Genotyping of HF Crossbred Cattle for ß-Casein Genes Using PCR-RFLP

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ABSTRACT

The world is going through the debate on hazardous effects of consumption of A1 milk on human health by producing of hazardous chemical compound like beta casomorphin-7 and how this is not the case with A2. The aim of the present study was to genotype the existing population of crossbred Holstein-Friesian (HF) cattle for polymorphism in â-Casein gene (CSN2) using PCR-RFLP technique. Total of 47 pluriparous cows of HF crossbred cattle were tested from Satara District (Maharashtra state). Genomic DNA was isolated from the blood samples. Isolated DNA samples were tested using PCR-RFLP technique. Restriction enzyme Dde I *was used for detection of polymorphism. In the present study it was observed that the gene frequencies of A1 gene and A2 gene are 0.6383 and 0.3617, respectively. The genotype frequency of A1A1 genotype was 0.28 and that of A1A2 genotype was 0.72. In the present study, no correlation was observed between milk traits and CSN2 genotypes of crossbred cows.*

Key words*: β -Casein; Beta casomorphin-7; Genotype; Crossbred Cattle; Polymorphism; PCR-RFLP; Satara;*

ß-Casein gene (CSN2) is localized at bovine chromosome 6 and its primary structure is composed of 209 amino acids with molecular mass is 23,946 to 24,097 Da (depending on the genetic variant (*Ribadeau and Dumas et al.,1972*). Beta-casein, a member of casein cluster, is the most polymorphic milk protein gene with 13 protein variants and the gene variants most common in Holstein-Friesian (HF) cattle are A1 and A2, the others (e.g. B, A3, C) being rare (*Roginski 2003*). Polymorphism in one of the beta-casein gene codons – CCT $\rightarrow$ CAT – causes substitution of proline (A2) by histidine (A1, B) in position 67 in the amino-acid sequence. It contains 5 serine-phosphate residues arranged in one phosphate center of 4 Ser-P residues in the region 14 to 21 and an additional single Ser-P in position 35. Therefore, it is calcium sensitive protein and claimed to be the most hydrophobic casein.

In addition, the B variant differs from the A1 variant in a substitution of arginine for serine at position 122. Importantly, it is the change to histidine at position 67 that has the potential to result in cleavage occurring upon digestion and a bioactive peptide, beta-casomorphin potentially being liberated (*Stewart et al., 1987; Damiani et al., 1992; Lien et al., 1992*). The beta-casein/ CSN2-A1 variant was associated with the incidence of diabetes mellitus type 1 (*Beales et al., 2002*), coronary heart disease and autism (*Elliot et al., 1988*). CSN2-A2 reduces the serum cholesterol and decreases concentration of LDL lipids which play an important role in prevention of a wide range of human vascular diseases. Some epidemiological studies indicated that consumption of CSN2-A1 may be associated with a higher occurrence of cardiovascular heart disease (CVD) and type I diabetes of in humans. However to our knowledge, there is no published record on CSN2 polymorphism in HF crossbreed cattle from Maharashtra region. This information is prerequisite for developing selection and breeding programs of local cattle population. Hence, the present study was undertaken with following objectives:

i. To study the polymorphism ß-casein gene in existing HF crossbred cattle using PCR-RFLP technique.

ii. To study the relation between milk production, milk fat percent with various genotype of ß-casein genes.

METHODLOGY

The study involved 47 crossbred HF cattle from Khandala Taluka, Satara, Maharashtra. The samples were collected from 5 organized (24) and unorganized farms (23).

DNA Isolation: Bovine genomic DNA was isolated by phenol-chloroform deproteinization and ethanol precipitation (*Sambrook et al., 1989*). 10 ml blood samples were collected in EDTA-coated vials. Deprotienization was carried out using proteinase K and digestion buffer enzyme for 2 hours. Phenol-chloroform technique was used for purification and isolation and the resultant product was washed with 70 per cent and 99.99 per cent ethyl alcohol. The DNA isolates were stored in 10×TE Buffer at -20^{0}C. DNA isolates were quantified by NanoDrop technique by finding out optimal ratio of absorbance at 260/280 nm.

PCR Amplification:DNA primers described by *McLachlan (2006)* were used to PCR amplification: (Forward primer) 5´- CCT TCT TTC CAG GAT GAA CTC CAG G-3´, (Reverse primer) 5´ - GAG TAA GAG GAG GGA TGT TTT GTG GGAGGC TCT- 3´. The PCR was carried out as previously described by *McLachlan, 2006*. The reaction mixture in the total volume 25 µl containing 1000 ng/ µl DNA, 1µl Taq polymerase (Fermentas), 3 mM MgCl2, 200 ìMdNTP, 5 pM of each primer, 0,006 mg BSA. The following amplification parameters were applied: 95^{o}C for 5 minutes followed by 30 cycles: 95°C for 40 seconds, 58°C for 60 seconds, 72°C for 90 seconds. The reaction was completed by the final synthesis 72°C for 10 minutes.

RFLP digestion : The PCR amplicon produced of CSN2gene was digested with *Dde I* restriction enzyme (Promega) for 1.5 hours under 37^{o}C. The Dde *I* recognizes the sequence C/TNAG and generates fragments with 5'- cohesive termini. Restriction digestion fragments were loaded on 3 per cent agarose gel containing 10 per cent ethidium bromide and the gel were analyzed in the UV rays.

Statistical analysis: Based on the results of genotyping for CSN2gene: genotypic frequencies, allelic frequencies and Hardy-Weinberg equilibrium (HWE) were as executed in POPGENE 32 version 1.32 software (*Yeh et al., 1999, http://www.ualberta.ca/~fyeh/fyeh)*. The WASP2 software by ICAR, Goa was used to find out association between the CSN2 gene genotypes and milk production traits.

RESULTS AND DISCUSSION

In present study the quantification of DNA by NanoDrop technique observed ratio of absorbance at 260/280 nm ranges from 1.6 to 2.0. PCR amplification showed the amplicon size of 121bp for above mentioned PCR conditions. It was found that after RE digestion A1 was not digested and fragment of 121bp remained intact and A2 allele was digested and showed fragments of 86bp & 35bp. This result is concurrent with the previous study (*Miluchova, et al., 2013***)**. RFLP of DNA samples revealed 13 samples out of 47 were of A1A1 genotype, whereas 34 samples were of A1A2 genotype. The gene frequency of A1 gene is 0.638 (63.8%) (Table1) and A2 gene is 0.362 (36.2%), whereas in genotyping studies in Slovak Spotted breed of cows by (*Miluchova, et al., 2013***)** revealed frequency of A1 and A2 alleles **(**0.2928) and (0.7072) respectively. The increased frequency of A1 allele in present study may be due to crossing of purebred HF cattle having more number of homozygous A1A1 genotype. It has been reported in previous research papers that 77 per cent dairy exotic breeds including HF carry A1A1 genotypes (*Keating et al., 2008*). In this study observed percentage of A1A1genotype is 27.66 per cent, that of A1A2 genotype it is 72.34 per cent and that of A2A2 genotype is 0.00 per cent in local crossbreed HF cattle and when it's compared to genotyping studies in Slovak Spotted breed of cows by (*Miluchova, et al.,2013***)** revealed genotypic frequency as A1A1 (0.1261), A1A2 (0.3334), A2A2 (0.5405). The lower percentage A1A1 genotype observed in this study may be because, there is predominance of A2 variant (0.987) in zebu cattle breeds (*Mishra et.al 2009*). The percentage of A2A2 genotype in HF crossbred animals was found to be 0.00 per cent which is most favored genotype for preventing above mentioned health hazards.

There was no significant difference observed between production traits *viz.* milk yield and fat per

cent for observed genotypes, as shown in Table 1. As shown in Table 2 the population under study does not follow Hardy-Weinberg equilibrium.

CONCLUSION

It is concluded that CSN2 is polymorphic gene in local HF crossbreed cattle, mostly of A1 gene (0.6383). More samples of the local HF crossbred cattle should be screened to assess the exact actual picture of field conditions concerning the prevalence of A1/A2 polymorphism possibly with other combinations of primers too for changing the dairy herd to more A2 milk producing cows may significantly improve the public health, along with higher productivity.

Table 1. Comparison of milk production traits in different genotypes

Production trait	A1A1 genotype	A1A2 genotype	Sig.
Milk yield (kg)	2719.237+41.423	2769.116+35.514	NS
Fat %	3.613+0.045	3.667+0.037	

Table 2. Frequency of genotypes and alleles along with Chi-square test for CSN2 gene in crossbred Holstein-Friesian

Genotypes			Gene/ Allele		χ^2 test (HWE)
A1A1	A1A2	A2A2	A1	A2	
0.28(13)	0.72(34)	0.00(00)	0.6383	0.3617	14.58^{NS}

HWE: Hardy-Weinberg equilibrium; NS: Non-significant; figures in bracket indicate number of animals.

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