Research paper

Genotyping of HF crossbred cattle breeding bulls for A1/A2 variant of β-casein gene

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ABSTRACT

The present study was carried out to genotype HF cross breeding bulls for A1/A2 variants of β-casein gene was studied by collecting the blood samples of 49 breeding bulls from Frozen Semen laboratory, Kharki, Pune, Frozen Semen Lab, Aurangabad and Bombay Gorakshak Mandali, Mumbai. Genomic DNA was isolated from the blood samples by phenol-chloroform method. DNA fragments of β-casein (CSN 2) gene were amplified by PCR using the suitable primers to amplify 121 bp products. PCR products were subjected for digestion with the *Dde*I restriction enzyme. After Restriction enzyme digestion the band pattern were observed on 4 per cents agar gel. The PCR-RFLP analysis revealed three band patterns (121bp, 86bp and 35bp) for A1A2 genotype and two band pattern (86bp and 35bp) for A2A2 genotype. The study showed two types of genotypes as A1A2 and A2A2 in the experimental population of HF crossbred breeding bulls. The genotype frequency of A1A2 and A2A2 was estimated as 65 and 35 per cent, respectively. Approximately gene frequency of A1 and A2 was calculated as 0.335 and 0.665, respectively. The present frequency status of A1 and A2 allele in the breeding bulls may be utilised for planning future breeding strategy in dairy cattle.

Keywords: Beta casein, A1/A2 variant, genotyping, breeding bulls

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INTRODUCTION

The world is trending with the A2 milk brand which is actually the 'Original milk' given by the dairy cows producing only A2 β-casein protein. Originally there was only A2 variant β-casein i.e. A2A2, before mutation occurred. This mutation occurred due to selective breeding for high milk production. The mutation resulted into 13 variants of β-casein gene as A1, A2, A3, A4, B, C, D, E, F, H1, H2, I and G. Among those variants A1 is considered as hazardous for human health. The hazardous health effects of A1 variant milk like diabetes mellitus type-1, autism, coronary heart disease, SIDS etc. A1 and A2 variants of β-casein protein differ in structure. A1 has histidine at 67th position and A2 has proline at the same position (Sharma et al. 2013). There is polymorphism at codon 67 of β-casein gene, C**C**T which codes for proline in A2 β-casein changes to C**A**T which codes histidine in A1 β-casein (Ganguly et al., 2013a). The structural difference in A1 and A2 β-casein leads to differential digestion by gut mucosa. The digestion of A1 β-casein in the gut by the action of digestive enzymes (Pepsin, pancreatic elastase etc.) results in the cleavage between histidine and adjacent amino acid and a bioactive peptide called beta casomorphin-7 (BCM-7) will be produced (Stewart et al., 1987; Lien et al., 1992). BCM-7 has opioid like activity and binds to opioid receptors and neural cells. It is thought that β-casein variant A1 play some role in the development of some human diseases like arteriosclerosis and type I diabetes as it yields the bioactive peptide BCM-7 (Kaminski et al., 2007).

A1 variant of β-casein gene was found in the milk of cattle breeds of Europe, USA, Australia and New Zealand. Holsteins and Ayrshires produces A1 milk as compared to other cattle breeds. In India, increased milk production has been obtained by

cross breeding with high milk yielding exotic cattle breeds. Therefore, there may chances of introduction of A1 in our population. Considering health hazards of A1variant of β-casein there is urgent need to find out the genotypes of breeding bulls for securing human health. The present study was carried out to detect the status of A1 and A2 allele in HF crossbred cattle breeding bulls population of Maharashtra state.

MATERIALS AND METHODS

Total 49 (39 HF crossbred, 2 indigenous and 8 Gir x HF)blood samples of breeding bulls were collected from Frozen Semen Laboratory, Kharki, Pune; Frozen Semen Lab., Aurangabad and Bombay gorakshak mandali, Betegaon. Genomic DNA was isolated by using phenol-chloroform method (Sam brook et.al. 2006). The primers set were used for PCR amplification of 121bp fragment of beta casein gene: F: 5´- CCT TCT TTC CAG GAT GAA CTC CAG G- 3´ and R: 5´ - GAG TAA GAG GAG GGA TGT TTT GTG GGA GGC TCT- 3´ (McLachlan, 2006).

The amplification was carried out at 58 ºC annealing temperature and the amplification parameters were used as: 95ºC for 5 minutes followed by 30 cycles: 95ºC for 40 seconds, 58ºC for 60 seconds, 72ºC for 90 seconds. The reaction was completed by the final synthesis 72ºC for 10 minutes. The PCR products were visualized in 1.7 per cent agarose gel. This PCR product of 121bp fragment of β-casein gene (exon 7) was then digested with DdeI restriction enzyme. Digestion was carried out in water bath at 37 ºC for 5 hours. The restriction fragments were then separated on 4 per cent agarose gel.

RESULTS AND DISCUSSION

Variant of β-casein gene was studied by various scientists. Lindersson et al. (1995) observed five genotypes of β-casein gene i.e. A1, A2, A3, A5 and B by using allele specific PCR. Similarly, Keating et al. (2008) evaluated difference in β-casein variant in bovine breeds by AS-PCR and relevance to BCM. Miluchova et al. (2009) studied Slovak Pinzgau cattle population for β-casein gene polymorphism and analysed genotype structure. While, Hanusova et al. (2010) detected allelic and genotypic frequencies of variants of β-casein (CSN2) in Holstein cows and bulls in Slovakia and analysed milk production traits of tested cows on the basis of their CSN2 genotypes.

In the present, study we amplified 121 bp fragment of beta casein gene exon VII (Figure 1). Similarly, Olenski et al. (2010) amplified 321 bp fragment of beta casein gene; Rangel et al. (2017) amplified 362 bp of exon 7; Miluchova et al. (2009) amplified 121 bp fragment of beta casein gene and did genotyping by PCR-RFLP method. Ramesha et.al. (2016) amplified 251 bp fragment of exon 7^{th} of β-casein gene in cattle and buffalo breeding bulls. Amplified fragment of 121 bp was further subjected to digestion with DdeI restriction enzyme. This digestion then resulted into two restriction patterns and was designated as A1A2, and A2A2 genotypes. The heterozygote A1A2 pattern had three fragments of sizes 121 bp, 86 bp and 35bp and A2A2 pattern had two fragments of sizes 86 bp and 35bp (Figure 2). The 35bp fragment was not visible in agarose gel. Out of 49 breeding bulls, 32 showed A1A2 genotype and 17 showed A2A2. Hence, the estimated allelic frequency was found to be 0.335 and 0.665 for A1 and A2 allele, respectively. The observed genotypic frequency for the genotypes A1A2 and A2A2 was, 0.65 and 0.35, respectively.

Present findings are partially in agreement with Shende et.al. (2017) reported only two genotypes A1A1 and A1A2 with frequencies 0.28 and 0.72 in HF crossbred cows by PCR-RFLP analysis of 121bp fragment. Whereas, Miluchova et.al. (2013) studied same fragment of beta casein gene and found three genotypes in HF crossbred cattle as A1A1, A1A2 and A2A2 with genotypic frequencies 0.1379, 0.4598 and 0.4023, subsequently and allelic frequency as A1 (0.2928) and A2 (0.7072). Whereas, Mishra et al. (2009) performed PCR-RFLP of 618 cattle including 15 zebu cattle breeds, 231 buffaloes including 8 river buffalo breeds were genotyped for β-casein gene and frequency data indicated the predominance of A1 in Bos taurus where as A2 variant in zebu cattle breeds while the river buffalo indicated only A2 variant. However, Hanusova et al. (2010) also amplified 121 bp of β-casein gene in 92 cows and 5 bulls. The frequencies of A1 and A2 allele of CSN2 in cows were 0.54 and 0.46. CSN2 genotypic frequencies in cows were A1A1 (0.13), A1A2 (0.83), A2A2 (0.04). Only

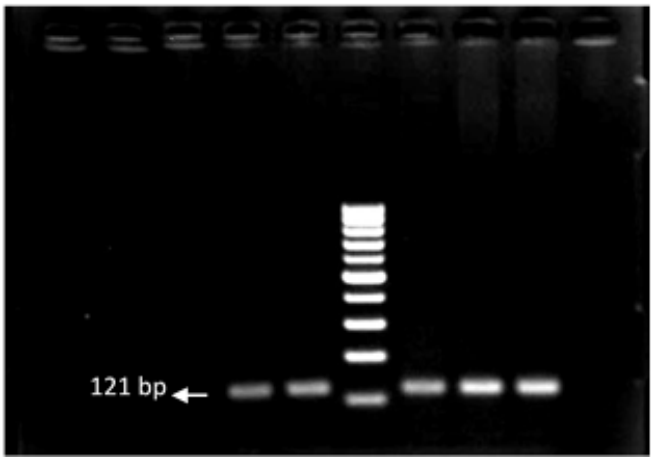

Figure 1: *PCR of 121bp fragment of beta casein gene. Lane 6th – Marker 100bp DNA Ladder*

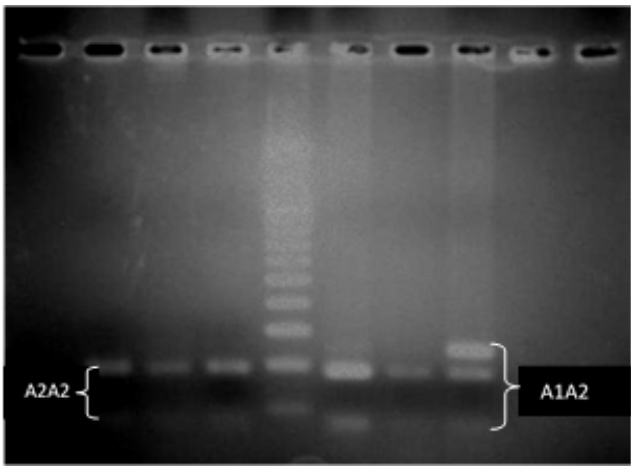

Figure 2: *PCR-RFLP of 121bp fragment of beta casein gene. Lane 5th – Marker 50bp DNA Ladder; 6th and 8th – genotype A1A2 (121bp, 86bp and 35bp); 2nd, 3rd, 4th and 7th – genotype A2A2 (86bp and 35bp)*

A1A1 and A1A2 genotypes with frequencies 0.20 and 0.80 were found in bulls. This genotypic result was nearly similar to present study result as we find two genotypes in breeding bull's population. High proportion of heterozygote individuals and genetic disequilibrium were recorded. Malarmathi et.al. (2014) observed high frequency of A2 allele in HF cross breed animal as 0.595. Sodhi et.al. (2012) reported predominance of the desirable A2 allele across all cattle types studied with a mean frequency of 0.645.

CONCLUSION

Considering the health hazards of A1 beta casein there is need to eliminate the A1 allele from the breeding populations of crossbred cattle in India. As New Zealand and Australia purely breeds A2 cows for production of A2 milk for future prospectus of human health. Thus, the present study was conducted mainly to know the status of A1/A2 variants in HF cross and other bulls used for semen production. Data generated from present study on current status of A1 and A2 type breeding bulls may be used in formulating suitable breeding plans in order to minimize undesired A1 allele in future generation.

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