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Divergence study of the α S2 casein haplotypes and its correlation with milk yield trait in Indian dairy cattle of Madhya Pradesh India

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Abstract

A numbers of casein polymorphisms exist in cattle also, making their analysis and association study quite complex. This study revealed the polymorphic variants and their association with milk production traits in Sahiwal and HF Crossbred cattle of Madhya Pradesh at α S2 casein gene (CSN1S2) gene locus. The RFLP analysis carried out in above both of the breeds of cattle revealed dissimilar genotypic patterns. The frequency of A allele was found to be highest as compared to B allele in above both breeds 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 both Sahiwal and HF crossbred breeds. The above result indicated that the populations of animals of above both 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 of HF crossbred breeds cow showed significantly higher milk yield per lactation than AA genotype of Sahiwal, whereas the AB genotype of HF crossbred showed significantly higher milk yield per lactation than its own AA genotype.

Keywords: α S2, casein gene, Sahiwal, HF cross breed

1. Introduction

As per the present study polymorphic variants and their association with milk production traits in Sahiwal and HF Crossbred cattle of Madhya Pradesh at α S2 casein gene (CSN1S2) gene locus. The milk casein genes in different breeds of cattle, are highly polymorphic genetic variants with numerous synonymous and non-synonymous mutations also. So far, 14 for alpha-S2-casein. This review provides a comprehensive overview on association study of genetic variants with milk production traits which explain expression of the casein genes also. In contrast to the low level of variation observed at the protein level, these DNA polymorphisms determine a high level of heterozygosity and, therefore, represent useful tools for genetic analyses since they can also be obtained without the need for gene expression. The high frequency of some casein protein variant like A in different cattle breeds and geographical regions might reflect specific breeding goals or adaptation to the environment. More genetic variability noticed because we linked old protein names with new ones that reveal. The haplotypes across the cluster of the four genetically linked casein genes are recommended as a valuable genetic tool for discrimination between breeds, managing genetic diversity within and between cattle populations, and breeding strategies. The enormous variation in the casein proteins and genes is crucial for milk production character like milk yield per lactation and daily milk yield. Through our current study of genetic polymorphism of alpha casein gene we show how contribute to improving the milk production trait.

2. Materials and Methods

2.1 Milk yield (MY)

Data of milk yield per lactation was collected from 50 Sahiwal and 50 HF crossbred cows.

2.2 Genomic DNA isolation

We have developed a standard protocols, to isolate DNA from blood described by modified John *et al.* (1991) [2].

2.3 Agarose gel electrophoresis

By 0.80% horizontal submarine agarose gel electrophoresis quality of DNA was assessed.

2.4 Concentration, purity and quality check of DNA

By the help of Nanodrop and spectrophotometer the concentration, purity and quality of DNA were measured.

2.5 Spectrophotometry

The Optical density (OD) value at 260 nm and 280 nm Spectrophotometer (Nanodrop 1000, Thermo Scientific). DNA samples with an OD 260/280 ratio of 1.70 to 1.90 were measured by using Nanodrop and further proceeded for agarose gel electrophoresis for quality check.

2.6 Casein gene primer sequence

The α S2-casein gene primers (F): 5'-TATGACATGTCGAGAAATGAG-3' (R): 5'-TTGGAACAATGCTATTAGGT T- 3'¹²⁶⁷ bp (Szymanowska *et al.* 2003)^[6] was used for the amplification of PCR product.

2.7 Polymerase Chain Reaction (PCR)

In the Polymerase Chain Reaction preprogrammed thermo cycler (Mastercycler gradient, Eppendorf) were used.

2.8 PCR- RFLP Assay

2.8.1 Restriction digestion

All the PCR products were digested by EcoRV restriction enzymes and then incubated at 37 °C for 3 hrs in the water bath.

2.8.2 Agarose gel electrophoresis of digested PCR products

After the digestion the digested PCR products were analyzed on 2.50% agarose gel. The mass ruler DNA ladder (100 bp-1000 bp) as a molecular size marker was used.

2.9 Sequencing

The sequences obtained from genotype were aligned using Clustal W. (Thompson *et al.*, 1994)^[4] and analyzed by using MEGA 6 software (Tamura *et al.*, 2004)^[7]. Aligned sequences were analyzed for group specific SNP marker.

2.10 Statistical analysis

2.10.1 Calculation of Gene and genotype frequencies:

Gene and genotype frequencies for different casein genes were estimated by using Popgene 32 (version1.32), Microsoft Windows-based freeware for population genetic analysis (Yeh *et al.*, 1999)^[5].

2.10.2 Association of various polymorphic variants of milk protein genes with Milk Yield (MY)

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

$$Y_{ijkl} = \mu + P_i + B_j + G_k + (PXB)_{ij} + (PXC)_{ik} + (PXD)_{jk} + (PXE)_{ijk} + e_{ijkl}$$

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

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

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

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

e_{ijkl} - is random error effect

2.10.3 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 above two breeds of cattle (Snedecor and Cochran, 1994)^[3].

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.

4. Association of α S2-Casein (CSN1S2) gene polymorphic variants with Milk yield per lactation.

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

4.1 Milk yield (MY) of different variants at α S2-casein gene (CSN1S2)/EcoRV locus in above both breeds of cattle

The least squares means for different genotypes of following both breeds of cattle breed has been presented.

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

Variants	Breeds	
	Sahiwal	HF crossbred
AA	1523.00 ^a ±39.00 (50)	1955.00 ^b ±112.00 (29)
AB	00±00 (00)	3176.00 ^c ±113.00 (21)
BB	0.00±0.00 (00)	0.00±0.00 (00)
Overall	1523.00 ^a ±39.00 (50)	2468.00 ^b ±111.00 (50)

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

The association of polymorphic variants of α S2-casein gene (CSN1S2) in both AA (1955.00^b±112.00) and AB (3176.00^c±113.00) genotype of HF crossbred breeds cow showed significantly higher milk yield per lactation than AA genotype (1523.00^a±39.00) of Sahiwal, whereas the AB genotype of HF crossbred showed significantly higher milk yield per lactation than its own AA genotype.

5. Discussion

The association of polymorphic variants of α S2-casein gene (CSN1S2) in both AA (1955.00^b±112.00) and AB (3176.00^c±113.00) genotype of HF crossbred breeds cow showed significantly higher milk yield per lactation than AA genotype (1523.00^a±39.00) of Sahiwal, whereas the AB genotype of HF crossbred showed significantly higher milk

yield per lactation than its own AA genotype. Contrary to these findings Hristov *et al.* (2014) ^[1] showed that the BB genotype determines higher milk production.

6. Conclusion

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 of HF crossbred breeds cow showed significantly higher milk yield per lactation than AA genotype) of Sahiwal, whereas the AB genotype of HF crossbred showed significantly higher milk yield per lactation than its own AA genotype.

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