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Casein Gene Polymorphism in Ladakhi Goat

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Abstract: *Alleles of αs_1 Casein (CSN1S1) locus are associated with milk protein production. Characterization of CSN1S1 locus in Indian goat breeds is important to evaluate their diversity for milk proteins production in different climatic zones of the country. The objective of present study is to analyze the variation at CSN1S1 locus in goats of Ladakh region by both genomics and protein level. Milk protein analysis was carried out in skimmed milk samples (n=40) of local goats by SDS-PAGE. SDS-PAGE revealed four milk protein genotypes (AA, BB, BE & AF) for CSN1S1 locus of which A/F allele was observed in lower frequency than B/E allele. Blood samples were collected from (n=34) local Ladakhi goats in their natural habitats and genomic DNA was isolated as per standard protocol. Genotyping was performed with allele-specific PCR. Genotyping of DNA samples showed five alleles (A, B, D, E & F) in frequency of 0.38, 0.27, 0.01, 0.27 and 0.04 respectively. Effective number of alleles, Observed number of alleles, expected homozygosity and Nei's expected heterozygosity were 3.28, 5.0, 0.324 and 0.696 respectively.*

Key words*: CSN1S1 locus, Casein polymorphism, Ladakhi goat, Indian goat, Goat genetic diversity*

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I. Introduction

Ladakh is located between 30º- 60º latitude and 75º-81º longitude in northern part of Himalaya, India, at the altitude of 3000 m to 6000 m from mean sea level. The harsh climatic conditions of the region includes low winter temperature (-35ºC), hot summers (35ºC), low partial pressure of oxygen (30% less), low relative humidity (30-45 %), high wind velocity, high influx of Infra Red (IR) and Ultra Violet (UV) radiations [1]. Cultivation of fodder crops is limited due to short cultivation period (April- September) and harsh agro-climatic conditions. Rainfall is sparse (30-40 mm/year) and the landscape is barren due to its aridity. Local goats of Ladakh thrive in this type of harsh and dry climatic condition. Changthangi breed of goat is known for producing Pashmina in Changthang region of Ladakh [2]. Information on other local goats of this region is lacking. First hand information gathered from local population indicates that, the goats are medium in size, produces Pashmina and milk in lesser quantity than Changthangi breed. Goats of this region have not been characterized for milk protein alleles.

The CSN1S1 locus has 16 co-dominant alleles and is associated with different rates of protein synthesis. The A, B and C alleles are associated with high level, E allele with medium-level, F and G alleles with low level of protein in the milk [3]. The distribution of different alleles at CSN1S1 locus has been investigated in European goat breeds at the genomic as well as protein levels [4, 5].

Casein variability in Indian goat breeds has been characterized by Rout et al. [6]. However, the study does not include the goats of ladakh region. It is important to compare the variability of αs_1-casein gene (CSN1S1 locus) in different Indian goat breeds of all the regions to determine the effect of αs_1-casein genotyping for genetic improvement, genetic diversity studies and for conservation programme.
Therefore, the present study aims to characterize the CSN1S1 locus in local goats of Ladakh, India.

II. Material and Methods

2.1 Sample collection and DNA isolation

Milk samples from 40 lactating goats were collected in their natural habitat. An effort was made to collect the samples from the same goats from which blood samples were taken. Blood samples for DNA isolation was collected from jugular vein of 34 unrelated goats in EDTA coated vaccutainer tubes. The goats belonged to two different villages of Nubra Valley of Ladakh. An effort was made to collect the samples from unrelated individuals on the basis of information provided by farmers. DNA was isolated from blood samples as per Thangaraj et al. [7]. Quality of isolated DNA was checked by Nano-drop spectrophotometer and submarine gel electrophoresis.

2.2 Genotyping

SDS-PAGE was carried out in skimmed milk samples. Gels were stained with Coomassie Brilliant Blue. Milk protein variants were determined by the molecular weight in gel documentation system (Alpa Innotech Corporation). DNA samples were analysed with allele-specific polymerase chain reaction (AS-PCR) and the amplified product was digested with Xmnl restriction enzyme. PCR was carried out in a 50µl reaction mixture containing 100 ng genomic DNA after optimization of amplification protocols. The amplification protocol was as follows: an initial cycle of 97ºC for 2 min, 60ºC for 45s and 72ºC for 2 min 30s; then 30 cycles of 94ºC for 45s and a final extension step 72ºC for 10 min. For restriction digestion 20µl of each PCR product was digested with 10 U of Xmnl endonuclease for overnight at 37ºC and digested products were analysed in 4% agarose gel, stained with ethidium bromide and analysed in a gel documentation system (Alpha Innotech Corporation, San Leandro, CA, USA).

2.3 Statistical analysis

Genepop [8] software was used to estimate allelic frequencies, expected heterozygosity, effective number of alleles and to verify Hardy-Weinberg equilibrium. The genotypes were observed by counting the patterns in gel documentation system.

III. Results

3.1 Milk protein variants in local Ladakhi goats

Milk protein genotyping was carried out by SDS-PAGE in 40 individual milk samples of Local Ladakhi goats. The electrophoresis pattern of individual milk samples is presented in Fig 1. SDS-PAGE genotyping revealed αs_1 (CSN1S1), αs_2 (CSN1S2), β (CSN2), κ- casein (CSN3) and whey proteins (β- LG and α- LA) fractions in Ladakhi goats. Table 1 shows the genotypic frequency of casein milk proteins. The αs_1 – casein 'A' allele (which is directly related to higher casein yield) was observed in lower frequency than B/E allele at CSN1S1 locus. The F variant was also observed but at low frequency in Local Ladakhi goat. The CSN1S2 locus showed monomorphic pattern in all the analysed samples. Similarly, there were no variations observed at β-casein locus. The κ- casein locus exhibited two alleles ($\kappa\text{-Cn}^A$ and $\kappa\text{-Cn}^B$), whereas, no allelic variation was observed in a β- LG locus. However, the β- LG showed a strong band, indicating higher expression of proteins as compared to other Indian goat breeds. α- LA locus exhibited monomorphic pattern in all the analyzed samples.

3.2 Molecular characterization of CSN1S1 locus in local Ladakhi goats

Genotyping of 34 individuals was carried out with the PCR-RFLP method. DNA samples were analyzed for the presence of different αs_1- casein allele by single AS-PCR. The amplified PCR product (224bp) was restriction digested with *Xmn I* restriction enzyme, which yielded four different variant groups of (150+63) bp, (161+63) bp, (223+150+63) bp and (212+63+50) bp respectively (fig 2,3,4). The pattern was identified as AA, AF, AD and BE genotypes respectively, whose frequencies have been given in Table 2. Genotyping at CSN1S1 locus showed that the BE genotype had the highest frequency in Local Ladakhi goats.

The expected homozygosity and heterozygosity of Local Ladakhi goats were 0.324 and 0.706 respectively. Nei's expected heterozygosity and average heterozygosity were 0.696 and 0.303 respectively. Effective and observed numbers of alleles were 3.284 and 5.0 in the studied population (Table 3).

B/E allele at αs_1- casein locus were further discriminated by AS-PCR using BE/R primer pair. Amplified products of 90 and 550 bp were identified as αs_1-CN B and αs_1- CN E alleles respectively. Out of the all genotypes (68), 58.82% of the individuals were identified as BB genotype and 29.41% individuals were identified as BE genotype (Table 4).

IV. Discussion

The goat CSN1S1 locus has been characterized by at least 16 alleles, which have been associated with different levels of protein synthesis. A first group of alleles (A, B1, B2, B3, B4, C, H, L, M) are related to a normal content of αs_1-Casein (about 3.6g/l), whereas alleles I and E are associated to an intermediate content (about 1.1g/l) and alleles F and G are related to a low level of αs_1-casein in the milk (about 0.45g/l). Alleles CSN1S1 N, 01 and 02 are 'null' alleles and have been associated with the apparent lack of αs_1-casein in milk [9].

Present study at the DNA and protein level showed presence of A, B, D, E and F alleles at αs_1-casein locus. The B and E alleles were observed in highest proportion. The discrimination of B and E variants, by use of BE (forward) and R (reverse) primer pair sufficed to detect one or both alleles with in same sample by ASA-PCR. The present study agreed well with the report of *Feligini et al.* [5]. Allele F and D were also observed in very low frequency. Allele D was observed in low frequency (0.01) in heterozygous form by DNA analysis but

not in SDS-PAGE, which needs further characterization. SDS-PAGE analysis also indicated that the B allele and E allele are in highest proportion as compared to allele A.

It is a known fact that, Indian goats are better producers of milk as well as protein in comparison to goats of Ladakh region. Indian breeds show A and B alleles in higher frequency, indicating the better allelic combination for the higher protein [10] in comparison to other goat breeds of France and Spain, whose predominant alleles are E and B [11]. The studied population had normal distribution of allelic frequency as is evidenced by the Nei's expected and average heterozygosity estimates.

V. Conclusion

From the present study, it may be concluded that goats of Ladakh region differ from many of the established Indian goat breeds at CSN1S1 locus. Reason may be different ancestry, or, evolutionary adaptation for harsh climatic conditions at the cost of milk production, as other livestock of this region are also poor in milk production.

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Tables and Figures

Table 1: Genotypic frequency of casein (milk protein) in Local Ladakhi goat

Locus	Observation	Frequency
αs_1-CN (CSN1S1)		
AA	14	0.35
BB	12	0.30
BE	10	0.25
AF	4	0.10
αs_2-CN (CSN2)		
AA	40	1
β-CN		
AA	40	1
κ-CN		
AB	40	1
β- LG		
AA	40	1
α- LA		
AA	40	1

Table 2: Genotypic and Allelic frequency for αs_1-CN (CSN1S1) locus in Local Ladakhi goats

Genotypic Frequency				Allelic Frequency				
AA	AF	AD	B/E	A	F	D	B	E
0.323	0.088	0.029	0.559	0.382	0.044	0.015	0.279	0.279

Table 3: Parameter at αs_1-CN (CSN1S1) locus in Local Ladakhi goats

Parameter	Observation
Number of allele	68
Observed number of alleles	5.0
Effective number of alleles	3.284
Expected homozygosity	0.324
Expected heterozygosity	0.706
Nei's expected heterozygosity	0.696
Average Heterozygosity	0.303

Table 4: B/E allelic frequency of in different goat breeds

S.No.	Breed	BE	B
1.	Local Ladakhi goat	29.41% (10)	58.82% (20)
2.	Jamunapari	-	93.75% (15)
3.	Barbari	25% (4)	68.75% (11)
4.	Black Bengal	-	80% (8)

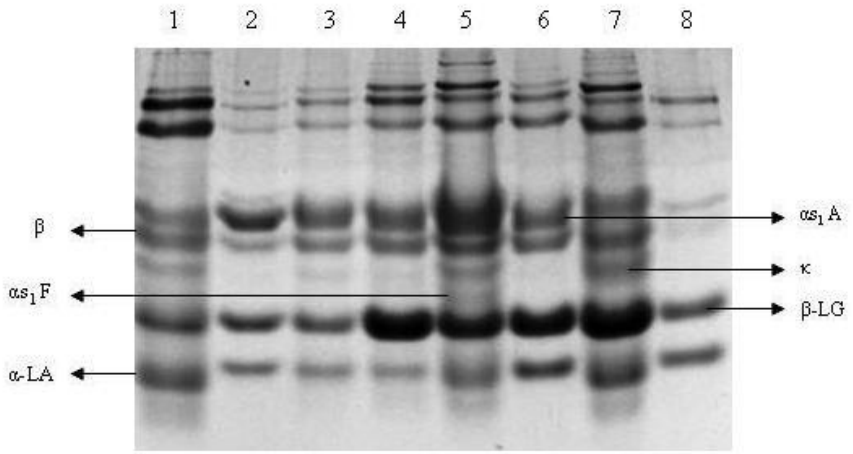

Fig. 1: SDS-PAGE profile of milk protein in goats, Lane 1 & 2 –Barbari,
Lane 3 & 4 – Black Bengal, Lane 5 & 6 – Local Ladakhi goat and Lane 7 & 8 - Jammunapari

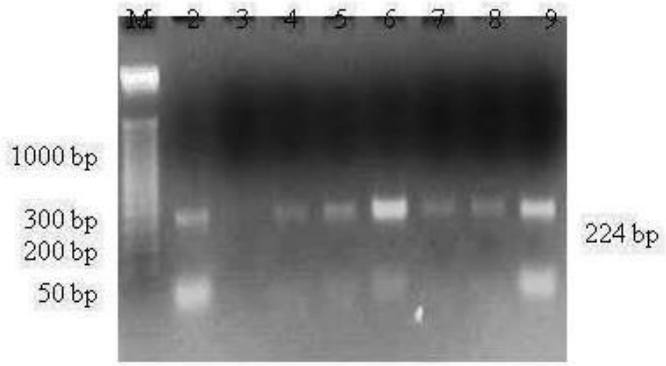

Fig. 2: PCR amplification of DNA region spanning from eight to the ninth intron of goat αs1-casein (CSN1) gene, Lane 1: Marker (50 bp), Lane 2-9: amplified PCR products

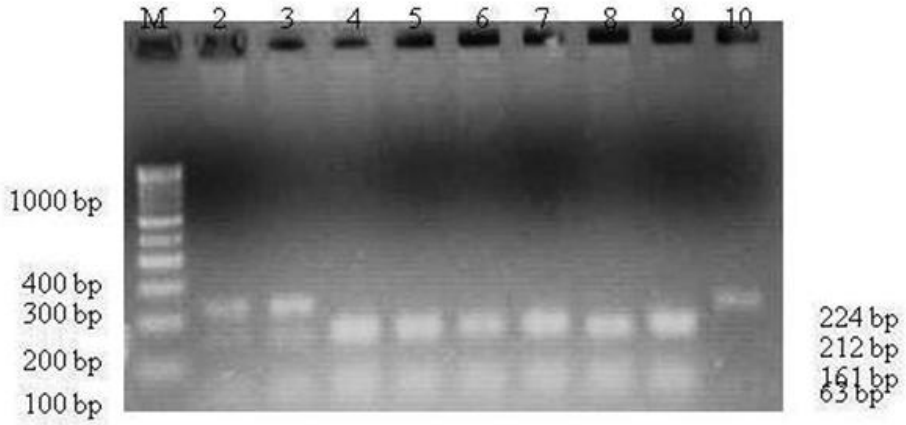

Fig. 3: *Xmn1*-RFLP genotypes of αs_1-casein (CSN1S1) gene in Local Ladakhi goat, Lane 1-Marker (100 bp), Lane 2-9- digested product and Lane 10- PCR product