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Comparative genomics study of different genetic variants of αs2-casein gene (CSN1S2) with milk production traits (lactose, SNF & density) in Malvi and Nimari cattle of Madhya Pradesh, India

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Abstract
αs2-casein is a primary milk protein. However, there has been little research focused on the effects of αs2- casein variants on milk. The αs2-casein (CSN1S2) play a major protein found in ruminant's milk, which is encoded by a highly polymorphic CSN1S2 gene present on chromosome 6. Research work was carried out on 50 animals each of Malvi and Nimari cow at College of Veterinary Science Jabalpur, Madhya Pradesh, India. The αS2 casein gene (1267 bp) was digested by EcoRV restriction enzyme yielding two genotypes *viz*., AA (1267 bp) and BB (1267/1150/117 bp) in Malvi and Nimari cow. Association study of different genotypes with milk composition traits revealed that the mean lactose % was significantly higher in Malvi compared to Nimari for A2A2 genotype. The difference of mean SNF % between Malvi, Nimari was found non-significant. The mean milk density (kg/L) was significantly higher in Nimari breed compared to Malvi.

Keywords: αS2, casein gene, malvi, nimari, breeds

1. Introduction
αS2 Casein genes are highly polymorphic and the high degree of variability has qualitative and quantitative effects on milk composition thereby affecting chemical, physical and technological properties of goat milk. The aim of this work was to evaluate the genetic polymorphisms of the αs2- casein (CSN1S2) gene and its association with different milk production traits in Malvi and Nimari breeds of cattle. Caseins are the main protein component of milk. In bovine species the entire casein gene cluster region spans about 250 kb on chromosome 6 (Hayes *et al*., 1993) [1]. Casein genes are highly polymorphic and the high degree of variability, together with post-translational modifi cations and differential splicing patterns, has qualitative and quantitative effects on milk composition. In cattle, at least 39 variants of the 4 casein proteins (αS1-, β-, αS2- and κ-casein) have been described to date. Many of these variants are known to affect milk-production traits, cheese-processing properties, and the nutritive value of milk. They also provide valuable information for phylogenetic studies. So far, the majority of studies exploring the genetic variability of bovine caseins considered European taurine cattle breeds and were carried out at the protein level by electrophoretic techniques. This only allows the identifi cation of variants that, due to amino acid exchanges, differ in their electric charge, molecular weight, or isoelectric point.

2. Material and Methods
2.1 Collection of milk samples
Collection of milk samples with economic traits about 100ml milk sample was collected from each of the above 100 cattle. The milk samples brought to the laboratory, maintaining cold chain and then Lactose (%), SNF (%) and Milk density (Kg/L) were determined.

2.2 Estimation of Lactose (%), SNF (%) and Milk density (Kg/L)
The Lactose (%), SNF (%) and Milk density (Kg/L) were analyzed by Milk analyzer of the Department of Veterinary Medicine, College of Veterinary Science & A.H., Jabalpur.

2.3 Blood Collection
5 ml blood sample was collected in EDTA coated vaccutainer aseptically from 50 animals of each of the four breeds *viz*. Malvi, Nimari, Sahiwal and HF crossbred cattle and brought to the laboratory, maintaining cold chain then processed for DNA isolation.

2.3.1 Genomic DNA isolation
1. Genomic DNA was extracted from venous blood as per the method described by John *et al.* (1991) [2] with minor modifications.
2. 5 ml blood from each lactating cow was collected from external jugular vein aseptically in sterile EDTA coated vaccutuiner (Vacutte Greiner bio-one Gmbh, Austria). After proper labeling, the samples were transported to the laboratory, maintaining cold chain for further analysis.
3. Blood (5 ml) was mixed with equal volume of solution-l (10mM Tris, 10mM KCI, 10mM MgCI2 of pH 7.6) in a 30 ml centrifuge tube. The composition of solution is given in Table 02.
4. 120 pi Nonidet P40 was added to lyses the cell in to the tube and the solution was mixed well by inverting the tubes several times.
5. Mixture was spinned at 4500 rpm for 50 min at 24 °C. The supernatant was discarded and the nuclear pellet was resuspended gently in 400 pi of solution-2 (10mM Tris, 10mM KCI, 10mM MgCl2, 0.50M NaCI, 2mM EDTA, of pH 7.6, 0.5% SDS) to lyses the nuclei. The composition of solution -2 is given in table 03.
6. Equal volume of Tris saturated phenol (400 pi) was added to the suspension and centrifuged at 14000 rpm for 25 min at 24 °C (Eppendorf Centrifuge).
7. The upper phase was transferred to a clean microfuge tube and equal volume (400 pi) of solution containing Tris saturated phenol (pH 8): chloroform: isoamyl alcohol (25:24:1) was added to the tubes.
8. The suspension was mixed thoroughly and spinned at 14000 rpm for 25 min. at 24 °C.
9. Subsequently, upper phase was again transferred to another microfuge tube and equal volume of chloroform: isoamyl alcohol (24:1) was added to the tube.
10. The suspension was mixed thoroughly and spinned at 14000 rpm for 30 min at 24 °C.
11. The upper aqueous phase was transferred to microfuge tube avoiding interface. The DNA was precipitated by adding 1/10th volume of sodium acetate (3M) and equal volume of chilled absolute ethanol. The tube was mixed gently.
12. The mixture was centrifuged at 14000 rpm for 50 min, at 4 °C to sediment the DNA and the ethanol was poured off carefully.
13. The precipitated DNA pellet was washed twice with 1ml of 70% ethanol by inverting the tubes at least for 15 minutes.
14. Ethanol was discarded and DNA pellet was allowed to air dry avoiding over drying.
15. The DNA pellet was resuspended in 500 pi of 0.3X TE buffer (pH 8.0) and incubated at 65 °C for 1 hr in a water bath.
16. The DNA sample was stored at -20 °C till further use

2.3.2 Setting of PCR Reaction: The PCR tubes were kept in a preprogrammed thermo cycler (Mastercycler gradient, Eppendorf) and set at the standardized reaction programme

2.4 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) [6] and analyzed by using MEGA 6 software (Tamura *et al*., 2004) [5]. Aligned sequences were analyzed for group specifi c SNP marker.

2.5 Statistical analysis
2.5.1 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) [7].

2.5.2 Association of various polymorphic variants of milk protein genes with Lactation length (LL)
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:

$$Yijkl = \mu + Pi + Bj+Gk+(PXB)ij +(PXG)ik+(BXG)jk+(PXBXG)ijk+ eijkl$$

Where,
Yijkl - is the Observed value of milk yield
μ - is the population mean
Pi - is the fi xed effect of parity
Bj - is the fi xed effect of breed
Gk - is fi xed effect of genotypes (k = 1, 2….)
(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
eijkl - is random error effect

2.5.3 Testing Hardy-Weinberg (H-W) equilibrium
The chi-square test (x2) was employed to test the status of Hardy-Weinberg equilibrium in the different population of four breeds of cattle (Snedecor and Cochran, 1994) [3].
To find out the association between the polymorphic variants/ genotypes of, αs2 -casein genes with milk production traits like, Lactose (%), SNF (%) and Milk density (Kg/L) in of Sahiwal and HF crossbred cattle by linear regression model was employed.

3. Results and Discussion
The frequency of A allele was found to be highest(100%) as compared to B allele(0.00) in above breeds of cattle under the study.

3.1 Association study
Lactose (%) of different variants at αs2-casein gene (csn1s2)/ ecorv locus in Malvi and Nimari breeds of cattle:

Table 1: Least squares means for Lactose (%) in the milk of Malvi and Nimari breeds of cattle at αS2-Casein (CSN1S2) gene locus

Variants	Breeds	
	Malvi	Nimari
AA	4.73^{a}±0.09 (22)	5.46^{b}±0.04 (31)
AB	5.02^{a}±0.08 (28)	5.72^{b}±0.18 (19)
BB	0.00±0.00 (00)	0.00±0.00 (00)
Overall	4.89^{c}±0.06 (50)	5.56^{a}±0.07 (50)

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

As shown in table 01, the mean lactose percent was significantly higher in Nimari than Malvi for both AA and AB genotyped animals. Among the above genotypes of both breeds of cattle, higher lactose percent was recorded in Nimari (5.72±0.18) for AB genotyped animals, while it was found significantly lower in Malvi (4.73±0.09) cattle for AA genotype (Table 01).

Table 2: Least squares means for SNF (%) in the milk of Malvi and Nimari breeds of cattle at αS2-Casein (CSN1S2) gene

Variants	Breeds	
	Malvi	Nimari
AA	7.86^{b}±0.16 (22)	8.86^{a}±0.15 (31)
AB	8.17^{b}±0.13 (28)	8.81^{a}±0.23 (19)
BB	0.00±00 (00)	0.00±0.00 (00)
Overall	8.03^{b}±0.11 (50)	8.84^{a}±0.13 (50)

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

Nimari cow showed significantly higher mean SNF percent in both genotypes of AA and AB compared to Malvi cows. So the mean SNF percent was recorded maximum in AA genotypes of Nimari breed (8.86±0.15) and minimum in AA genotype of Malvi cow (7.86±0.16). In accordance to the above findings, Szymanowska *et al*. (2004) [4] showed that the AA genotype determine higher lactose and SNF percent in Polish Black and White cattle.

Table 3: Least squares means for milk density (kg/L) of different breeds of cattle at αS2-Casein (CSN1S2) gene

Variants	Breeds	
	Malvi	Nimari
AA	1. 03^{a}±0.08 (22)	1.04^{b}±0.08 (31)
AB	1.03^{a}±0.09 (28)	1.05^{b}±0.07 (19)
BB	0.00±00 (00)	0.00±0.00 (00)
Overall	1.03^{a}±0.09 (50)	1.04^{b}±0.08 (50)

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

The mean milk density (kg/L) was significantly higher for AB genotype of Nimari cows compared to AB and AA of Malvi breed. So the mean milk density (kg/L) was observed maximum in Nimari (1.05±0.07) for AB genotype and minimum in Malvi (1.03±0.08) for AA genotype animals, (Table 03).

4. Conclusion

In last to conclude PCR-RFLP analysis of αs2-Cn gene (1267bp) with EcoRV RE revealed two genotypes *viz*., AA (1267/1267bp) and AB (1267/1150/117bp) in Malvi and, Nimari breeds of animals. The frequency of A allele was found to be highest as compared to B allele in above both breeds of cows under the study. Association study of different genotypes with milk composition traits revealed that the mean lactose percent was significantly higher in Malvi breed of animal compared to Nimari for AB genotyped animals as compared to AA genotype. Nimari cow showed significantly higher mean SNF percent in both genotypes of AA and AB compared to Malvi cows. The mean milk density (kg/L) was significantly higher for AB genotype of Nimari cows compared to AB and AA of Malvi breed

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