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POLYMORPHISM IN EXON 7 OF *β-LACTOGLOBULIN* AND EXON 5 OF *STEAROYL COENZYME A DESATURASE* GENES IN GOATS

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ABSTRACT

Kerala has two native breeds of goats namely Attappady Black and Malabari. These breeds, along with Malabari crossbreds formed the material for study. Reported polymorphisms in exon 7 of *β-Lactoglobulin (βLG)* and exon 5 of *stearoyl soenzyme A desaturase* (SCD) were screened to find out the genotypic and allelic frequencies in particular goat populations and to observe an association if any, with milk protein and fat percentage in goat milk. DNA was isolated from blood samples of 347 animals representing three goat populations. Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) was conducted for two loci namely *βLG* and *SCD.* PCR-RFLP of *βLG* gene fragment using *SacII* enzyme revealed three genotypes namely, CC, CD and DD. The frequency of CC genotype was very low in all the populations, whereas DD genotype had high frequency in Attappady Black and crossbred populations (0.58 and 0.67, respectively) and CD genotype was high in Malabari populations (0.70). Frequency of D allele (0.76) was higher than C allele (0.24) in all three populations. PCR-RFLP of *βLG* gene revealed significant (P<0.01) association between genotypes and genetic groups of goats. However, no association was established between genotypes and milk protein percentages. PCR-RFLP of exon 5 of *SCD* gene using *RsaI* enzymes showed a monomorphic banding pattern, supporting the reports that there is less genetic variation in the coding region of *SCD* gene in goats.

Key words: *β-Lactoglobulin gene,* Goats, Milk constituents*,* Single nucleotide polymorphism, *Stearoyl Coenzyme A Desaturase gene*

Goats are recognized as an important livestock species, mainly due to their ecological adaptation and acceptance of goat products by all communities (Siliankove, 2000). Native goat breeds of Kerala include Malabari and Attappady Black. Dual purpose Malabari goats are commonly found in the northern districts of Kerala. Attappady Black goats, originating from the hilly terrains of the Attappady region in Palakkad district, are noted for their hardy nature and disease resistant capacity (Thomas et al., 2011). Though milk production potential is poor, they are recognized as a distinct meat breed of Kerala (Stephen et al., 2005). Malabari crossbreds, maintained in University Goat and Sheep Farm, Mannuthy, Kerala has the inheritance of Malabari, Saanen, Alpine and Boer breeds and has resulted from four decades of breeding and selection.

Goat milk is gaining much popularity among consumers. Milk proteins are generally classified as caseins (80% of the total proteins) and whey proteins (18% of total milk protein). Whey proteins contained two major proteins, α-lactalbumin and *β*-lactoglobulin. Polymorphism of *β*-lactoglobulin (*βLG)* and its association with milk composition and milk yield was reported by Kumar et al. (2006) in Indian goats and by

Agaoglu et al. (2012) and Atehortua et al. (2012) in exotic goat breeds. The iron-containing enzyme stearoyl coenzyme A desaturase (SCD) is reported to be involved in the biosynthesis of monounsaturated fatty acids. An association of *SCD* genotypes with average milk and protein yields was studied in Alpine goats by Crepaldi et al. (2013). In the present study, the reported polymorphisms in exon 7 of *β*LG and exon 5 of *SCD* were screened using restriction enzymes, to find out the genotypic and allelic frequencies in specific goat populations and to find out an association, if any, with milk protein and fat percentage in goat milk.

MATERIALS AND METHODS

A total of 347 goats belonging to Malabari, Attappady Black and Malabari crossbreds formed the material for study. Blood was collected from the jugular vein of each animal into sterile ethylene diamine tetra acetic acid coated vacutainers and DNA was extracted by phenol-chloroform method (Sambrook and Russell, 2001). To amplify exon 7 and 3`UTR of *β*LG and exon 5 of SCD genes following primers were used (Agouglu et al., 2012; Yahyaoui et al., 2004):

*β*LG-exon 7	Forward	AGTGTAGAAGGGACAGCCCAGC
	Reverse	GTGGAATGACACATGGAGAGGG
SCD-exon 5	Forward	AGTGTAGAAGGGACAGCCCAGC
	Reverse	GTGGAATGA CACATGGAGAGGG

PCR was performed using 10 p*M* each of diluted primers, 200µM of 10 m*M* dNTPs, 1 unit of Taq DNA polymerase, 1.2 m*M* $MgCl_2$, 10X polymerase buffer and 100 ng of template DNA, made up to final concentration of 20 µl using ultra filtered Millipore water. PCR was done in a thermal cycler (Applied Bio-Systems) with conditions of initial denaturation at 94°C for 3 min, followed by 35 cycles of denaturation, annealing and extension at 94°C for 30 sec, 59.6°C for 30 sec for *β*LG and 64.6°C for 30 sec for SCD, 72°C for 30 sec, respectively. A final extension was done at 72°C for 5 min. Theamplicons obtained from different breeds of goats were randomly selected, sequenced and evaluated using Basic Local Alignment Search Tool (BLAST) to ascertain the identity of gene segments and to locate the polymorphic sites if any, which formed the recognition site for restriction endonucleases. Reaction components for Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (RFLP) included 200 ng of PCR products with the needed amount of restriction enzyme and 1X buffer, made up to 30 µl. For *βLG* gene, 20 units of *SacII* enzyme and for SCD gene, 1 unit of *RsaI* enzyme were added and incubated for 37°C for 2 h and 37°C for 15 min, respectively. After restriction digestion the samples were checked in 2.0% agarose gels in a Gel doc system (Bio-Rad, USA). Upon electrophoresis, the segments resolved in the gel and the genotypes were recorded and marked against each individual. Genotypic and allelic frequencies were calculated for three populations under study.

Milk from 65 lactating Malabari, Attappady Black and Malabari crossbred goats of University Goat and Sheep Farm, Mannuthy were collected during early, middle and late lactation to estimate milk fat and protein percentage by electronic Milk Analyser (MRC Scientific Instruments). The chosen site was polymorphic for *βLG* genes and its associations were worked out using following General Linear Model for non-orthogonal data by SPSS Ver. 21:

$$y_{ijkl} = \mu + b_i + s_j + g_k + e_{ijkl,}$$

Where, y_{ijkl} = milk protein % of ijklth animal, b_i = effect of ith breed (i = 1 to 3), s_j =effect of jth stage of lactation (j = 1 to 3), g_k = effect of kth *βLG* genotype (k = 1, 2, 3) and e_{ijkl} = random error

Chi square test was conducted on cross tabulated data to find out the association of genotypes with genetic groups of goats using SPSS Ver. 21.

RESULTS AND DISCUSSION

PCR amplification of *ßLG gene fragments* resulted in a 426 bp amplicon which was commercially sequenced and BLAST analysis confirmed the identity of the segment as exon 7 and 3'end of *βLG* gene. The results of restriction digestion *Sac II* enzyme are presented in Plate 1. Homozygous CC (347, 79 bp), heterozygous CD (426, 347 and 79 bp) and homozygous DD (426 bp) genotypes were obtained, which were scored as 1, 2 and 3. Paired analysis of CC and DD sequences revealed a mismatch at 78th

position of the amplicon. It was an established C→A mutation at 81st bp of exon 7 which led to the disappearance of recognition site CCGCGG of *SacII* enzyme, hence the difference in banding patterns. This was further confirmed using a chromatogram pattern (Plate 2 and 3). Pena et al. (2000) reported two polymorphisms in exon 7 of *βLG* gene and one of the polymorphic sites allowed a PCR-RFLP genotyping using *SacII* enzyme. Agaoglu et al. (2012) reported *SacII* polymorphism of *βLG* gene in Honamli, Hair and Saanen goats and Atehortua et al. (2012) reported *βLG* polymorphism in goats of Antioquina, Colombia. In buffalo, *βLG* polymorphism was also reported by Patel et al. (2007).

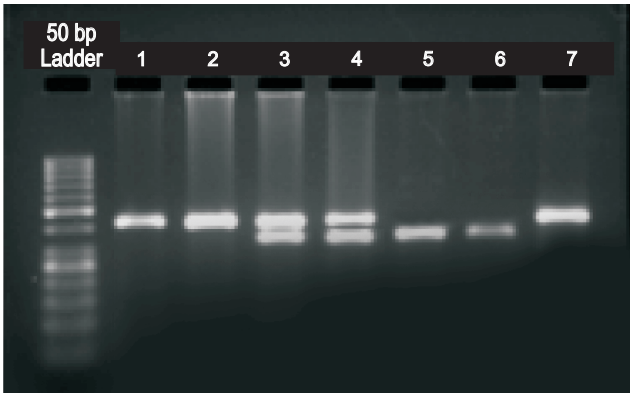

Plate 1. PCR product and RFLP alleles of βLG gene fragment resolved in 2% agarose gel (Lane 1: 50 bp ladder, lane 2, 3: CC genotype, lane 4, 5: CD genotype, lane 6, 7: DD genotype, lane 8- PCR product)

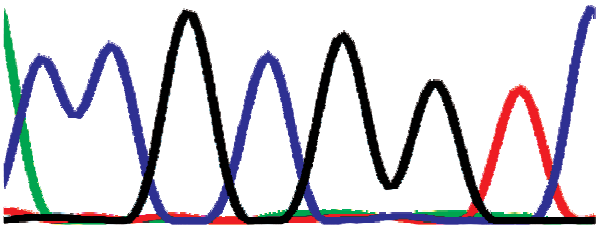

Plate 2. Snapshot of chromatogram showing CCGCGG in βLG gene sequence (CC genotype)

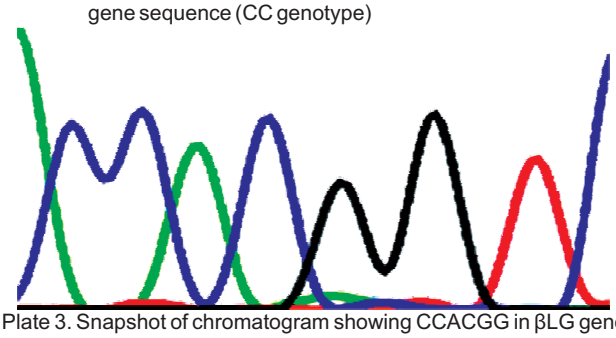

Plate 3. Snapshot of chromatogram showing CCACGG in βLG gene sequence (DD genotype)

Genotypic and allelic frequencies for *βLG* gene fragment are presented in Table 1. The frequency of CC genotype was very low in all the populations, whereas DD genotype had high frequency in Attappady Black and crossbred populations (0.58 and 0.67, respectively) and CD genotype was high in Malabari populations (0.70). The overall frequencies of three genotypes CC (S_2S_2), CD (S_1S_2) and DD (S_1S_1) were 0.03, 0.42 and 0.55, respectively. Allelic frequencies of C and D were 0.24 and 0.76 (Table 1). Similar results were given by Elmaci et al. (2009) in Turkish hair goats, where S_2S_2 frequency (0.11) was found to be lower than S_1S_1 (0.45) and S_1S_2 (0.44). They also showed that frequency of S_2 allele (C allele) at *βLG* locus was lower compared to frequency of S_1 (D) allele which was also in agreement with the present study. Agaoglu et al. (2012) also reported similar frequencies for Hair and Saanen goats. Consistent with the result of this study, French and Spanish goats also showed higher S_1 allele frequency than S_2 (Pena et al., 2000). The genotype frequency of S_2S_2 (CC) in the present study was comparatively much lower than that of other reports. The results disagreed with the findings of Kumar et al. (2006) who stated that S_2 allele frequency was higher than S_1 allele frequency in Indian goats.

Table 1. Frequency of genotypes and alleles at *β*-lactoglobulin locus in goats

Goat (n) population	Genotypic frequency			Allelic frequency	
	CC	CD	DD	C	D
Attappady Black (80)	0.05	0.37	0.58	0.24	0.76
Crossbred (98)	0.03	0.30	0.67	0.18	0.82
Malabari (169)	0.05	0.70	0.25	0.40	0.60
Overall (347)	0.03	0.42	0.55	0.24	0.76

The protein percentages in milk were 4.87±0.39, 4.66±0.11 and 4.82±0.11 for CC, CD and DD genotypes, respectively. No significant association was established between *βLG* genotype and milk protein per cent. Similarly, the stages of lactation and genetic groups failed to produce significant changes in protein per cent in milk. Cross tabulation of data for *βLG* genotypes and genetic groups revealed significant (P<0.01) association among them (Table 2). While the DD genotype was predominant in

Attappady Black and crossbred groups of goats, Malabari goats had more of CD genotype. But no significant difference in protein percentages was noticed between genotypes. This might be because of the reason that the three different genetic groups of goats revealed similar milk protein percentages in this study.

Table 2. Cross tabulation of data for *βLG* genotypes and genetic groups of goats

βLG genotype	Attappady Black	Crossbred	Malabari	Total
CC	4 (5.0)	3 (3.1)	4 (2.4)	11 (3.2)
CD	30 (37.5)	29 (29.6)	88 (52.1)	147 (42.4)
DD	46 (57.5)	66 (67.3)	77 (45.5)	189 (54.4)

Values in parentheses indicates percentage; Chi square = 14.75 significant at P<0.01

An amplicon of 447 bp was obtained on amplification of exon 5 of SCD gene (Plate 4). Amplicons were commercially sequenced and identity of *SCD* confirmed by BLAST analysis. On restriction digestion with *RsaI* enzyme all individuals showed the similar banding pattern of 238, 111 and 98 bp (Plate 4). The sequenced fragment revealed two cutting sites for enzyme *RsaI* (GTAC) at 98th and 208th position of amplicon. Of these two, the second one (208th bp of amplicon) was an established single nucleotide polymorphism (SNP), G→T at 112th bp of exon 5. But the populations under this study were monomorphic, with the presence of cutting site GTAC for all individuals, thus giving the same banding pattern.

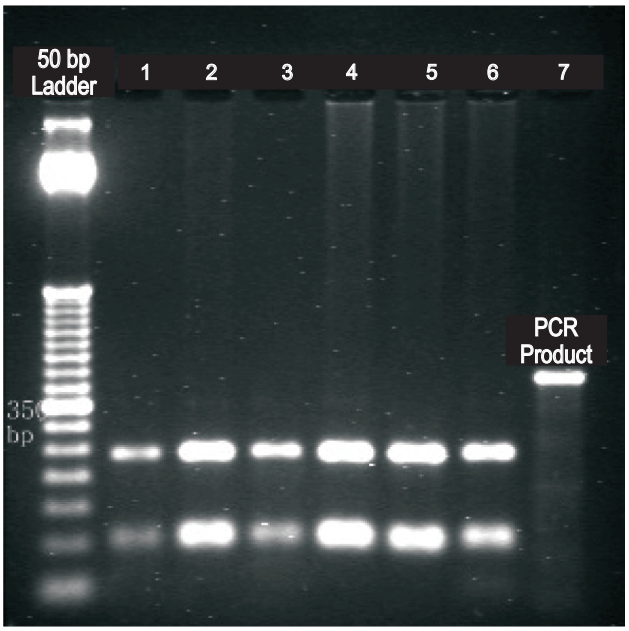

Plate 4. PCR product and RFLP alleles of SCD gene fragment resolved in 2% agarose gel (Lane 1 to 6: monomorphic bands, lane 7: PCR product)

Similarly, lack of variability of coding region of *SCD* in goats and sheep was also reported by Bernard et al. (2001) and Garcia-Fernandez et al. (2009), respectively. Zhang et al. (2010) identified six SNPs in intron 3, intron 4 and exon 6 of caprine *SCD* gene of Xuhuai, Boer and Haimen breeds, thus identifying one SNP in the coding region of gene. Thus, it is suggestive that coding region of *SCD* has less genetic variation in goats. On the contrary, several studies reported significant associations between polymorphisms in bovine *SCD* gene and the fatty acid compositions of meat and milk (Taniguchi et al., 2004; Moili et al., 2007). In Alpine dairy goats, Crepaldi et al. (2013) reported that the TGT deletion located on the 3' untranslated region (3' UTR) of the *SCD* gene had significant effects on average milk and protein yields. To conclude, PCR-RFLP of exon 7 of *βLG* gene fragment in goats, using *SacII* enzyme revealed three genotypes which had significant association with genetic groups and not with the milk protein percentage. PCR-RFLP of exon 5 of the *SCD* gene using *RsaI* enzymes showed a monomorphic banding pattern, supporting the reports that there is less genetic variation in the coding region of *SCD* gene in goats.

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