breed. Saanen is the largest of the goat dairy breeds. Mature weight is around 68 kg or more, with bucks weighing over 91 kg. This breed identifies as "Queen of the Dairy Goats" because it produces approx. 3.8 litres milk a day [American Dairy Goat Association 2012]. The goats considered in this study are the first herd of Alpine and Saanen breeds that entered Iran for mating and improving native goat breeds.

In this paper, determination of the genetic polymorphism of κ-casein gene in Alpine and Saanen goats is presented using single-strand conformation polymorphism (SSCP) as a first step for more investigations upon this gene and its association with economical traits.

Material and methods

The blood samples were withdrawn randomly from 34 Alpine (4 male and 30 female) and 42 Saanen (2 male and 40 female) goats reared in a private farm, Golestan province, Iran. DNA was extracted using standard salting – out method [Miller *et al*. 1988]. The quality and quantity of extracted DNA were measured spectrophotometrically and by electrophoresis on 1% agarose gel.

Polymerase Chain Reaction (PCR) was carried out, using the Personal Cycler™ thermocycler (BIOMETRA, Germany) and the PCR Master Kit (CINNA Gen Inc., Iran). Each reaction mixture consisted of 12.5 µl of the master mix, 1 µl of the DNA solution (50 to 100 ng/µl), 1 µl of each primer (5 pmol/µl) and deionized water making up a final volume of 25 µl.

For amplifying a 416 bp fragment from the exon IV of the κ-casein gene primers described by Barracosa [1996] were used: κ-CSN- F (5'-GAGAAAGATGAAAGATTCTTCG-3') and κ-CSN- R (5'-GCTTCTGGATTATCTACAGTG-3'). An initial denaturation

step was applied at 95°C for 5 min followed by 32 cycles of denaturation at 95°C for 30 sec, annealing at 58°C for 1 min and extension at 72°C for 1 min, and a final extension of 72°C for 8 min.

K-casein gene variants were identified by PCR-SSCP method. For SSCP analysis, 5 μL of each amplification product were added to 15 μL of denaturizing solution (95% formamide, 10 mM NaOH, 0.05% xylene cyanol and 0.05% bromophenol blue). The samples were heat-denatured at 95°C for 5 min, immediately chilled on ice and loaded onto 12% polyacrylamide gel (39:1). The gels were run at 240-300 V for 10 – 14 h, at 4°C. The electrophoresis was carried out in a vertical unit in 1x TBE buffer. The gels were stained with silver nitrate to observe the conformational patterns. Allele and genotype frequencies and chi-square test to assess the goodness of fit to Hardy-Weinberg equilibrium expectations for the distribution of genotypes were calculated with Pop-Gene software (V 1.31) – Yeh *et al*. [1997].

Results and discussion

The PCR correctness was assessed by electrophoresis of each sample (4 μl) on 1.3% (w/v) agarose gel (Fig. 1). Moreover, the PCR-SSCP for *CSN3* gene was carried out on polyacrylamide gel and was found in two (K1 and K2) different conformational patterns for *CSN3* gene (Fig. 2). The frequencies of banding patterns of *CSN3* gene are presented in Table 1.

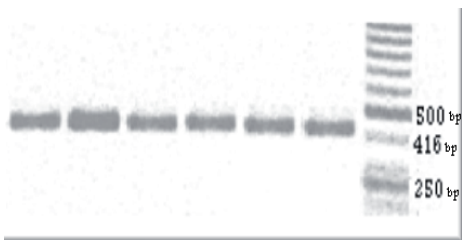

Fig. 1. Electrophoresis of a 416 bp fragment of *CSN3* gene on 1.3% agarose gel.

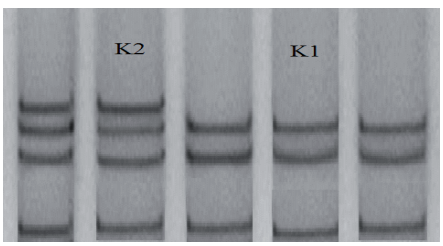

Fig. 2. SSCP analysis of the 416 bp fragment of *CSN3* gene on 12% polyacrylamide gel after silver staining.

Table 1. The frequencies of banding patterns of *CSN3* gene in Alpine and Saanen goats

Animal/Gene	Banding patterns	Alpine frequency	Saanen frequency
CSN3	K1	0.91	0.76
	K2	0.09	0.24

Table 2 shows the results of chi-square test for Hardy-Weinberg equilibrium value of *CSN3* gene in the animals studied. No significant difference between the observed and expected frequencies of *CSN3* gene. So, these results confirm the Hardy-Weinberg equilibrium in populations investigated.

Table 2. Chi-Square test for HWE in *CSN3* genotype in Alpine and Saanen goats

Breed	Genotype	Observed(O)	Expected(E)	$(O-E)^2/E$	chi^2
Alpine	K1	31	29.6	0.04	0.48
	K2	3	4.4	0.44	
Saanen	K1	32	33	0.03	0.13
	K2	10	9	0.1	

*P<0.05.

Despite the crucial role of *CSN3* in the micelles structure and clotting process, very few studies have been devoted to its effect on milk composition. In this study, two conformational patterns (K1 and K2) were identified for *CSN3* gene in both breeds. Present results at the *CSN3 locus* are in accordance with earlier studies by Yahyaoui *et al*. [2003] who detected two patterns for this gene in Alpine goats. In addition, Feligini *et al*. [2002] and Caravaca *et al*. [2009] identified two alleles of this gene in Italian goat breeds and Murciano-Granadina goats. Also, similar results were reported by Ceriotti *et al*. [2004] and Ahani Azari *et al*. [2011] in which the PCR-SSCP analysis of exon 4 of this gene showed two conformational patterns in three Italian sheep breeds and Dalagh sheep, respectively. However, Yahyaoui *et al*. [2001, 2003] identified three alleles for this gene in Saanen goat. Kumar *et al*. [2009] studied polymorphism of *CSN3* by SSCP method and revealed three conformational patterns in Indian goats. Other authors observed different *CSN3* polymorphisms in the goat [Patel *et al*. 2011, Kiplagat *et al*. 2010, Sztankóová *et al*. 2009] and sheep breeds [Mandal *et al*. 2008, Staiger *et al*. 2010, Giambra *et al*. 2010].

Further studies upon different goat populations are necessary to establish the distribution of these alleles and their effects on the quality and functional properties of milk.

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