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TC Tolenkhomba
Assistant Professor, Department of Animal Genetics and Breeding, College of Veterinary Sciences and Animal Husbandry, Central Agricultural University, Selesih, Aizawl, Mizoram, India

Prava Mayengbam
Associate Professor, Department of Veterinary Physiology and Biochemistry, College of Veterinary Sciences and Animal Husbandry, Central Agricultural University, Selesih, Aizawl, Mizoram, India

BR Yadav
Principal Scientist & National Fellow, Dairy Cattle Breeding Division, National Dairy Research Institute, Karnal, Haryana, India

Corresponding Author:
TC Tolenkhomba
Assistant Professor, Department of Animal Genetics and Breeding, College of Veterinary Sciences and Animal Husbandry, Central Agricultural University, Selesih, Aizawl, Mizoram, India

Milk protein genes polymorphism in Sahiwal cattle

TC Tolenkhomba, Prava Mayengbam and BR Yadav

Abstract
The polymerase chain reaction techniques (PCR-RFLP and ARMS-PCR) were used for amplification of DNA fragments specific to milk protein loci in 107 Sahiwal cattle maintain at NDRI, Karnal. In the Sahiwal population, the frequency of κ-casein allele A (0.88±0.02) was observed predominantly than the B allele (0.12±0.02). The allele A (0.98±0.01) of β-casein was found in a higher frequency than the B allele (0.02±0.01) in the population. The A allele (0.61±0.04) was found in a higher frequency than B (0.39±0.04) in αS1-casein gene. Among two alleles observed in α-lactalbumin locus, B allele (0.89±0.02) was found in higher frequency than A allele (0.11±0.02). In the β-lactoglobulin locus, B allele (0.84±0.02) was found predominantly than A allele (0.16±0.02). The observed homozygosity and heterozygosity of the milk proteins loci in the population were within the Hardy-Weinberg expectation as revealed by the chi-square value.

Keywords: milk protein genes, PCR-RFLP, ARMS-PCR, polymorphism, Sahiwal

Introduction
In the livestock industry, improvement of milk production through selective breeding is one of the very useful tools. The Conventional methods being used for selective breeding are time consuming and expensive. However, recent development in the identification of molecular markers specially the candidate genes associated with milk production traits of an animal have allowed the selection of animals even before phenotypes are expressed and independent of age and gender [1].

Milk protein polymorphisms have great significance in production traits, reproduction efficiency and adaptation capacity of the cattle, as well as detection of influences on milk nutritional and technological properties [2, 3, 4]. Milk protein components which are mainly affected by genetic factors [5, 6] have an important influence on milk production traits [7]. Milk proteins are usually divided into two major groups, the whey protein (comprising α-lactalbumin and β-lactoglobulin) and whole casein (comprising four different native caseins (Cn): αs1-Cn, αs2-Cn, β-Cn and k-Cn) [8]. Molecular techniques had been tested and used for the detection of variation or polymorphisms existing among individuals in the population for specific regions of the DNA [9, 10].

India and its neighbouring countries are homelands to various breeds of cattle from which most of the elite breeds of bovines have developed. Sahiwal is one of the most important milch breeds of zebu cattle that originated and available in India. This study was undertaken to find out polymorphism in milk protein loci using molecular techniques in Sahiwal cattle.

Materials and Methods
Blood samples were collected in heparin coated vacutainer tubes from the jugular vein of 107 Sahiwal cattle maintained at Cattle Yard, National Dairy Research Institute (NDRI), Karnal. DNA was isolated from blood following the standard phenol-chloroform extraction method [11]. PCR-RFLP was done to identify the genotypes of κ-casein, α-lactalbumin and β-Lactoglobulin loci using the reported primers [12, 13, 14]. Amplification Refractory Mutation System-Polymerase Chain Reaction (ARMS-PCR) was used to identify the genotypes of β-Casein and αS1-Casein [Rincon and Medrano (2003)]. The PCR amplification was carried out in a 25 µl volume containing 2.5 µl of 10X PCR buffer, 2mM of $MgCl_2$, 200 µM of each dNTP, 5 pM of each primer (5 pM each of outer and 3 pM each of inner primers for ARM-PCR), 2 U Taq DNA polymerase and 60 ng genomic DNA.

The following cycles were applied: at 95 ºC/5 min, followed by 35 cycles of – 95 ºC/30 sec, at annealing temperature for 45 sec, 72 ºC/30s and a final extension at 72 ºC for 10 min. The amplified DNA digested with a restriction enzyme and ARMS-PCR amplified products were separated in 3% agarose gel and visualized under Ultra violet light. The allele frequencies of all the loci were calculated and the population was tested for Hardy Weinberg equilibrium.

Results and Discussions

PCR-RFLP analysis of κ-Casein locus

The κ-casein is one of the abundant milk proteins forming the calcium-dependant aggregates termed micelles and has effects on the manufacturing properties of milk.

A 437 bp corresponding to 5' – and 3' – terminus of exon IV and intron IV was PCR amplified. The digestion of amplified fragments with *Hind*III yielded two types of restriction patterns and accordingly genotypes were identified. One *Hind*III restriction site was found in position 346 of the amplified fragment of allele B. The A allele showed a 437 bp fragment since there was no restriction site for the enzyme. The digested B allele produced a 346 bp and 91 bp fragment. The heterozygous kappa casein AB genotype yielded a restriction pattern of three (437 bp, 346 bp and 91 bp) fragments (Figure 1).

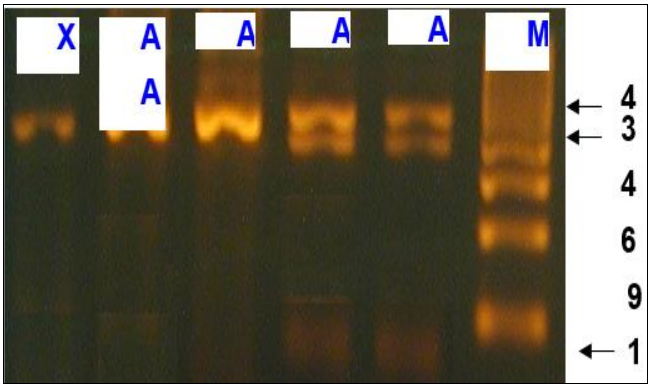

Fig 1: Genotypes of 437 bp kappa casein gene amplified product digested with *Hind* III revealed by PCR-RFLP in 3% agarose gel

The frequency of A and B alleles were 0.88±0.02 and 0.12±0.02 respectively (Table 1). The genotypes AA and AB were found in 84 animals (0.76) and 26 animals (0.24), respectively. The genotype BB could not be found in any of the animals under study. The present was found similar to the findings from Friesian Holstein cattle where the frequency of A allele is 0.78 - 0.85[15]. Higher frequencies of A allele (0.62 - 0.63) were reported from Egyptian Baladi and Holstein cattle3 [2], Turkey native cattle, Anatolian black and East Anatolian red cattle (0.75 - 0.78) [16]. In a study, the frequency of κ-CN allele (0.81) was higher than B allele (0.19) in Palestinian Holstein- Friesian cattle [17]. The genotypic frequencies observed in the population were within the Hardy-Weinberg expectation.

ARMS-PCR analysis of β-casein locus

The β-casein family constitutes up to 45% of the casein of bovine milk. There were two mismatches at –2 in 3′ ends in both inner primers. Two alleles were observed due to mutation on position 149 of the amplicon. The B allele was produced due to mutation in nucleotide position 8267 from Cytosine (C) to Guanine (G). The amplified products of genotypes AA and AB animals yielded two (338 and 217 bp) and three (338, 217 and 177) fragments, respectively (Figure 2). It was found that A allele was found in a predominantly high frequency (0. 98±0.01) than that of B allele (0.02±0.01). Among the genotypes, AA was observed in 106 animals, whereas AB genotype was observed in 4 animals only (Table 1). The genotype BB was not found in any of the animals in the population. In accordance with the present finding, the frequency of β-casein A variants was 0.94 in Kangayam cattle [18]. The genotypic frequencies observed in the population were within the Hardy-Weinberg expectation for the β-casein locus in agreement with other studies.

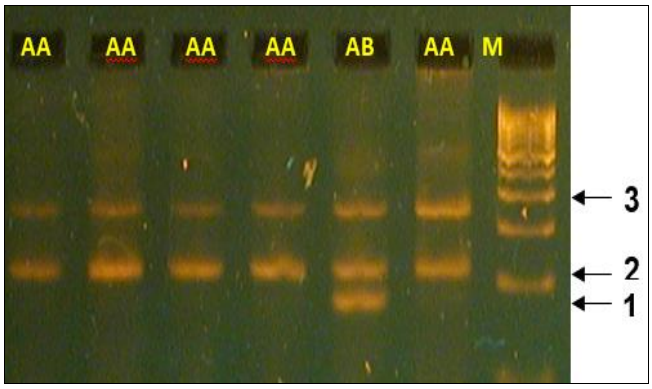

Fig 2: Genotypes of β-casein gene revealed by ARMS-PCR in 3% agarose gel

ARMS-PCR analysis of αs1-casein locus

There were two mismatches at –2 in 3′ ends in both primers. Two alleles were observed due to mutation on position 100 of the amplicon. The B allele was produced due to mutation in nucleotide position 1956 from Arginine (A) to Guanine (G).

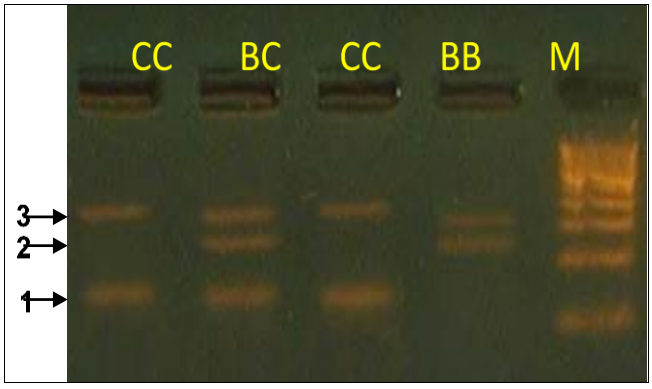

Fig 3: Genotypes of αS1-casein gene revealed by ARMS-PCR in 3% agarose gel

The amplified products of genotypes AA, AB and BB animals yielded two (310 and 236 bp), three (310, 236 and 130 bp) and two (310 and 130 bp) fragments, respectively (Figure 3). It was found that B allele was found in a higher frequency (0.61±0.04) than that of B allele (0.39±0.04) in the population. Among the αs1-casein genotypes, BB has observed in 55 (0.50) animals whereas AB genotype was observed in 23 (0.21) animals. The genotype BB was found in 32 (0.29) animals in the population. The present findings are in agreement with the reported predominance of the αs1-casein B allele in the zebu population in contrast with the high frequency of the αs1-casein B allele in *Bos taurus* breeds [19]. The other variant αs1-casein D allele was reported in Jersey cows from the Netherlands [20]. This variant was not found in

Sahiwal population. This asymmetric distribution in zebu and European cattle may be explained by the different processes of domestication to which these animals were submitted. The genotypic frequencies observed in αs1-casein locus in the population were not within the Hardy-Weinberg expectation.

PCR-RFLP analysis of α-lactalbumin locus

The α-lactalbumin is a constituent of lactose-synthetase, the enzyme responsible for the synthesis of lactose. Because of its association with lactose production, α-lactalbumin is thought to play a role in regulating milk volume. A 309 bp fragment of α-lactalbumin locus was amplified corresponding to exon I and a part of 5′ flanking sequence and intron I.

Two allelic variants A and B were observed in the present study in Sahiwal. The digestion of amplified fragments with *Msp*I yielded two types of restriction patterns due to underlying changes in codon 10 and respective genotypes were identified accordingly. The restriction digests of BB and AB animals yielded two (220 and 89 bp) and three (309, 220 and 89) fragments, respectively (Figure 4).

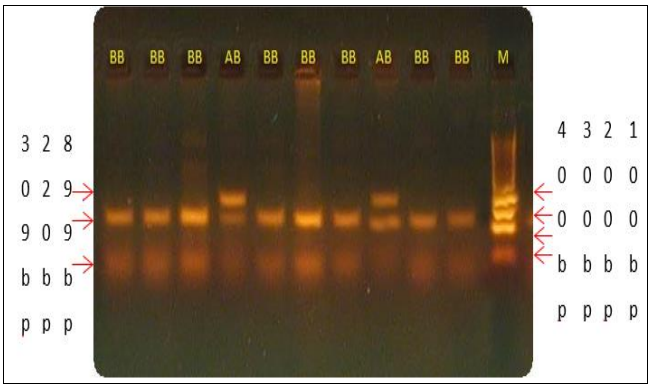

Fig 4: Genotypes of 309 Bp amplified product of α-lactalbumin gene digested with Msp I revealed by PCR-RFLP in 3% agarose gel

It was found that the B allele was found in high frequency (0.89±0.02) than that of A allele (0.11±0.02). Among the genotypes, BB genotype was observed in high frequencies (0.78) whereas AB genotype had the frequency of 0.22 in the population (Table). The genotype AA could not be found in any of the animals in the population. In an analysis of milk whey protein fraction by starch gel and polyacrylamide gel electrophoresis, a higher frequency of α-lactalbumin A variants (0.621±0.0275) was reported in Kangayam cattle [18]. Among several European breeds of *Bos taurus* cattle, α-lactalbumin was found to be monomorphic with the fixation of the α-lactalbumin B allele [21]. However, *Bos taurus* cattle of Podolic breed, like *Bos indicus*, were polymorphic at α-lactalbumin locus [22]. On the contrary, α-lactalbumin A allele was found to occur at a higher frequency (0.95 to 0.96) in some of the *Bos taurus* breeds of Roumanian and Russian origin [23]. A third α-lactalbumin variant, named C, was reported in Bali (banteng) cattle (*B. javanicus*) [24]. Until now, there is no evidence of this third allele neither in *B. taurus* nor in *B. indicus*.

PCR-RFLP analysis of β-lactoglobulin locus

The β-lactoglobulin is the major whey protein in cow milk. A 262 bp fragment of β-lactoglobulin gene was amplified corresponding to exon IV. The digestion of amplified fragments with HaeIII yielded two types of restriction patterns and accordingly genotypes were identified. The A allele showed 153 and 79 bp fragments. The digested B allele produced 109, 79 and 74 bp fragments. The heterozygous β-lactoglobulin AB genotype yielded a restriction pattern of 153 bp, 109 bp and 79/74 bp fragments (Figure 5).

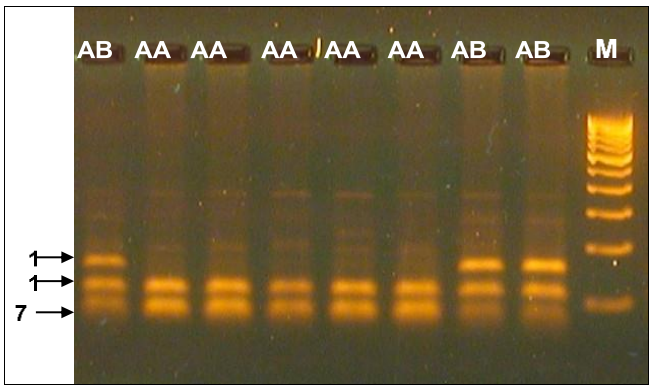

Fig 5: Genotypes of 262 bp β-lactoglobulin gene amplified product digested with *Hae* III revealed by PCR-RFLP in 3% agarose gel

The β-lactoglobulin B allele was found predominantly with the frequency of 0.84±0.02 in the population. The genotypes BB and AB were found in 75 (0.68) animals and 35 animals (0.32), respectively. The genotype AA was not found in any of the animals under study. The present finding further confirmed the results previously reported in Indian Zebu cattle [25]. The genotypic frequencies observed in the population were within the Hardy-Weinberg expectation for the β-lactoglobulin locus

Table 1: Gene and genotypic frequencies of milk protein loci in Sahiwal cattle

Parameter		Locus				
		κ-Casein	**β-Casein**	**αS1-Casein**	**β-lactoglobulin**	**α-Lactalbumin**
Allele	A	0.88±0.02	0.39±0.04	0.39±0.04	0.16±0.03	0.11±0.02
	B	0.12±0.02	0.61±0.04	0.61±0.04	0.84±0.03	0.89±0.02
Genotype	AA	0.96	0.29	0.29	0	0
	AB	0.04	0.21	0.21	0.33	0.22
	BB	0	0.50	0.50	0.67	0.78
Observed Heterozygosity		0.23	0.04	0.21	0.33	0.22
Expected Heterozygosity		0.21	0.04	0.48	0.27	0.20
Chi-square value		1.79^{NS}	0.03^{NS}	26.07*	3.96^{NS}	1.63^{NS}

^{NS}Non-significant, *Significant at $P<0.05$

Heterozygosity in the Milk Proteins Loci

The unbiased and maximum likelihood estimates of heterozygosity in Sahiwal cattle are presented in Table. The observed heterozygosity ranged from 0.04 to 0.48 with the mean heterozygosity value of 0.24. The highest and lowest heterozygosity values were observed in αS1-Casein and β-

Casein loci respectively. The observed homozygosity and heterozygosity of the milk proteins loci in the population were within the Hardy-Weinberg expectation as revealed by the chi-square value.

The findings contribute to a better understanding of the distribution of milk protein variants in Sahiwal cattle. Further, the information may help in the breeding process and effective selection procedures.

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