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Research Article

DETECTION OF ê-CASEIN AND â-LACTOGLOBULIN VARIANTS IN HOLSTEIN FRIESIAN CATTLE BY PCR-RFLP

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ABSTRACT

A polymerase chain reaction–Restriction Fragment Length Polymorphism assay was performed on genomic DNA extracted from blood samples of 281 pure Holstein Friesian bulls/ bull calves, to detect allelic variants of the bovine kappa casein and beta-lactoglobulin gene responsible for milk production traits. A 350 bp fragment of kappa casein and 247 bp fragment for beta-lactoglobulin was amplified and digested with Hinf I and Hae III restriction enzymes respectively. Two types of alleles A and B and three types of genotypes AA, BB and AB for kappa casein and beta-lactoglobulin were observed in the study which are useful markers for milk production traits on which bull can be evaluated and selected for future breeding programmes.

Key words: PCR-RFLP, ê-casein, â-lactoglobulin, cattle, Holstein Friesian

The black and white Friesian cattle are the most numerous and productive among the three hundred or more cattle breeds in the world today. There are very few countries where it is not present as a pure breed or used for crossbreeding (Jasiorowski *et al.*, 1998). Normally cow's milk contains 3-5% protein, out of which 80% is casein and 20% is whey protein. Beta-lactoglobulin (â-lactoglobulin) is one of two major whey proteins, found in the milk of animals including cattle, sheep, dogs, and pigs. The second major classification of milk protein such as êappa-casein (ê-casein) with whey 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 occurs in several different variants due to genetic variations within and between various species. It has been confirmed by several authors that ê-casein gene variant "B" would be more desirable than "A" variant since it is linked with higher casein, total protein as well as fat content in the milk (Kroeker *et al.*, 1985) and higher cheese yielding capacity (McLean *et al.*, 1984) as well as better coagulation property in terms of rennet clotting time and curd firmness (Marzialli and Ng-Kwai-hang, 1986).

Two major genetic variants of ê-casein such as A and B have been identified. Variants A has threonine (ACC) and aspartic acid (GAT) amino acid at position 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 ê-casein gene (Pinder *et al.*, 1991). Similarly, â-lactoglobulin is also found in a number of genetic variants of which A and B are predominant. The variants differ by two amino acids substitutions in the polypeptide chains and two single nucleotide substitutions in the â-lactoglobulin. Variant A has aspartic acid (GAT) and valine (GTG) at 64 and 118 positions whereas variant B has glycine (GGT) and alanine (GCG). Milk produced by â-lactoglobulin AA-genotype has been found to contain more lactoglobulin, less casein and less fat than that obtained from BB cows (Van der Berg *et al.*, 1992). The objective of this study was to determine ê-casein and â-lactoglobulin, the known genotypes for milk traits, in pure Holstein Friesian (HF) dairy breed in India.

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MATERIALS AND METHODS

Blood samples were collected from 281 pure HF bulls/bull calves from different sperm stations/farms across the country. The DNA was extracted from blood cells by phenol chloroform method as described by Sambrook *et al*. (1989). The quality and quantity of DNA was estimated using spectrophotometer and agarose gel electrophoresis.

PCR-RFLP assay for ê-casein genotype: For detection of ê-casein genotypes, 350 bp DNA fragment was amplified by polymerase chain reaction (PCR) 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'). PCR mixture contained 1X PCR buffer, 0.4mM dNTPs, 1U of *Taq* DNA polymerase, 0.4 pM each of sense and antisense primers, 100ng DNA, 2.5 mM $MgCl_2$ and sterilized distilled water to make a final volume of 25 ìl. The PCR reaction included the following steps: predenaturation for 3 minutes at 94°C followed by 30 cycles at 94°C for 30 seconds, 58°C for 1 minute, 72°C for 2 minutes and final extension of 10 minutes at 72°C. The amplified PCR product was digested by using *Hinf* I and 1X reaction buffer at 37° C for overnight. The digested products were loaded and visualized on 4% agarose gel after staining with ethidium bromide.

PCR-RFLP assay for â-lactoglobulin genotypes: Similarly, detection of â-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' respectively. PCR mixture contained 1X PCR buffer, 0.4 mM dNTPs, 1U of Taq DNA polymerase, 0.4 pM each primer, 100ng DNA, 2.5 mM $MgCl_2$ and sterilized distilled water to make a final volume of 25ìl. The PCR reaction included the following steps: Predenaturation for 3 minutes at 94^{o}C followed by 30 cycles of 94^{o}C for 1½ minutes, 58°C for 1 minute; 72°C for 2 minutes and final extension for 10 minutes at 72°C. The amplified PCR product was digested by using *Hae* III in 1X reaction buffer at 37°C for overnight. The digested products were loaded on 4% agarose gel and visualized after staining with ethidium bromide.

RESULTS AND DISCUSSION

Electrophoretic analysis of isolated genomic DNA using 0.8% agarose gel followed by observation on UV transilluminator revealed sharp high molecular weight bands of DNA that indicates that DNA was of good quality and suitable for PCR-RFLP analysis. The ratio of the optical density estimated at 260 nm and 280 nm by using UV spectrophotometer, was ranging from 1.7 to 1.9, indicating good quality of DNA without contamination. The restriction digestion analysis of 350 bp PCR product of ê-casein indicates the presence of three types of restriction patterns. In the first pattern, three fragments 134, 132, 84 bp were observed (showing two bands because of overlapping of two fragments of 134 and 132 bp) while in the second pattern two fragments 266 and 84 bp were observed. The third pattern produced four fragments 266, 134, 132, 84 bp, (showing three bands because of overlapping of two fragments of 134 and 132 bp) which was the coupling of first and second pattern, in other words it is a heterozygote (Fig 1). Hence the first pattern was assigned as genotype AA, second pattern as genotype BB and the third as genotype AB. Similarly, the restriction digestion analysis of the 247 bp PCR product of â-lactoglobulin also revealed three types of restriction pattern, two fragments of 148, 99 bp, two fragments of 99, 74 bp (overlapping of two bands of 74 bp) and three fragments of 148, 99, 74 bp respectively. Third pattern of three fragments of 148, 99 and 74 bp is a heterozygote. (Fig 2) Hence the first and second group were assigned as genotype AA, and genotype BB, and third was designated as genotype AB.

The study reveals that AA genotype (73.7%) for ê-casein was very high than BB genotype (2.1%) and almost three times higher than AB genotype (24.2%) indicating that $2/3^{rd}$ HF population has allele (A) for less casein production (Table). Similarly AA genotype (14.2%) for â-lactoglobulin was far less than

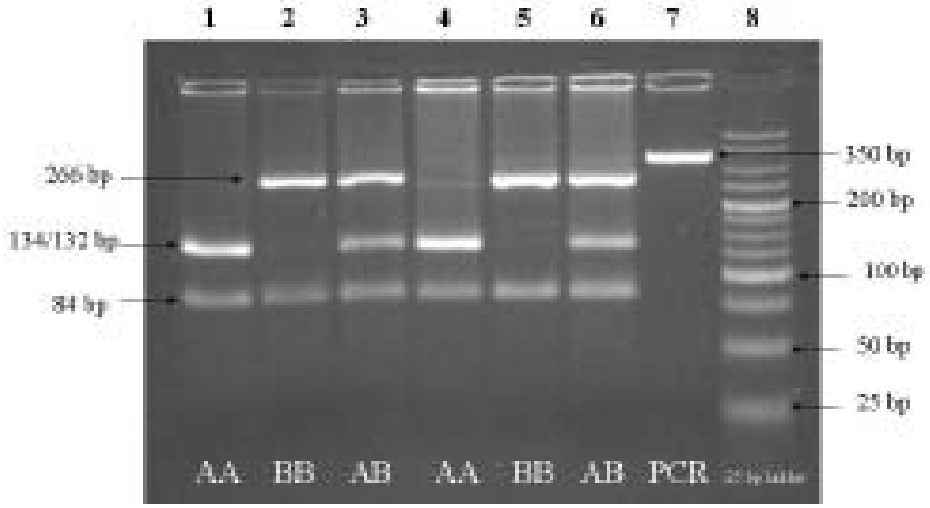

Fig 1. Electrophoretogram of *Hinf* I digested PCR product generated by amplification of genomic DNA, using *ê*- casein specific primers.
Lane # 1 & 4; 134, 132 & 84 bp bands indicate AA-genotypes
Lane # 2 & 5; 266 & 84 bp bands indicate BB-genotypes
Lane # 3 & 6; 266, 134, 132 & 84 bp bands indicate AB-genotypes
Lane # 7; 350 bp of PCR product, Lane # 8; 25 bp DNA ladder

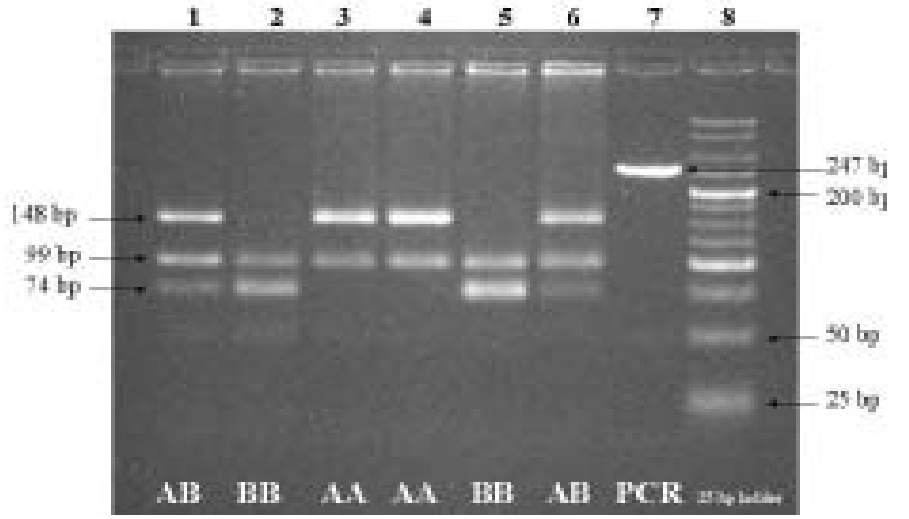

Fig 2. Electrophoretogram of *Hae* III digested PCR product generated by amplification of genomic DNA, using â-lactoglobulin specific primers.
Lane # 1 & 6; 148, 99 & 74 bp bands indicate AB-genotypes
Lane # 2 & 5; 99 & 74 bp bands indicate BB-genotypes
Lane # 3 & 4; 148 & 99 bp bands indicate AA-genotypes
Lane # 7; 247 bp of PCR product, Lane # 8; 25 bp DNA ladder

BB genotype (41.9%) and AB genotype (43.8%) indicating that Indian HF population has favourable allele for whey protein as well as fat production, as B- allele is favorable for higher fat percentage. The ê-casein and â-lactoglobulin are proteins expressed in milk and due to their polymorphism, may serve as informative molecular markers for yield, composition and technological properties of milk (Medrano *et al*., 1990, Ron *et al*., 1994, Sabour *et al*., 1996). The *ê*-casein B allele was reported to have a favourable and significant effect on both milk and milk protein yield (Mao *et al*., 1992). Relationship between the allele and high protein content of milk as well as the technological properties of milk has also been reported by Sabour *et al*. (1996). Milk produced by â-lactoglobulin AA-genotype cows found to contain

Table
Genotypes of ê-casein and â-lactoglobulin estimated in Holstein Friesian cattle

Animal no.	Genotype					
	ê-casein			â-lactoglobulin		
	AA	BB	AB	AA	BB	AB
Total (281)	207	6	68	40	118	123
Per cent	73.7	2.1	24.2	14.2	41.9	43.8

more lactoglobulin, less casein and less fat than that obtained from BB-genotyped cows (Hill *et al*., 1993, Van der Berg *et al.*, 1992) showed that milk produced by BB genotype cows yielded significantly more cheese than that by AA-genotype cows. In Polish Black and White cattle, cows of ê-casein AA genotype were characterized by higher overall milk production, while those of AB and BB genotype yielded milk with higher protein content (Walawski *et al*., 1994).

In general, B variant of both proteins have been recognized as superior for milk quality in European cattle breeds. Thus, it may be concluded that ê-casein and â-lactoglobulin genotype when used as genetic markers in selection programmes, may moderately but significantly contribute to the improvement of milk production trait in cattle. Genetic improvement by selection based on breeding value, though provides potential to enhance the performance of animals but it is a time consuming exercise as generation interval is very high and one has to wait for selection till the phenotypic expression of the desired traits. Moreover, as milk traits are limited to females, it is not possible to select male cattle on the basis of their own performance. Selection based on markers not only minimizes problem but also they are more reliable and animals can be selected at an early age for breeding programme. Therefore, three types of genotypes AA, BB, and AB and two types of alleles A and B for ê-casein and â-lactoglobulin were observed in the study which are useful markers for milk production traits on which bull can be evaluated and selected for future breeding programmes.

REFERENCES

Hill, J.P. (1993). The relationship between â-lactoglobulin phenotype and milk composition in New Zealand dairy cattle. *J. Dairy Sci*. **76**: 282-286.

Jasiorowski, H.A., Stolzman, M. and Reklewski, Z. (1998). The International Friesian Strain Comparison Trail. A publication of FAO. pp. 1-18.

Kroeker, E.M., Ng-Kwai-Hang, K.F., Hayes, J.F. and Moxley, J.E. (1985). Effect of environmental factors and milk protein polymorphism on composition of casein fraction in bovine milk. *J. Dairy Sci.* **68**: 1752-1757.

Mao, I.L., Buttazozonil, G. and Aleandri, R. (1992). Effects of polymorphic milk protein genes on milk yield and composition traits in Holstein cattle. *Acta Agriculturae Scand. Section A (Anim. Sci.)* **42**: 1-8.

McLean, D.M., Graham, E.R.B., Ponzoni, R.W. and Mckenzie, H.A. (1984). Effects of milk protein genetic variants on milk yield and composition. *J. Dairy Res.* **51**: 531-546.

Marzialli, A.S. and Ng-Kwai-Hang, K.F. (1986). Effects of milk composition and genetic polymorphism on coagulation properties of milk. *J. Dairy Sci.* **69**: 1793-1798.

Medrano, J.F. and Aquilar-Cordova, E. (1990). Polymerase chain reaction amplification of bovine â-lactoglobulin genomic sequences and identification of genetic variants by RFLP analysis. *Anim. Biotech*. **1**: 73-77.

Pinder, S.J., Perry, B.N., Skidmore, C.J. and Savva, D. (1991). Analysis of polymorphism in the bovine casein genes by use of polymerase chain reaction. *Anim. Genet*. **22**: 11-20.

Ron, M., Yoffe, O., Ezra, E., Medrano, J.F. and Well, J.L. (1994). Determination of milk protein genotype on production traits of Israeli Holsteins. *J. Dairy Sci*. **77**: 1160-1173.

Sabour, M.P., Lin, C.Y., Lee, A.J. and McAllister, A.J. (1996). Association between milk protein variants and genetic values of Canadian Holstein bulls for milk yield traits. *J. Dairy Sci*. **79**: 1050-1056.

Sambrook J, Fritsch E.F. and Maniatis T. (1989). Molecular Cloning: A Laboratory Manual, (2nd edn.) Vol. 3, Cold Spring, Harbour Laboratory Press, New York.

Van der Berg, G., Escher, J.T.M., D.E. Konning, P.J. and Bovenhuis, H. (1992). Genetic polymorphism of κ-casein and β-lactoglobulin in relation to milk composition and processing. *Netherland Milk Dairy J*. **46**: 145-168.

Walawski, K., Sowinski, G., Czarnik, U. and Zabolewicz, T. (1994). Beta– lactoglobulin and kappa-casein polymorphism in relation to production traits and technological properties of milk in the herd of Polish Black and White cows. *Genitica Polonica* **12**: 93-108.