Beta-casein (CSN2) polymorphism in Ongole (Indian zebu) and Frieswal (HF × Sahiwal crossbred) cattle

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Realizing the significant association of A1 and A2 β-casein (CSN2) polymorphism with health promoting properties and milk performance traits, the present investigation was undertaken to explore A1/A2 polymorphism in Indian zebu breed, Ongole (Bos indicus) and crossbred, Frieswal (HF × Sahiwal) cattle using allele-specific (AS)-PCR. Thirty eight Ongole cow, 124 Frieswal heifers and 48 Frieswal bulls were included in the study. The frequency of A2 allele was observed to be 0.94 in Ongole, 0.68 in Frieswal heifers; and 0.56 in Frieswal bull populations. Although all the three genotypes were observed in Frieswal bulls and heifers; however, no animal of A1A1 genotype was found in Ongole cattle and only four animals were of heterozygous genotype (A1A2). Considering the fact of healthfulness of A2 milk as well as positive relationship of A2 allelic variant with milk performance traits in different cattle breeds, the existing variability in β-casein (A1/A2) locus in Frieswal population may be exploited in near future for genetic selection. However, association of A2 variant with performance traits needs to be addressed before selection of Frieswal bulls at the time of birth.

Keywords: Bos indicus, β-casein polymorphism, Frieswal crossbred cattle, Ongole indigenous cattle.

Introduction

Bovine milk contains four caseins, namely, α-s1- (CSN1S1, 39-46%), α-s2- (CSN1S2, 8-11%), β- (CSN2, 25-35%), and κ-casein (CSN3, 8-15% of total caseins)1. β-Casein is the most polymorphic milk protein gene with 13 known protein variants. The most frequently observed forms of β-casein in dairy cattle breeds are A1 and A22. The difference between the A1- and A2-β-casein variants is a single amino acid substitution (CCT → CAT) at the 67th residue of the 209 amino acid chain. This difference in structure results in A1-β-casein preferentially releasing an opioid peptide called β-casomorphin-7 (BCM-7) upon digestion3, which may lead to adverse physiological effects. BCM-7 is an exorphin having potential to elicit opioid activity via its affinity to both mu- and delta-opiate receptors on a range of tissues and organs including the digestive tract4, respiratory5 and immune systems6,7. Furthermore, BCM-7 has been reported to catalyse the oxidation of LDL8. Bovine BCM-7 has been implicated in potential risk of human ischemic heart diseases9,10, diabetes9,11,12, atherosclerosis13, and sudden infant death syndrome3, autism and schizophrenia14.

Besides health promoting properties, the A2 variant has also been reported to have positive relationship with milk performance traits especially protein and milk yield15,16,17, whereas A1 variant showed the opposite influence18,19. Initial report indicates the predominance of A2 allelic variant in 15 zebu cattle breeds and 8 breeds of river buffaloes in India20; however, screening of crossbred population in India has not yet been undertaken for CSN2 polymorphism. According to 18th Livestock Census, 200721, India possesses the largest number of cattle population (199 million), which constitute nearly 12.7% of the world’s total cattle population, comprising of about 166 million indigenous and 33 million crossbred. India continues to remain the largest milk producer of 112 MMT in the world (Animal Husbandry Statistics 2010, http://dahd.nic.in/dahd/ upload/ BAHS_2010.pdf).

Cattle contribute to about 43 per cent of total milk, of which 20 per cent is produced by indigenous cows and 23 per cent by crossbreds and exotic cows. Moreover, country’s crossbred population is increasing very rapidly (Livestock census, 2007)21. To evolve a national milch breed capable of producing 4000 kg milk in a mature lattation of 300 days with 4% butter fat, the Frieswal population is being
developed at Military Farms of the country by crossing Holstein Friesian (HF) and Sahiwal breeds. After seven generations of interbreeding, the population has achieved a production level of 3550 kg in 4th lactation. Currently, more than 17,000 Frieswal females are available at 37 Military farms of India.

Considering the healthfulness of A2 milk as well as positive relationship of A2 allelic variant with milk performance traits in different cattle breeds, the present investigation was undertaken to explore the existing variability in β-casein (CSN2) A1/A2 polymorphism in Indian zebu breed, Ongole (B. indicus) and crossbred, Frieswal (HF × Sahiwal) cattle using allele-specific (AS)-PCR.

**Materials and Methods**

A total of 210 animals, including 38 Ongole cow, 124 Frieswal heifers and 48 Frieswal bulls, were included in the study. Genomic DNA was isolated from the whole blood using standard phenol chloroform extraction method\(^{20}\). Primers were designed using Primer 3 software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) from NCBI database sequence (M55158.1). The genomic specificity of the primers was tested using the Primer-BLAST program (http://www.ncbi.nlm.nih.gov/tools/primer-blast/). AS-PCR was carried out using a forward primer carrying either A (IGBhF: 5′ CTT CCC TGG GCC CAT CCA 3′) or C (IGBpF: 5′ CTT CCC TGG GCC CAT CCC 3′) and at the 3′ end a common reverse primer (IGBR: 5′ AGA CTG GAG CAG AGG CAG AG 3′) to amplify a 244 bp fragment (Fig. 1a). Primer pairs IGBhF-IGBR and IGBpF-IGBR were intended to pick histidine (A1) and proline (A2) specific amplicon, respectively. PCR was carried out from a starting template of approx 50 ng of genomic DNA in a final reaction volume of 25 µL containing 1× Taq DNA polymerase buffer (Sigma), 1.5 mM MgCl\(_2\) (Sigma), 200 µM dNTPs (Sigma), 0.5 µM of each primer and 1 U Taq polymerase (Sigma). PCR conditions were: initial denaturation at 94°C for 5 min, followed by 5 cycles of 94°C for 30 sec, 66°C for 30 sec and 72°C for 30 sec; thereafter 30 cycles of 94°C for 30 sec, 64°C for 30 sec and 72°C for 30 sec and a final extension 72°C for 5 min. PCR products were visualized in 1.0% agarose gel. Further, to validate the sequence, a pair of primers (A1 A2 Seq F: 5′ CCA GGA TAA AAT CCA CCC CT 3′; A1 A2 Seq R: 5′ AGG GAA GGG CAT TTC TTT GT 3′) was designed from NCBI database sequence (M55158.1) to amplify a 202 bp fragment (Fig. 1b) of exon VII encompassing the polymorphic site. Representative amplicons were sequenced directly using automated DNA sequencer by Sanger’s dideoxy chain termination method. Gene (allele) and genotype frequencies were calculated\(^{22}\).

**Results and Discussion**

A large number of evidence has specifically been linked A1-β-casein to a range of illnesses since it preferentially releases an opioid peptide called BCM-7 (β-casomorphin-7) upon digestion. In the present investigation, an AS-PCR was employed to explore A1/A2 polymorphism in Indian zebu breed Ongole (Bos indicus) and crossbred Frieswal (HF × Sahiwal) cattle. Although all the three genotypes, viz., A1A1, A1A2 and A2A2, were observed in Frieswal heifer and bulls (Fig. 1c), no animal of A1A1 genotype was found in Ongole cattle and only four animals were of heterozygous genotype (A1A2). AS-PCR was found to be very effective in distinguishing A1 and A2 alleles. The genotypes were further confirmed by nucleotide sequencing.

In Ongole cattle, genotype frequencies were 0.0 (A1A1), 0.11 (A1A2) and 0.89 (A2A2). However, the corresponding frequencies in Frieswal heifer were 0.12, 0.40 and 0.48 and, in Frieswal bulls, were 0.23, 0.42 and 0.35 for A1A1, A1A2 and A2A2, respectively (Table 1). The frequency of A2 allele

![Fig. 1 (a-c)—(a) Schematic representation of amplification of A1 and A2 beta-casein (CSN2) allelic variants using AS-PCR; (b) A 202 bp fragment of exon VII encompassing the polymorphic site, amplified and sequenced for confirmation of genotypes; & (c) Frieswal animals of different genotypes (A1A1, A1A2 & A2A2) have been shown, where A1 and A2 specific PCR product are indicated at the bottom of the photograph.](image-url)
was 0.94, 0.68 and 0.56 in Ongole cow, Frieswal heifer and Frieswal bull populations, respectively. Allelic distribution of β-casein locus observed in Frieswal heifer and bull populations in the present investigation offers sufficient scope for modulation of gene frequency through planned breeding. However, because of very high frequency to almost fixed nature of A2 allele, A1/A2 polymorphism may not be a suitable candidate for selection purpose in Ongole and other well defined Indian cattle breeds because of very high frequency to almost fixed nature of A2 allele. However, considering the increasing trends of crossbred cattle population in India and frequency distribution of A1/A2 alleles in Frieswal population, elimination or selection against A1 allelic variants may be suggested to reduce the negative effect of A1 milk. Moreover, it will be interesting to study the association of A2 polymorphism with production traits specially milk and protein yield in crossbred Frieswal population.

### Acknowledgement

Authors are grateful to the Directors, Project Directorate on Cattle and Frieswal Project, Meerut for providing all the necessary facilities to carry out the present investigation.

### References

8. Torreilles J & Guerin M C, Casein-derived peptides can promote human LDL oxidation by a peroxidase-dependent and

<table>
<thead>
<tr>
<th>No.</th>
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<th>Total no. of samples</th>
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<tr>
<td>1</td>
<td>Ongole</td>
<td>38</td>
<td>A1 A1: 0.68</td>
<td>0.40</td>
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<td></td>
<td></td>
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<td>A1 A2: 0.11</td>
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<td></td>
<td></td>
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<td>A2 A2: 0.23</td>
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<tr>
<td>2</td>
<td>Frieswal heifers</td>
<td>124</td>
<td>A1 A1: 0.68</td>
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<td></td>
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<td></td>
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<td>A2 A2: 0.32</td>
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<tr>
<td>3</td>
<td>Frieswal bulls</td>
<td>48</td>
<td>A1 A1: 0.68</td>
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In conclusion, we have successfully genotyped 210 animals including 38 Ongole cow, 124 Frieswal heifers and 48 Frieswal bulls for β-casein locus employing allele specific PCR technique. It is obvious that A1/A2 polymorphism may not be a suitable candidate for selection purpose in Ongole and other well defined Indian cattle breeds because of very high frequency to almost fixed nature of A2 allele. However, considering the increasing trends of crossbred cattle population in India and frequency distribution of A1/A2 alleles in Frieswal population, elimination or selection against A1 allelic variants may be suggested to reduce the negative effect of A1 milk. Moreover, it will be interesting to study the association of A2 polymorphism with production traits specially milk and protein yield in crossbred Frieswal population.

The frequency of A1 allele in different exotic breeds generally varies between 0.01-0.06 (Guernsey), 0.09-0.22 (Jersey), 0.31-0.66 (Holstein), 0.43-0.72 (Ayrshire) and 0.71 (Danish Red). In the present investigation, A1 allele frequency, in Frieswal heifers (A2) and Friesian bulls (A4), has been observed to be very close to Holstein. Recently, comparable frequencies of A1 (0.35) and A2 (0.65) alleles has also been reported in Polish Holstein-Friesian bulls. A2-β-casein variant has been found to exhibit a positive relationship with milk performance traits, especially of protein and milk, as shown by reports on Finnish Ayrshire, New Zealand Ayrshire and Polish HF. The A2 variant also increases breeding value for milk and protein yield and decreases breeding value for milk fat percentage. Likewise, a negative influence of A1 variant on performance traits in Israeli HF was observed. Recently, an association of casein haplotypes in Norwegian Red Cattle was reported, where C allele (determining A2 protein variant) of β-casein was found to be a potential marker for higher protein and milk yield.

Presently, BCM-7 has been implicated in a range of undesirable effects on the digestive system and on some immune and neurological functions. It has also been experimentally shown that the level of BCM-7 in β-casein A1 hydrolysed milk was four times higher than in A2 milk. Considering the above facts as well as positive relationship of A2 variant with milk performance traits in different cattle breeds, the existing genetic variability in β-casein (A1/A2) locus of Frieswal population may be exploited in near future for genetic selection. However, association of A2 variant needs to be established with performance traits in this population before taking final decision.

### Table 1—Gene and genotype frequency of β-casein gene

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14 Sun Z & Cade J R, A peptide found in schizophrenia and autism causes behavioral changes in rats, Autism, 3(1999) 85-95.


