Alpha-S1 Casein Gene Polymorphism and Association with Milk Production Traits in Sahiwal and Holstein Frisian Crossbred Cattle

Akhilesh Pandey and Mohan Singh Thakur*

Department of Animal Genetics and Breeding, Nanaji Deshmukh Veterinary Science University, Jabalpur, Madhya Pradesh, INDIA

*Corresponding author: MS Thakur; Email: drmohansingh@gmail.com

Received: 30 March, 2018 Revised: 18 May, 2018 Accepted: 22 May, 2018

ABSTRACT

The present research work was conducted on 100 lactating cows comprising 50 each of Sahiwal and HF Crossbred cattle. All the screened in Sahiwal (n = 50) and HF Crossbred (n = 50) cows revealed monomorphic pattern, only, AA genotype (310 bp) was found at αs1-casein gene (CSN1S1)/HindIII locus. The allelic frequency for allele A in the screened animals of Sahiwal and HF Crossbred cattle was 1.00 and for allele B was 0.00. The population was found under HWE at this locus (χ²=0.00 NS). Milk yield and Daily milk yield was noticed significantly higher in HF Crossbred as compared to Sahiwal cattle.

Keywords: Casein gene, lactating cows, milk yield, PCR- RFLP

The αS1-casein (CSN1S1) is localized in bovine chromosome 6. It is a loose flexible medium sized protein consists of two hydrophobic regions connected by a hydrophilic region. It has phosphate groups located in the hydrophilic region and most of the calcium associated with is exists at the phosphate groups (Ferranti et al., 1998). Nucleic acid sequences have been reported for αS1 like casein mRNAs from cow, sheep, rat and guinea pigs. They are found to be divergent sequences with a large number of point mutations Ferranti et al. (1998). It has an important role in the capacity of milk to transport calcium phosphate and is organized at 5'-terminus of casein cluster. Till now, 9 variants (A-I) have been reported in the coding region of αS1-CN (Kishore et al., 2013).

MATERIALS AND METHODS

Animals and DNA isolation

The present research work was conducted on 100 lactating cows comprising 50 each of Sahiwal and HF Crossbred cattle. The blood samples were collected randomly from a total of 50 animals of Sahiwal cattle breed maintained in Livestock Farm Anjora (Durg) Chhattisgarh and 50 HF Crossbred cattle maintained at Livestock Farm, College of Veterinary Science and A.H., Jabalpur and Private Dairy Farm at Pariyat, Jabalpur.

Genomic DNA was extracted by John et al. (1991) method. The concentration and purity of DNA was checked by Nanodrop Spectrophotometer (ND-1000). The integrity of the DNA was examined by agarose gel (0.8%) electrophoresis and visualized the gel under UV transilluminator using Gel documentation system (Gel-doc Bio-Rad, USA).

Gene and genotype frequencies were estimated using Pogepne 32 (version 1.32), Microsoft Windows-based freeware for population genetic analysis (Yeh et al., 1999).

The data related to milk production was recorded from 50 each of Sahiwal and HF Crossbred cattle (i.e., identification number, parity, lactation length, milk yield and daily milk yield etc.). The data was subjected to least squares analysis of variance (Snedecor and Cochran, 1994).

Amplification of αs1-casein gene (CSN1S1)

The αS1-casein (CSN1S1) gene of 310 bp was amplified...
using a specific primer pairs (CSN1S1/start F: 5’-TGCATGTTCTCATAATAACC-3’ and CSN1S1/stop R: 5’-GAAGAAGCAGCAAGCTGG-3’; Mir et al., 2014). PCR amplification was carried out in a total volume of 25 μl that contained 3.0 μl of genomic DNA (90 nmoles), 12.5 μl 2X PCR mastermix (Fermentas, USA), 1.0 μl of each primer (forward and reverse) and 7.5 μl of DNAase free water. The cycle conditions included an initial period of denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 54.5°C for 1 min and extension at 72°C for 10 min. The PCR product was checked by agarose gel (2.0%) electrophoresis in 0.5X TAE buffer at constant voltage of 90 volt for 50 minutes after staining with EtBr. The mass ruler DNA ladder (100 bp-1000 bp) as a molecular size marker was used for sizing of the DNA bands. The amplified product in the gel was visualized by UV transilluminator and photographed using Gel documentation system (Gel-doc Bio-Rad, USA).

Restriction digestion of amplified PCR product
The amplified PCR products of all the samples were digested by restriction enzyme (RE) HindIII at 37°C for overnight in water bath. The RE digested PCR products were analyzed on 2.5% agarose gel in 0.5X TAE buffer at constant voltage of 90 volt for 50 minutes after staining with EtBr. The mass ruler DNA ladder (100 bp-1000 bp) as a molecular size marker was used for sizing of the DNA bands. The amplified product in the gel was visualized by UV transilluminator and photographed using Gel documentation system (Gel-doc Bio-Rad, USA).

RESULTS AND DISCUSSION

Gene and Genotypic frequencies at αs1-casein (CSN1S1) gene locus
The genotypic and allelic frequencies of αs1-casein gene (CSN1S1) in Sahiwal and HF Crossbred cattle are presented in table 1. In the present study, all the screened populations of Sahiwal (n=50) and HF Crossbred (n=50) revealed only the one type of genotype (AA) with 100 per cent frequency. The allelic frequency for allele A in Sahiwal and HF Crossbred cattle was 1.00 and for allele B was 0.00. Contrary to above findings, Silva and Lama (1997) and Caroli et al. (2008) reported predominance of B allele over C allele in HF, Brazilian Zebu cattle and Carora cattle, respectively.

Table 1: Distribution of gene and genotypic frequency of αs1-casein (CSN1S1) variants in different breeds of cattle

| Breeds | Genotype Freq | χ² value | Gene Freq |
|--------|---------------|----------|-----------|
|        | AA            | AB       | BB        | A          | B          |
| Sahiwal|               |          |           |            |            |
|        | 1.00          | 0.00     | 0.00      | 0.00NS     | 1.00        | 0.00        |
| (50)   | (0)           | (0)      |           |            | (0)         | (0)         |
| HFC    |               |          |           |            |            |
|        | 1.00          | 0.00     | 0.00      | 0.00NS     | 1.00        | 0.00        |
| (50)   | (0)           | (0)      |           |            | (0)         | (0)         |

NS-Non-significant, Freq- frequencies, HFC- HF Crossbreds

Chi-square values for testing correspondence between observed and expected genotypic frequencies at this locus were found to be non-significant in Sahiwal, and HF Crossbred cattle, indicating that the populations of these two cattle breeds under study were in Hardy-Weinberg equilibrium (Table 1). In accordance to above findings, Golijow et al. (1999) reported that the populations of Argentine Creole and Argentine Holstein were found in HWE for CSN1S1 loci. These facts indicate that the artificial selection is not disturbing the equilibrium of gene frequencies in the milk production related loci in these breeds of cattle.

Association of αs1-Casein (CSN1S1) gene polymorphic variants with milk yield
All the screened animals of Sahiwal and HF crossbred cattle were found monomorphic for αs1-Casein (CSN1S1) gene/HindIII locus (Fig. 1 and 2). Therefore, the association study of different CSN1S1 gene variants with milk production traits could not perform.

The effect of breed was found significant (P<0.01) for daily milk yield (DMY) and milk yield per lactation (MY) trait. The mean DMY and MY in Sahiwal and HF Crossbred cattle have been presented in table 2. Only AA genotype was observed in all the animals of Sahiwal and HF Crossbred cattle. The mean MY of AA
The fixation of favourable allele (A) might be due to high milk producing nature of HF crossbred and Sahiwal cattle. Consequently, we could not establish any association of the observed polymorphism with milk production traits.

Contrary to these findings, Hristov et al. (2014) showed that the BB genotype determines higher milk production. The results revealed in the present study are in accordance with the previous reports.

Fig. 1: PCR-RFLP/HindIII assay of αS1 gene showing genotype pattern in 2.5% agarose gel of Sahiwal cattle. M: 100bp DNA ladder, Lanes: 1-12

Fig. 2: PCR-RFLP/HindIII assay of αS1 gene showing genotype pattern in 2.5% agarose gel of HF Crossbred cattle. M: 100bp DNA ladder, Lanes: 1-13

Table 2: Mean Milk Yield per lactation (L) and daily milk yield in different breeds of cattle at αs1-Casein (CSN1S1) gene locus

| Variants | Milk yield/lactation (litres) | Daily milk yield (litres) |
|----------|------------------------------|--------------------------|
|          | Sahiwal                      | HF crossbred             | Sahiwal                      | HF crossbred             |
| AA       | 1523.00 ± 39.00 (50)         | 2468.00 ± 111.00 (50)    | 5.77 ± 0.14 (50)             | 7.62 ± 0.39 (50)         |
| AB       | 0.00 ± 0.00 (0)              | 0.00 ± 0.00 (0)          | 0.00 ± 0.00 (0)              | 0.00 ± 0.00 (0)          |
| BB       | 0.00 ± 0.00 (0)              | 0.00 ± 0.00 (0)          | 0.00 ± 0.00 (0)              | 0.00 ± 0.00 (0)          |
| Overall  | 1523.00 ± 39.00 (50)         | 2468.00 ± 111.00 (50)    | 5.77 ± 0.14 (50)             | 7.62 ± 0.39 (50)         |

Means bearing the different superscript differ significantly (p<0.01), Numbers in the parentheses denotes number of animals.

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as reported by Szymanowska et al. (2004) in Polish Black and White cattle.

CONCLUSION

In the present study, we could not establish any association between genotype and milk production trait because these cattle were found homozygous for αs1-Casein (CSN1S1) gene/HindIII locus. The population of HF Crossbred and Sahiwal cattle was in HWE at this locus. The DMY and MY was noticed higher in HF Crossbred compared to Sahiwal cattle.

ACKNOWLEDGEMENTS

The authors are thankful to Vice Chancellor, NDVSU, Jabalpur (M.P.) for providing necessary facilities during entire research work at this esteemed University. The Livestock Farm, Anjora, Durg (CG) and Instructional Livestock Farm Complex, College of Veterinary Science and A.H., Jabalpur and Private Dairy Farm at Pariyat, Jabalpur (M.P.) are gratefully acknowledged for providing help during sample and data collection.

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