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Allelic Frequency of Kappa-Casein and Beta-Lactoglobulin in Indian Crossbred (*Bos taurus* × *Bos indicus*) Dairy Bulls

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Abstract: A polymerase chain reaction – restriction fragment length polymorphism test was performed on DNA samples extracted from blood samples of 256 Holstein Friesian crossbred and 112 from Jersey crossbred bulls/bull calves to detect allelic frequency of the bovine kappa-casein and beta-lactoglobulin gene responsible for milk production traits. A 350 bp fragment of kappa-casein and a 247 bp fragment of beta-lactoglobulin were amplified and digested with *Hinf*I and *Hae* III restriction enzymes, respectively. In the study, 2 types of alleles A and B and 3 types of genotypes AA, BB, and AB for kappa-casein and beta-lactoglobulin were observed. The results indicated that allelic frequency of kappa-casein for A-allele (0.66) was higher compared to B-allele (0.34). Similarly, B-allele frequency (0.72) of beta-lactoglobulin was much higher compared to A-allele (0.27) in crossbred dairy bulls. Kappa-casein and beta-lactoglobulin are useful markers for milk production traits on which bulls can be evaluated and selected for future breeding programmes.

Key Words: Jersey, Holstein Friesian, Kappa-casein, Beta-lactoglobulin, PCR-RFLP, genotype

Introduction

Crossbreeding programmes initiated during the 1950s in India between indigenous and exotic cattle mainly with Holstein Friesian (HF) and Jersey have boosted milk production many fold. Normally cow's milk contains 3%-5% protein, of which 80% is casein and 20% is whey protein. Beta-lactoglobulin is 1 of 2 major whey proteins found in the milk of animals including cattle, sheep, dogs, and pigs (but is not found in humans, mice, and other mammalian species). It occurs in several different variants due to genetic variations within and between various species. These whey proteins and the caseins, a second major classification of milk proteins, such as kappa-casein, are a source of minerals and amino acids for the young. These proteins play a crucial role in the coagulation and curdling of milk. This role in coagulation is also important to humans in that it is a required component in the production of cheese.

It has been confirmed by several authors that kappa-casein gene variant 'B' would be more desirable than 'A' variant since it is linked with higher casein, total protein, and fat content in the milk (1), and higher cheese yielding

capacity (2), as well as better coagulation in terms of rennet clotting time and curd firmness (3).

Two major genetic variants of Kappa-casein, A and B, were identified. Variant A has threonine (ACC) and aspartic acid (GAT) amino acid at positions 136 and 148, respectively. In variant B, isoleucine (ATC) substitutes threonine and aspartic acid is substituted by alanine (GCT). These differences result from a single base mutation in the Kappa-casein gene (4). Similarly, Beta-lactoglobulin is also found in a number of genetic variants in which A and B variants are predominant. The variants differ by 2 amino acid substitutions in the polypeptide chains and 2 single nucleotide substitutions in the Beta-lactoglobulin. Variant A has aspartic acid (GAT) and valine (GTG) at positions 64 and 118, whereas variant B has glycine (GGT) and alanine (GCG). Milk produced by LGB AA-genotype was found to contain more lactoglobulin, less casein, and less fat than that obtained from BB cows (5). The objective of this study was to determine the genotypes and allelic frequency of kappa-casein and beta-lactoglobulin in crossbred dairy bulls, mainly HF × Zebu and Jersey × Zebu, in India.

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Materials and Methods

Blood samples were collected from 368 crossbreds including 256 HF crossbred and 112 from Jersey crossbred bulls/bull calves from different sperm stations/farms across the country. The DNA was extracted from blood cells by phenol chloroform method (6). The quality and quantity of DNA were estimated using a spectrophotometer and agarose gel electrophoresis.

PCR-RFLP assay for kappa-casein genotypes

For detection of kappa-casein genotypes, a 350 bp DNA fragment was amplified by polymerase chain reaction (PCR), which was set by adding sense primer (5' ATC ATT TAT GGC CAT TCC ACC AAA G 3') and antisense primer (5'GGC CAT TTC GCC TTC TCT GTA ACA GA 3'). The PCR mixture contained 1 X PCR buffer, 0.4 mM dNTPs, 1 U of *Taq* DNA polymerase, 0.4 pM each of sense and antisense primer, 100 ng of DNA, 2.5 mM MgCl₂, and sterilised distilled water to make a final volume of 25 µl. The PCR reaction included the following steps: predenaturation for 3 min at 94 °C followed by 30 cycles 94 °C for 30 s, 58 °C for 1 min, 72 °C for 2 min, and a final extension of 10 min at 72 °C. The PCR product of 350 bp was seen on 4% agarose gel. The amplified PCR product was digested using *Hinf*I and 1 X reaction buffer at 37 °C overnight. The digested product was loaded and visualised on 4% agarose gel after staining with ethidium bromide.

PCR-RFLP assay for beta-lactoglobulin genotypes

Similar to kappa-casein, for detection of beta-lactoglobulin genotypes, a 247 bp DNA fragment was amplified by PCR, which was set by adding sense and antisense primers: 5' TGT GCT GGA CAC CGA CTA CAA AAA 3' and 5' GCT CCC GGT ATA TGA CCA CCC TCT 3'. The PCR mixture contained 1 X PCR buffer, 0.4 mM dNTPs, 1 U of *Taq* DNA polymerase, 0.4 pM of each primer, 100 ng of DNA, 2.5 mM MgCl₂, and sterilised distilled water to make a final volume of 25 µl. The PCR reaction included the following steps: predenaturation for 3 min at 94 °C followed by 30 cycles of 94 °C for 1.5 min, 58 °C for 1 min, 72 °C for 2 min, and a final extension for 10 min at 72 °C. The PCR product of 247 bp was visualised on 4% agarose gel. The amplified PCR product was digested using *Hae* III and 1 X reaction buffer at 37 °C overnight. The digested product was loaded and visualised on 4% agarose gel after staining with ethidium bromide.

Results

Electrophoretic analysis of the isolated DNA using 0.8% agarose gel followed by observation on a UV Transilluminator revealed sharp high molecular weight bands of DNA, which indicates that DNA was of good quality and suitable for PCR-RFLP analysis. The visual estimation revealed that the concentration of DNA is about 200 µg/ml. The optical density (OD) values at 260 nm and 280 nm obtained by UV spectrophotometer were used for estimating the quality and quantity of the isolated DNA. The restriction digestion analysis of 350 bp PCR product of kappa-casein indicates the presence of 3 types of restriction pattern. In the first pattern, 3 fragments (134, 132, 84 bp) were observed, while in the second pattern 2 fragments (266 and 84 bp) were observed. The third pattern produced 4 fragments (266, 134, 132, 84 bp), which was the coupling of the first and the second pattern; in other words, it is a heterozygote (Figure 1). Hence the first pattern was assigned as genotype AA, the second pattern as genotype BB, and the third as genotype AB. The restriction digestion analysis of the 247 bp PCR product of beta-lactoglobulin indicates the presence of 3 types of restriction pattern; 2 fragments of 148 and 99 bp, 2 fragments of 99 and 74 bp (74 bp fragment was created due to the overlapping of 2 bands that are 2x) and 3 fragments of 148, 99, and 74 bp were observed. The third pattern of the 3 DNA fragments of 148, 99, and 74 bp was coupling of the first and the second patterns; in other words, it is a heterozygote (Figure 2). Hence the first was assigned as genotype AA, the second as genotype BB, and the third as genotype AB.

The Table reveals that the AA genotype frequency (0.44) of kappa-casein was higher than that of BB genotype (0.12) and was similar to AB genotype (0.44). Similarly AA genotype frequency (0.06) of beta-lactoglobulin was far lower than that of BB genotype (0.50) and AB genotype (0.44). The allelic frequency of kappa-casein for A-allele (0.66) was almost 2 times higher than that of B-allele (0.34), indicating that two-third crossbred breeding bulls have allele (A) for less casein production. Similarly the B-allele frequency (0.73) was much higher than A-allele (0.27) for beta-lactoglobulin, indicating that one-third crossbred bulls have B-allele for higher fat production.

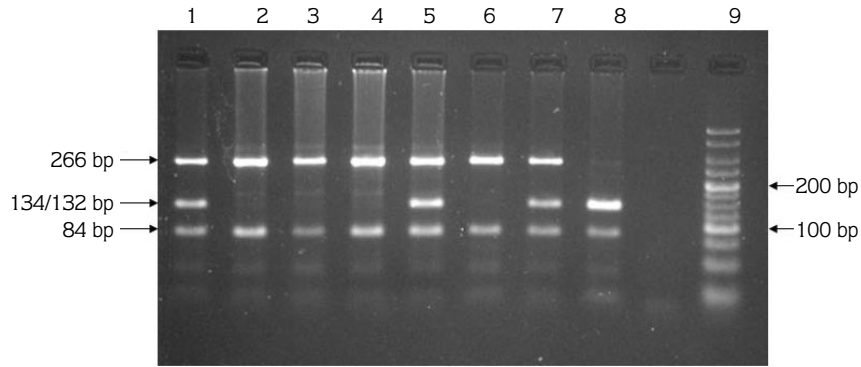


Figure 1. Electrophoretogram of *Hinf* I digested PCR product generated by amplification of genomic DNA using kappa-casein specific primers. Lane # 1,5 & 7-AB Genotype; lane # 2,3,4 & 6-BB Genotype; lane # 8-AA Genotype; lane # 9-24 bp DNA marker.

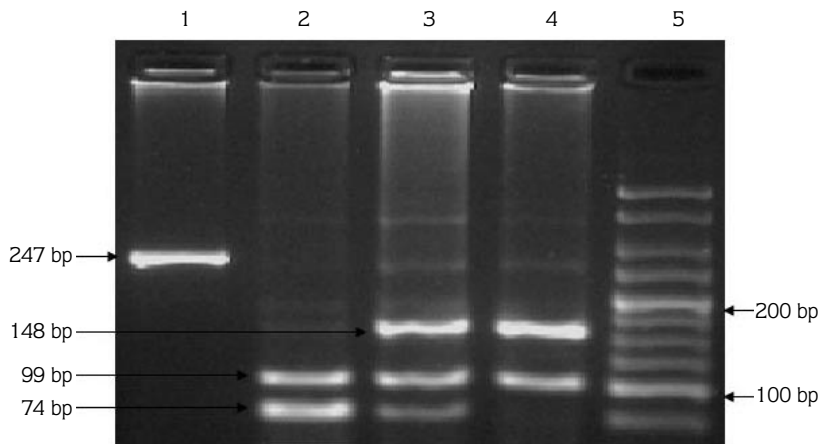


Figure 2. Electrophoretogram of *Hae* III digested PCR product generated by amplification of genomic DNA using Beta-lactoglobulin specific primers. Lane # 1; PCR Product-247 bp, lane # 2; BB Genotype, lane # 3; AB Genotype, lane # 4; AA Genotype, lane # 5; 25 bp DNA marker.

Table. Genotypes, genotype frequencies (in parentheses), and allele frequencies in crossbred bulls (n = 368).

Animal breed	No. of bulls	Genotype (genotype frequency)						Allele frequency			
		Kappa-casein			Beta-lactoglobulin			Kappa-casein		Beta-lactoglobulin	
		AA	BB	AB	AA	BB	AB	A	B	A	B
HF crossbred	256	138 (0.54)	25 (0.10)	93 (0.36)	12 (0.05)	133 (0.52)	111 (0.43)	0.72	0.28	0.26	0.74
Jersey crossbred	112	23 (0.21)	18 (0.16)	71 (0.63)	07 (0.07)	52 (0.46)	53 (0.47)	0.52	0.48	0.30	0.70
Total	368	161 (0.44)	43 (0.12)	164 (0.44)	19 (0.06)	185 (0.50)	164 (0.44)	0.66	0.34	0.27	0.73

Discussion

The frequency of alleles for kappa-casein and beta-lactoglobulin in crossbred populations depends on the frequencies of their dams and sires (*Bos taurus* and *Bos indicus*) that are primarily used for crossbreeding. This could be because of HF and zebu cattle as a study revealed the higher allelic frequency of A-allele (0.86) for kappa-casein and B-allele (0.64) for beta-lactoglobulin (7) in the HF population. In a previous study, indigenous cattle of different breeds also revealed that the frequency of A-allele was 0.78 for kappa-casein whereas the frequency of B-allele was 0.66 for beta-lactoglobulin (8). On the other hand, high allelic frequency of B-allele (0.65) for both kappa-casein and beta-lactoglobulin (0.66) were observed in a Jersey cattle population (9).

Kappa-casein B allele was reported to have a favourable and significant effect on both milk and milk protein yield (10). The relationship between the allele and high protein content of milk as well as the technological properties of milk were also reported (11). The fact that milk produced by beta-lactoglobulin AA-genotype cows

was found to contain more lactoglobulin, less casein, and less fat than that obtained from BB-genotyped cows (2,5,12) showed that milk produced by BB genotype cows yielded significantly more cheese than that produced by AA-genotype cows. In Polish Black and White cattle, cows of kappa casein AA genotype were characterised by higher overall milk production, while those of AB and BB genotype yielded milk with higher protein content (13).

In general, B variant of both proteins was recognised as superior for milk quality in European cattle breeds. Thus, it may be concluded that kappa-casein and beta-lactoglobulin genotypes, when used as genetic markers in selection programmes, may moderately but significantly contribute to the improvement of milk production traits in cattle. A selection based on markers not only minimises problems but also they are more reliable, and animals can be selected at an early age for breeding programmes. Three types of genotypes (AA, BB, and AB) and 2 types of alleles (A and B) were observed in the study that are useful markers for milk production traits on which bull can be evaluated and selected for future breeding programmes.

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