Molecular characterization of A1/A2 Beta-casein Alleles in Vrindavani crossbred and Sahiwal cattle

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ABSTRACT

In the present investigation Vrindavani cross bred and indigenous Sahiwal cows were genotyped for A1/A2 beta-casein alleles. Present genotypic study on A1/A2 Beta-casein variants was conducted in a population of 660 cattle which comprises of 354 Vrindavani and 306 Sahiwal cows. Out of them 354 Vrindavani cows were maintained at Cattle and Buffalo Farm, IVRI, Izatnagar and 306 Sahiwal cows under study were maintained at Kamdhenu Gaushala, Noormahal, Jalandhar. In Vrindavani cows, all three type of genotype were observed viz., A1A1, A1A2 and A2A2. Genotypic frequencies of A1A1, A1A2 and A2A2 genotypes were 0.11, 0.47 and 0.42 respectively. The frequency of A1 and A2 allelic frequency in this population was 0.35 and 0.65 respectively in Vrindavani cows. In Sahiwal cows, only two types of genotypes were observed viz., A1A2 and A2A2. Genotype frequencies for A1A2 and A2A2 genotypes were 0.13 and 0.87 in Sahiwal cattle. The allelic frequencies for A1 and A2 were 0.06 and 0.94 respectively in Sahiwal cows. The results revealed that maximum genotypic frequency observed was A1A2 (0.47) followed by A2A2 (0.42) and A1A1 (0.11) in Vrindavani cattle while in Sahiwal cattle maximum genotypic frequency of A2A2 (0.87) followed by A1A2 (0.13) respectively. The observed heterozygosity (0.47) and PIC value (0.35) pointed towards the existence of medium genetic variability in the tested population.

Key words: A1, A2, PIC, Sahiwal, Vrindavani, V%

INTRODUCTION

Milk is a common source of food in our diet, which is made up of two types of protein groups i.e. casein and whey protein. Bovine milk protein constitutes 80 per cent of casein (Niki et al., 1994; Martein et al., 1994) and rest of 14 per cent whey protein (McLachlan, 2001; Roginsky, 2003). There are four casein types viz: alpha S1 (CSN1S1, 39–46%), alpha S2 (CSN1S2, 8–11%), beta (CSN2, 25–35%), and kappa (CSN3, 8–15%) casein (Eigel et al., 1984; Roginsky, 2003). Beta-casein is the degradation product of gamma-casein (Ostersen et al., 1997; Miller et al., 1990). Whey proteins mainly include: beta lactoglobulin, alpha lactalbumin, glycomacropeptides, immunoglobulins, bovine serum albumin and small proportion of minor proteins like lactoperoxidases, lysozyme and lactoferrin (Farrell et al., 2004). One of the major proteins present in cow milk is bovine beta casein (CSN2) and it varies depending on genetic makeup of cows. Bovine beta-casein is encoded by single autosomal gene that occupy approximately 200 Kb on the sixth chromosome. Bovine beta casein molecule is made up of 209 amino acid protein (Hanusova et al., 2010) that constitute about 30 per cent of the total bovine milk protein. Due to natural genetic Mutation, beta casein can be present as one of the two major types A1 or A2. Other variants are A3, B, C, D, E, F, H1, H2, I and G (Roginsky, 2003). The major difference between A1 and A2 types of beta casein is an amino acid substitution where A1 variant has histidine amino acid and while A2 variant has proline amino acids at the 67th residue of beta casein protein chain (Hanusova et al., 2010). The structural difference between these two types beta-caseinis due to enzymatic digestion of protein in the gut by digestive enzymes mainly pepsin, pancreatic elastase and leucine amino peptidase resulting in the cleavage between histidine and adjacent amino acid successively releasing bioactive peptide, beta casinomorphin-7 (BCM-7) (Elliott et al., 1999, Lien et al., 1992; Stewart et al., 1987). The amino acid difference between these two variants is CAT in A2 genotype and CCT in A1 genotype (Swaisgood, 1992). This BCM-7 (Tyr-Pro-Phe-Pro-Gly-Pro-Ile) having morphine-like activity (Brantl et al., 1979), and has been shown to stimulate in vitro Proliferation of human T cell lymphocyte (Gill et al., 2000). It has also cytomodulatory properties (Meisel and Bockelmann 1999). These peptides are inactive within the sequence of the parent protein that is, A2 variant containing proline at position 67 (Kaminski et al., 2007). These peptides are active in A1 variant containing histidine at position 67 in a peptide chain. Epidemiological studies have found a correlation between A1 beta casein milk
intake and the incidence of certain human diseases like arteriosclerosis, type I diabetes etc (Kaminski et al., 2007). This BCM-7 has been related associated with highly risk of human ischemic heart diseases (McLachlan, 2001; Laugesen and Elliott, 2003), insulin-dependent diabetes (Elliott et al., 1999; Laugesen and Elliott, 2003) atherosclerosis (Tailford et al., 2003) and sudden infant death syndrome (Sun et al., 2003).

Thus screening of A1 allele in cattle population is essential with respect to human health point of view. Keeping this fact in mind, Vrindavani crossbred and Sahiwal indigenous cattle breed population screened by the PCR-RFLP method to explore the A1 allele frequency in the cattle population.

MATERIALS AND METHODS

Present study was conducted on 660 animals which comprised 354 Vrindavani crossbred strain (Holstein-Friesian, Brown Swiss, Jersey and Hariana cattle) and 306 Sahiwal milk cattle breeds maintained at Cattle and Buffalo farm ICAR-IVRI, Izatnagar, Bareilly and Kamdhenu Gaushala, Nurmahal, Jalandhar Punjab, respectively.

About 5 ml of blood were collected from each animal in a sterile 15 ml polypropylene centrifuge tube (Vacutainer) containing ethylene diamine tetra acetic acid (EDTA) as anticoagulant (1 mg/ml of blood) and stored at -20 °C until DNA extraction. Bovine genomic DNA was extracted from whole blood by using phenol chloroform extraction method described by Sambrook and Russell (1989). The purity and concentration of the DNA samples were assessed by picodrop (Picodrop Ltd, Cambridge, UK). The OD\textsubscript{260/280} value of 1.7 to 1.9 was considered as pure DNA for further analysis.

This extracted DNA was used in polymerase chain reaction (PCR) for genotyping of animal using the concept of amplification created restriction site (ACRS-PCR) (Raies et al., 2009). The amplified DNA product sizes were 121 bp were digested with 2 units of the DdeI restriction enzyme (Fermentas). Restriction digestion products of 121 bp were digested with 2 units of the DdeI restriction enzyme (Fermentas). Restriction digestion fragments were loaded on 3% agarose gel (Invitrogen) containing 1 × TBE buffer at 120 V for 5 min, followed by 90 V for 2 hours and then gel was visualized under UV transilluminator and documented using gel documentation system to record the result.

STATISTICAL ANALYSIS

The genotype and allelic frequencies at A1/A2 locus were calculated by direct counting method i.e. by counting the number of bands appearing in the gels and the variations of the allelic frequencies among the three groups were analyzed by the Chi (÷2) - square test of significance as described by Snedecor and Cochran (1994) considering the allelic frequencies in a 2×2 table.

The gene and genotype frequencies were calculated by using the following formulae.

\[
\text{Proportion of animals with particular genotype} = \frac{\text{Proportion of animals with particular genotype}}{\text{Total number of animals}}
\]

\[
\text{Frequency of an allele} = \frac{(2 \times \text{no.of homogygote}+\text{no.of heterogygote})}{2 \times \text{total no.of individuals}}
\]

Effectiveness of allele incidence was evaluated with following parameters: theoretical heterozygosity (He), experimental heterozygosity (Ho), polymorphism information content (PIC), expected homozygosity (E), effective number of alleles (ENA), level of possible variability realization (V %).

**Experimental heterozygosity (He)** (Nei., 1973)

\[
\text{He exp}=1-\Sigma(p^2 + q^2)
\]

**Polymorphism information content (PIC)** (Bolstein et al., 1980)

\[
\text{PIC} = 1-\Sigma(P_i^2+q^2)\Sigma_{i=1}^{n-1} X \Sigma_{j=i+1}^{n} X 2 P_i X P_j
\]

**Expected homozygosity (E)** (Crowand Kimura., 1970)

\[
E = \Sigma P_i^2
\]

**Effective number of alleles (ENA)** (Crowand Kimura, 1970)

\[
\text{ENA} = \frac{1}{p^2+q^2}
\]
Level of possible variability realization (V %) (Crow and Kimura., 1970)

\[
V = \frac{1 - E}{1 - 1/N} \times 100
\]

RESULTS AND DISCUSSION

Genotype and Allele Frequencies of beta – Casein gene (CSN2) in Vrindavani and Sahiwal cattle: Allele A1 produced 121 bp fragments and A2 produced 86 bp and 35 bp fragments in PCR-RFLP. The genotype and allele frequencies of beta - casein A1A2 in Vrindavani crossbred and Sahiwal cattle are presented in Table 1. In the population included in study all three genotypes i.e. A1A1, A1A2 and A2A2 in Vrindavani cattle, while in Sahiwal cattle only two types of genotypes i.e. A1A2 and A2A2 were observed.

There were 40 animals having homozygous genotype A1A1 (121 bp), 166 having heterozygous genotype A1A2 (121 bp, 86 bp and 35 bp) and 148 animals of Vrindavani having homozygous genotype A2A2 (86 bp and 35 bp). The frequency of A1A1, A1A2 and A2A2 genotype in Vrindavani were 0.11, 0.47 and 0.42 respectively. The A1 and A2 allelic frequency in this population were 0.34 and 0.65 respectively. The results revealed that maximum genotypic frequency of A1A2 (0.47) was observed followed by A2A2 (0.42) and A1A1 (0.11) in Vrindavani crossbred strain. Since the breeding records of Vrindavani cattle population were not available so that the exotic inheritance level could not be determined. It may be presumed that due to almost complete fixation of A2 allele in the native breeds, the frequency of A2A2 genotype is higher than A1A2 and A1A1 genotype frequencies. The presence of A1A1 genotype in the population indicates that these animals could belong to F2 and above crossbred generation which were backcrossed with exotic bulls resulting in A1A1 genotype.

There is no previous report available on genotyping of Vrindavani crossbred cattle. The allelic frequency in Vrindavani crossbred cattle population was 0.35 and 0.65 respectively. More than 0.56 A1 variants in crossbred cattle were also reported by several workers (Ganguly et al., 2013b, Bech et al., 1990, Ehrmann et al., 1997, Ikonen et al., 1997 and Hanusová et al., 2010) however less A1 variants in crossbred were reported by Ganguly et al., 2013a, Sodhi et al., 2011, Kaminski et al., 2007, Olenski et al., 2010, Malarmathi et al., 2014, Kaminski et al., 2006, Winkelman and Wickham (1997, Manga et al., 2006 and Jaisawal et al., 2014). The value of gene homozygosity (Ho), gene heterozygosity (He) and polymorphism information content (PIC) were found to be 0.47, 0.45 and 0.3515 respectively. The results revealed that Vrindavani crossbred cattle population harbour intermediate homozygosity and PIC values (Table 2).

In this study, Sahiwal breed was having only two type of genotypes viz., A1A2 and A2A2. Out of them only 40 animals having heterozygous genotype A1A2 (121bp, 86 bp and 35 bp) and majority of 266 animals of Sahiwal having homozygous genotype A2A2 (86 bp and 35 bp) were found. There was no single animal of Sahiwal breed having A1A1 homozygous genotype. The frequency of A1A1, A1A2 and A2A2 genotype in Sahiwal were 0.00, 0.13 and 0.87 respectively. The A1 and A2 allelic frequency in this population were 0.065 and 0.93 respectively. The results revealed that more than 0.87 Sahiwal cattle having A2A2 genotype and remaining 0.13 were having A1A2 heterozygous genotype. The ratio of A1 beta - casein variants in Sahiwal cattle was ranged only 0.11 to 0.14 in the present population of these breeds. A similar finding in Ongole cattle was reported by Ganguly et al., 2012. However Mishra et al., 2009 reported no beta casein A1 variants in Sahiwal cattle breeds. But they reported that in Malnad Gidda indigenous cattle breed were having 0.19 β-casein A1 variants. Malarmathi et al., 2012 did not find A1 variants in Kangeyam breed. Muhammed and Stephens (2012) found 0.34 β- casein A1 variants in Vechure and 0.79 in Kasargode indigenous cattle. The value of gene homozygosity (Ho), gene heterozygosity (He) and polymorphism information content (PIC) were found to be 0.13, 0.118 and 0.3575 respectively. The results revealed that Sahiwal cattle population have lower homozygosity and PIC values (Table 2).

The expected homozygosity for gene CSN2 is in these populations ranged from 0.11 to 0.45. There was a corresponding decrease in the level of possible variability (11.31 and 45.62, respectively) which corresponds to the

<table>
<thead>
<tr>
<th>Name of Breed</th>
<th>Source</th>
<th>Number of observations (N)</th>
<th>Genotype Frequency</th>
<th>Gene Frequency</th>
<th>(\chi^2)</th>
<th>P</th>
<th>d.f. = 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vrindavani</td>
<td>Cattle and Buffalo Farm</td>
<td>N=354</td>
<td>0.11 (N=40)</td>
<td>0.47 (N=166)</td>
<td>0.42 (N=148)</td>
<td>0.35</td>
<td>0.65</td>
</tr>
<tr>
<td>Sahiwal</td>
<td>Kamdhenu Gaushala, Nurmahal Jalandhar Punjab</td>
<td>N=306</td>
<td>0.0 (N=0)</td>
<td>0.13 (N=40)</td>
<td>0.87 (N=266)</td>
<td>0.06</td>
<td>0.94</td>
</tr>
</tbody>
</table>

Table 2: Effectiveness of alleles for beta - casein (CSN2) gene in Vrindavani and Sahiwal breed of cattle

<table>
<thead>
<tr>
<th>Locus</th>
<th>Alleles</th>
<th>Breed</th>
<th>H</th>
<th>H</th>
<th>PIC</th>
<th>E</th>
<th>ENA</th>
<th>V%</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSN2</td>
<td>A1;A2</td>
<td>Vrindavani</td>
<td>0.47</td>
<td>0.455</td>
<td>0.3515</td>
<td>0.545</td>
<td>1.8348</td>
<td>45.62</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sahiwal</td>
<td>0.13</td>
<td>0.1128</td>
<td>0.1064</td>
<td>0.8872</td>
<td>1.1271</td>
<td>11.31</td>
</tr>
</tbody>
</table>
effective number of alleles (1.1271 in Sahiwal and 1.8348 in Vrindavani).

**CONCLUSION**

The present investigation results of Sahiwal cattle breed revealed that more than 94% animals were having beta-casein A2 variants, which prevented the production of harmful BCM-7 opioid during human gastric and gut enzymatic digestion. Hence it is here mentioned that our indigenous cattle genetic resources are not having 100% beta-casein A2 variant and crossbred cattle are having 100% β-casein A1 variant which was a myth in public. Therefore dairy cattle breeders should start genotyping of all bulls for A2A2 β-casein variants so that frequency of β-casein A2 variant will increase in future.

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**Conflict of Interest Statement:** The authors declare that they have no conflict of interest.

**REFERENCES**


