ANALYSIS OF β-CASEIN GENE FOR A1 AND A2 GENOTYPE USING ALLELE SPECIFIC PCR IN KANGEYAM AND HOLSTEIN FRIESIAN CROSSBRED CATTLE IN TAMIL NADU

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ABSTRACT

Milk is one of the most important protein diet to the human population. However, in last few decades, presence of A1 β-casein in milk was associated with important issues associated with range of illnesses in human being. In this study a total of 85 cattle blood samples (Kangeyam and HF crossbred) were analysed for A1 β-casein gene based on AS-PCR. A1/A2 genotype frequency data indicated that 37% were A2 homozygous (A2A2), 17% were A1 homozygous (A1A1) and 46% heterozygous (A1A2) in HF crossbred cattle. The pure Kangeyam (Bos indicus) cattle breed had only A2 gene and showed only A2A2 genotype, which produce safer A2 milk for the human consumption. The Holstein Friesian cross breed animal also showed mostly of A2 gene (0.595).

Key words: β-casein gene, AS-PCR, A1 and A2 genotype

Milk is one of the primary protein diet to the human population. Cow milk protein contains 25-30% β-casein, which contains 209 amino acids. Bovine β-casein (CASB) gene belongs to the cluster of 4 casein genes αS1, αS2, β and K, located on chromosome number 6 (Rijnkels, 2002). β-casein has 12 genetic variants A1, A2, A3, B, C, D, E, F, H1, H2, I, and G out of which A1 and A2 are common and B is the less common genetic variant (Farell et al., 2004). A2 β-casein is recognized as the original β-casein protein because it existed before a mutation caused the appearance of A1 α-casein in European cattle (Bos taurus) a few thousand years ago. β-Casomorphin – 7 (β-CM-7) is the compound released while digestion of A1 β-casein but A2 β-casein does not produce the compound. β-CM-7 is a strong opioid, which is 10 fold stronger than morphine. Bell et al. (2006) reported that the β-CM-7 can potentially affect numerous opioid receptors in the cardiovascular, respiratory, nervous system and immune system in human. β-CM-7 release is due to minor variation in β-casein A1, possessing the amino acid histidine at residue

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67 of the mature protein. The A2 variant has proline in that position and it does not favor the production of this protein fragment by gastric and gut enzyme during human digestion (Bell et al., 2006). This is due to single nucleotide polymorphism (SNP) of β casein gene. The gene possess 67th codon as CCT (A2), for the wild type β casein and the codon as CAT (A1) results in the production of mutated type β casein (Veena et al., 2013). A1 β-casein, significantly associated with type 1 diabetes mellitus (DM-1), ischemic heart disease (IHD), autism and including other immune suppression activities in human being (Bell et al., 2006 and Truswell, 2005).

Genetic characterization of β-casein gene for the majority of Bos taurus breeds indicated that they are carrier of A1 β-casein variants in most of the dairy breeds except few taurine breeds like Jersey and Gurensy having A2 β-casein variant (Kaminski et al. 2007). Mishra et al. (2009) reported that A2 variant is predominant in Zebu cattle breed. Country related differences in the β-casein allele frequencies are observed which may be reflective of local breeding policies, some cross-breeding and most prominently targeted breeding for increased milk production traits (Aschaffenburg 1963). There is no report on the frequency of bovine β-casein variants in the bovine population of Tamil Nadu. In this study, an attempt was made to analyse the frequency of the A1 allele of β-casein gene in Holstein-Friesian cross bred and Kangeyam cattle.

A total of 85 cattle, including 63 Holstein Friesian cross breed cattle and 22 Kangeyam cattle from the organized farms were included in this study. Blood samples were collected from each animal in sterile polypropylene tubes containing EDTA as an anti-coagulant. Genomic DNA was isolated from the blood sample using standard guanidine hydrochloride method (Montgomery and Sise, 1990). The purity and concentration of the DNA samples were assayed by picodrop (Picodrop Ltd, Cambridge, UK). The OD260/280 value of 1.7 to 1.9 was considered as pure DNA for further analysis.

Allele-specific PCR (AS-PCR) was carried out using the primers reported by Ganguly et al. (2013a); forward primers with either A (IGBhF 5’ CTT CCC TGG GCC CAT CCA 3’) or C (IGBpF 5’ CTT CCC TGG GCC CAT CCC 3’) at the 32 end and a common reverse primer (IGBR 5’ AGA CTG GAG CAG AGG CAG AG 3’) to amplify a 244 bp fragment of ß-casein gene. Primer pairs IGBhF-IGBR and IGBpF-IGBR were intended to give rise to Histidine (A1) and Proline (A2) specific amplicon, respectively. PCR was carried out with approximately 50 ng of genomic DNA in a final reaction volume of 25 µl containing 12.5 µl of Ampliqon Taq DNA Polymerase Master Mix red (2X) and 0.5 iM of each primer. PCR cyclic condition was done with initial denaturation at 94°C for 5 min, followed by 5 cycles of 94°C for 30 sec, 64°C for 30 sec and 72°C for 30 sec; thereafter 30 cycles of 94°C for 30 sec, 62°C for 30 sec and 72°C for 30 sec and a final extension of 72°C for 5 min. PCR products were visualized after electrophoresis in 1.5% agarose gel. Further, the PCR products were validated by sequencing using the pair of primers reported by Ganguly et al. (2013a); (A1A2 Seq Forward: 5’ CCA GGA TAA AAT CCA CCC CT 3’; A1A2 Seq Reverse: 5’ AGG GAA GGG CAT TTC TTT GT 3’) to amplify a 202 bp fragment of exon VII (figure 1B and 2).
Occurrence of the A1 variant of α-casein has much interest in the last decade due to the bioactive peptide β-CM-7 which is liberated from this A1 β-casein during enzymatic digestion. In the present study, an AS-PCR was carried out to explore the presence of A1A2 polymorphism in Tamil Nadu native cattle breed Kangeyem and Holstein Friesian cross breed (HF X Local cattle) cattle. Electrophoretic analyses of the AS-PCR ampliqons from cattle and their genotype (Figure 1A) and the gene and genotype frequency results are listed in Table 1. Among these, only A2A2 genotype was observed in Kangeyam cattle and not a single animal had A1A1 and A1A2 genotype, which is concurred with the findings of Mishra et al. (2009). In case of gene frequency, Mishra et al. (2009) observed similar trend in Kangeyam cattle population as 0 and 1 for A1 and A2, respectively. While all the three genotypes viz., A1A1, A1A2 and A2A2, were found in the HF cross bred cattle and genotype frequency were 0.17, 0.46 and 0.37 respectively. Ganguly et al. (2013b), found that the β-casein gene frequency in Frieswal cattle population as 0.15(A1A1), 0.41(A1A2) and 0.44 (A2A2). The allele and genotype frequencies of β-casein in crossbred cattle population in Kerala were found as 0.46(A1), 0.56(A2) and 0.32(A1A1), 0.28(A1A2), 0.40(A2A2) (Muhammed and Stephen 2012) respectively. The A1 and A2 gene frequency was 0.405 and 0.595 respectively in HF cross bred cow. The A1 α-casein gene frequency ranges were reported as 0.31 – 0.66 in Holstein cattle (Kaminski et al., 2007), 0.32 to 0.44 in Frieswal cattle (Ganguly et al., 2013a). A1 and A2 gene frequencies have been reported as 0.35 and 0.65 respectively in Polish HF bull (Olenski et al., 2010). In the present study the data of A1 and A2 gene frequencies in the HF cross are 0.405 and 0.595 respectively (Table 1) and similar to that of Kaminski et al. 2006 and Winkelmen and Wickham (1997).

The presence of A1 gene in crossbred cattle leads to production of A1 milk and this is considerably associated with the issues related to human health. The A1A2 hypothesis is significantly more important for public health, if the clinical implications of A1 α-casein on human health are proved. If a cow/bull is with A2A2 genotype, she/he is guaranteed to pass on the A2 allele to their progeny. Similarly, an A1A1 cow/bull is guaranteed to pass on the A1 allele. In the present study the amplification and sequencing of α casein gene for differentiating homozygous A2A2, heterozygous A1A2, or homozygous A1A1 was also carried out. There is a possibility to eliminate the frequency of A1 allele by screening and selection of young and proven bulls, which are having only A2 allele for further breeding. It may be necessary to monitor the breeding bulls to avoid the over spreading of A1 allele through artificial insemination. Since crossbred animals have a major role in total milk production in India, screening the exotic bulls before utilizing for breeding purpose is more important to draw a sound breeding policy to produce healthy A2 milk production. The aim of changing the dairy herd to more A2 milk producing cows may significantly improve the public health, along with higher productivity.
Fig 1:  **(A)** Allele-specific PCR showing the presence of 3 genotypes (genotype in parenthesis). Lane 1, 3, 5: A1 specific PCR. Lane 2, 4, 6: A2 specific PCR.  
**(B)** PCR amplicon of exon VII β-casein gene polymorphic site.
Fig 2. Result of Nucleotide sequence shows 99% identity (Accession M64756.1, AY899917.1) and 98% identity (Accession JX273430.1, JQ408387.1, JQ408385.1, JX273429.1, JQ408388.1, etc.,)

Table I. Genotype and Gene frequencies of â-casein gene in cattle.

<table>
<thead>
<tr>
<th>Breeds</th>
<th>No of samples</th>
<th>Genotype frequency</th>
<th>Gene frequency</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>A1A1</td>
<td>A1A2</td>
</tr>
<tr>
<td>HF cross</td>
<td>63 (n=11)</td>
<td>0.17</td>
<td>0.46</td>
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<tr>
<td>Kangeyam</td>
<td>22 (n=0)</td>
<td>0</td>
<td>0</td>
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REFERENCES


