

ANALYSIS OF BETA-CASEIN GENE VARIANTS IN BUFFALO
OF BOCHASAN FARM THROUGH PCR-RFLP

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ABSTRACT

Bovine milk and dairy products play a significant role in traditional diets worldwide. Bovine milk protein consists of 80% casein and 20% whey. β -casein is the most common hydrophobic casein found in bovine milk. It has 2 frequent variants, A1 and A2. The only structural variation between the two beta-caseins is the location of amino acid 67, where histidine (CCT) in A1 is substituted by proline (CAT) in A2. The study was undertaken to analyze bovine β -casein variants in a total of 105 Jaffarabadi buffalo of Bochasan farm using polymerase chain reaction-restriction fragment length polymorphism. Genomic DNA was successfully isolated using Phenol-Chloroform-Isoamyl alcohol method. Quality and quantity analysis was done by Agarose gel electrophoresis and Nanodrop respectively. The DNA was amplified using PCR that yielded 121bp fragment. Genetic polymorphism was detected by digesting PCR products using restriction endonuclease *Dde* I. Restriction digestion with *Dde* I revealed two genotypes: A1A2 and A2A2. The genotypic frequencies of A1A2 and A2A2 genotypes were 0.25 and 0.75 respectively. A1A1 genotype was absent in the studied population.

The allelic frequency was 0.13 for A1 allele and 0.87 for A2 allele. These results confirmed that Jaffarabadi buffaloes predominantly have A2 milk.

Keywords: *Bubalus bubalis*, buffaloes, Beta-casein, genotype, allele frequency, milk, PCR-RFLP

INTRODUCTION

Bovine milk and dairy products play a significant role in traditional diets worldwide (Tokarchuk *et al.*, 2023). Buffalo contributes 48.34% to the total milk production of India (Indian Council of Agricultural Research). The protein content in buffalo milk is 3.87 gm approximately. Beta casein makes up about 35% (w/w) of total bovine casein protein. It has 209 amino acid residues and is more soluble in water than other caseins, particularly at low temperatures (Post *et al.*, 2012). Bovine milk includes 15 genetic variants (A1, A2, A3, B, C, D, E, F, G, H1, H2, I, J, K and L), the most well-known and common of which are A1 and A2 (Fan *et al.*, 2021). A1 variant of beta-casein is a consequence of a mutation that causes differences in the β -casein protein due to a single nucleotide

polymorphism in the gene CCT (A2, proline) to CAT (A1, histidine) at the 67th residue in amino acid chain. Molecular markers play an important role in the selection of animals for the improvement of milk and meat production in both sexes (Yadav *et al.*, 2017). A2 variant reduces serum cholesterol (Venn *et al.*, 2006). In some countries like New Zealand and Australia, A2 milk is preferred over A1 milk. Jaffarabadi buffaloes are the heaviest of all the Indian breeds. They have gigantic bodies with horns forming ring-like structures. They weigh around 800 kg. The average milk production is 2238 kg with average fat of 7 to 9%.

MATERIALS AND METHODS

Experimental materials

A total of 105 Jaffarabadi buffalo blood samples was collected from Shree Hari Dairy Farm, Bochasan, Anand, Gujarat. The blood samples were collected from the jugular vein. All the animals were unrelated and selected at random.

Genomic DNA isolation

Genomic DNA was extracted by phenol-chloroform-isoamyl alcohol method as described by Patel *et al.* (2017) with minor modifications. In brief, 3 ml of heparinized blood was mixed with equal volume of Solution 1 (10 mM Tris HCl, 10 mM KCl, 10 mM MgCl₂ and 20 µL of Nonidet P40). The solution was mixed well and kept at -20°C and the nuclear pellet was spinned down. The supernatant was discarded and the pellet was resuspended in 800 µL of Solution 2 (10 mM Tris HCl, 10 mM KCl, 10 mM MgCl₂, 0.5 M NaCl, 2 mM EDTA and 0.5% SDS) along with this 5 µL of proteinase K and incubated at 60°C overnight. Next day, DNA was extracted by treating the mixture

with 500 µL of equilibrated phenol and 200 µl Phenol: Chloroform: Isoamyl alcohol (25:24:1). The DNA was precipitated by double volume of chilled ethanol and washed with 70% ethanol. The pellet was dried and resuspended in nuclease free water. The quality of isolated genomic DNA was checked electrophoretically using 1% agarose gel. The quantity of the isolated genomic DNA was checked by NanoDrop spectrophotometer (Thermo scientific NanoDrop 2000).

Polymerase Chain Reaction (PCR)

A pair of bovine oligonucleotide primers (Forward primer- 5'- CCT TCT TTC CAG GAT GAA CTC CAG G -3' and Reverse primer- 5'- GAG TAA GAG GAG GGA TGT TTT GTG GGA GGC TCT-3' described by McLachlan *et al.* (2006) in buffaloes were used in the present study to amplify 121 bp exon on VII segment of beta-casein locus.

The PCR was executed in a final reaction volume of 25 µL. The mixture was denatured for 5 minutes at 95°C, and PCR was run for 30 cycles of 95°C for 30 seconds (Denaturation), 63°C for 30 seconds (Annealing), 72°C for 30 seconds (Extension). Final extension was carried out for 5 minutes at 72°C.

RFLP and Agarose Gel Electrophoresis

The 10 µL of amplified PCR products were digested with 4 units of *Dde* I in a final reaction volume of 20 µL. The reaction mixture was incubated overnight at 37°C in water bath. The digested products were loaded on 3% agarose gel containing 1X TBE buffer and electrophoresed at 120 volts for 60 to 70 minutes. The gel was analyzed under a UV trans-illuminator and compared with 50 bp ladder.

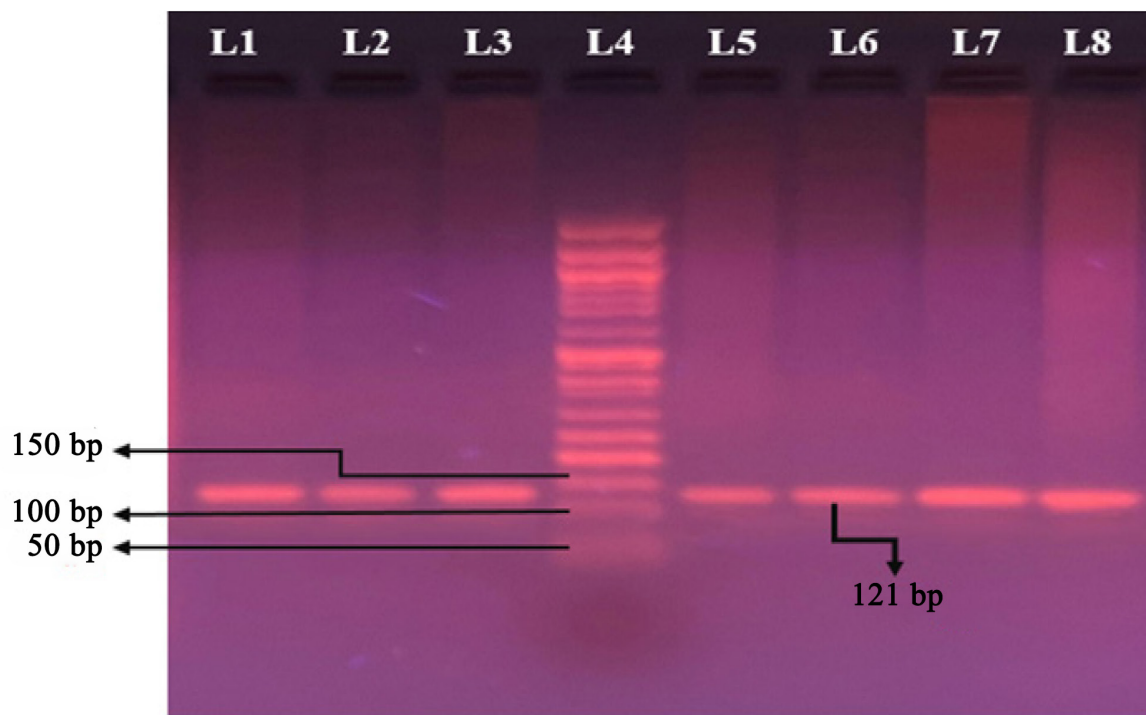


Figure 1. Electrophoresis pattern of PCR products generated by amplification of CSN2 gene on 2% agarose gel. Lanes 1-3: S37 to S39; Lane 4: 50 bp DNA ladder; Lanes 5-8: S40 to S43.

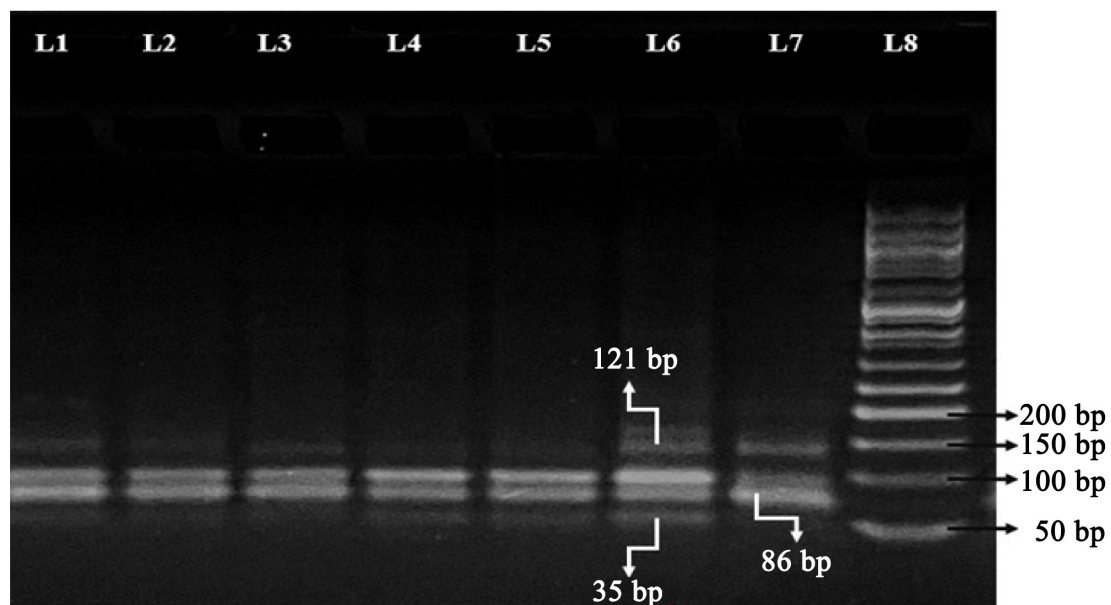


Figure 2. PCR-RFLP pattern of CSN2 gene on 3% agarose gel. Lane 1-7: S37, S38, S39, S40, S41, S42, S43 respectively. Lanes 8: 50 bp DNA ladder.

Table 1. Genotypic frequency of A1 and A2 allele of CSN2 gene.

Total no. of animals	Genotype frequency			Total
	A1A1	A2A2	A1A2	
105	00	0.75	0.25	1.0

Table 2. Allelic frequency of A1 and A2 allele of CSN2 gene.

Allelic frequency		Total
A1	A2	
0.13	0.87	1.0

RESULTS AND DISCUSSIONS

Electrophoretic examination of extracted DNA using 1% agarose gel followed by visualization under UV trans-illuminator found high molecular weight sharp bands, indicating good DNA quality which were appropriate for PCR and RFLP analysis. The purity of all the 105 DNA samples was confirmed by Nanodrop (Nanodrop 1000 by Thermo Scientific, USA). The purity of DNA was confirmed by taking the absorbance ratio at A260/280. The ratio varied between 1.25 to 2.38. The concentration of DNA in the samples varied between 30 to 250 ng/μl. PCR products of all the 105 samples produced the expected 121 bp fragments on 2% agarose gel electrophoresis. The restriction digestion analysis of 121 bp PCR product of beta casein usually have three type of patterns; i) A1A1 genotype yield one fragment 121bp, ii) A2A2 genotype yield two fragments 35 bp and 86 bp and iii) A1A2 genotype yield three fragments of 35 bp, 86 bp and 121 bp, which arise on coupling of first and second pattern i.e. it is heterozygous. However, the restriction digestion analysis of beta casein in all the Jaffarabadi buffaloes yielded two genotypes A2A2 and A1A2.

In the population of Jaffarabadi breed homozygous genotype A1A1 was not found, but heterozygous genotype A1A2 (26 buffaloes) and homozygote genotype A2A2 (79 buffaloes) were observed. The genotypic frequencies of A1A2 and A2A2 genotypes were 0.25 and 0.75 respectively. A1A1 genotype was absent in the studied population. The allelic frequency was 0.13 for A1 allele and 0.87 for A2 allele. These results confirmed that Jaffarabadi buffaloes predominantly have A2 milk.

Mishra *et al.*, 2009; Ramesha *et al.*, 2016 discovered similar results after analyzing 231 and 72 buffaloes from nine distinct breeds, respectively. Formaggioni *et al.*, 1999; Jaiswal *et al.*, 2014 studied the confirmation of the A2A2 genotype in buffaloes supports evidence that A1 allele was only present in cattle 10,000 years ago, with the A2 allele serving as the ancestor. The Buffalo Breeder Association gives a purity label to dairy products made exclusively from buffalo milk. The fact that buffalo has A2A2 genotype for beta-casein assures that products carrying the purity species seal are likewise A2. As there are very few reported information related to beta casein gene polymorphism in buffaloes especially

Jaffarabadi buffalo. This study provides useful information related to pattern of A1 and A2 allele in Jaffarabadi buffalo to the animal breeders. The metabolism of variant A2 is different from A1 milk as it does not release bioactive peptide BCM-7 that reduces the serum cholesterol which plays a key role in prevention of human vascular diseases. As a result, the buffalo A2 milk is healthier than A1 milk (Fan *et al.*, 2021).

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