

Polymorphism at Kappa-casein gene in Barbari goats studied by PCR-SSCP

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Barbari the dual-purpose breed, is found in several districts of Uttar Pradesh like Etah, Aligarh, Agra, Mathura, and in some adjoining parts of Rajasthan. This breed is known for its meat, milk and prolificacy. In any livestock breeding, the primary focus of selection has been to improve milk yield and meat production and usually, it is achieved using classical quantitative genetics. Several potential candidate genes were recognized that affect polygenic traits such as milk and meat production. The caseins (α₁, α₂, β, and κ) comprise the major protein component of ruminant milk and are secreted in the form of stable calcium phosphate micelles. The assembly of caseins into micelles allows maintaining low viscosity of the milk despite the high protein concentration. The casein micelles are of functional importance for protein and mineral nutrition of the offspring, and for determining the physical properties of milk. It is widely accepted that kappa-casein gene plays a crucial role in stabilizing the micelles by coating a structurally different protein layer. Kappa casein is widely polymorphic in bovine species with 9 genetic variants characterized to date. The variants were found with variable frequency in all analyzed populations including zebu (*Bos indicus*) and yak (*Bos grunniens*) (Ng-Kwai-Hang and Grosclaude 1992). The impact of bovine κ-casein variants A and B on milk production traits were extensively studied and most results agree in indicating that milk from cow's genotyped κ-casein BB contains higher proportions of fat, proteins, and caseins than milk derived from κ-casein AA cows (revised in Ng-Kwai Hang 1998). Alternatively, a PCR-SSCP procedure has also been described (Barroso *et al.* 1998, Prinzenberg *et al.* 1999).

Single-strand conformation polymorphism (SSCP) analysis was used to detect mutations (Orita *et al.* 1989). This technique is based on the principle that changes in the nucleotide sequence of a PCR product affect its single-strand conformation. Molecules differed by a single base have different conformers under non-denaturing conditions and movement is also different.

The aim of this study is the evaluation of genetic variability of Kappa-casein gene for sequence variation using PCR-SSCP protocol. The resulting information may be utilized for association of these variants with milk and meat production traits in these goats.

Sample collection and DNA extraction: Purebred *Barbari* goats from the flock of the Institute (Makhdoom) were used. The blood samples (8–10 ml each) were collected from 100 animals of *Barbari* flock. The blood samples were collected in 10 ml vacutainer tubes and stored in deep freezer at –20°C for further analysis. Genomic DNA was isolated using standard procedure of proteinase-K digestion (Sambrook *et al.* 1989). After checking the quality and quantity of DNA, it was diluted to a final concentration of 50 ng/ml in highly purified water, and was stored at 4°C.

PCR amplification: Primers were designed as per the gene bank accession number AY027868. Kappa-casein gene amplified by PCR using the 2 primer pairs is shown in Table 1. Polymerase chain reaction (PCR) was carried out using about 50–100 ng genomic DNA in a 25 ml reaction volume on advanced primus 96 PCR machine. The reaction mixture consisted of 200 mM each of dATP, dCTP, dGTP and dTTP, 50 nM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100,

Table 1. SSCP fragments at κ-casein gene and PCR analysis parameters

Fragment	Product length	Primer sequence Base pairs (5'–3')	T _m value (°C)	Primers (pmol)
kcn-3	469	Atgtatctgtcattcttgagggttc 26	56.00	10pmol/l
		Ctcataaaaatcaacacaacttagcc 26	56.00	10pmol/ml
kcn-4	459	Tgtgctgagtaggtatccttagtatgg 27	61.00	10pmol/ml
		Gcgttgctctcttgatgtctccttag 27	61.00	10pmol/ml

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1.5 mM MgCl₂, 0.75 unit Taq DNA polymerase and 10 pmol/ml of each primer. The thermo cycling conditions employed

were initial denaturation of 95°C for 5 min, 30 cycles of 95°C for 30 sec and annealing at 57–64°C for 30 sec, and extension at 72°C for 45 sec followed by final extension at 72°C for 5 min, and finally 10°C. An amplified product was analyzed by electrophoresis on 1.5% agarose gel at 100V for 20 min using ethidium bromide staining.

SSCP analysis: PCR products were resolved on 12% polyacrylamide gel. After standardization 30 ml of PAGE mixture was prepared by adding 12 ml of acryl amide and bisacrylamide, 3 ml of 10X TBE, 14 ml of water, 40 µl TEMED and 400 µl ammonium per sulphate (APS). The polymerized gel was given a pre-run at 375V in 1 X buffer for 30 min in vertical gel electrophoresis system. An aliquot of 10 µl of each amplified product was mixed with 8 µl of loading dye (95% formamide + 0.05 g bromophenol blue + 0.05 g xylene cyanol) and 8 µl of denaturing solution (10 mM EDTA + NaOH). The denatured products were loaded carefully in gel. The electrophoresis was carried out for 10 h at 375 V and 6–8°C in 1% TBE buffer. The gels were stained in silver nitrate (Bassam *et al.* 1991). The PCR-SSCP gels were scored based on differential conformation and movement patterns of each of the single strand of amplified DNA (Orita *et al.* 1989).

PCR products from 113 animals were resolved by SSCP and the results are presented in Table 2 and Figs 1,2. The PCR products amplified with primer 1 resolved into 2 different conformational patterns. The frequencies of those conformational patterns are 53.84 and 46.15%. The SSCP analysis of the region based on primer 2 also revealed 2 conformational patterns. The frequencies of those patterns ranged from 38.46 to 61.53%.

The polymorphism at these regions may affect production traits including milk production and milk quality. The SSCP analysis of the κ -casein gene revealed high level of polymorphism across different exonic and intronic regions in the flock of Barbari. The bovine κ -casein BB genotype was also associated with the production of milk with superior manufacturing properties, e.g. shorter rennet coagulation time, formation of a firmer curd, and in higher cheese yield (Schaar 1984, Marzali and Ng Kwai Hang 1986). This effect is associated with the milk casein micelles (Morini *et al.* 1975) resulting in a larger micelle surface area which allows the formation of a firmer and more consistent curd.

Table 2. The band patterns and their frequency at different fragments of κ -casein in Barbari goats

Fragment	No.of pattern	Band pattern	Pattern frequency (%)
kcn-3	2	A	53.84%
		B	46.15%
kcn-4	2	A	38.46%
		B	61.53%

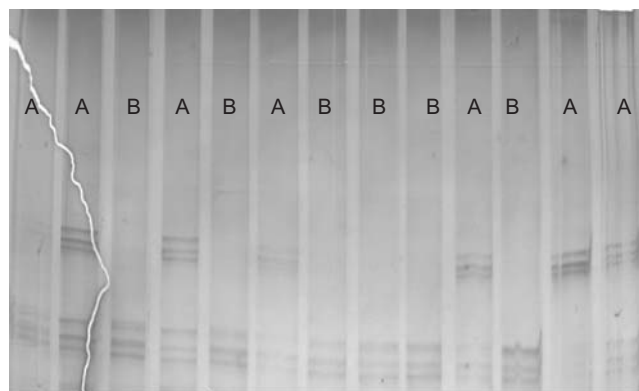


Fig 2. SSCP patterns of Kappa-casein Exon 4

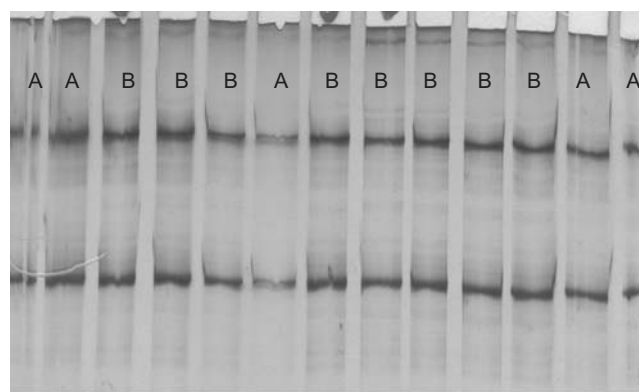


Fig. 1. SSCP patterns of Kappa-casein Exon 3.

The present preliminary results reinforce the idea that SSCP analysis is a valuable tool for the establishment of allelic variants in the genes that express κ -casein associated with production traits. These allelic variants may further need to be confirmed through sequencing of them. Upon their confirmation, the association studies of single nucleotide polymorphisms (SNPs) and/or haplotypes with production traits may be carried out and if found significant, may be used as genetic markers for the improvement of this flock.

SUMMARY

The present study was conducted to evaluate the genetic variability of kappa-casein gene for sequence variation using PCR-SSCP protocol. The preliminary results showed that SSCP analysis is a favourable tool for the establishment of allelic variants in the genes that express κ -casein associated with production traits.

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