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Kappa casein gene polymorphism in Zalawadi goats

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Kappa casein protein variants were analysed in blood samples of 50 unrelated Zalawadi goat breeds by PCR-RFLP techniques with *Hae*III restriction enzyme. Two primers (5′-TCC CAA TGT TGT ACT TTC TTA ACA TC-3′) and (5′-GCG TTG TCC TCT TTG ATG TCT CCT TAG-3′) were used as forward and reverse primers, respectively for the amplification of *CSN3* gene. The resultant PCR product observed at 645 bp was digested with restriction endonuclease *Hae*III, and the resultant fragments of 416 bp and 229 bp after separation by electrophoresis were visualized and documented by gel documentation system, which indicated the presence of Kappa casein AA genotype in population. The band sizes were compared with molecular size marker and found to be of the same size in all the 50 samples.

Keywords: *CSN3* gene, *Hae*III, PCR-RFLP, polymorphism, Zalawadi goat

The kappa casein fraction of goat milk plays a crucial role in the formation, stabilization and aggregation of the casein micelles and thus affects the technological as well as nutritional properties of milk[1-7]. In goats, the three calcium-sensitive casein genes (αs1, αs2 & β) are polymorphic and alleles are associated with strong differences in their level of expression. Calcium-sensitive casein genes seem to have evolved from a primitive casein gene through gene duplication[8]; however, it appears that kappa casein is not related structurally and evolutionary to these genes. Kappa casein is structurally related to γ-fibrinogen[9,10]. It has been demonstrated that the *kappa casein (CSN3)* gene possesses the highest degree of conservations[11]. However, high intra species variability has also been reported for *CSN3* gene in cattle[12] and goat[13]. The kappa casein protein variants in goats were established and confirmed at the protein[14,15] and DNA[15-19] level. A total of 14 DNA variants have been identified in the domestic goats[19,20] and showed that the number of alleles identified in the domesticated goat has increased to 16, of which 13 are protein variants and 3 are silent mutations, involving a total of 15 polymorphic sites in *CSN3* gene (exon 4). As *CSN3* gene plays an important role in forming casein micelle and in maintaining lactation, it is necessary to analyse genetic polymorphism at kappa casein locus in Zalawadi goats.

Based on the information provided by farmers, blood samples were collected from 50 unrelated Zalawadi goats from natural habitat belonging to Kapadvanj taluka of Khera district, Gujarat, India. DNA was extracted from cellular part (WBCs) of blood samples using the standard protocol published[21].

Surendranagar district located between 22° to 23.45° North latitudes and 69.45º to 72.15º East longitudes in the state of Gujarat is the home tract of Zalawadi goats. The goats are large in size and their coat is black with long coarse hairs. Ears are long, wide, leaf-like and drooping. Both sexes have long twisted horns, pointed upward. In female, the udder is well developed with large conical teats. The average daily milk yield recorded is 2.02±0.18 kg in 197.2±5.8 d of lactation[22]. The average daily milk yield at fortnightly interval ranged from 1.09 (first parity) to 1.42 L (fourth parity) with an average of 1.17±0.09 L in 6.57±0.31 months. The average productive life of these Zalawadi goats was reported to be 5 to 6 lactations[23].

The forward primer (5′-TCC CAA TGT TGT ACT TTC TTA ACA TC-3′) and reverse primer (5′-GCG TTG TCC TCT TTG ATG TCT CCT TAG′-3′) were used for the amplification of the *CSN3* locus exon 4 region (encoding the mature kappa casein protein)[17]. The PCR reaction was performed in a 25 µL final volume containing 12.5 µL of 2× PCR Master Mix, 7.5 µL of deionised water, 1.0 µL of forward primer, 1.0 µL of reverse primer and 3.0 µL of goat genomic DNA. Thermal cycling

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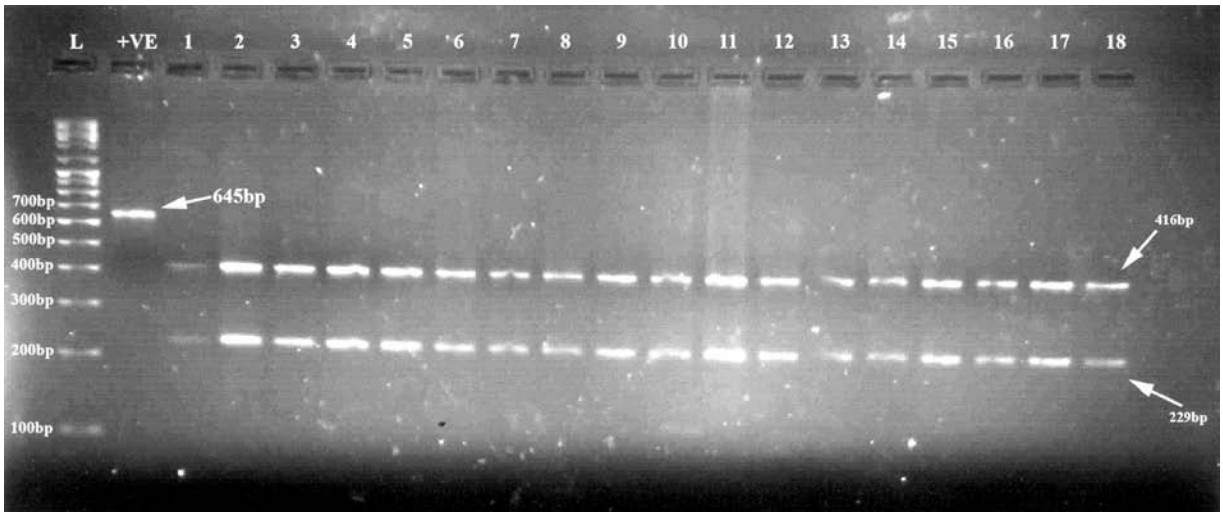

Fig. 1—Agarose gel showing restriction fragmentation pattern of *kappa casein* (*CSN3*) gene by using enzyme *Hae*III on PCR products (645 bp) [L: Molecular size marker (100 bp DNA step ladder); +VE: PCR product of *CSN3* gene (645 bp); & Lanes 1 to 18: Samples showing AA genotype with 416 and 229 bp restriction fragments]

condition was: 95°C for 5 min, 30 cycles of 95°C for 45 sec, 56°C for 45 sec and 72°C for 1 min, with a final extension at 72°C for 10 min. About 10 µL of the PCR product was digested with 5 units of the restriction endonucleases *Hae*III (Fermentas, Hanover, MD) overnight at 37°C. The resultant fragments were separated by electrophoresis in a 2% agarose gel stained with ethidium bromide. The bands were visualized under UV light and documented by gel documentation system (SynGene Genius Bioimaging System, UK). The band sizes judged by Gene tool were compared with molecular size marker and recorded.

PCR amplification of exon 4 of *CSN3* locus was carried out in 50 Zalawadi goat breed. PCR amplified product was observed as 645 bp (Fig. 1). The band sizes were judged by Gene tool and comparing with molecular size marker and recorded to be of the same size in all the 50 samples. The restriction analysis by *Hae*III enzyme produced two fragments of 416 bp and 229 bp (Fig. 1), indicating the presence of *kappa casein* AA genotype in the population. The polymorphic site consists of a single nucleotide substitution A to G at position 242 of the exon 4 and produces an amino acid substitution Asp/Gly[18]. Identical *Hae*III RFLP patterns, was observed in Indian goats like Barbari, Marwari, Beetal, Surti and local goats of Madhya Pradesh state showing absence of polymorphism in *CSN3* gene[24]. In Montefalcone goats, polymorphic *Hae*III patterns for SNP242 were observed. Allele 242A produced two fragments (416 bp and 229 bp), while alleles 242G generated three fragments (366, 30 & 229 bp) on *Hae*III RFLP digestion of *CSN3* exon 4[25]. In Egyptian breeds, an additional variant E was observed over and above variant A, and allele E displayed restriction pattern consisting of fragments 366, 229 and 50 bp[26].

The casein monomorphism observed in our study at locus exon 4 must be considered along with the known variants of other casein loci, as reported by previous workers, to make the information on casein haplotypes valuable for inclusion in the genetic improvement programmes of Indian goat breeds.

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