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Status of association of α S2 casein gene with daily milk yield (DMY) trait in Sahiwal and HF crossbred cow of Madhya Pradesh, M.P, India

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Abstract

Study related to present study implicate that the cattle dissimilar genotypic patterns of RFLP analysis of α S2 casein gene (CSN1S2) gene carried out in both Sahiwal and HF cross breeds of cow. Absence of restriction site at both the alleles that resulted in the appearance of single compact bands of size 1267bp was referred to as genotype AA known as monomorphic and noticed in Sahiwal cows. The samples exhibiting three fragments (1267bp/ 1150bp/ 117bp) were denoted as AB genotype and noticed in HF Cross bred cow. AA and AB genotypes were observed in HF Cross bred. 1.00, 0.00, and 0.00 in Sahiwal and 0.58, 0.42 and 0.00 in HF crossbred cattle, respectively and the respective gene frequency for A and B alleles were found to be 1.00 and 0.00 in Sahiwal and 0.79 and 0.21 in HF crossbred cattle. The frequency of A allele was found to be highest as compared to B allele in above both breeds of cattle under the study. Non-significant value of Chi-square was observed for testing genotypic frequencies at this locus in both Sahiwal and HF crossbred breeds. Hardy-Weinberg equilibrium was noticed in the above mentioned populations of animals. With respect of daily milk yield both AA and AB genotype of HF crossbred breeds cow showed significantly higher daily milk yield than AA genotype of Sahiwal, whereas the AB genotype of HF crossbred showed significantly higher daily milk yield than its own AA genotype.

Keywords: α S2, casein gene, sahiwal, HF cross breeds

1. Introduction

As per Priyanka Priyatharsini *et al.* (2018) [9] Milk protein constitutes 36% -casein, 27% peptides and amino acids. κ Casein, 9% also contains immunoglobulins, hormones, growth factors, cytokines, nucleotides, peptides, polyamines, enzymes and several others bioactive peptides. The National Bureau of Animal Genetic Research (NBAGR) demonstrated the superior milk quality of Indian cattle breeds. After scanning 22 cattle breeds, scientists concluded that in five high milk-yielding native breeds – Red Sindhi, Sahiwal, Tharparkar, Rathi and Gir – the status of A2 allele of the beta casein gene was 100 per cent. According to Pandey *et al.* (2020) [1] Indian breed Malvi and Nimari of Madhya Pradesh district also have 100 per cent A2 gene and genotypic frequency. In other Indian breeds it was around 94 per cent, compared to only 60 per cent in exotic breeds like Jersey and HF. The enormous variation in the casein proteins and genes is crucial for milk production character like milk yield per lactation and daily milk yield. Asim *et al.* (2022) [5] the most frequent genotype was AB (50.0 percent) followed by BB (43.3 percent), while the least common was AA (6.9 percent) in the 30 Friesian X Bunaji cows. This is attributed to the fact that most selection program favoured the AB and BB genotypes which influenced milk yields or production in dairy cattle.

2. Materials and Methods

2.1 Daily Milk Yield (DMY): Daily milk yield data was collected from 50 Sahiwal and 50 HF crossbred.

2.2 Genomic DNA isolation: Genomic DNA was extracted from venous blood as per the method described by John *et al.* (1991) [3] with minor modifications.

2.3 Concentration, purity and quality check of DNA

The concentration, purity and quality of DNA were checked by Nanodrop spectrophotometer and agarose gel electrophoresis.

2.4 Casein gene primer sequence

The α S2-casein gene primers (F): 5'-TATGACATGTGCGAGAAATGAG-3'
(R): 5'-TTGGAACAATGCTATTAGGT T- 3'1267 bp (Szymanowska *et al.* 2003) [10] was used for the amplification of PCR product.

2.5 Polymerase Chain Reaction (PCR)

The PCR tubes were kept in a preprogrammed thermo cycler (Mastercycler gradient, Eppendorf) and set at the standardized reaction programme. Initial denaturation (5 minutes) and final denaturation (1 minute) temp. Was 94°C 60°C annealing temp. (1 min.) Was 60°C where extension (1 minute) and final extension (5 minutes) temp. was 72°C

2.6 PCR- RFLP Assay

2.6.1 Restriction digestion

All the PCR products of α S2 casein gene were digested by EcoRV restriction enzymes. The reaction mixture was spanned for few seconds for uniform mixing and then incubated at 37°C for 3 hrs in the water bath.

2.7 Sequencing

Sequencing of amplicon was done for the confirmation of genotype of the cattle. The sequences obtained from genotype were aligned using Clustal W. (Thompson *et al.*, 1994) and analyzed by using MEGA 6 software (Tamura *et al.*, 2004). Aligned sequences were analyzed for group specific SNP marker.

2.8 Statistical analysis

2.8.1 Calculation of Gene and genotype frequencies: Gene and genotype frequencies for different casein genes under study were estimated using Popgene 32 (version1.32), microsoft Windows-based freeware for population genetic analysis (Yeh *et al.*, 1999) [8].

2.9 Association of various polymorphic variants of milk protein genes with daily milk yield per day

Association study of various polymorphic variants of milk protein genes for lactation length data were subjected to least squares analysis of variance employing following linear model:

$$Y_{ijkl} = \mu + P_i + B_j + G_k + (PXB)_{ij} + (PXG)_{ik} + (BXG)_{jk} + (PXBXG)_{ijk} + e_{ijkl}$$

Where,

Y_{ijkl} - is the Observed value of milk yield

μ - is the population mean

P_i - is the fixed effect of parity

B_j - is the fixed effect of breed

G_k - is fixed effect of genotypes ($k = 1, 2, \dots$)

$(PXB)_{ij}$ - is interaction effect of parity and Breed

$(PXG)_{ik}$ - is interaction effect of parity and genotypes

$(BXG)_{jk}$ - is interaction effect of Breed and genotypes

$(PXBXG)_{ijk}$ - is interaction effect of parity, breed and genotypes

e_{ijkl} - is random error effect

2.10 Testing Hardy-Weinberg (H-W) equilibrium

The chi-square test (χ^2) was employed to test the status of Hardy-Weinberg equilibrium in the different population of four breeds of cattle (Snedecor and Cochran, 1994) [4].

3. Results

The frequency of A allele was found to be highest as compared to B allele in above both breed of cattle under the study. 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 breeds of cattle. The above result indicating that the populations of animals of above breeds were in Hardy-Weinberg equilibrium at this locus.

3.1 Association of α S2-Casein (CSN1S2) gene polymorphic variants with Daily milk yield trait

The polymorphic variants of α S2-casein gene (CSN1S2) in Sahiwal and HF crossbred breeds of cattle and their association with daily milk yield (DMY), have been studied as below:

3.2 Least squares means for Daily Milk yield (DMY) in Sahiwal and HF crossbred at α S2-Casein (CSN1S2) gene locus

The analysis of variance for different genotypes of above both cattle breed has been presented in table 01. The effect of genotypes was found significant ($P < 0.01$) for DMY trait. The mean DMY (DMY) in Sahiwal and HF crossbred cattle has been presented in table 01.

Table 1: Least squares means for DMY (L) in Sahiwal and HF crossbred breeds for α S2-Casein (CSN1S2) gene locus.

Variants	Sahiwal	HF crossbred
AA	5.67 ^b ±0.14 (50)	5.81 ^b ±0.30 (29)
AB	0.00±0.00 (00)	10.11 ^a ±0.43 (21)
BB	0.00±0.00 (00)	0.00±0.00 (00)
Overall	5.77 ^b ±0.14 (50)	7.62 ^a ±0.39 (50)

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

The mean DMY(L) of AB genotyped HF crossbred (10.11±0.43) was significantly higher than its own AA genotype (5.81±0.30) and AA genotypes of Sahiwal breed (5.77±0.14) also (Table 02) whereas the both AA genotype of Sahiwal and HF Crossbred (5.81^b±0.30) showed non-significant variation with each other.

4. Discussion

The mean DMY(L) of AB genotyped HF crossbred (10.11±0.43) was significantly higher than its own AA genotype (5.81±0.30) and AA genotypes of Sahiwal breed (5.77±0.14) also (Table 02) whereas the both AA genotype of Sahiwal and HF Crossbred (5.81^b±0.30) showed non-significant variation with each other. The results revealed in the present study are in accordance as reported by Szymanowska *et al.* (2004) [6] in Polish Black and White cattle.

5. Conclusion

PCR-RFLP analysis of α S2-Cn gene (1267bp) with *EcoRV* RE revealed two genotypes *viz.*, AA (1267/1267bp) and AB (1267/1150/117bp) in HF crossbred animals, whereas, only AA (1267/1267 bp) genotype was observed Sahiwal cattle. All the screened animals of Sahiwal was found monomorphic

at α 2-Cn/*EcoRV* gene locus. The frequency of A allele was found to be highest as compared to B allele in above both breed of cattle under the study. 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 breeds of cattle. The above result indicating that the populations of animals of above breeds were in Hardy-Weinberg equilibrium at this locus. The association of polymorphic variants of α S2-casein gene (CSN1S2) in both AA and AB genotype the mean DMY(L) of AB genotyped HF crossbred was significantly higher than its own AA genotype and AA genotypes of Sahiwal breed also (Table 01) whereas the both AA genotype of Sahiwal and HF Crossbred showed non-significant variation with each other.

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