POTENTIAL INFLUENCE OF KAPPA (κ) CASEIN GENE AND ITS ASSOCIATION WITH MILK QUALITY TRAIT (SNF) IN MALVI AND NIMARI BREED OF COW OF M P, INDIA.

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ABSTRACT:

Present study revealed that polymorphic variants and their association with milk production traits at κ-casein gene (CNS3) locus in Malvi and Nimari, cattle. Association of polymorphic variants of κ -Casein (CSN3) gene revealed that the frequencies of AA are higher as compared to AB and BB genotypes. The frequency of A allele was found to be highest as compared to B allele in all the 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 Malvi, Nimari breeds of cattle, indicating that the populations of these animals were in Hardy-Weinberg equilibrium at this locus. The analysis of variance showed significant (P<0.01) effect of breed on SNF (%).The mean SNF per cent of Malvi was significantly lower than Nimari cattle. The maximum SNF per cent was noticed in Nimari breed of cattle.

Keywords: - K casein gene, SNF, Malvi, Nimari.   .

INTRODUCTION :

The term 'total Solids' (TS) refers to the quantity of SNF plus fat present in milk. It may range from 12 to 16 per cent, depending on its source. For cow milk, TS is 12 per cent (3.5%F and 8.5% SNF) while for buffalo milk it ranges between 15 and 16 per cent (6-7% F and 9% SNF).This protein is encoded by a trait locus at chromosome 6 and comprises a sequence of 162 amino acids (Rijnkels, 2002) and this protein represents up to 12% of the total casein in bovine milk .The two main k-casein variants, A and B, differ in the amino acids at positions 136 (Thr for Ile) and 148 (Asp for Ala) (Alexander et al., 1988). Several polymorphisms have been detected in the open reading frame reviewed by (Caroli et al. 2006) and in non-coding regions such as the 5'-flanking region of the casein genes (Cosenza et al. 2007, 2008) Reports on the association of k-casein genetic variants and milk composition have somewhat conflicting results Ng-Kwai-Hang et al. (1990) reported changes in milk protein concentrations due to the k-casein genotypes A and B. However found no effect of k-casein variants on milk crude protein, while others reported higher contents of protein Stevanovic et al. (2000) and caseins in allele B, k-casein cow milk.

MATERIAL AND METHODS :

Collection of milk samples with economic traits: After the collection of milk samples was brought to the laboratory, maintaining cold chain and then SNF % in the milk were determined.

Estimation of Lactose, SNF and milk density: The data of then SNF % were analyzed by Milk analyzer.

Blood Collection

5 ml blood sample was collected in EDTA coated vaccutainer aseptically from 50 animals of each of Malvi and Nimari breeds and maintaining cold chain then processed for DNA isolation.

Genomic DNA isolation:

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As per the method described by John et al. (1991) with minor modifications Genomic DNA was extracted from blood .

Agarose gel electrophoresis:

0.80% horizontal submarine agarose gel electrophoresis performed for Quality check of DNA.

Concentration, purity and quality check of DNA :

Taken the help of Nanodrop, spectrophotometer and agarose gel electrophoresis.

Casein gene primer sequence:

The κ -casein gene primers (F): 5'-GCTGAGCAG GTATCCTAGTTAT-   3'   (R):   5'   - CTTCTTTGATGTCTCCTTAGAG - 3', 443 bp Schlieben et al. (1991) was used for the amplification of PCR product.

Polymerase Chain Reaction (PCR)

Setting of PCR Reaction:

The PCR tubes were kept in a pre-programmed thermo cycler where the Initial denaturation (5 minutes) and final denaturation (1 minute) temp. was 940C and 600C anniling temp.(1 min.) Was 600C where extension(1 minute) and final extension(5 minutes) temp. was 720C

Agarose gel electrophoresis of PCR reaction product :

To confirm the targeted PCR amplification the PCR products were analyzed on 2.00 % agarose gel. The mass ruler DNA ladder (100 bp - 1000 bp) as a molecular size marker was used for sizing of the DNA bands.

PCR- RFLP Assay:

All the PCR products of k casein gene were digested by HindIII restriction enzymes in water bath at 37°C for 3 hrs.

Agarose gel electrophoresis of digested PCR products:

Digested PCR products were analyzed on 2.50 % agarose gel (5 µl of PCR product mixed with 1 µl of gel loading dye).

Sequencing:

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.

Association of various polymorphic variants of milk protein genes with SNF (%) :

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

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

Testing Hardy-Weinberg (H-W) equilibrium:

Hardy-Weinberg equilibrium tested by the chi-square test $(x2)$ in above mentioned both breeds of cattle (Snedecor and Cochran, 1994).

Association study conducted between the polymorphic variants/ genotypes of, k -casein genes with milk SNF (%) of Malvi and Nimari crossbred cattle by linear regression model .

RESULTS :

SNF (%)

The results of least squares analysis of variance presented in table 01, showed that the effect of breed was significant, however the genotype, parity, breed x genotype and breed x parity interactions were non-significant for SNF (%) trait.

SNF (%) in milk of different variants at κ-Casein (CSN3) gene locus in   Malvi and Nimari breeds of cattle.

The least square means for SNF (%) in Malvi and Nimari breed cattle have been presented in table 02. The AA genotyped animals in above both breeds of cattle revealed   non-significant difference with AB genotype. The AA genotype of Nimari (8.86±0.15) showed significantly higher SNF (%) in the milk than both AA (8.06±0.12) and AB (7.95±0.21) genotypes of Malvi breed of cattle (Table 02).

Means bearing the different superscript differ significantly (p<0.05), Values in parentheses are number of animals.

DISCUSSION :

The frequency of A allele was found to be highest as compared to B allele in all the four 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 Malvi, Nimari and Sahiwal breeds of cattle, indicating that the populations of these animals were in Hardy-Weinberg equilibrium at this locus.

The analysis of variance showed significant (P<0.01) effect of breed on SNF (%).The mean SNF per cent of Malvi was significantly lower than Nimari, cattle. The maximum SNF per cent was noticed in Nimari breed of cattle. Our findings agree with the results of Szymanowska et al. (2004) who reported higher lactose (%) and SNF (%) for AA genotyped Polish Black and White cattle.

CONCLUSION:

It is concluded that the frequencies of AA is higher as compared to AB and BB genotypes. The frequency of A allele was found to be highest as compared to B allele in all the four 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 Malvi, Nimari breeds of cattle, indicating that the populations of these animals were in Hardy-Weinberg equilibrium at this locus. The analysis of variance showed significant (P<0.01) effect of breed on SNF (%) traits. The maximum SNF per cent was noticed in Nimari breed of cattle.

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Table 01: Least square analysis of variance for SNF (%) at κ-Casein (CSN3) gene locus in Malvi, Nimari cattle.

	MS	F-value
Breeds	1.87	3.05*
Genotype (Variants)	0.54	0.87[NS]
Parity	0.61	0.99[NS]
Breed x Genotype	0.40	0.65 [NS]
Breed x Parity	0.90	0.90[NS]
Genotype x Parity	0.64	1.05[NS]
Error	0.61	-

*significant (p<0.05), NS- Non-significant

Table 02: Least squares means for SNF (%) in different breeds at κ-Casein (CSN3) gene locus.

Variants	Malvi	Nimari
AA	8.06[c]±0.12 (36)	8.86[a]±0.15 (33)
AB	7.95[c]±0.21 (14)	8.80[ab]±0.26 (17)
BB	0.00±0.00 (0)	0.00±0.00 (0)
Overall	8.03[b]±0.18 (50)	8.84[a]±0.19 (50)

Means bearing the different superscript differ significantly (p<0.05), Values in parentheses are number of animals.