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Different genetic variants' of αs1-casein gene (CSN1S1) and their association with lactose, SNF and milk density

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Abstract
The aim of this study was to know the polymorphic variants and their association with milk production traits in Malvi, Nimari, Sahiwal and HF crossbred cattle of Madhya Pradesh during 2016 to 2017 at αS1 casein gene (CSN1S1) gene locus. The analysis of variance for different genotypes of αs1-casein gene in four breeds of cattle showed the significant effect of breed (P<0.01) for lactose (F=16.43**), SNF (F=10.58**) and Density (F=25.74**) traits of the milk. The mean lactose percent was significantly higher in Nimari (5.56±0.07) as compared to Malvi (4.89±0.06) and Sahiwal (5.25±0.07). However, the mean lactose percent in milk of Sahiwal (5.25^{b}±0.07) and HF crossbred cattle (5.39^{ab}±0.07) showed non-significant difference. The mean SNF percent of Malvi (8.03^{b}±0.11) was significantly lower than Nimari (8.84^{a}±0.13), Sahiwal (8.74^{a}±0.12) and HF crossbred cattle (8.54^{a}±0.08), whereas the maximum SNF percent was noticed in Nimari. In density observation, significantly higher mean density of milk was recorded in Nimari (1.04^{a}±0.09) and HF crossbred (1.04^{a}±0.09) as compared to Sahiwal (1.03^{b}±0.10) and Malvi (1.03^{c}±0.08) breeds of cattle.

Keywords: Genotypes, protein, fat, Malvi, Nimari, Sahiwal, HF crossbred

Introduction
Milk naturally possessing many chemical and physical components. All milks contain the same kind of constituents but in varying amount. Milk contain two types of protein casein and whey protein. Caseins that make up about 80% of the milk proteins, consisting of four proteins Alpha S1 (CSN1S1, 39-46% of total caseins), alpha S2 (CSN1S2, 8-11%), beta (CSN2, 25-35%), and kappa (CSN3, 8- 15%). Whey proteins have two major fractions alpha-lactalbumin and beta-lactoglobulin [1]. It has phosphate groups located in the hydrophilic region and most of the calcium associated with is exists at the phosphate groups. Milk composition is economically important to milk producers and processors and nutritionally important to consumers. It has been known for years that variations in milk composition occur; however, the composition of milk marketed nationally has been rather constant over the last 15 years, averaging 3.6 percent fat, 3.2 percent protein, and 4.7 percent lactose [8]. This is probably partly because of the prominence of the Holstein breed and the pricing of milk based on fat concentration. Factors affecting milk composition such as breed, genetic variation within breed, health, environment, management practices, and diet may occur. However, the SNF showed significant difference (P<0.05) among the milk of buffalo (8.79%) and of goat milk (8.92%) [3]. In contrast, the SNF of milk samples obtained from cow and sheep (9.17 and 9.71%, respectively). Milk proteins polymorphism has been of great interest in animal breeding and dairy industry due to its relationships with production traits, milk composition and quality and its potential use in genetic selection of bovine breeds [2].

Objective: To study the association of genetic variant's of αs1-casein gene (csn1s1) with Lactose, SNF and Milk density in Malvi, Nimari, Sahiwal and HF crossbred cattle.

Materials and Methods
The research work was conducted during 2016 to 2017 on 200 lactating cows comprising 50 each of Malvi Nimari, Sahiwal and HF crossbred cattle. The data and sample of Malvi collected from the Government Cattle Breeding Farm, Agar, (MP) and Nimari from the Government Cattle Breeding Farm, Rodia, Khargon (MP), Sahiwal from Livestock Farm,

College of Veterinary Science and A.H., Anjora (Durg) and Govt. Sahiwal Breeding Farm, Anjora (Durg) and HF crossbred from Livestock Farm, College of Veterinary Science and A.H., Jabalpur and Private Dairy Farm at Pariyat, Jabalpur.

Identification number, Parity, Lactation length and Lactation yield of each animal under study, were recorded. About 100ml milk sample from each cow are collected in the sterilized tube and mixed with 0.8% formalin and then 5 ml blood sample was collected from same cow in EDTA coated test tube. Collected samples are maintained in cold chain during transportation and in laboratory. In first phase of research the milk samples are processed for Lactose (%), SNF (%) and density (Kg/L) analysis and they were analyzed by milk analyzer. In 2nd phase DNA isolation, PCR, RFLP, and genotyping was done by standardized procedure and in last step PCR product sent for sequencing.

Sequencing and analysis

Sequencing of amplicon was done for the confirmation of genotype of the cattle. The sequences obtained from genotype were aligned using Clustal W [5] and analyzed by using MEGA 6 software [4]. Aligned sequences were analyzed for group specific SNP marker.

Statistical analysis

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 [7].

Association of various polymorphic variants of milk protein genes on Milk yield (MY), Daily milk yield (DMY), Protein (%), Fat (%), Lactose (%), SNF (%) and Milk density (Kg/L)

Association study of various polymorphic variants of milk protein genes for Milk yield (MY), Daily milk yield (DMY), Protein (%), Fat (%), Lactose (%), SNF (%) and Milk density (Kg/L) data were subjected to least squares analysis of variance employing following linear model [9]:

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

Where,

Yijkl - is the Observed value of milk yield

μ - is the population mean

Pi - is the fixed effect of parity

Bj - is the fixed effect of breed

Gk - is fixed 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

Correlations between various traits (Milk yield and Milk composition traits) for different genotypes, breeds wise

To find out the association between the polymorphic variants/ genotypes of αs1 casein genes with milk production traits like, Milk yield (MY), Daily milk yield (DMY), Protein (%), Fat (%), Lactose (%), SNF (%) and Milk density (Kg/L) in of Malvi, Nimari, Sahiwal and HF crossbred cattle, Karl Pearson correlation method was employed [10].

Results and Discussion

Lactose (%) in milk of different breeds of cattle

The source of variation, mean sum of squares along with F-value for different breeds has been presented in Table 1. The effect of breed was found significant ($P<0.01$) for milk lactose percent trait. The mean lactose percent in the milk of Malvi, Nimari, Sahiwal and HF crossbred cattle has been presented in Table 2.

Table 1: Analysis of variance for Lactose (%) in Malvi, Nimari, Sahiwal and HF Crossbred cattle.

Source of Variance	DF	MS	F-Value
Breeds	3	4.06	16.43**
Error	196	0.25	
Total	199		

** Highly significant ($p<0.01$)

The mean lactose percent was significantly higher in Nimari (5.56±0.07) as compared to Malvi (4.89±0.06) and Sahiwal (5.25±0.07). However, the mean lactose percent in milk of Sahiwal and HF crossbred cattle showed non-significant difference (Table 2).

Table 2: Mean Lactose (%) in milk of different breeds at αs1-Casein (CSN1S1) gene locus.

Variants	Breeds			
	Malvi	Nimari	Sahiwal	HF crossbred
AA	4.89^{c}±0.06 (50)	5.56^{a}±0.07 (50)	5.25^{b}±0.07 (50)	5.39^{ab}±0.07 (50)
AB	0.00±00 (00)	0.00±00 (00)	0.00±00 (00)	0.00±00 (00)
BB	0.00±00 (00)	0.00±00 (00)	0.00±00 (00)	0.00±00 (00)
Overall	4.89^{c}±0.06 (50)	5.56^{a}±0.07 (50)	5.25^{b}±0.07 (50)	5.39^{ab}±0.07 (50)

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

SNF (%) in milk of different breeds of cattle

The source of variation, mean sum of squares along with F-value for different breeds has been presented in Table 3. The effect of breed was found significant ($P<0.01$) for SNF percent in milk. The mean SNF percent in milk of Malvi, Nimari, Sahiwal and HF crossbred cattle has been presented in Table 4.

Table 3: Analysis of variance for SNF (%) in Malvi, Nimari, Sahiwal and HF Crossbred cattle

Source of Variance	DF	MS	F-Value
Breeds	3	6.54	10.58**
Error	196	0.62	
Total	199		

** Highly significant ($p<0.01$)

The mean SNF percent in Malvi, Nimari, Sahiwal and HF crossbred cattle were found to be 8.03±0.11, 8.84±0.13, 8.74±0.12 and 8.54±0.08 percent, respectively. The mean SNF percent of Malvi was significantly lower than Nimari, Sahiwal and HF crossbred cattle. The maximum SNF percent was noticed in Nimari breed of cattle (Table 4). The present findings agree with the results of Szymanowska *et al.* (2004) [4] who reported higher lactose (%) and SNF (%) for AA genotyped Polish Black and White cattle.

Table 4: Mean SNF (%) in milk of different breeds at αs1-Casein (CSN1S1) gene locus.

Variants	Breeds			
	Malvi	Nimari	Sahiwal	HF crossbred
AA	8.03^{b}±0.11 (50)	8.84^{a}±0.13 (50)	8.74^{a}±0.12 (50)	8.54^{a}±0.08 (50)
AB	0.00±00 (00)	0.00±00 (00)	0.00±00 (00	0.00±00 (00
BB	0.00±00 (00)	0.00±00 (00)	0.00±00 (00)	0.00±00 (00)
Overall	8.03^{b}±0.11 (50)	8.84^{a}±0.13 (50)	8.74^{a}±0.12 (50)	8.54^{a}±0.08 (50)

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

Density (kg/L) in milk of different breeds of cattle

The source of variation, mean sum of squares along with F-value for different breeds has been presented in table 5. The effect of breed was found significant (P<0.01) for milk density (kg/L). The mean density in milk of Malvi, Nimari, Sahiwal and HF crossbred cattle has been presented in Table 6.

Table 5: Analysis of variance for Density (Kg/L) in Malvi, Nimari, Sahiwal and HF Crossbred cattle

Source of Variance	DF	MS	F-Value
Breeds	3	264.22	25.74**
Error	196	10.27	
Total	199		

** Highly significant (p<0.01)

As shown in Table 6, significantly higher mean density was recorded in Nimari (1.04±0.09) and HF crossbred as compared to Sahiwal and Malvi.

Table 6: Mean Density (kg/L) in milk of different breeds at αs1-Casein (CSN1S1) gene locus.

Variants	Breeds			
	Malvi	Nimari	Sahiwal	HF crossbred
AA	1.03^{c}±0.08 (50)	1.04^{a}±0.09 (50)	1.03^{b}±0.10 (50)	1.04^{a}±0.09 (50)
AB	0.00±00 (00)	0.00±00 (00)	0.00±00 (00	0.00±00 (00
BB	0.00±00 (00)	0.00±00 (00)	0.00±00 (00)	0.00±00 (00)
Overall	1.03^{c}±0.08 (50)	1.04^{a}±0.09 (50)	1.03^{b}±0.10 (50)	1.04^{a}±0.09 (50)

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

Conclusion

As per the analysis of variance for genotype of αs1-casein gene in four breeds of the cattle the significant effect of breed (P<0.01) for lactose, SNF and Density traits of milk was noticed,. The mean lactose percent was significantly higher in Nimari as compared to Malvi and Sahiwal. However non-significant difference observed in the mean lactose percent in milk of Sahiwal and HF crossbred cattle. The mean SNF percent of Malvi was significantly lower than Nimari, Sahiwal and HF crossbred cattle, whereas the maximum SNF percent was noticed in Nimari. In density observation, significantly higher mean density of milk was recorded in Nimari and HF crossbred as compared to Sahiwal and Malvi breeds of cattle.

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