# STUDIES ON GENETIC POLYMORPHISM OF ALPHA S1 CASEIN GENE IN NANDIDURGA GOATS

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**DECEMBER, 2014**

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*Thesis submitted to the*

***Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar***

*In partial fulfilment of the requirements*

*For the award of the degree of*

## MASTER OF VETERINARY SCIENCE

**in**

## ANIMAL GENETICS AND BREEDING

***By***

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## CERTIFICATE

This is to certify that the thesis entitled **"*STUDIES ON GENETIC POLYMORPHISM OF ALPHA S1 CASEIN GENE IN NANDIDURGA GOATS*"** submitted by **Mr. UMESHA KAVALI, ID No. MVHK1203** in partial fulfilment of the requirements for the award of the degree of **MASTER OF VETERINARY SCIENCE** in **ANIMAL GENETICS AND BREEDING** of the Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar, is a record of bonafide research work carried out by him during the period of his study in this University, under my guidance and supervision and the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles.

Bangalore
December, 2014

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Major Advisor
Professor & Head

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**(Dr. V. GIRISH KUMAR)**

*Affectionately Dedicated*

*To*

*My Beloved Parents*

*And*

*Family Members*

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# LIST OF ABBREVIATIONS

| | | |
|---|---|---|
| BLAST | : | Basic Local Alignment Search Tool |
| BLASTn | : | Nucleotide BLAST |
| bp | : | base pair |
| DNA | : | Deoxy ribo nucleic acid |
| dNTPs | : | Deoxy ribo nucleotide triphosphate |
| EDTA | : | Ethylene Diamine Tetra Acetic Acid |
| FAO | : | Food and Agricultural Organization |
| g | : | Gram |
| hr | : | Hour |
| KCL | : | Potassium Chloride |
| kb | : | Kilo base |
| M | : | Molar |
| µg | : | Micro gram |
| MgCl2 | : | Magnesium Chloride |
| mg | : | milli gram |
| µl | : | Micro litre |
| ml | : | Milli litre |
| µm | : | Micro moles |
| mM | : | Milli moles |
| ng | : | Nano gram |
| NCBI | : | National Centre for Biotechnology Information |
| OD | : | Optical Density |
| p.mol | : | Pico moles |
| PCR | : | Polymerase Chain Reaction |
| RFLP | : | Restriction Fragment Length Polymorphism |
| rpm | : | Revolutions per minute |
| *Taq* | : | *Thermus aquaticus* |
| TBE | : | Tris- boric acid-EDTA |
| TE | : | Tris- EDTA |

# Introduction

# I. INTRODUCTION

Goat rearing occupies an important place in augmenting the economy of our country as it provides livelihood to lakhs of people. Goats are multi-utility animals, producing meat, milk, skin and hair. Goat meat is low in cholesterol and fat and free from religious taboos unlike pork. Goats can adapt to varying environmental conditions. They have tremendous ability to survive and often thrive on sparse vegetation unsuitable for feeding of other livestock. Goats can be profitably raised with low investment under intensive and most extensive forms of nomadic grazing. Goat rearing requires low cost and is hence suited for landless labourers and marginal farmers (Bhat and Khan, 2008).

The population of goat continues to increase at an average rate of about 3.4 per cent per year. The direct contribution of goats to the Indian economy is estimated at Rs. 59.74 billion annually. The slaughter byproducts, skin and fiber of goats provide raw material to consumer industries such as leather and textiles. In India the total goat population is 140.05 million (Anon, 2007). India has 23 recognized goat breeds.

The goat population of Karnataka is 61.52 lakhs as per 2007 census, of which 6, 21,967 are classified as Nandidurga goats (Anon., 2007) constituting around 10 per cent of total goat population of the Karnataka.

Nandidurga goat is a non-descript goat, distributed in Mandya, Tumkur, Chitradurga and Davanagere districts of Karnataka. White is the most predominant color (74.7 %) followed by black (2.3 %) and mixed (23 %) with black/ brown patches (Azaruddin, 2011).

Osmanabadi is one of the popular goat breeds of India and are reared for both milk and meat purpose. Bidri goats are mainly reared for meat purpose.

Goat milk is gaining popularity among consumers as it fetches the farmers a premium price compared to cow milk. Milk protein is one of the important milk quality traits of goat milk and mammalian milk protein consists of casein and whey proteins. The important types of caseins are α-S1, α-S2, β and k. Several studies on milk proteins indicated that there exist variations in these traits. Studying such variations is important for improvement of their production potential. In the recent past, DNA based techniques have been used for polymorphic studies, as this focuses on the genetic polymorphism of candidate genes and their application in marker studies. Polymerase Chain Reaction (PCR) amplification followed by Restriction Fragment Length Analysis (RFLP) is one such approach.

Milk protein variations are considered as one of the important markers for milk production traits. Variations at Alpha S1Casein gene have been analyzed by various workers (Grosclaude *et al*., 1987; Ramunno *et al*., 2000). The association of Alpha S1Casein gene polymorphism with milk yield has been reported in several recognized breeds of goats in India and across the world. However such studies are scanty in non-descript goats like Nandidurga.

Keeping the above points in view, the present study was taken up with the following objectives:

1. To amplify the Alpha S1Casein gene of Nandidurga goats by Polymerase Chain Reaction.
2. To study the polymorphic patterns of the above gene by RFLP and DNA sequence analysis.

# Review of Literature

# II REVIEW OF LITERATURE

## 2.1 Nandidurga goat

Nandidurga goat is a non-descript breed of Karnataka and is distributed in Davanagere, Chitradurga, Tumkur and Mandy districts of Karnataka state. The animals of this breed are predominantly white in colour followed by black and white or brown colour and an admixture of all these coat colors. Most of the animals are horned and horns are oriented backwards and downwards. The ears are pendulous, forehead is convex and has no beard and wattles (Azharuddin, 2011).

Osmanabadi breed is mainly distributed around Osmanabad region of Maharashtra. It is characterized by tall and large sized body with predominantly black coat color, long and pendulous ears and long horns.

Bidri is a non-descript goat of Karnataka. It is found in Bidar, Aurad, Bhalki, Humanabad and Basavakalyan taluqs of Bidar district. It is a small sized goat with black coat color with occasional brown and white spots. Most of the animals have straight forehead with curved horns. Wattles and beards are usually absent. Tail is of medium length.

## 2.2 Molecular markers for genome analysis

A Molecular marker is defined as any stable and inherited variation detectable or measurable by a suitable technique and which can be used to detect presence of specific genotype or phenotype, which is otherwise not measurable or very difficult to detect. A piece of genetic material that bears or produces a distinctive feature is generally

considered to be a marker, usually a mutant gene, and can be either dominant or recessive (Strickberger, 1985).

'Molecular markers' or 'genomic markers' originated during the recent past due to advances made in the field of molecular biology. A large amount of data have been generated since the first demonstration of RFLP (Grodzicker *et al.,* 1974), and are used as markers in human genetics (Botstein *et al*., 1980), in genetic improvement of plants (Beckman and Soller, 1983; Burr *et al.,* 1983) and animals (Beckman and Soller, 1983).

The use of PCR (Saiki *et al*., 1988) to amplify a DNA sequence of interest and subsequent restriction enzymes analysis (RFLP) of the amplified product represented a milestone in this endeavor (Pinder *et al*., 1991). An unlimited number of genetic polymorphisms at DNA sequence level have provided a number of genomic markers such as RFLP's (Botstein *et al*., 1980), Mini satellites or Variable Number of Tandem Repeats (VNTR) (Jeffrey *et al*., 1985; Nakamura *et al*., 1987), Mini satellites (Litt and Lutty, 1989; Weber and May, 1989; Tautz, 1990; Fries *et al.*, 1990) and RAPD markers (Williams *et al*., 1990; Welsh and McClelland, 1990).

Beuzen *et al.* (2000) opined that the use of molecular markers to define the genetic makeup (genotype) and predict the performance of animal is a powerful aid in animal breeding. The selection based on these markers is known as Marker Assisted Selection (MAS). Several candidate genes for milk qualities have been studied by various authors. α- S1 casein gene is one such candidate gene found to be associated with milk quality.

### 2.3 Polymerase Chain Reaction (PCR)

Polymerase Chain Reaction PCR) was developed by Mullis *et al*. (1986). This is an *in vitro* method for the enzymatic synthesis of specific sequences of DNA. The reaction normally uses two oligonucleotide primers that hybridize to opposite strands that flank the target DNA sequence to be amplified. The elongation of the primers is catalyzed by Taq DNA polymerase that is isolated from thermophilic eubacterium *Thermus aquaticus*. A repetitive series of cycles involving template denaturation, primer annealing and extension of annealed primers by Taq DNA polymerase results in an exponential accumulation of specific DNA fragment.

#### 2.3.1 Deoxy Ribo nucleoside Triphosphates (dNTPs)

PCR is normally performed with dNTP concentration around 100 µm, although at lower dNTP concentration (10 - 100 µm), Taq DNA polymerase has a higher fidelity. Innis and Gelfand (1990) suggested that the four dNTPs should be used at equivalent concentrations to minimize disincorporation errors, and the lowest possible dNTP concentration appropriate for the length and composition of the target sequence must be used.

#### 2.3.2 PCR buffer and magnesium concentration

PCR buffer is an important component of the PCR technique and may affect the outcome of amplification. In particular, the concentration of $MgCl_2$ has a profound effect on the specificity and yield of amplification. Generally, excess $Mg^{2+}$ will reduce the yield. Innis and Gelfand (1990) recommended a PCR buffer that contains 10-15 mM Tris HCl with pH between 8.3 and 8.8 at 20 °C. A magnesium concentration of 1.5 - 2.0 mM

is optimal for most PCR products generated with Taq DNA Polymerase. Optimization normally involves supplementing the magnesium concentration in 0.5 or 1.0 mM increments (Sambrook and Russell, 2001).

### 2.3.3 Annealing temperature

Annealing temperature is one of the important thermal cycling parameters. It is the temperature at which primer anneals to template DNA. This temperature varies from primer to primer and is to be determined based on number of bases in the primer and per cent of GC content in the primer. A thumb rule formula, viz., $Tm = (G + C) \times 4 + (A + T) \times 2$ is generally used for approximation of this temperature (Sharrocks, 1994).

### 2.3.4 DNA template

Use of high quality, purified DNA templates greatly enhances the success of PCR reactions. Optimum concentration of template DNA per reaction could vary substantially from typical conditions (100 ng per reaction) depending on the primer - template combination use (Sambrook and Russell, 2001).

### 2.3.5 Primers used for PCR Amplification of Alpha S1 CSN or α- S1 CSN gene

As recommended by Innis and Gelfand (1990), primer concentration between 0.1 and 0.5 µM are considered optimum. Sequences not complementary to the template can be added to the 5' end of the primers. These exogenous sequences become incorporated into the double stranded PCR product and provide a means of introducing restriction sites or regulatory elements at the ends of the amplified target sequence (Roux, 1995).

Generally, the primers used in PCR are in between 20 - 30 nucleotides in length and with higher GC content, which allows a reasonably high annealing temperature to be used. Ideally, PCR primers should have a 40 - 60 per cent GC content and a 3'- terminal "G/C clamp" (at least one or two 3' G's and or C's); be similar in size (18 - 25 bases), Tm values and nucleotide ratios; and be free of repetitive motifs, palindromes, excessive degeneracy and long stretches of poly - purines or poly - pyrimidines.

Primer-dimer is an amplification artefact often observed in the PCR product, especially when many cycles of amplification are performed on a sample containing very few initial copies of template. It is a double – stranded fragment, whose length is very close to the sum of the two primers and appears to occur when one primer is extended by the polymerase over the other primer.

**2.4 Restriction Fragment Length Polymorphism (RFLP)**

RFLP refers to inherited differences in sites of restriction enzymes that result in differences in length of the fragments produced by cleavage with relevant restriction enzymes. Genetic Polymorphism is the coexistence of multiple alleles at a locus and any site at which multiple alleles exist as stable components of the population. Polymorphism in the genome can be detected by comparing the restriction maps of different individuals and a difference in restriction maps between two individuals is a Restriction Fragment Length Polymorphism or RFLP (Lewin, 2000). It can be used as a genetic marker; instead of examining some feature of the phenotype, a genotype as revealed by restriction map is assessed.

### 2.4.1 Restriction Endonuclease

Restriction Endonucleases (RE) is class of endonuclease enzymes which cleave DNA at specific nucleotide sequences. In some bacteria they form part of defensive mechanism against infection by bacteriophage in which the bacterium will methylate specific sites in its own DNA to prevent cleavage by its restriction enzyme which is still able to destroy foreign unmethylated DNA. Different enzymes, as obtained from a different range of organisms, have different specificities, often recognizing four or six base pair sequences. Because of this specificity, restriction enzymes cleave a sample of DNA into defined polynucleotide fragments which can be separated upon electrophoresis according to their length. This procedure is sufficiently sensitive to be able or detect a difference of one base pair in certain circumstances and hence the enzymes are highly essential and a prerequisite for the procedure of genetic manipulation.

### 2.4.2 Principles of RFLP

RFLPs occur as a result of DNA base changes, deletions, insertions or rearrangements that either create, eliminate or translocate restriction enzyme cleavage site. A point mutation, deletion or insertion can create or abolish the recognition site for a particular RE at the locus and there by changes the size of the resulting restriction fragments. Inversion on other hand, changes the distance between a pair of RE sites and therefore, causes changes in size of restriction fragment. The changes in DNA sequences associated with an allelic change at a locus can be visualized by the altered mobility of restriction fragments of gel electrophoresis (Theilmann *et al.,* 1989)

Individuals carrying different allelic variants of the gene will show different distribution patterns. These differences in band numbers and sizes result from change in fragment size and are termed as Restriction Fragment Length Polymorphisms (RFLP). Such variants are inherited in a Mendelian fashion and since gene expression is not required for RFLP analysis, variation in the flanking regions or introns of genes may also be detected. Consequently, the RFLP approach for analysis of genomic variation is potentially powerful (Theilmann *et al*., 1989).

**2.5 Milk proteins**

Interest in milk proteins has been growing at an amazing pace, particularly in the last few years because of its nutritional, technological and economic reasons. Although both quality and quantity of milk protein differ between the species and breeds as well as between individuals and within breed, the milk proteins in general, are divided into two classes; Caseins and Whey proteins. The caseins, which account for 80 per cent of total milk proteins, exist as four fractions *viz*., Alpha S1 which is also denoted as α-S1, α-S2, β and κ Casein. Whey proteins which constitute about 20 per cent of total milk proteins, comprises of β-lactoglobulin, α-lactalbumin, serum albumin and immunoglobulins.

Goat caseins show a complex qualitative and quantitative variability, characterized by several genetic polymorphisms and multiple post-translational modifications. Different transcriptional and post-transcriptional mechanisms control casein gene expression, dramatically affecting the technological properties of milk (Martin *et al.*, 1999).

### 2.5.1 Casein genes

Casein genes are organized as a cluster in the order s1-casein (CSN 1S1), casein (CSN2), s2-casein (CSN1S2) and k-casein (CSN3) (Ferretti *et al.,* 1990; Threadgill and Womack, 1990). In goats the entire casein gene cluster region spans about 250 kb on chromosome 6 (Hayes *et al.,* 1993). Furthermore CSN1S1 and CSN2 are only 12 kb apart (Leroux and Martin, 1996).

### 2.5.2 Alpha S1 (α-S1 Casein)

Among Caseins, α-S1 represents more than 40 per cent in bovine milk (Farrel *et al.,* 2004) while in goat milk it ranges from 5 to 25 per cent due to occurrence of polymorphism in CSN1 S1 gene (Boulanger *et al*., 1984). Moioli *et al.* (2007) opined that the protein content in goat milk is affected by polymorphism in Alpha S1 gene resulting in significant cheese yield difference.

### 2.5.3 Genetic polymorphism of α- S1 Casein

First demonstration of the genetic polymorphism of Alpha S1 CSN done by starch gel electrophoresis. The two electrophoretic bands were named A and B in order of their decreasing mobility; variant B is diffused in all the species.

Wilkins and Xie (1997) found a mutation in Alpha S1 CSN gene. This mutation gives rise to the same Alpha S1 'A' variant with the deletion of exon 6. The Alpha S1 CSN gene is present in the region of intron 8, exon 9 and intron 9 (Bras, 2009). Sahar Ahmad (2006) reported that the Egyptian goat breeds carried low percentage of homozygous alleles required for cheese industry.

### 2.5.4 Alleles of Alpha S1 CSN

Grosclaude *et al*. (1987) detected 7 alleles at the Alpha S1 casein in Alpine & Saanen goat breeds. Grosclaude and Martin (1997) reviewing the various works on Alpha S1 casein reported that 17 different alleles had been identified that can be associated different concentrations of Alpha S1 CSN in the milk. The alleles are A, B1, B2, B3, B4, C, H, L, M, E, I, F, D, G, O1, O2 and N. Neveu *et al.* (2002) reported the presence of another allele O3 at the Alpha S1 casein locus.

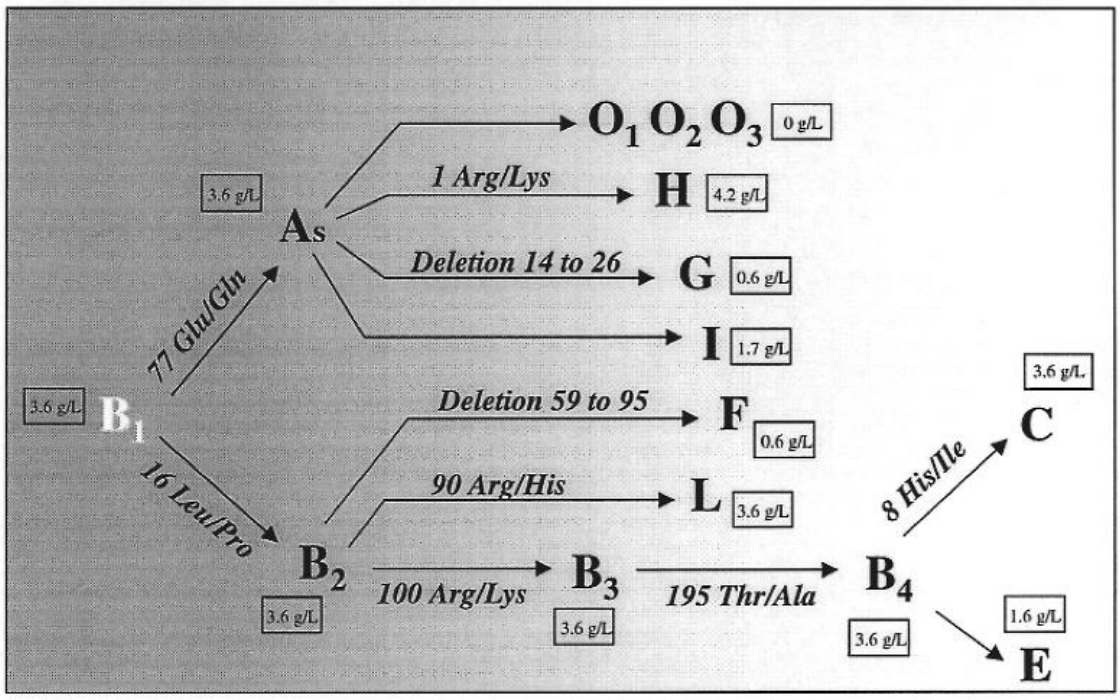



**Fig. 2.1** Phylogeny of goat $\alpha_{s1}$-Cn alleles: molecular differences between corresponding variants and $\alpha_{s1}$-Cn content in milk, per allele (Neveu *et al.*, 2002)

### 2.5.5 Alpha S1 Casein gene amplification

Ramunno *et al*. (2000) amplified this gene with the aim of developing a method for simultaneous typing for the deletion at the ninth exon and the 11 bp insertion in the

downstream intron. Through PCR-RFLP, they observed a/a, b/b, c/c, d/d, a/b, a/c, a/d, b/c, b/d and c/d genotypes in 180 unrelated goats. Their PCR protocol included initial cycle of 97 °C for 2 minutes, 60 °C for 45 seconds and 72 °C for 2 minutes 30 seconds extension for 30 cycles and final extension at 72 °C for 10 minutes.

Sahar Ahmed in 2006, studied Egyptian goat using PCR-RFLP technique and identified ten genotypes similar to that of Ramunno *et al*. (2000). Marini *et al*. (2011) studied Alpha S1casein gene polymorphism in Malaysian goat breeds through PCR method and observed that all these goat breeds showed high frequency of A, B and C allele of Alpha S1 casein gene.

Sztankoova *et al.* (2012) conducted study on two endangered goat breeds of Czech Republic by means of PCR - RFLP and observed allelic frequencies of 0.658 and 0.597 in F allele, and 0.269 and 0.303 in A allele in the two breeds, respectively.

Faruk *et al.* (2013) studied polymorphism of α-S1 gene in goats of Turkey by PCR-RFLP method and determined frequencies of A, B, F and N alleles as 0.375, 0.632; and 0.367, 0.208; 0.017, 0.009; 0.242, 0.000, respectively, in Siirt and Kilis goat populations. Their PCR protocol included; initial denaturation at 94 °C for 15 minutes followed by 10 cycles of 94° C for 30 seconds, 60 °C for 30 seconds, decreasing 1 °C in each cycle and 72 °C for 30 seconds and 25 cycles of 94 °C for 30 seconds, 50 °C for 30 seconds, 72 °C for 30 seconds and final extension step of 72 °C for 10 minutes.

Markovic *et al.* (2009) studied 98 goats of Balkan goat breeds and identified AA, AF and FF genotypes, and A and F alleles through PCR which included following

protocol; denaturation at 94 °C for one minute, annealing at 58 °C for 45 seconds and extension at 72 °C for 1 minute 30 seconds.

### 2.5.6 Association of Alpha S1 Casein gene with production traits

Amongst the different proteins, the concentration of α-S1 Casein in goat milk is an important variable and can be attributed to the α-S1 Casein genotype of the goat. In turn, α-S1 Casein concentrations have been positively correlated with the amount of total solids, total protein and casein in goat milk and have been related to increase in cheese yield, coagulation time and firmness of the curd (Ambrosoli *et al.,* 1988; Pirisi *etal.,* 1994; Clark and Sherbon, 2000).

The high expressing or strong and desirable alleles for cheese making (A, B1, B2, B3, B4, C, H, L and M) produce 3.5 g/L of α-S1 Casein per allele (Brignon *et al.,* 1989; Chianese *et al.,* 1997; Martin *et al.,* 1999; Bevilacqua *et al.,* 2002). The intermediate alleles (E and I) each produce 1.1 g/L of α-S1 Casein (Martin *et al.,* 1999). The low expressing or weak and undesirable alleles for cheese making (F, D and G) produce only 0.45 g/L of α-S1 Casein (Martin *et al.,*1999) and non expressing or null alleles (O1,O2 and N) produce no α-S1 Casein (Cosenza *et al.,*2003). The H, I, L and M variants are rare and have been identified in local southern Italian breeds (Chianese *et al.,* 1997, Bevilacqua *et al.,* 2002).

Grosclaude *et al*. (1987) observed a strong positive correlation between Alpha S1 casein and the whole casein contents of goat milk. They detected 7 alleles at the Alpha S1 casein in Alpine & Saanen goat breeds and found out that total solids, fat, gross yield

were significantly higher in cheese made from goat milk containing high Alpha S1 casein.

Leroux *et al.* (1990) conducted a study on Alpine and Saanen goat breeds and observed that alleles A, B and C had higher expression levels with 3.6 g/L of Alpha S1 casein protein, whereas E allele had intermediate expression level with 1.6 g/L of Alpha S1 casein protein. Both D and F alleles were having low expression level with 0.6 g/L of Alpha S1 casein protein.

Remeuf (1993), in a study on two Greek indigenous goat breeds (Attaki and Skopoles) and Alpine and Saanen breeds of goats, reported that caprine Alpha S1casein gene affected protein and fat composition of milk.

Ramunno *et al.* (2000) concluded that polymorphism at the CSN1S1 makes differences in the level of protein synthesis. Their study revealed that alleles A, B and C with high expression affect 3.6 g/L of α- S1 casein protein, while E with intermediate level affects 1.1 g/L of protein. Likewise, F and G alleles with low expression level affect 0.45 g/L of protein.

Amigo *et al*. (2000) and Bevilacqua *et al*. (2002) found that Alpha S1 casein gene has a relationship with productive traits, milk composition and milk quality.

Sahar Ahmad (2006) observed that Egyptian goat breeds carried low percentage of homozygous alleles required for cheese industry. According to Moioli *et al*. (2007) protein content in goat milk is affected by polymorphism in Alpha S1 casein gene, resulting in significantly different cheese yield.

Kumar *et al*. (2007) conducted Alpha S1 casein gene polymorphism study among Indian goats such as Jamnapari, Jakhrana, Beetal, Barbari, Marwari, Osmanabadi, Chegu, Sirohi, Black Bengal, Gaddi, Surti and local goats of Uttar Pradesh and Madhya Pradesh by using PCR-RFLP technique and observed allele A with frequency of 0.68 to 1.00, B with a frequency of 0.10 to 0.23 and F with frequency of 0.005. In their study, allele A was predominant. They observed genotypes AA, BB, AD and AF.

**Fig. 2.2. Nandidurga kid**

**Fig. 2.3. Osmanabadi doe**

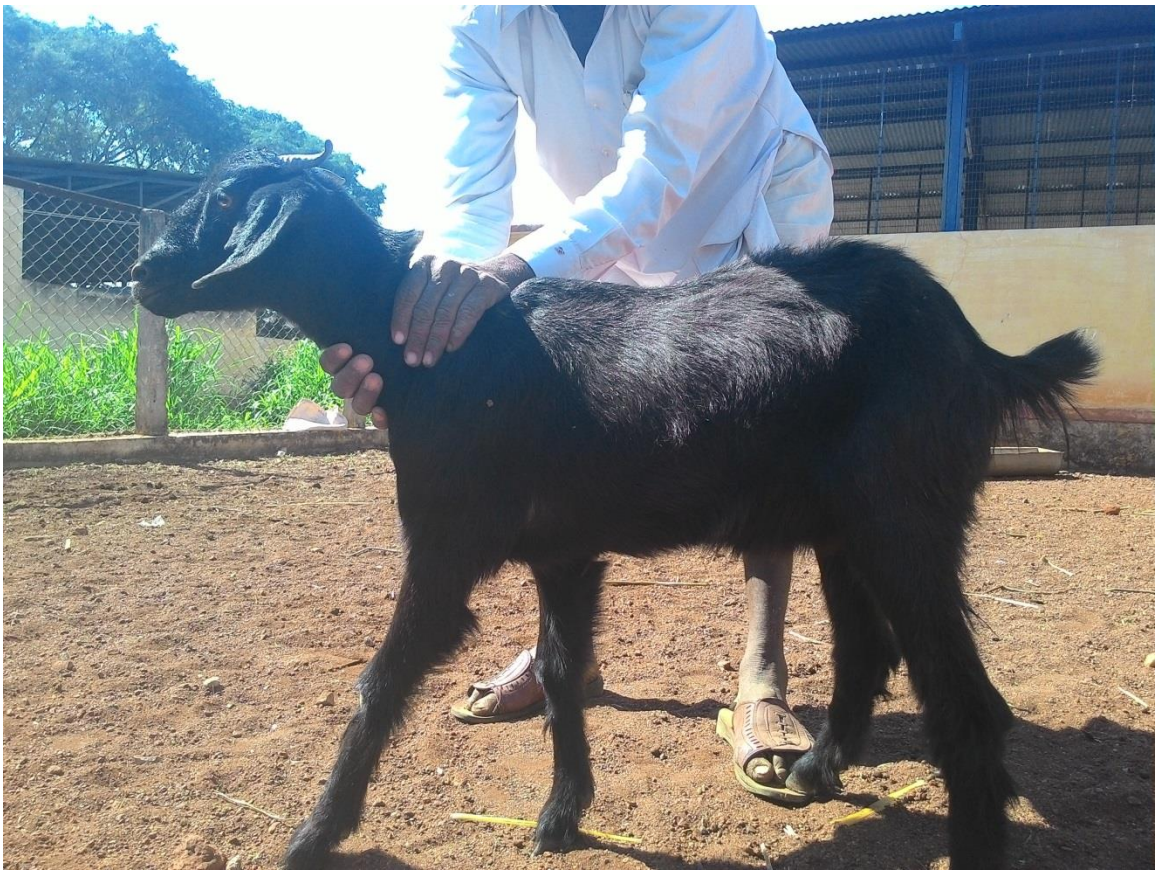


**Fig. 2.4. Bidri buck**

# Materials and Methods

# III. MATERIALS AND METHODS

## 3.1 Materials

### 3.1.1 Experimental Animals

The present study was conducted in Nandidurga goats, randomly selected from the flocks belonging to farmers of Challakere taluk of Chitradurga district and Sira taluk of Tumkur district, whereas Bidri and Osmanabadi goats were selected randomly from Bidar district of Karnataka.

### 3.1.2 Chemicals and Reagents

The chemicals and reagents used in the present study were of molecular biology grade and are presented in Annexures 1 and 3.

### 3.1.3 Primers

The primers for the amplification of desired gene in this study were procured from Sigma (Sumana Enterprises), Bengaluru.

### 3.1.4 Glassware and Plastic ware

All the glassware were thoroughly cleaned and sterilized as per the standard procedures. Micro-centrifuge tubes and micro tips were autoclaved before use.

### 3.1.5 Equipment

The main equipments used in this study are presented in the Annexure 2.

## 3.2 Methods

### 3.2.1 Blood Collection

About 10 ml of blood was collected from each animal aseptically from jugular vein in a vacutainer tube containing 0.5 per cent EDTA. The samples were later stored at 4 °C and DNA was isolated within 24 hrs.

### 3.2.2 DNA Extraction (High salt method)

DNA was isolated from the collected blood samples using a modified High salt method (Miller *et al*., 1988) as follows:

1. Ten ml of blood sample was taken in a 15 ml centrifuge tube into which 5 ml of RBC lysis buffer was added, inverted several times and incubated at 4 °C with occasional mixing.
2. The tube was centrifuged at 4,000 rpm at room temperature for 10 minutes and the Buffy coat was removed along with small quantity of RBC and plasma and transferred to another centrifuge tube. The Buffy coat was resuspended in four volumes of RBC lysis buffer and kept at 4 °C for five minutes with occasional mixing. The mixture was centrifuged at 2,000 rpm for five minutes. This step was repeated three to four times until a clear nuclear pellet was obtained.
3. The resulting pellet was suspended in a five ml of TE (pH 8.0) buffer and carefully disturbed using a micropipette.

4. Proteinase-K and Sodium Dodecyl Sulphate (SDS) were added at final concentrations of 0.2 µg/ ml (20 µl) and 0.5 per cent (0.5 ml), respectively. The mixture was incubated at 37 °C for overnight with occasional mixing.

5. After the incubation, two ml of 5M NaCl solution was added and mixed thoroughly. One volume of Chloroform: Iso amyl alcohol (24:1) was added and mixed thoroughly by brief vortexing on a cyclomixer for one minute. The mixture was centrifuged at 6,000 rpm for 10 minutes. The upper aqueous phase was transferred carefully to a fresh tube using a Pasteur pipette. The above step was repeated twice to get clear aqueous phase.

6. Finally, two volumes of chilled absolute alcohol (ethanol) was added to the supernatant containing DNA at room temperature and inverted several times until the DNA was precipitated.

Precipitated DNA strands were transferred to a micro centrifuge tube containing one ml of ice-cold, 70 per cent ethanol and centrifuged at 2,000 rpm for five minutes. Supernatant was discarded and the pellet was air-dried. Dried DNA pellet was resuspended in 400µl TE buffer and kept at 65 °C for 15 minutes. The samples were then stored at -20 °C till further use.

### 3.3 Determination of Quality and Quantity of Genomic DNA

The quantity and purity of DNA were checked using UV-Spectrophotometer. About 2 µl of DNA was dissolved in 400 µl of TE buffer and optical density (OD) values were measured at 260 nm and 280 nm with distilled water as blank. DNA samples having

the OD value between 1.7 and 2.0 were considered better for PCR reaction. The concentration of DNA was estimated using the following formula.

Concentration of DNA (ng) = OD 260 nm x 50 x 100 (dilution factor)

The stock solution of DNA was then kept in freezer at - 20 $^{\circ}$C till further use.

**3.4 Agarose gel electrophoresis**

Agarose gel electrophoresis (0.8 per cent) was carried out to confirm the quality of isolated DNA. Required amount of Agarose was dissolved in 1 X Tris boric acid EDTA (TBE) buffer to make up to a final concentration of 0.8 per cent. The Agarose was melted in a microwave oven. Ethidium bromide (0.5 µg/ ml) was added to the Agarose gel solution and allowed to cool sufficiently. The gel tray was sealed on either side using adhesive tape and the comb was inserted into the gel. The melted Agarose solution was poured into the gel tray carefully, avoiding formation of air bubbles.

After the gel had polymerized, the comb and the seal on either side of the gel tray were removed carefully. The gel tray was kept in an electrophoresis tank and 1X TBE buffer was poured to submerge the gel in the tank. The DNA samples were mixed at 1/6$^{th}$ volume of 6 X gel loading buffer and loaded into the wells using a pipette. The gel was electrophoresed in 1 X TBE buffer at 100 V at room temperature for half an hour. The gel was visualized under UV transilluminator and photographed using gel documentation system. The quality of the DNA was assessed based on the bands developed. Good quality DNA was used for further study.

### 3.5 Polymerase Chain Reaction (PCR)

#### 3.5.1 PCR amplification of Alpha S1 CSN gene

Part of intron eight, exon nine and part of intron nine region of Alpha S1 CSN gene was amplified by polymerase chain reaction.

#### 3.5.2 PCR primers

The details of the oligo nucleotide primers (Ramunno *et al*., 2000) used for PCR are presented in Table 3.1.

**Table 3.1 Primers used for amplification of selected region of Alpha S1CSN gene**

| Primer | Primer sequence |
|---|---|
| Forward | 5'- TTCTAAAAGTCTCAGAGGCAG-3' |
| Reverse | 3'- GGGTTGATAGCCTTGTATGT-5' |

#### 3.5.3 Composition of PCR reaction mixture

PCR was carried out in a reaction mixture of 25 µl. A master mix for minimum of 10 samples was prepared and aliquoted 24 µl in each PCR tube and finally one µl of genomic DNA was added to make a final volume of 25 µl. The composition of reaction mixture for PCR is given in Table 3.2.

**Table 3.2 Composition of PCR mix**

| Sl. No. | Components | Quantity (µl) |
|---|---|---|
| 1. | Sigma water | 17.5 |
| 2. | Buffer (10 X) with $MgCl_2$ | 2.5 |
| 3. | 2.5 Mm dNTPs (100 µM each) | 1.0 |
| 4. | Forward Primer (395 µl/100uM) | 1.0 |
| 5. | Reverse Primer (461 µl/ µM) | 1.0 |
| 6. | DNA template (50 ng/ µl) | 1.0 |
| 7. | *Taq* DNA Polymerase (3U/ µl) | 1.0 |
| | **Total Volume** | 25.0 |

### 3.5.4 Preparation of template DNA for PCR

The stock DNA was then diluted with autoclaved distilled water to arrive at a final concentration of 50 ng/ µl. This was designated as the working solution.

### 3.5.5 PCR Methodology

1. PCR tubes containing mixture were quickly spun at 10,000 rpm for few seconds.
2. Allele specific PCR conditions were optimized to amplify specific region of gene. Thermal cycles were repeated 30 times to obtain sufficient PCR amplification for PCR-RFLP analysis (Table 3.3).

After the above reaction, the PCR tubes were removed from the thermal cycler and placed on ice till further use. The amplified products were checked in 1.5 per cent

Agarose gel prepared with 1 X TBE buffer. 5µl of amplified products were mixed with 1µl of 6 X gel loading dye and loaded into the wells.

**Table 3.3 Thermal cycling conditions**

| Sl. No. | Step | Temperature (° C) | Time (min) |
|---|---|---|---|
| 1. | Initial denaturation | 94 | 2 |
| 2. | Denaturation | 94 | 1 |
| 3. | Annealing | 60 | 1 |
| 4. | Extension | 72 | 2 |
| | Repeated the cycle for 30 times | | |
| 5. | Final Extension | 72 | 10 |
| 6. | Hold | 4 | ∞ |

### 3.5.6 Agarose Gel Electrophoresis to verify PCR product amplification

1. The required amount of Agarose (0.45 g) was weighed in a conical flask and 30 ml of 1X TBE buffer was added to prepare 1.5 per cent solution. The solution was completely melted in a microwave.
2. The solution was allowed to cool to 60 ° C and Ethidium bromide (0.5µg/ ml) was added and mixed thoroughly. The comb was positioned 1 mm above the plate so that a complete well was formed when the Agarose was added.
3. The gel was allowed to set for 30 - 45 minutes at room temperature. Both the comb and tape were removed carefully and the gel was mounted in the electrophoresis tank with TBE buffer.

4. Five µl of PCR product from each tube was mixed with 1µl of 6X gel loading dye and electrophoresed on 1.5 per cent Agarose gel containing ET Br at 100 V for 45 minutes in 0.5X TBE buffer.
5. A DNA marker (100 bp DNA ladder) was loaded and run along with the samples in the same gel.
6. After 45 minutes, the power supply was turned off and the lid was removed from the gel tank. The gel was visualized for the presence of a single compact band of expected size under the UV Trans – illuminator and photographed using gel documentation system.
7. The quality of the DNA was assessed based on the bands developed. Good quality DNA was used for further analysis for PCR - RFLP technique.

### 3.6 Restriction Enzyme used for PCR-RFLP

The restriction endonuclease used for PCR-RFLP was *Xmn* I, which is isolated from *Xanthomonos manihotis* species and recognizes the sequence GAAGC/AGTTC and generates fragments with blunt ends.

#### 3.6.1 Restriction digestion of PCR products

The *Xmn* I restriction enzyme was utilized to determine the polymorphism in the amplified PCR products. The digested fragments were resolved on Agarose gel electrophoresis. The components for restriction digestion of PCR product with *Xmn* I is given in Table 3.4. Immediately after addition of restriction enzyme at the rate of 0.5 µl,

the digested products were placed in 0.2 ml PCR tubes and incubated overnight at 37 °C in a water bath. They were later subjected to Agarose gel electrophoresis.

**Table 3.4 Optimized RE digestion mixture**

| Sl. No. | Reaction components | Quantity (µl) |
|---|---|---|
| 1. | Sigma water | 25.0 |
| 2. | 10 X buffer for RE | 5.0 |
| 3. | RE | 0.5 |
| 4. | PCR product | 20.0 |
| | Total volume | 50.0 |

### 3.6.2 Agarose gel electrophoresis of RFLP products

The restriction enzyme digested PCR products were electrophoresed on 4 per cent Agarose gel. The restriction pattern resolved by Agarose gel electrophoresis was photographed by Gel documentation system and the fragment sizes were determined in comparison with DNA molecular weight marker.

## 3.7 Determination of gene frequency and genotype frequency

The genotype was determined by scoring the bands under the gel doc system and the gene and genotype frequencies calculated.

## 3.8 Sequencing of PCR products

Representative samples showing different RFLP patterns were selected for sequencing. PCR products were sent to Genotypic Pvt. Ltd, Bengaluru for sequencing and later analyzed.

### 3.8.1 DNA sequence analysis

The DNA sequence was analyzed by Basic Local Alignment Search Tool (BLAST) at National Centre for Biotechnology Information (NCBI) website for homology. The query sequence was compared with the database available in the public domain.

# Results

# IV. RESULTS

The present study was aimed at polymorphic analysis of Alpha S1CSN gene in Nandidurga goats from Tumkur and Chitradurga districts and Osmanabadi and Bidri goats from North Karnataka.

## 4.1 Isolation of genomic DNA

The DNA was isolated from blood samples of 85 Nandidurga goats, 35 Osmanabadi and 50 Bidri goats by adopting the High salt DNA extraction procedure of Miller *et al.* (1988).

### 4.1.1 Quantity and quality of DNA

On an average, about 300 µg of pure genomic DNA was extracted from each blood sample of 10 ml. The OD 260/ 280 nm ratio ranged between 1.7 and 1.9., which indicated good purity. The quality of isolated DNA was confirmed by Agarose gel electrophoresis (0.8 per cent). The DNA was observed to be of high molecular weight and appeared as single band without sheared fragments (Figs. 4.1 to 4.3).

## 4.2 PCR amplification of Alpha S1 CSN gene

The PCR reactions were set up for the amplification of part of intron 8, exon 9 and part of intron 9 region of α S1 CSN gene from genomic DNA using oligo nucleotide primers (Ramunno *et al.*, 2000).

### 4.2.1 Standardization of PCR procedure

The PCR technique was initially optimized for the different PCR components, viz., template DNA concentration, dNTPs concentrations and annealing temperatures. One µl of genomic DNA, 200 µM dNTPs and annealing temperature of 60 °C was found to be optimal to amplify the desired PCR product. Ten pico mole of each of the primers per reaction volume of 25 µl was used throughout the experiment as an optimum primer concentration. For complete and uniform amplification, 30 cycles of PCR was found to be ideal.

The oligo nucleotide primers employed by Ramunno *et al.* (2000) were used for the amplification of Alpha S1 CSN gene by PCR technique. PCR products of approximately 213 bp or 224 bp were amplified among the animals studied (Figs. 4.4 and 4.5).

### 4.3 PCR-RFLP analysis of Alpha S1CSN gene using *Xmn*I

Both the PCR amplified products of 213 and 224 base pair length were digested by restriction endonuclease enzyme *Xmn* I. The 213bp product was cut into two fragments of sizes 150 bp and 63 bp. All the samples tested yielded similar results. The allele exhibiting these two fragments was denoted as A and animal was genotyped as AA. The 224 bp product was cut into two fragments of size 161 bp and 63 bp. The allele exhibiting these two fragments was denoted as B and animal was genotyped as BB. This indicated the presence of only one restriction endonuclease site for *Xmn* I, which was also confirmed by DNA sequencing. These RFLP patterns are presented in Fig. 4.6.

The RFLP analysis carried out in the Osmanabadi and Bidri goats revealed similar results as observed in Nandidurga goats. Upon RFLP analysis with *Xmn*I enzyme, similar genotypic patterns as observed in the Nandidurga goats were also revealed in the animals studied in the two goat breeds but with different frequencies. These RFLP patterns are presented in the Figs. 4.7 and 4.8.

### 4.4 Allelic and genotypic frequencies

The allelic patterns of α- S1 CSN obtained in Nandidurga goats are presented in Table 4.1. Out of the 85 blood samples analyzed, 76 were of A type and nine were of B type. The frequencies were 0.894 and 0.116 for A and B alleles, respectively, as well as for the genotypes AA and BB (Table 4.2). Of the 35 Osmanabadi goats, 31 exhibited AA type and 4 were of BB type and the frequencies were 0.886 and 0.114 for A and B alleles as well as for the two genotypes, respectively (Tables 4.5 and 4.6). Amongst the 50 Bidri goats, 47 animals were of AA type and 3 were of BB type and the corresponding frequencies were 0.94 and 0.06 (Tables 4.3 and 4.4).

### 4.5 DNA sequencing

Based on RFLP pattern the allele A and B was selected for DNA sequencing. DNA sequencing was outsourced to Genotypic Pvt. Ltd, Bengaluru.

#### 4.5.1 DNA Sequence analysis

DNA sequencing revealed the insertion of eleven nucleotides (CGTAATGTTTC) in ninth intron which was responsible for BB genotype. The sequence data was subjected to NCBI BLAST and was compared with other data

available in the public domain for goat and other species. It was confirmed that the amplified product was that of α- S1 CSN gene. The BLAST analysis of the DNA sequence of th α- S1 CSN gene fragment revealed 100 per cent identity with that of *Capra hircus* gene encoding α- S1 CSN. The BLAST analysis also had 90 per cent identity with that of *Ovis aries*, 88 per cent identity with *Bos taurus* and 95 per cent identity with *Bubalus bubalis and* 85% with *Bubalus bubalis.* (Table 4.7 to 4.9).

**Table 4.1: Allelic patterns of Alpha S1 CSN gene digested with XmnI restriction endonuclease enzyme in Nandidurga goat**

| Sl. No. | Allele type | Fragment size (bp) | Number of animals (n=85) |
|---|---|---|---|
| 1. | AA | 150, 63 | 76 |
| 2. | BB | 161, 63 | 9 |

**Table 4.2: Genotype and allelic frequency of Alpha S1 CSN gene/ *Xmn*I polymorphism in Nandidurga goat**

| Genotype | Observed frequency | Alleles | Allelic frequency |
|---|---|---|---|
| AA | 0.894 | A | 0.894 |
| BB | 0.116 | B | 0.116 |

**Table 4.3: Allelic pattern of Alpha S1 CSN gene digested with *Xmn*I restriction endonuclease enzyme in Bidri goat**

| Sl. No. | Genotype | Fragment size (bp) | No. of animals |
|---|---|---|---|
| 1. | AA | 150, 63 | 47 |
| 2. | BB | 161, 63 | 3 |

**Table 4.4: Genotype and allelic frequency of Alpha S1 CSN gene/ XmnI polymorphism in Bidri goat**

| Genotype | Frequency | Alleles | Allelic frequency |
|---|---|---|---|
| AA | 0.94 | A | 0.94 |
| BB | 0.06 | B | 0.06 |

**Table 4.5: Allelic pattern of Alpha S1 CSN gene digested with XmnI restriction endonuclease enzyme in Osmanabadi goat breed**

| Sl. No. | Genotype | Fragment size (bp) | No. of animals |
|---|---|---|---|
| 1 | AA | 150,63 | 31 |
| 2 | BB | 161,63 | 4 |

**Table 4.6: Genotype and allelic frequency of Alpha S1 CSN gene/ XmnI polymorphism in Osmanabadi goat breed**

| Genotype | Observed frequency | Alleles | Allelic frequency |
|---|---|---|---|
| AA | 0.886 | A | 0.886 |
| BB | 0.114 | B | 0.114 |

**Table 4.7: Result of BLASTn showing the percentage identity of the part of intron 8, exon 9 and part of intron 9 region of the Alpha S1 CSN gene locus of Nandidurga goat with other species**

| Accession No. | Description | Max. score | Total score | Query coverage (per cent) | Max. Identity (per cent) |
|---|---|---|---|---|---|
| AJ504710.2 | *Capra hircus* csn1S1 gene for alpha s1 casein, allele A, exons 1-19 | 394 | 394 | 100 | 100 |
| X56462.1 | *Capra hircus* as1-casein gene, partial cds, exons 9-11 | 394 | 394 | 100 | 100 |
| JN560175.1 | *Ovis aries* alpha s1 casein (CSN1S1) gene, complete cds | 287 | 287 | 100 | 90 |
| X59856.2 | *Bos taurus* csn1s1 gene for alpha-S1-casein | 261 | 261 | 100 | 88 |
| EF133464.1 | *Bubalus bubalis* alpha-S1 casein (CSNS1) gene, exon IX and partial cds | 241 | 241 | 72 | 95 |
| FJ429670.1 | *Bison bison* alpha s1 casein (CSN1S1) gene, intron 9 | 134 | 134 | 61 | 85 |

**Table 4.8: Result of BLASTn showing the percentage identity of the part of intron 8, exon 9 and part of intron 9 region of the Alpha S1 CSN gene locus of Bidri goat with other species**

| Accession No. | Description | Max. score | Total score | Query coverage (per cent) | Max. Identity (per cent) |
|---|---|---|---|---|---|
| X56462.1 | *Capra hircus* as1-casein gene, partial cds, exons 9-11 | 394 | 394 | 100 | 100 |
| AJ504712.2 | *Capra hircus* csn1S1 gene for alpha s1 casein, allele N, exons 1-19 | 387 | 387 | 100 | 99 |
| FJ440848.1 | *Ovis aries* East Friesian Milk sheep a- s1 CSN gene, CSN1S1-H allele, partial cds | 145 | 145 | 36 | 100 |
| FJ440847.1 | *Ovis aries* Merino land sheep alpha s1CSN1S1 gene, C" allele, exons 7 through 9 and partial cds | 145 | 145 | 36 | 100 |
| X59856.2 | *Bos taurus* csn1s1 gene for alpha-S1-casein | 261 | 261 | 100 | 88 |
| EF133464.1 | *Bubalus bubalis* alpha-S1 casein (CSNS1) gene, exon IX and partial cds | 241 | 241 | 72 | 95 |

**Table 4.9: Result of BLASTn showing the percentage identity of the part of intron 8, exon 9 and part of intron 9 region of the Alpha S1 CSN gene locus of Osmanabadi goat with other species**

| Accession No. | Description | Max. score | Total score | Query coverage (per cent) | Max. Identity (per cent) |
|---|---|---|---|---|---|
| AJ504710.2 | *Capra hircu*s csn1S1 gene for alpha s1 casein, allele A, exons 1-19 | 394 | 394 | 100 | 100 |
| X59856.2 | *Bos taurus* csn1s1 gene for alpha-S1-casein | 261 | 261 | 100 | 88 |
| JN560175.1 | *Ovis aries* alpha s1 casein (CSN1S1) gene, complete cds | 287 | 287 | 100 | 90 |
| FJ440847.1 | *Ovis aries* Merino land sheep alpha s1CSN1S1 gene ,C" allele, exons 7 through 9 and partial cds | 145 | 145 | 36 | 100 |
| FJ429670.1 | *Bison bison* alpha s1 casein (CSN1S1) gene, intron 9 | 134 | 134 | 61 | 85 |

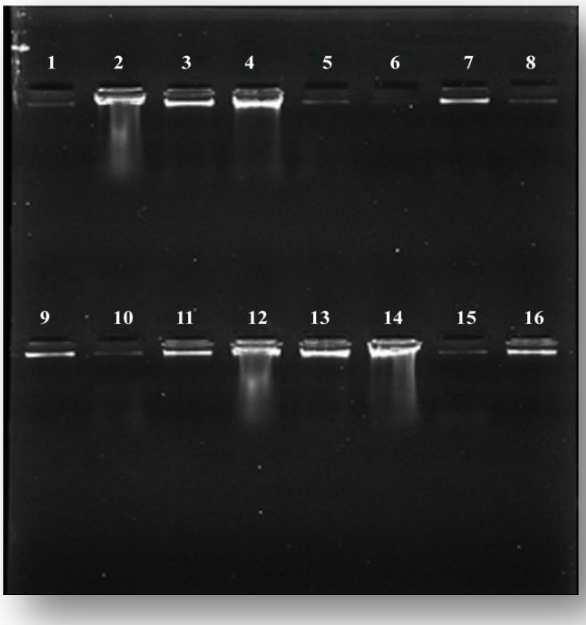



**Fig. 4.1. Agarose gel electrophoresis of genomic DNA of Nandidurga goat**

**Lanes 1-16; Genomic DNA**

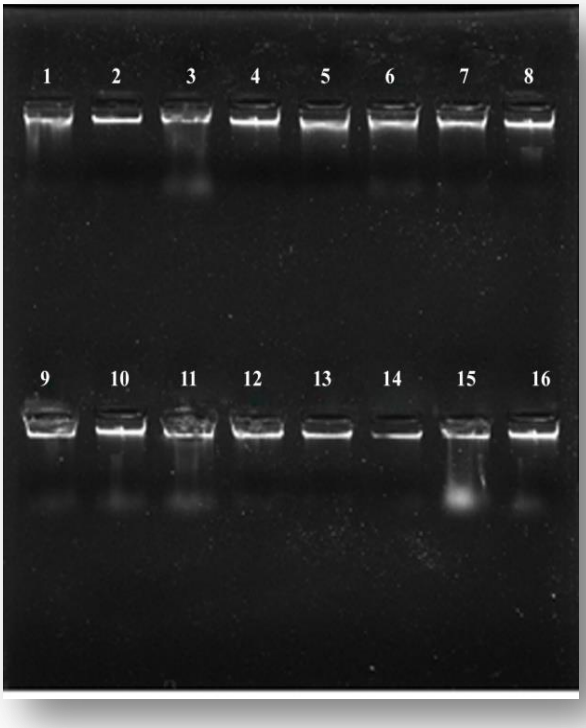



**Fig. 4.2. Agarose gel electrophoresis of genomic DNA of Bidri goat**

**Lanes 1-16; Genomic DNA**

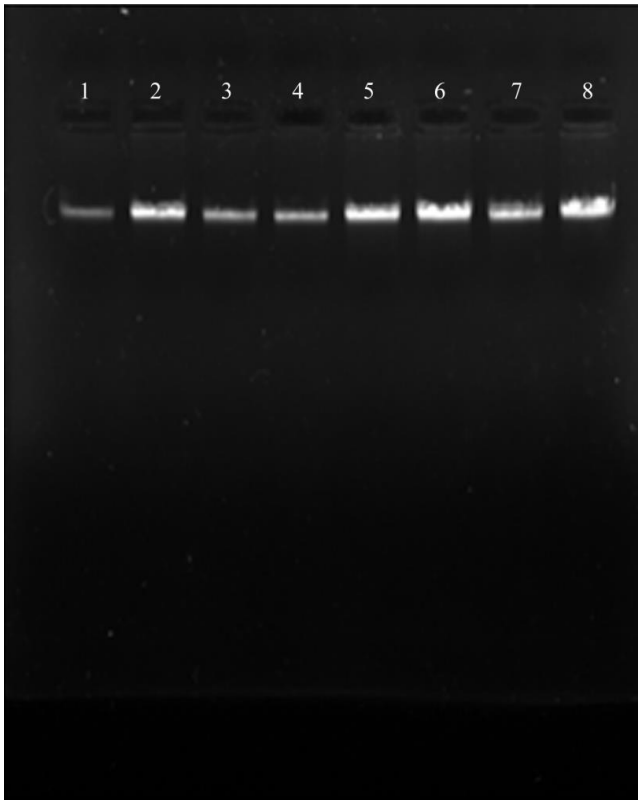



**Fig. 4.3. Agarose gel electrophoresis of genomic DNA of Osmanabadi goat**

**Lanes 1-8: Genomic DNA**

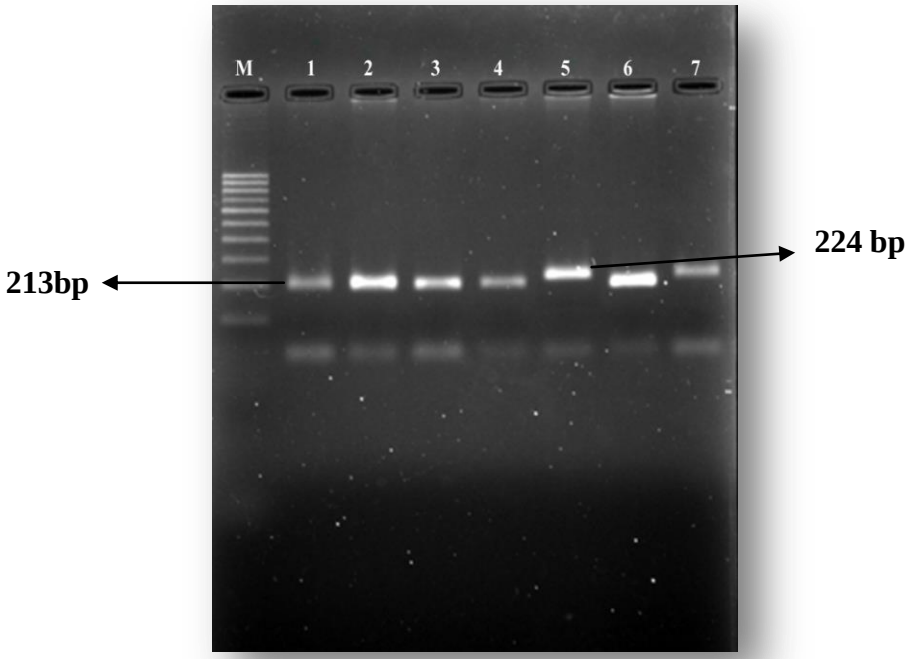



**Fig. 4.4. PCR amplification products of Alpha S1 CSN gene of Nandidurga goat**

**Lane M : 100 bp DNA ladder**

**Lanes 1-7 : PCR amplicons**

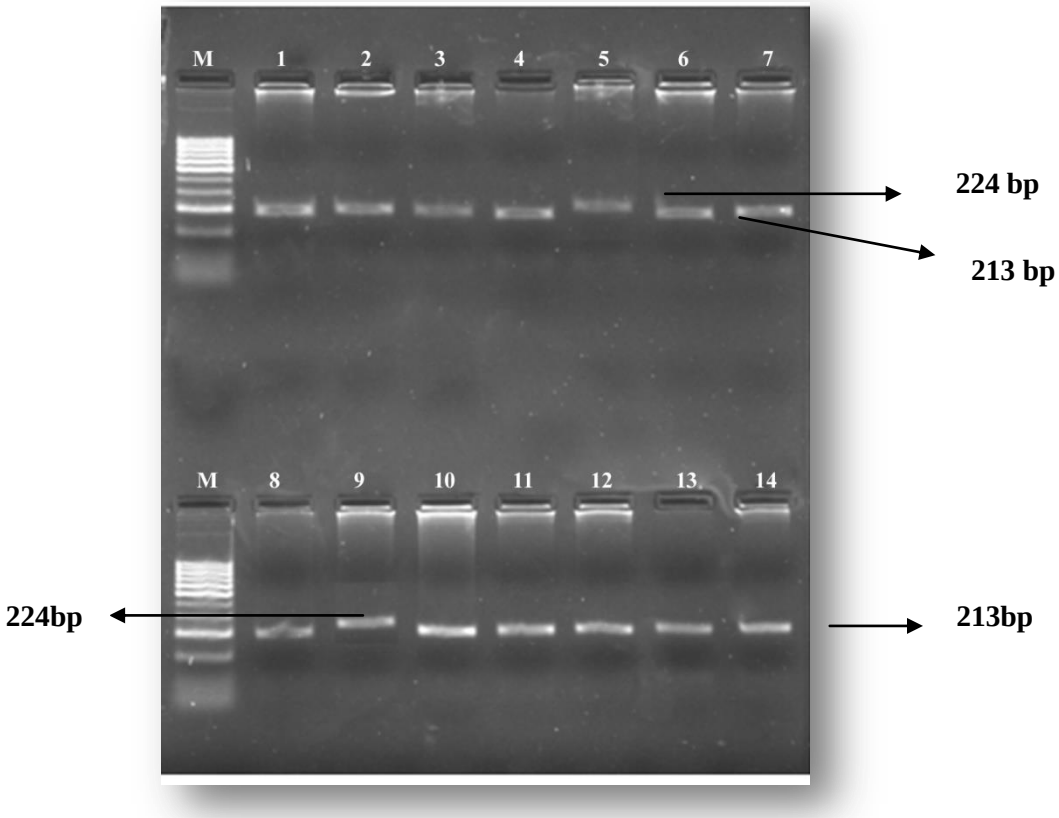



**Fig. 4.5. PCR amplification products of Alpha S1 CSN gene of Bidri and Osmanabadi goat**

**Lane M : 100 bp DNA ladder**

**Lanes 1-7 : PCR amplicons of Bidri goat samples**

**Lanes 8-14 : PCR amplicons of Osmanabadi goat samples**

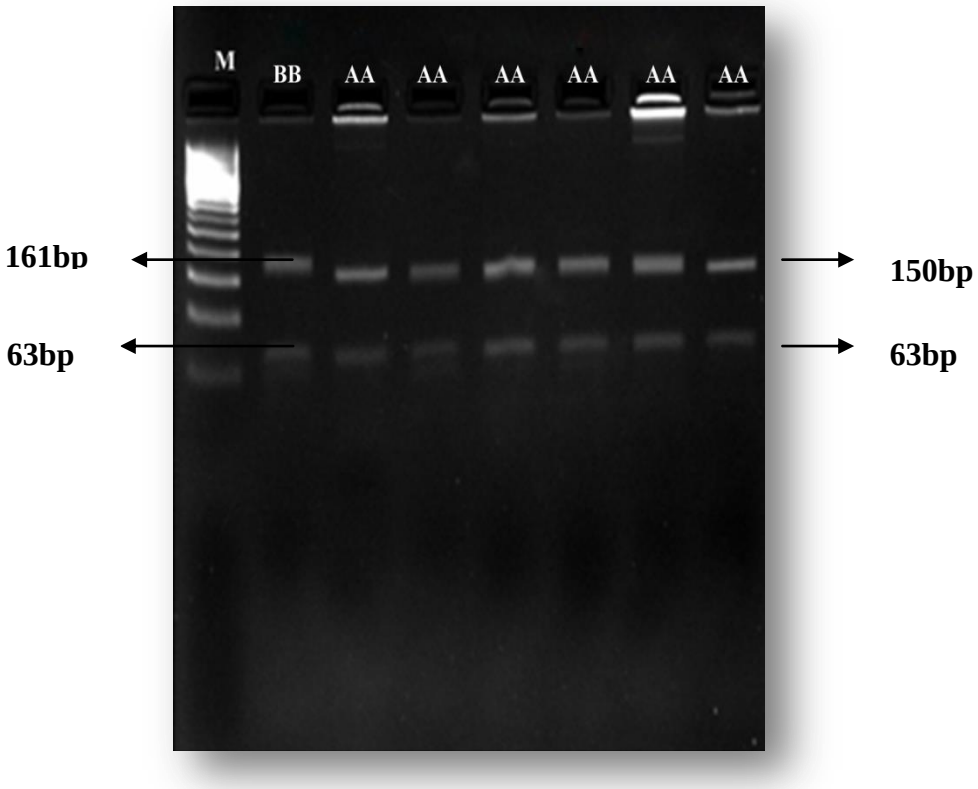



**Fig. 4.6. PCR-RFLP of Alpha S1 CSN gene with XmnI in Nandidurga goat**

**M- 50 bp DNA ladder**

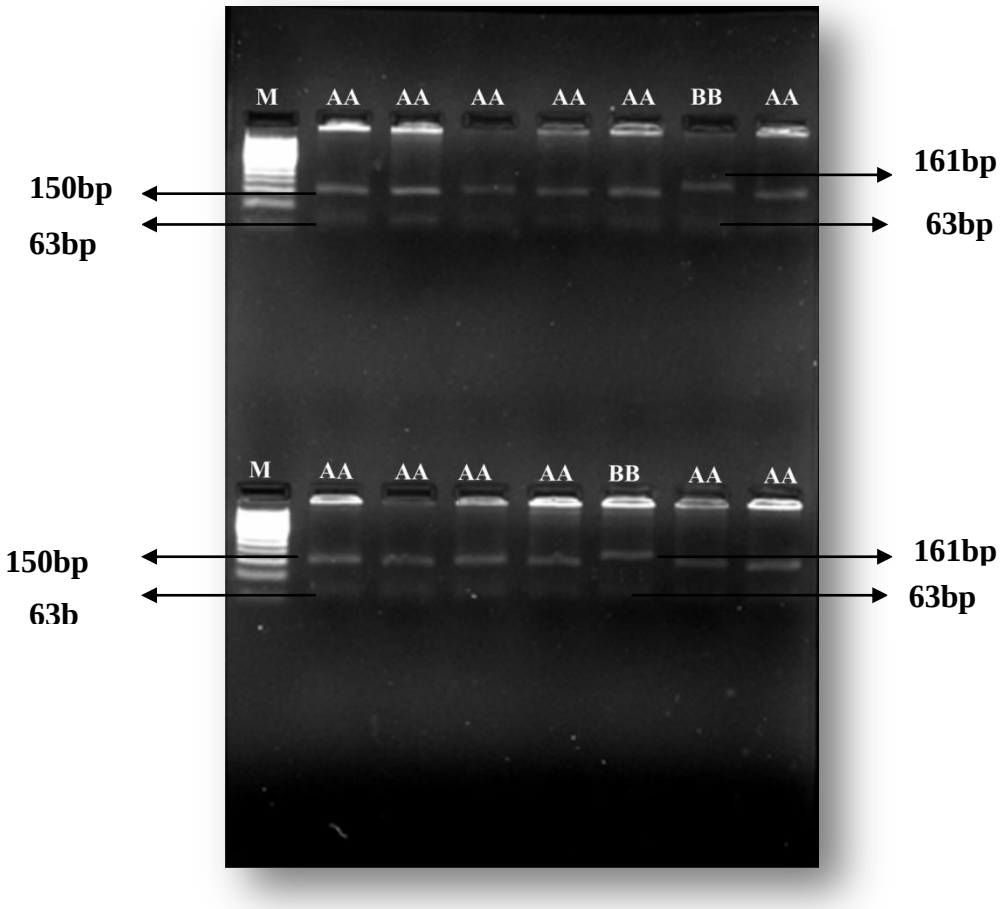



**Fig. 4.7. PCR-RFLP of Alpha S1 CSN gene with XmnI in Bidri**

**M- 50 bp DNA ladder**

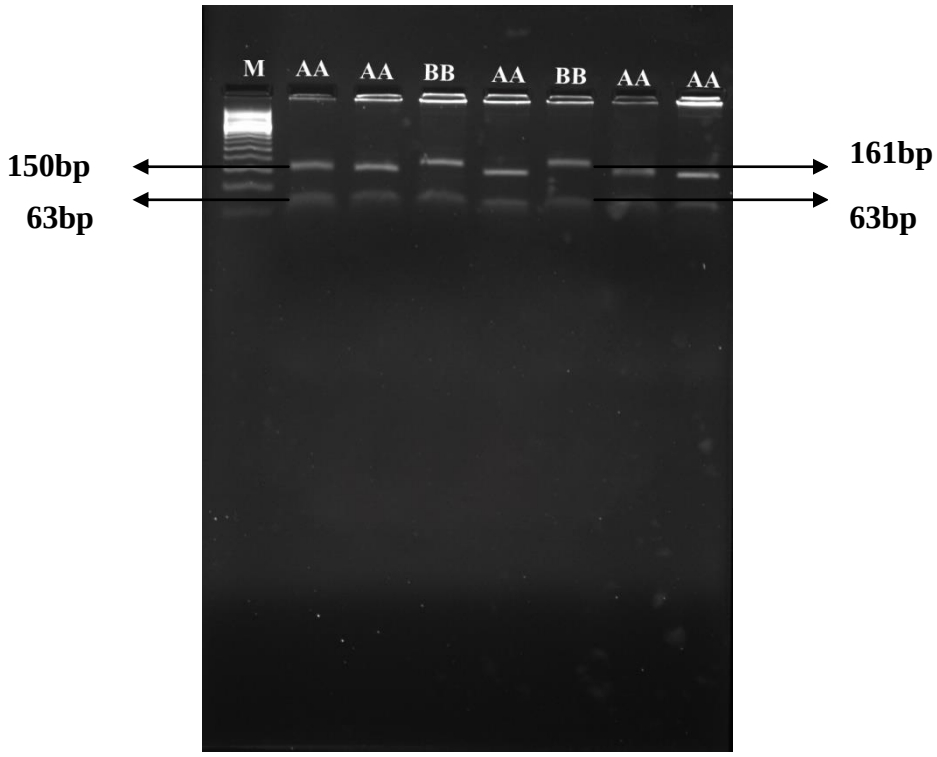



**Fig. 4.8. PCR-RFLP of Alpha S1 CSN gene with Xmn I in Osmanabadi goat**

**M- 50 bp DNA ladder**

# Discussion

# V. DISCUSSION

Milk quality or composition, among other traits, is an important economic trait to be considered for selection and breeding for genetic improvement among goats. Studies in the recent past have been concentrated on the milk quality with more emphasis being laid on the presence or absence of certain components in relation to human health. Casein genes have been found to determine milk composition, milk yield and milk processing qualities.α-S1 CSN is considered as a vital marker gene influencing milk traits and variation in locus of this gene would provide other criteria of early selection in goat. The present study was therefore taken up with the objective of studying the existence of genetic polymorphism of α- S1 CSN gene in Nandidurga, a non-descript goat variety of Karnataka. The study also included Osmanabadi, a recognized goat breed also seen in some parts of the state as well as Bidri goats which are prevalent in North Karnataka.

## 5.1 Isolation of genomic DNA

Several techniques are available to isolate genomic DNA from blood, milk and other biological specimens. Depending upon the facilities available, various techniques are followed by different laboratories. The Miller's High salt method is safer to phenol-chloroform method since it does not involve phenol, which is a corrosive. The High salt method yield has also been recorded to yield more DNA as compared to phenol-chloroform method (Annapoorani, 1996), as it avoids the loss of DNA in phenol phase.

Thus, the High salt method adopted by Miller *et al.* (1988) was used in the present study to isolate genomic DNA from blood samples. This procedure was found to be

suitable for isolation of DNA from blood samples of goat (Folch *et al.*, 1994), sheep (Montgomery and Sise, 1990) and cattle (Gwakisia *et al.*, 1994; Gelhaus *et al.*, 1995).

### 5.1.1 Yield and quality of DNA

Using the High salt method, about 290 to 310 µg of pure genomic DNA was extracted from each of 10 ml blood sample.

Arshan Shahid (2013) and Aiswarya Venu (2013) reported yield of about 300 µg of DNA per 10ml of blood from Nandidurga goats by using Miller's high salt method, in the same laboratory.

However, Chitra (2002) reported yield of 231.09 ±11.65 µg of DNA from 5 ml of goat blood. The ratio of optical density at 260 and 280 nm was more than 1.7. Mathew (2004) and Seena (2006) reported yields of 350.05 ± 10.05 µg and 357.05 µg of DNA, respectively, from 5 ml of blood of Malabari goats.

Similarly, Montgomery and Sise (1990) reported yield of 640 µg DNA from 20 ml of sheep blood with high salt method. Mitra (1994) obtained higher yields of 343 to 693 µg with an average of 403 µg per 10 ml of cattle and buffalo blood.

Senthil (1995) reported 450 µg to 800 µg with an average of 625 µg per 15 ml of cattle blood using similar protocol. Annapoorani (1996) obtained a yield of DNA ranging from 210 to 602 µg with an average of 400 µg per 10 ml of buffalo blood.

Aravindakshan (1997) reported pure DNA in the range of 246 to 572 µg with an average of 360 µg per 10 ml of cattle blood and 300 to 707 µg with an average 452 µg

per 10 ml of buffalo blood. The quality of the extracted DNA was found to be satisfactory.

Thus the variations may be attributed to the conditions existing in the different laboratories.

### 5.2 PCR-RFLP of Alpha S1 CSN gene

#### 5.2.1 PCR technique

The PCR technique as described by Ramunno *et al.* (2000) was followed for amplifying selected region of Alpha S1 CSN gene.

#### 5.2.2 Amplification of α-S1 CSN gene by PCR

The PCR conditions are vital for successful amplification of target sequence in the DNA and for obtaining specific quantity of desired product.

The optimum parameters used for the amplification of the α-S1 CSN gene are given Table 3.3. Annealing temperature and time optimized in this study was 60 °C for 60 seconds for 30 cycles. Bozkaya *et al.* (2013) and Marini *et al*. (2011) reported annealing temperature of 60 °C for 30 seconds optimum for amplifying the α- S1 CSN gene whereas, Bozidarka *et al*. (2009) found annealing temperature of 58 °C for 45 seconds optimum for amplification. Similarly, Ramunno *et al.* (2000) and Soares *et al.* (2009) reported 60 °C for 45 seconds and 62 °C for 45 seconds ideal for amplifying this gene, respectively. The variation in annealing temperature between laboratories indicates that the annealing temperature needs to be standardized for different laboratory conditions.

### 5.2.3 Alpha S1 CSN gene polymorphism

The specific region of α-S1 CSN gene was amplified by using standardized PCR protocol. The size of amplified products was not the same for all animals studied indicating variability of the α-S1 CSN gene region in the goats studied.

### 5.2.4 Restriction patterns

The PCR-RFLP technique was followed to detect variation within the amplified genomic portion. The restriction enzyme *Xmn*I which has a higher potential for detecting polymorphism was used to digest the PCR amplicons. Two patterns were resolved from XmnI restriction endonuclease digested PCR amplicons.

### 5.2.5 Restriction patterns with *Xmn*I

Upon digestion**,** the 213 bp PCR products were yielded two fragments of sizes 150 bp and 63 bp. This pattern was observed in all the animals of three breeds showing 213 bp PCR products. This locus was assumed to be controlled by one allele, A, which resolved two fragments. This RFLP pattern was named as AA type. The RE cleavage of PCR product of size 224 bp length resulted in two fragments of size 161 bp and 63 bp. This pattern was observed in all the animals of three breeds showing 224 bp PCR products. This pattern was presumed to be controlled by one allele, B, Which resolved two different fragments. The restriction pattern was designated as BB type. Study indicated the presence of one restriction endonuclease site in the animals studied.

Leroux *et al.* (1990) observed alleles A, E, F, O and O’ and genotypes AA, AF, AE, AO and EE in Alpine and Saanen goat breeds through PCR - RFLP technique.

Sahar Ahmed (2006) studied genetic polymorphism of Egyptian goats such as Zaraibi, Baladi, Sahrawl and Damascus and noticed alleles A, B, D and C with genotypes AA, BB, DD, AC and BD in Damascus breed. In other three goat breeds genotype AC and BD were seen. BD genotype was predominant in Zaraibi and Sahrawl whereas genotype AC was predominant in Baladi goat breed.

Chessa *et al.* (2007) through PCR - SSCP conducted genetic study in 8 different goat breeds of different continent of the World and found alleles A and B in all breeds. Allele F was noticed only in Hair goat and Nubian while allele B' found only in Yerkoy, an Angora goat and Hair goat.

Maltova *et al.* (2007) conducted polymorphic study on Czech goat breeds namely Brown short hair and White short hair by using PCR - RFLP technique. They observed alleles F (0.658; 0.597), A (0.269; 0.303), E (0.084; 0.085) and E1 (0.019; 0.015) in Brown short hair and White short hair, respectively.

Marie *et al.* (2007) made study on Alpine and Saanen goat breeds by means of PCR-RFLP method in which allele E with frequencies of 0.35 ; 0.48 and F with frequencies of 0.3;0.28 in Alpine and Saanen respectively. However, allele A or B were not observed in these two breeds.

Kumar *et al.* (2007), conducted polymorphism study among Indian goats such as Jamnapari, Jakhrana, Beetal, Barbari, Marwari, Osmanabadi, Chegu, Sirohi, Black Bengal, Gaddi, Surti, local goat of Uttar Pradesh and Madhya Pradesh by using PCR - RFLP technique and observed alleles A with frequency of 0.68 to 1.0, B with a frequency

of 0.1 to 0.23and F with frequency of 0.005 In their study allele A was predominant. They observed genotypes AA, BB, AD and AF. AB genotype was not found. This study is in agreement with the present study in that allele A was the predominant and genotype AB was not found. However, alleles D and F were not observed in present study.

Similarly, Bozidarka *et al.* (2009) in a study on two flocks of Balkan goat breeds observed flock I has allele A (0.664) and F (0.336) where as flock II had allele A with frequency 0.557 and F with 0.423. They reported presence of genotypes AA (44 per cent; 33.3 per cent), FF (11.3 per cent; 18 per cent) and AF (44.6 per cent; 48.8 per cent) in these goat flock I and II, respectively.

Marini *et al.* (2011) studied polymorphism in Boer, Boer-feral, Katjang and Jamnapari of Malaysia using PCR-RFLP technique and observed alleles A, B,C and F among which predominant alleles were A and C.

Bozkaya *et al.* (2013), in a polymorphism study in Kilis, Sanliurfa and Siirt goat breeds of Turkey by PCR-RFLP technique, reported alleles A, B, F and N where A was the most dominant allele in all three breeds. They observed genotypes AA, AN, AB, NN and BN.

In the present study, only two alleles, A and B and two genotypes, AA and BB, were found in the three goat flocks studied. The presence of A and B alleles is in conformity with the reports of earlier workers on α-S1 CSN gene in goats. However, the other alleles such as E, C, D, F, O and O', as well as other resultant genotypes reported in the other breeds were not observed in the present study. These variations indicate a

breed wise distribution of the Alpha S1CSN gene in the different goats**.** The absence of AB genotype is in agreement with study of Kumar *et al*. (2007) in several Indian goat breeds. This needs to be verified by crossing parents with AA and BB genotypes and studying their offspring.

**5.3 Restriction Fragment Length**

In the present study, allele A with two fragments of sizes 150 and 63 bp and allele B with two fragments of sizes 161 and 63 bp were identified.

However, Bozkaya *et al* (2013) identified genotypes AA, AB, AN and BN with corresponding fragment length of 150, 63; 161, 63; 212 and 212, 63. Similarly Ramunno *et al.*(2000) observed genotypes AA, BB, CC, DD, AB, AC, AD and BC with corresponding fragment lengths of 150,63; 161,63; 212, 223; 161,150,63; 212,150,63; 223,150,63 and 212,161,63. Likewise, Kumar *et al.* (2007), observed genotypes AA, BB, AD and AF with a corresponding fragment lengths of 150, 63; 161, 63; 212, 150, 63 and 223, 150, 63. Thus the present study is in conformity with these earlier reports. However, only genotypes AA and BB were observed in the present study. The RE XmnI had been used by all the above workers.

Sahar Ahmad (2006) identified genotypes AA, BB, DD, AC and BD with corresponding restriction fragment length of 150, 161, 223, 150 and 212 and 161 and 223bp.

**5.4 Casein alleles and protein production:**

Ramunno *et al.* (2000) classified the different alleles into three groups based on expression levels. The alleles A, B and C were put into high expression, allele E into intermediate and alleles F and G into low level groups, with corresponding levels of 3.5 g/ L, 1.1g/L and 0.45 g/L of Alpha s1 casein protein in milk.

Balteanu (2011), in a study conducted on Carpathian goat breed, observed that genotype AA significantly influenced yield of protein, fat, casein and cheese, as compared to genotypes EE and FF.

Several earlier workers (Martin *et al.,* 1999; Leroux *et al.,* 1990; Roncada *et al.,* 2002) have opined that the milk of animals with strong alleles such as A, B and C is associated with increased casein content, and can be utilized for commercial dairy industry, where as mild alleles such as D, F, E, O, O', N have lesser influence on casein protein synthesis and is more suitable for allergic people.

However, it should be noted that the predominance of allele A provides other criteria of selection in addition to traditional selection methods in small ruminants since A allele is associated with higher expression level of Alpha S1 CSN protein (Ramunno *et al.*, 2000).

**5.5 DNA sequence analysis of α-S1 CSN gene**

The amplified products of part of intron 8, exon 9 and intron 9 of Alpha S1 CSN gene in Nandidurga goat, Bidri goat and Osmanabadi goat was sequenced at DNA sequencing facility, Genotypic Technology Pvt. Ltd Bengaluru. Addition of 11

nucleotides cgtaatgtttc in ninth intron region as revealed by DNA sequencing probably resulted in allele B.

The DNA sequencing data was subjected to NCBI BLASTn. The sequence of the present study was compared with that available in public domain. All sequences shared high homology to the published caprine Alpha S1 CSN gene sequences. A 100 per cent homology was observed between *Capra hircus* sequence published and the present sequence data. There was 90 per cent homology with Ovine Alpha S1 CSN gene, 88 per cent with that of Cattle and 95 per cent with Buffalo Alpha S1 CSN gene.

This is one of the first studies in non-descript goats like Nandidurga and Bidri. To validate this finding, further study with large number of samples in these goats is essential to confirm genetic polymorphism and to know the association if any with the milk composition traits.

## CONCLUSION

1. The Alpha S1 CSN gene polymorphism was carried out for the first time in Nandidurga and Bidri goats and the animals were found to possess alleles A and B and genotypes AA and BB. No AB genotypes were observed in these animals.

2. The presence of allele A in higher frequency may be suggestive of association with higher expression level of Alpha S1 CSN protein. Further studies are thus warranted on a larger scale in support of this supposition.

# Summary

# VI. SUMMARY

The present study was conducted with the objective of analyzing the genetic variation at Alpha S1 CSN gene region of Nandidurga non-descriptive goat of Karnataka by using PCR - RFLP technique. Genomic DNA was isolated from blood samples of 85 Nandidurga, 35 Osmanabadi and 50 Bidri goats. DNA was extracted using high salt technique and the quantity and quality of DNA as determined by Spectrophotometer and Agarose gel electrophoresis was satisfactory.

Two oligo nucleotide primers Alpha S1 CSN F and Alpha S1 CSN R were used to amplify a part of intron 8, exon 9 and intron 9 of Alpha S1 CSN gene. Amplification was carried out with initial denaturation of template DNA at 94 °C for 2 minutes, followed by 30 cycles of denaturation at 94 °C for 1 minute, annealing at 60 °C for 1 minute and extension at 72 °C for two minutes. A final extension of 72 °C for 10 minutes was included in the PCR protocol. Each 25 µl PCR reaction consisted of 100 ng of genomic DNA, 10 pmol of each primer, 100 µM of dNTPs, 3 units *Taq* DNA polymerase and 2.5 µl of 10X assay buffer with $MgCl_2$.

Restriction analysis was carried out using 10 units of restriction endonuclease *Xmn*I to detect the genetic variation at 37 °C for 14 hours of incubation. The digested product was run on 4 per cent agarose gel along with 50 base pair DNA molecular weight marker. The RFLP analysis in blood samples of Nandidurga, Bidri and Osmanabadi goats revealed two restriction patterns with two alleles namely A and B. The ‘A’ allele yielded two fragments of sizes 150 bp and 63 bp. Similarly ‘B’ allele yielded two fragments at

161bp and 63bp due to insertion of eleven base pairs. Two genotypes, AA and BB, were identified in goat populations.

The gene frequencies of A allele were 0.894, 0.886 and 0.960 in Nandidurga, Osmanabadi and Bidri goats, respectively. Frequency of allele B was 0.116, 0.114 and 0.06, respectively.

The sequence analysis indicated that there was high homology between the present result and the published caprine Alpha S1 CSN gene sequences.

Alleles A and B are associated with increased casein protein synthesis. Hence Nandidurga, Bidri and Osmanabadi goats can be reared for increased protein. This study is the first of its kinds carried out in non-descript Nandidurga goat and Bidri goat. However further study with large number of unrelated goat samples is essential to confirm the association of this gene polymorphism with milk production traits if any.

# Bibliography

# VII. BIBLIOGRAPHY


AMBROSOLI, R., DI STASIO L. and. MAZZOCCO, P., 1988. Content of αs1- casein and coagulation properties in goat milk. *J. Dairy Sci*. **71**: 24–28

AMIGO, L., RECIO and RAMOS, M., 2000. Genetic polymorphism of milk protein; its effect on technological properties of milk: A review. *Int. Dairy Sci*., **11**: 135-149

ANNAPOORANI, C., 1996. Genome analysis by RFLP and RAPD in buffaloes. *MVSc Thesis*, submitted to the Tamil Nadu Veterinary and Animal Sciences University, Chennai, India

ANON., 2007. 18th Quinquennial Indian Livestock Census, Department of Animal Husbandry and Dairying, Ministry of Agriculture, New Delhi

ARAVINDAKSHAN, T.V., 1997. Genome analysis in cattle and buffaloes by RFLP and RAPD techniques, *PhD thesis* submitted to the Tamil Nadu Veterinary and Animal Sciences University, Chennai, India

ARSHAN SHAHID, A., 2013. Studies on genetic polymorphism of Beta lactoglobulin gene in Nandidurg goat. *MVSc Thesis*, submitted to Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar, Karnataka, India

AYSHWARYA, R.V., 2013. Genetic Polymorphism of Major Histocompatibility Complex Class II DRB genes in Nandidurg goats. *MVSc Thesis*, submitted to Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar, Karnataka, India

AZARUDDIN, N., 2011. Characterization of Nandidurga breed of goat. *MVSc* thesis submitted to KVAFSU, Bidar

BALTEANU, V., 2011. Effect of alpha S1 casein polymorphism on milk composition and cheese making efficiency in Carpathian goat breed. Bul. Univ. Agri. Sc. and Vet. Med., **68**: 71-76

BECKMAN, J.S. and SOLLER, M., 1983. Restriction fragment length polymorphism in genetic improvement: Methodologies, mapping and costs. *Theor. Appl. Genet.*, **67**: 35-43

BEUZEN, N.D., STEAR, M.J. and CHANG, K.C., 2000. Molecular markers and their use in animal breeding. *The Vet. J.*, **160**: 42-52

BEVILACQUA, C., FERRANTI, P., GARRO, G., VELEI, C., LAGONIGRO, R., LEROUX, C., PIEREOLA, E., ADDEO, F., PILLA, F., CHIANESE, L. and MARTIN P., 2002. Inter-allelic recombination is likely responsible for the occurrence of a new variant (alpha s1-casein variant in the goat species). *European J. Biochem*., **269**: 1293-1303

BHAT, P.N. and KHAN, B.U., 2008. A Text book on Goat production, pp 158-171

BOTSTEIN, D., WHITE, R.L., SKOLNICK, M. and DABIS, R.W., 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphism. *Ame*rican *J. Hum. Genet*., **32**: 324-333

BOULANGER, A., GROSCLAUDE, F. and MAHE, M.F., 1984. Alpha S1and α-s2 casein polymorphism in goats. *Genet. Select. Evol.*, **16**: 157-175

BOZIDARKA, M., MARKOVIC, M., JOVONOVIC, S. and KROFINOVIC, M., 2009. Gene frequencies of caprine alpha S1 casein polymorphism in Montenegrin Balkan goat breed. *Acta Veterinaria,* **59**: 613-619

BOZKAYA, F., GURLER S. and YERTURK, M., 2013. Genetic variability of CSN 1S1 gene in goat populations raised in south eastern region of Turkey. *Kafka's Univ Bet Fak Derg.*, **19**(1): 147-152

BRAS, R., 2009. Polymorphism of alpha s1-casein gene in a dairy goat herd in the south-eastern region of Brazil. *Zootec*., **38**(6): 112-115

BRIGNON, G., MAHE, M. F., GROSCLAUDE, F. and. RIBADEAU-DUMAS. B., 1989. Sequence caprine αs1-casein and characterization of those of its genetic variants which are synthesized at a high level, αs1-CnA, B and C. *Protein Seq. Data Anal*. **2**: 181–18

BURR, B., EVOLA, S.V., BURR, F.A. and BECKMANN, J.S., 1983. The application of restriction fragment length polymorphism to plant breeding. In: *genetic engineering principles and methods*. *Edit*: J.K. SETLOW and A. HOLLAENDER *Edn*: 5, Plenum Press, New York, *pp* 45-49

CHESSA, S., CHIATTO, F., RIGNANESE, D., IBEAGHA, M.E., OZBEYAZ, C., HASSAN, Y.A., BAIG, M.M., ERHARDT, G. and CAROLI, A., 2007. The casein genes in goat breeds from different continents; analysis by PCR-SSCP. *Ital. J. Anim. Sci.,* **6**: 7

CHIANESE, L., FERRANTI, P., GARRO, G., MAURIELLO, R. and ADDEO, F., 1997. Occurrence of three novel αs1-casein variants in goat milk.Pages 259–267 in Proc. Int. Dairy Fed.-Fed. Int. Laiterie Semin.Milk Protein Polymorphism II. *Int. Dairy Fed*., Palmerston North, New Zealand

CHITRA, R., 2002. Polymorphism of growth hormone gene in Malabari goats (*Capra hircus), M.V.SC thesis,* submitted to the Kerala Agricultural University, Thrissur, Kerala, India

CLARK, S. and SHERBON, J. W., 2000. Alphas1-casein, milk composition and coagulation properties of goat milk. *Small Rumin. Res*. **38**: 123–134

COSENZA, G., ILLARIO, R.., RANDO, A., DI GREGORIO, P., MASINA, and. RAMUNNO, L., 2003. Molecular characterization of the goat CSN1S01 allele. *J. Dairy Res*. **70**: 237–240

FARRELL, H.M., JMENEZ FLORES, R. and BLECK, G.T., 2004. Nomenclature of the proteins of cow milk. 6th revision. *J. Dairy Sc.*, **87**: 1641-1674

FARUK BOZKAYA, SUKRU GURGLER, MUGDAT YERTURK., 2013. Genetic variability of CSN1S1 gene in goat populations raised in South eastern Region of Turkey. *Kafkas Univ Vet Fak Derg.***19** : 147-152.

FERRETTI, L., LEONE, P. and SGARAMELLA, V., 1990. Long range restriction analysis of the bovine casein genes. *Nucleic Acids Res.*, **18**: 6829-6833

FOLCH, J.M., COLL, A. and SANCHEZ, A., 1994. Complete sequence of the caprine β-lacto globulin. *J. Dairy Sci*., **77**:3493-3497

FRIES, R.., EGGEN, A. and STRANZINGER, G., 1990. The bovine genome contains polymorphic microsatellite. *Genomics.*, **8**: 403-406

GELHAUS, A., SCHNITTGER, L., MEHLITZ, D., HORSTMANN, R.D. and MAYER, C.G., 1995. Sequence and PCR-RFLP analysis of 14 novel BoLA-DRB3 alleles. *Anim. Genet*., **26**: 147-153

GRODZICKER, T., WILLIAMS, J., SHARP, P. and SAMBROOK, J., 1974. Physical mapping of temperature sensitive mutations of adenovirus. *Quant. Bio*., **39**: 439-446.

GROSCLAUDE, F., MAHE, M.F., BRIGNON, G., DI-STASIO, L. and JEUNET, R., 1987. A Mendalian polymorphism underlying quantitative variation of goat αS1-casein. *Genet. Sel*., **19**: 399-412

GROSCLAUDE, F. and MARTIN. P., 1997. Casein polymorphism in the goat. in Proc. *Int. Dairy Fed*., Pages 241–253

GWAKISIA, P.S., KEMP, S.J. and TEALE, A.J., 1994. Characterization of Zebu cattle breed in Tanzania using random amplified polymorphic DNA markers. *Anim. Genet*., **25**: 89-94

HAYES, H., PETIT, C., BOUNIOL and POPESCU, P., 1993. Localization of the α-s2 casein gene to the homologous cattle, sheep and goat chromosome by in situ hybridization. *Cytogenet. Cell* Genet., **64**: 282-285

INNIS, M.A. and GELFAND, D.H., 1990. Optimization of PCR in: PCR Protocols: A guide to methods and applications. *J. Dairy Sci.,* **37**: 1141-1146

JEFFERYS, A.J., BROOKSFEILD, J.F.N. and SAMEONOFF, R., 1985. Positive identification of an immigration test case using human DNA fingerprinting. *Nature*, **317**: 801-819

KUMAR, A., ROUT, P.K., MANDOL, A. and ROY, R., 2007. Identification of the CSN 1S1 allele in Indian goats by the PCR-RFLP method. *Animal.,* **8**: 1099-1104

LEROUX, C., MARTIN, P., MAHE, F.M., LEVEZIEL, H., and MERCIER, C.J., 1990. Restriction fragment length polymorphism identification of goat alpha S1casein alleles: a potential tool in selection of individuals carrying alleles associated with a high level protein synthesis. *Anim. Genet.,* **21**: 341-351

LEROUX, C. and MARTIN, P., 1996. The caprine Alpha S1and β-casein genes are 12-kb apart and convergently transcribed. *Anim. Genet.,* **27**:93

LEWIN, B., 2000. *Genes VIII.* Oxford University Press, London

LITT, M. and LUTTY, J.A., 1989. A hyper variable microsatellite revealed by in vitro amplification of dinucleotide repeat within the cardiac muscle action gene. *American J. Hum. Genet.,* **44**: 397-401

MAGA, A.E., DAFTARI, P., KULTZ, D. and PENEDO, T.C.M., 2009. Prevalence of alpha S1 casein genotypes in American dairy goats. *J. Anim. Sci.,* **87**: 3464-3469

MALTOVA, V., SZTANKOOVA, Z., and MALA, G.,2007. Genetic polymorphism at the CSN1S1 gene in two Czech goat breeds. *Czech.J.Anim.Sci.,* **52**(7) : 199-202.

MARINI, A.B.M., RASHID, A.B., MUSADDIN, K. and ZAWAWI, I., 2011. Study of Alpha S1CSN gene polymorphism in Katjang, Jamnapari, Boer and Boer-feral goats in Malaysia. *J. Trop. Agric. and Fd. Sc.*, **39** (1): 121-124

MARTIN, P., OLLIVIER-BOUSQUET, M. and GROSCLAUDE, F., 1999. Genetic polymorphism of caseins: A tool to investigate casein micelle organization. *Int. Dairy J*. **9**: 163–171

MATHEW, B., 2004. Growth and survivability of GH/Mspl genotypes in Malabari goats. *M.V.Sc. Thesis*, submitted to the Kerala Agricultural University, Thrissur, Kerala, India

MILLER, S.A., DYKES, D. and POLESKY, H.F., 1988. A sample salting out procedure for extraction of DNA from humen nucleated cells. *Nuclein Acids Res.*, **16**: 12-15.

MITRA, A., 1994. Studies on restriction fragment length polymorphism in cattle and buffaloes. *PhD Thesis*, NDRI, Karnal, India

MOIOLI, B., ANDREA, M.D. and PILLA, F., 2007. Candidate gene affecting sheep and goat milk quality. *Small Ruminant Res.*, **68**: 179-192

MONTGOMERY, G.W. and SISE, J.A., 1990. Extraction of DNA from sheep white blood cells. *New Zealand J. Agri. Res*., **33**: 437-441

MARKOVIC BOZIDARKA, MARKOVIC M., JOVANOVIC, S. and KRAFINOVIC, M.., 2009. Gene frequencies of caprine Alpha S1 casein polymorphism in Montenegrian Balkan goat Breed. Acta Veterinaria., ***59****: 6134-619.*

MULLIS, K., FALCOONA, F., SCHARF, S., SAIKI, R., HOM, G. and ERLICH, H., 1986. Specific enzymatic amplification of DNA in vitro; The polymerase chain reaction. Cold spring Harbor Symposia on Quantitative Biology.,**1100**: 263-273.

NAKAMURA, Y., LOPPERT, M., CONNEL, P., WOLFF, R., HOLM, T., CULVER, M., MARTIN, C., FUJIMOTO, E., HOFF, M., KUMLIN, E. and WHITE, R., 1987. Variable number of tandem repeat (VNTR) markers for human gene mapping. *Science.,* **235**: 1616-1622

NEVEU, C., RIAUBLANC, A., MIRANDA, G.,2002. Is the apocrine milk secretion process observed in the goat species rooted in the perturbation of the intracellular transport mechanism induced by defective alleles at the alpha s1 Cn locus? *Reproductive Nutrition Development.* **42**: 163-172.

PINDER, S.J., PERRY, B.N., SKIDMORE, C.J. and SARVAL, D., 1991. Analysis of polymorphism in the bovine casein genes by use of the polymerase chain reaction. *Anim. Genet*., **22**: 11-20

PIRISI, A., COLIN, O., LAURENT, F., SCHER, J. and PARMENTIER., M., 1994. Comparison of milk composition, cheese making properties and textural characteristics of the cheese from two groups of goats with a high or low rate of αs1-casein synthesis. *Int. Dairy J*. **4**: 329–345

RAMUNNO, L., COSENZA, G., PAPPALARDO, M., PASTORE, N., GALLO, D., GREGORIO, D.P. and MASINA, P., 2000. Identification of the goat $CSN1S1^{F}$ allele by means of PCR-RFLP method. *Intern. Societ. Anim. Genet.,* **31**: 333-346

RAMUNNO, L., G. COSENZA, A. RANDO, A. PAUCIULLO, R. ILLARIO, D. GALLO, DI BERARDINO D. and MASINA, P., 2005. Comparative analysis of gene sequence of goat CSN1S1 F and N alleles and characterization of CSN1S1 transcript variants in mammary gland. *Gene,* **345**: 289-299

REMEUF, F., 1993. Influence of genetic polymorphism of Caprine Alpha S1Casein gene on physic-chemical and technological properties of milk. *Ital. J. Anim. Sci.,* **73**: 549-557

RONCADA, P., GAVITAGHI, A., LIBERATORI, S., CANAS, B., BINI, L. and GREPPI, G., 2002. Identification of caseins in goat milk. *Proteomics.,* **2**: 723-726

ROUX, K., 1995. Optimization and trouble shooting in PCR. Manual supplement from: PCR *methods and applications.,* **20**: 185-193

SAHAR AHMED., 2006. Identification of Egyptian goat alpha S1casein alleles using PCR-RFLP method. *International Journal of Dairy Science,* **1**: 27-31

SAIKI,R.K., GELFAND, D.H., STOFFEL, S., SCHARF, S.J., HIGUCHI, R., HORN,G.T., MILLIS,K.B. and ELRICH, H.A.,1998. Primer-directed amplification of the DNA with a thermos stable DNA polymerase. *Science* ,**239**: 487-491.

SAMBROOK, J. and RUSSEL, D. W., 2001. *Molecular cloning*. A laboratory manual, *Edn*: 3rd, Cold Spring Harbor Laboratory Press. Cold Spring Harbor, NY

SEENA, T.X., 2006. Polymorphism of Ovine fecundity gene linked microsatellite markers in Malabari goats. *M.V.Sc Thesis*, submitted to the Kerala Agricultural University, Thrissur, Kerala, India

SENTHIL, M., 1995. Studies on restriction fragment length polymorphism (RFLP) in cattle. *M.V.Sc. Thesis*, submitted to the Tamil Nadu Veterinary and Animal Sciences University, Chennai, India

SHARROCKS, A.D., 1994. The design of primers for PCR. In: PCR technology-Current innovations (eds. H.G. Griffin and A.M. Griffin), CRC Press., *Boca Raton: pp* 5-11

SOARES, M.A.M., RODRIGUES, T.T.M., MONGOL, P.G., RIBEIRO, C.F.L. SILVA, C.L.J. and BRANCALHAO, C.M.R., 2009. Polymorphism of alpha S1casein gene in dairy goat herd in the South eastern region of Brazil. *R. Bras. Zootec.*, **38**: 80-85

STRICKBERGER, M. W., 1985. Genetics, *Edn*: 3rd, Prentice-Hall Pvt. Ltd., New Delhi

SZTANKOOVA, Z., MALTOVA, V. and MALA, G., 2001. Genetic polymorphism at the CSN 1s1 gene in two Czech goat breeds. *Czech. J. Anim. Sci.*, **52**: 199-202

TAUTZ, D., 1990. Genomic fingerprinting goes simple. *Bio Essays*, **12**: 44-46

THEILMANN, J.L., SKOW, L.C., BAKER, J.F. and WOMACK, J.E., 1989. Restriction fragment length polymorphisms for growth hormone, prolactine osteonectin, α-crystalline, γ- crystalline, fibronectin and 21- steroid hydroxylase in cattle. *Anim. Genet*., **20**: 257-266

THREADGILL, D.W. and WOMACK, J.E., 1990. Genomic analysis of the major bovine milk protein genes. *Nucleic Acid Res.*, **18**: 6935-6942

WEBER, J., and MAY, P.E., 1989. Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. *American J. Hum. Genet.,* **44**: 388-395

WELSH, J., and McCLELLAND, M., 1990. Finger printing genomic using PCR with arbitrary primers. *Nucleic Acid Res.,* **18**: 7213-7218

WILLIAMS, J.G.K., KUBELIK, A.R., LIVAK, K.J., RAFALSKI, J.A. and TINGEY, S.V., 1990. DNA polymorphism amplified by arbitrary primers is useful as genetic markers. *Nucleic Acid Res*., **18**: 6531-6535

WILKINS, R.J. and XIE, TAO.,1997. Two distinct gene mutations – one milk proteins polymorphisms. The example of the alpha s1 casein A variant. *International Dairy Federation.***9702** : 330-333.

# Abstract

# VIII. ABSTRACT

A study was conducted with the objective of analyzing the genetic variation at Alpha S1 Casein gene region in Nandidurga non-descriptive goats of Karnataka by using PCR - RFLP technique. Genomic DNA was isolated from blood samples of 85 animals. The genetic diversity of caprine Alpha S1CSN locus was investigated by PCR - RFLP by amplifying part of intron 8, exon 9 and part of intron 9 of the Alpha S1 CSN gene. Restriction endonuclease enzyme *Xmn*I was used to detect the genetic variation of the experimental unit in Alpha S1 CSN gene**.** Upon PCR - RFLP analysis, two patterns were observed which allowed the identification of two alleles A and B, and two genotypes, AA and BB. Further PCR - RFLP study in 35 Osmanabadi and 50 Bidri goats prevalent in North Karnataka revealed similar observations. Ironically AB type was not observed in any of these goats. The sequence analysis indicated that there was a high homology between the present result and the published caprine Alpha S1CSN gene sequences. There was query coverage to the extent of 100 per cent. This is one of the first studies in non-descript goats. Presence of predominant 'A' allele among these goats provides other selection criteria in addition to traditional selection methods, for increased milk protein.

# Annexures

# IX. ANNEXURES

## ANNEXURE 1

**Chemicals:**

| Chemical | Source |
|---|---|
| Absolute alcohol | Merck |
| Acetic acid | BDH-E, Merck (India) Ltd. |
| Agarose | SRL, Mumbai. |
| Ammonium chloride | SRL, Mumbai |
| Ammonium per Sulphate | SRL, Mumbai |
| Boric acid | SRL, Mumbai |
| Bromophenol blue | SRL, Mumbai |
| Chloroform | SRL, Mumbai |
| dNTPs | Bangalore Genie Pvt Ltd. |
| EDTA | SRL, Mumbai |
| Ethidium bromide | SRL, Mumbai |
| Iso amyl alcohol | BDH-E, Merck (India) Ltd |
| Potassium chloride | SRL Mumbai |
| Sodium chloride | Merck |
| SDS | SRL, Mumbai |

**Enzymes:**

| | |
|---|---|
| Proteinase-K | Bangalore Genie Pvt Ltd |
| Taq DNA polymerase | Amnion Pvt Ltd |
| RE | New England Biological |

**Molecular size markers:**

| | |
|---|---|
| 50 base pair DNA ladder | Bengaluru Genie Pvt. Ltd. |
| 100 base pair DNA ladder | Amnion Pvt. Ltd. |

# ANNEXURE-2

## Laboratory equipment:

| Equipment | Suppliers/Firm |
|---|---|
| Bench top centrifuge | Remi, India |
| Electric balance | Sartorius, Switzerland |
| Gel Documentation system | Bio Rad |
| Gel drier | Bio Rad |
| Micro centrifuge | Eppendorf |
| Micropipettes | Nichipet EX |
| pH meter | Global, India |
| Sequencing Electrophoresis apparatus | Bangalore Genie Pvt Ltd |
| Spectrophotometer | Eppendorf |
| Submarine electrophoresis unit | Bangalore Genie Pvt Ltd |
| Thermal cycler | Bio Rad |
| UV Transilluminator | Cleaver Scientific Ltd |

# ANNEXURE-3

## A. Solution or reagents for DNA isolation

**EDTA (0.5M)**

Disodium Ethylene Diamine Tetra Acetic acid (EDTA): 18.61 g

Triple glass distilled water to make up to : 100ml

pH adjusted to 8.0, autoclaved and stored at room temperature

**Sodium Dodecyl Sulphate (SDS)**

Sodium Dodecyl Sulphate : 10 g

Triple glass distilled water to make up to : 100 ml

**5M NaCl**

Sodium chloride : 29.27 g

Triple glass distilled water to make up to : 100 ml

Filtered and stored at room temperature

**Tris-EDTA buffer (TE)**

Tris (hydroxy methyl) amino methane (10mM) pH 7.6 : 0.12114 g

EDTA (0.1Nm) : 0.03722 g

Triple glass distilled water to make up to : 100ml

pH adjusted to : 8.0

**RBC lysis buffer**

Ammonium chloride (1.7M) : 9.1 g

Triple glass distilled water to make up to : 1000 ml

pH adjusted to 8.0, kept at 4°C

**B. Solutions or Reagents for checking of DNA samples:**

**Tris acetate EDTA (TAE) buffer (50X)**

Triple (hydroxy methyl) amino methane (10mM) pH 7.6: 48.4 g

Glacial acetic acid : 11.42 ml

0.5 M EDTA (pH 8.0) : 20 ml

Triple glass distilled water to make up to : 1000ml

**Agarose (0.8%)**

Agarose : 0.24 g

Triple glass distilled water to make up to : 30 ml

**Agarose (1.5%)**

Agarose : 0.45 g

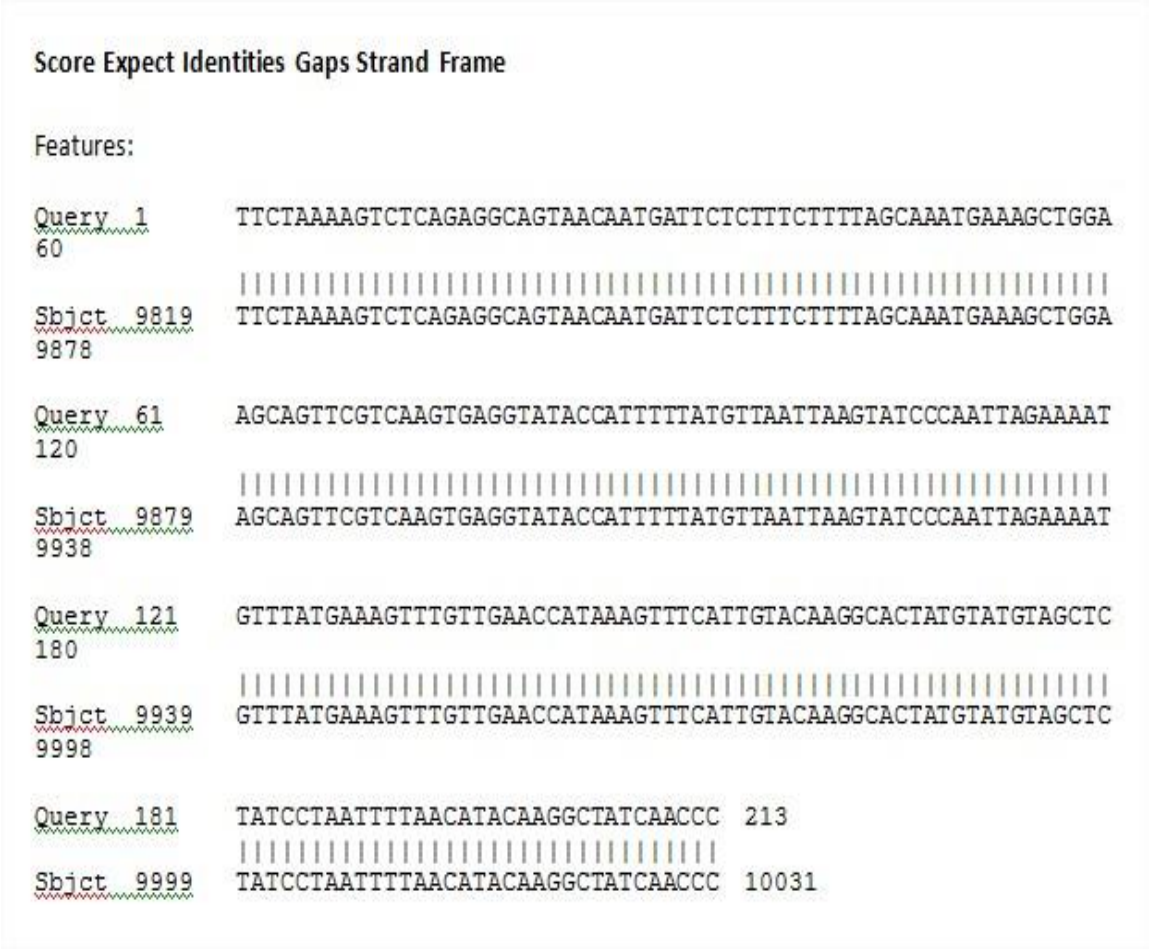
TAE (1X) to make up to : 30 ml

**Agarose (3.0%)**

Agarose : 0.9 g

TAE (1X) to make up to : 30 ml

# ANNEXURE 4



Score Expect Identities Gaps Strand Frame

Features:

```
Query  1      TTCTAAAAGTCTCAGAGGCAGTAACAATGATTCTCTTTCTTTTAGCAAATGAAAGCTGGA
60

Sbjct  9819   TTCTAAAAGTCTCAGAGGCAGTAACAATGATTCTCTTTCTTTTAGCAAATGAAAGCTGGA
9878

Query  61     AGCAGTTCGTCAAGTGAGGTATACCATTTTTATGTTAATTAAGTATCCCAATTAGAAAAT
120

Sbjct  9879   AGCAGTTCGTCAAGTGAGGTATACCATTTTTATGTTAATTAAGTATCCCAATTAGAAAAT
9938

Query  121    GTTTATGAAAGTTTGTTGAACCATAAAGTTTCATTGTACAAGGCACTATGTATGTAGCTC
180

Sbjct  9939   GTTTATGAAAGTTTGTTGAACCATAAAGTTTCATTGTACAAGGCACTATGTATGTAGCTC
9998

Query  181    TATCCTAATTTTAACATACAAGGCTATCAACCC  213

Sbjct  9999   TATCCTAATTTTAACATACAAGGCTATCAACCC  10031
```

**BLASTn of allele A of α-S1 Casein gene region of Nandidurg goat with that of *Capra hircus* (AJ 504710.2) on NCBI Gene Bank**

# ANNEXURE 5

| Score | Expect | Identities | Gaps | Strand | Frame |
|---|---|---|---|---|---|
| 396 bits(214) | 8e-107() | 221/224(99%) | 1/224(0%) | Plus/Plus | |

Features:

```
Query  1      TTCTAAAAGTCTCAGAGGCAGTAACAATGATTCTCTTTCTTTTAGCAAATGAAAGCTGGA
60

Sbjct  9821   TTCTAAAAGTCTCAGAGGCAGTAACAATGATTCTCTTTCTTTTAGCAAATGAAAGCTGGA
9880

Query  61     AGCAGTTCGTCAAGTGAGGTATACCATTTTTATGTTAATTAAGTATCCCAATTAGAAAAT
120

Sbjct  9881   AGCAGTTCGTCAAGTGAGGTATACCATTTTTATGTTAATTAAGTATCCCAATTAGAAAAT
9939

Query  121    GTTTATGAAAGTTTGTTGAACCATAAAGTTTCCGTAATGTTTCATTGTACAAGGCACTAT
180

Sbjct  9940   GTTTATGAAAGTTTGTTGAACCATAAAGTTTCCGTAATGTTTCATTGTACAAGGCACTAT
9999

Query  181    GTATGTAGCTCTATCCTAATTTTAACATACAAGGCTATCAACCC  224

Sbjct  10000  GTATGTAGCTCTATCCTAATTTTAACATACAAGGCTATCAACCC  10043
```

**BLASTn of allele B of α-S1 Casein gene region of Nandidurg goat with that of *Capra hircus* (AJ 504711.2) on NCBI Gene Bank**