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## Different genetic variants' of $\alpha$ s1-casein gene (csn1s1) and their association with lactation length (days) fat (%) and protein (%)

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**Abstract**

In the aim of association study of different genetic variants' of  $\alpha$ s1-casein gene (csn1s1) and their association with lactation length (days) fat (%) and protein (%), the analysis of variance for different genotypes in four breeds of cattle has been observed that the effect of genotypes was found significant 74.31\*\* ( $P < 0.01$ ) for lactation length (LL) trait. Significantly higher LL (Days) was recorded in AA genotype of HF crossbred  $329.70^a \pm 8.93$  while the lowest LL was noticed in AA genotype of Nimari  $202.42^d \pm 3.67$ . The mean LL between AA and AB genotype of Malvi, Sahiwal and HF crossbred cattle showed non-significant difference. The higher mean Fat (%) was noticed for AA genotype than AB genotype of Malvi and Nimari however in HF crossbred higher Fat (%) was observed for AB genotype. The mean Fat% between AA and AB genotypes showed non-significant difference in Malvi, Sahiwal and HF crossbred cattle but the mean protein per cent was significantly higher in Malvi  $3.36^b \pm 0.05$  and Nimari  $3.74^a \pm 0.07$  and non-significantly higher in HF crossbred for AB genotype as compared to AA genotype. Among the different genotypes of all four breeds of cattle, significantly higher protein (%) was recorded in Nimari for AB genotyped animals.

**Keywords:** Genotypes, protein, fat, Malvi, Nimari, Sahiwal, hf crossbred

**Introduction**

Alpha S1-casein represents the major protein fraction (31%) among the bovine milk proteins (caseins and whey) and constitutes up to 40% of total casein. It has phosphate groups located in the hydrophilic region and most of the calcium associated with it exists at the phosphate groups. Nucleic acid sequences have been reported for  $\alpha$ s1 like casein mRNAs from cow, sheep, rat and guinea pigs. They are found to be divergent sequences, with a large number of point mutations [2].

It is a calcium sensitive and highly phosphorylated protein. It has an important role in the capacity of milk to transport calcium phosphate and is organized at 5'-terminus of casein cluster located on bovine chromosome 6 (BTA6). The distribution of genotypes of all four milk protein systems was different from breed to breed [1]. For Jersey cows, significant differences in the gene were found for alpha-s1, k-casein and beta-Lg. For SDM cows a change in the K- casein frequency occurred whereas for RDM cows no change was found. Linkage between some of the casein loci was found within all three breeds. They also reported the predominance of genotype AA, with frequency 64 and 74 per cent in RDM and SDM cows, respectively. The proportion of phenotype variance explained (PPVE) by each SNP was estimated according based on some of the parameters of the linear mixed models used [9]. Genotypes with fewer than six observations were excluded (Table S1). P-nominal (Pnominal) values were corrected for multiple testing using the proportion of false positives (PFP); [10] approach.

**Materials and Methods**

The research work was conducted on 200 lactating cows comprising 50 each of Malvi Nimari, Sahiwal and HF crossbred cattle. Identification number, Parity, Lactation length and Lactation yield of each animal under study, were recorded. About 100ml milk sample from each cow were 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 Protein (%), Fat (%), Lactose (%), SNF (%) and Milk density (Kg/L)

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analysis and they were analyzed by milk analyzer. In 2<sup>nd</sup> phase the genotyping was done after DNA isolation, PCR, RFLP, and sequencing from standardized procedure.

### 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 [10] and analyzed by using MEGA 6 software [5]. 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),

Yijkl	-	is the Observed value of milk yield
$\mu$	-	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  $\alpha$ s1 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.

#### Lactation Length (LL) in 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 LL trait. The mean LL (days) per lactation in Malvi, Nimari, Sahiwal and HF crossbred cattle has been presented in Table 2.

**Table 2:** Mean Lactation Length (days) in different breeds at  $\alpha$ s1-Casein (CSN1S1) gene locus.

Variants	Breeds			
	Malvi	Nimari	Sahiwal	HF crossbred
AA	301.08 <sup>b</sup> ±5.20 (50)	202.42 <sup>d</sup> ±3.67 (50)	267.86 <sup>e</sup> ±6.41 (50)	329.70 <sup>a</sup> ±8.93 (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	301.08 <sup>b</sup> ±5.20 (50)	202.42 <sup>d</sup> ±3.67 (50)	267.86 <sup>e</sup> ±6.41 (50)	329.70 <sup>a</sup> ±8.93 (50)

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

#### Fat (%) 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 fat (%) trait. The mean fat per cent in milk of Malvi, Nimari, Sahiwal and HF crossbred cattle has been presented in Table 4.

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

Source of Variance	DF	MS	F-Value
Breeds	3	13.92	12.58**
Error	196	1.11	
Total	199		

\*\* Highly significant ( $P < 0.01$ )

Microsoft Windows-based freeware for population genetic analysis [8].

#### 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:

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

Where,

**Table 1:** Analysis of variance for LL (days) in Malvi, Nimari, Sahiwal and HF Crossbred cattle

Source of Variance	DF	MS	F-Value
Breeds	3	149847.00	74.31**
Error	196	2016.00	
Total	199		

\*\* Highly significant ( $P < 0.01$ )

The mean LL for Malvi, Nimari, Sahiwal and HF crossbred cattle were found to be 301.08±5.20, 202.42±3.67, 267.86±6.41 and 329.70±8.93 days, respectively. The significantly higher LL was noticed in HF crossbred cattle, while it was noticed minimum in Nimari breed of cattle (Table 2).

The mean fat% in milk of Malvi, Nimari, Sahiwal and HF crossbred cattle were found to be 2.85±0.13, 3.04±0.16, 3.23±0.15 and 2.03±0.16 per cent, respectively. The significantly lowest fat per cent was observed in HF crossbred cattle as compared to Malvi, Nimari and Sahiwal breeds of cattle. Among three indigenous breeds of cattle, mean fat per cent was non-significantly higher in Sahiwal breed of cattle (Table 4). Ikonen *et al.* [3] reported in Brown Swiss cattle that Fat (%) in the milk increases by presence of B allele in the population.

**Table 4:** Mean Fat (%) in milk of different breeds at  $\alpha$ s1-Casein (CSN1S1) gene locus.

Variants	Breeds			
	Malvi	Nimari	Sahiwal	HF crossbred
AA	2.85 <sup>a</sup> ±0.13 (50)	3.04 <sup>a</sup> ±0.16 (50)	3.23 <sup>a</sup> ±0.15 (50)	2.03 <sup>b</sup> ±0.16 (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	2.85 <sup>a</sup> ±0.13 (50)	3.04 <sup>a</sup> ±0.16 (50)	3.23 <sup>a</sup> ±0.15 (50)	2.03 <sup>b</sup> ±0.16 (50)

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

### Protein (%) 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 protein per cent trait. The mean protein per cent in milk of Malvi, Nimari, Sahiwal and HF crossbred cattle has been presented in Table 6.

**Table 5:** Analysis of variance for Protein (%) in Malvi, Nimari, Sahiwal and HF Crossbred cattle

Source of Variance	DF	MS	F-Value
Breeds	3	1.44	10.30**
Error	196	0.14	
Total	199		

\*\* Highly significant ( $P<0.01$ )

**Table 6:** Mean Protein (%) in different breeds at  $\alpha$ s1-Casein (CSN1S1) gene locus.

Variants	Breeds			
	Malvi	Nimari	Sahiwal	HF crossbred
AA	3.36 <sup>b</sup> ±0.05 (50)	3.74 <sup>a</sup> ±0.07 (50)	3.59 <sup>a</sup> ±0.05 (50)	3.69 <sup>a</sup> ±0.04 (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	3.36 <sup>b</sup> ±0.05 (50)	3.74 <sup>a</sup> ±0.07 (50)	3.59 <sup>a</sup> ±0.05 (50)	3.69 <sup>a</sup> ±0.04 (50)

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

### Conclusion

Effect of genotypes was found significant ( $P<0.01$ ) for lactation length (LL) trait and significantly higher LL (Days) was recorded in AA genotype of HF crossbred while the lowest LL was noticed in AB genotype of Nimari. The mean LL between AA and AB genotype of Malvi, Sahiwal and HF crossbred cattle showed non-significant difference. The higher mean Fat (%) was noticed for AA genotype than AB genotype of Malvi and Nimari however in HF crossbred higher Fat (%) was observed for AB genotype. The mean Fat% between AA and AB genotypes showed non-significant difference in Malvi, Sahiwal and HF crossbred cattle but the mean protein per cent was significantly higher in Malvi and Nimari. Among the different genotypes of all four breeds of cattle, significantly higher protein (%) was recorded in Nimari for AB genotyped animals.

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The mean protein per cent was significantly lower in Malvi (3.36±0.05) as compared to Nimari (3.74±0.07); Sahiwal (3.59±0.05) and HF crossbred (3.69±0.04) cattle. However, the mean protein per cent among Nimari, Sahiwal and HF crossbred cattle did not differ significantly (Table 06). Similar to above findings McLean, *et al.* [4] and Szymanowska *et al.* [7] reported that the AA genotype showed higher protein (%) in Argentinian Holstein cattle and Polish Black and White cow. Contrary to above findings Ikonen *et al.* [3] reported the positive association of B allele with milk protein (%) in Brown Swiss cattle.

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