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## Characteristics of kappa-casein (*k*) genetic Variants and its association with lactation length (LL), milk fat and milk protein traits in Sahiwal and HF cross bred of cow in MP, India

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### Abstract

Present study revealed that polymorphic variants and their association with milk production traits at  $\kappa$ -casein gene (CNS3) locus in Sahiwal and HF Crossbred cow. The frequency of A allele was found to be higher 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 Sahiwal breed of cattle, indicating that the populations of these animals were in Hardy-Weinberg equilibrium at this locus, while the Chi-square value was found to be significant ( $p < 0.05$ ) for HF crossbred cattle revealing the Hardy Weinberg disequilibrium at this  $\kappa$ -casein (CSN3) gene locus. Association of polymorphic variants of  $\kappa$ -Casein (CSN3) gene with lactation length (LL), Fat (%) and protein (%) revealed that the mean LL of AA genotype of Sahiwal was significantly higher than AB genotype, while this difference was found to be non significant between AA and AB genotypes of HF crossbred cattle. With respect to mean value of Fat (%) There was a non significant difference between AA and AB genotypes of Sahiwal and HF crossbred cows, while the breed wise difference was found significant in HF crossbred with Sahiwal. Comparatively lower fat (%) was noticed in AA genotype of HF crossbred. The least square means for protein (%) in Sahiwal and HF crossbred cattle showed non-significant difference between AA and its own AB genotype.

**Keywords:** K casein gene, lactation length, fat, protein

### 1. Introduction

The milk yield and its composition are due to many factors such as breeds, genetic information, environments and other managerial factors (Stoop *et al.*, 2009). Breeding and managerial strategies to increase quality and quantity of dairy milk production traits. Many authors are identified significant association of gene variants of many genes with milk traits in cows and noticed that molecular markers based selection may improve genetic quality of herds (Miluchová *et al.*, 2014) [12]. The knowledge of genetic structure *viz.*, allelic and genotypic frequencies at particular locus can be incorporated under selection program for enhancing herd production and profitability. The Kappa casein is positioned at chromosome number BTA6 and it is most important protein among the four milk casein proteins. Its most of the protein-coding region located in exon consists of five exons spread over about 13.06 kilobases. Polymorphic studies of this gene has been intensely conducted on many cattle breeds (Ceriotti *et al.*, 2004; Dadhich *et al.*, 2006; Jōudu *et al.*, 2007; Alipanah *et al.*, 2008) [3, 16, 8, 10]. Polymorphic Variants of kappa casein, A and B are most common and important (Malik *et al.*, 2000; Bonvillani *et al.*, 2000) [15, 1]. Its allelic variants may be used as a candidate marker in increasing milk production and its composition (Özdemir and Doğru, 2005) [11]. Some studies reported that BB genotype was more associated with milk traits (Rachagani and Gupta, 2008) while other studies suggested AA genotype as most favourable for animal selection (Curi *et al.*, 2005; Alipanah *et al.*, 2008) [14, 10]. Additionally, due to the association with dairy products specially cheese making, allele B is preferable in cattle breeding programmes in several countries (Azevedo *et al.*, 2008) [2]. In order to reconcile these inconsistent reports and to obtain more reliable solution, an updated evidence-based approach is utmost necessary. In various data analysis design Meta-analysis was planned for summarizing genetic structure and association of gene polymorphisms of kappa casein gene with milk traits in cows populated over larger geographical area.

**2. Material and Methods**

**2.1 Collection of milk samples with economic traits:** about 100 ml milk sample was collected each from 50 cows of Sahiwal and 50 cows of HF Crossbred. The milk samples brought to the laboratory, maintaining cold chain and then Lactation Length (LL), Fat (%) In Milk Protein (%) were determined.

**2.2 Estimation of then Fat (%) and Milk Protein (%)**

The data of Lactation Length (LL) already recorded but Fat (%) and Milk Protein (%) 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 Sahiwal and HF Crossbred brought to the laboratory, maintaining cold chain then processed for DNA isolation.

**2.4 Genomic DNA isolation**

Genomic DNA was extracted from venous blood as per the method described by John *et al.* (1991) [7] with minor modifications.

**2.5 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 (Thompson *et al.*, 1994) [21] and analyzed by using MEGA 6 software (Tamura *et al.*, 2004) [20]. Aligned sequences were analyzed for group specific SNP marker.

**2.6 Statistical analysis**

**2.6.1 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) [24].

**2.6.2 Association of various polymorphic variants of milk protein genes on LL, Protein (%), Fat (%),**

Association study of various polymorphic variants of milk protein genes for LL, Protein (%), Fat (%) data were subjected to least squares analysis of variance employing following linear model:

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

- Where,
- Y<sub>ijkl</sub> - is the Observed value of milk yield
  - μ - is the population mean
  - P<sub>i</sub> - is the fixed effect of parity
  - B<sub>j</sub> - is the fixed effect of breed
  - G<sub>k</sub> - is fixed effect of genotypes (k = 1, 2,...)
  - (PXB)<sub>ij</sub> - is interaction effect of parity and Breed
  - (PXG)<sub>ik</sub> - is interaction effect of parity and genotypes
  - (BXG)<sub>jk</sub> - is interaction effect of Breed and genotypes
  - (PXBXG)<sub>ijk</sub> - is interaction effect of parity, breed and genotypes
  - e<sub>ijkl</sub> - is random error effect

**2.7 Testing Hardy-Weinberg (H-W) equilibrium**

The chi-square test (x<sup>2</sup>) was employed to test the status of Hardy-Weinberg equilibrium in the different population of four breeds of cattle (Snedecor and Cochran, 1994) [18].

**2.8 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, αs2, β and κ-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.

**3. Results and Discussion**

**3.1 Polymorphism at κ-casein gene (CNS3) locus using PCR-RFLP**

Allelic and genotypic frequencies were estimated using Popgene 32(version 1.32), Microsoft Windows-based freeware for population genetic analysis and the population was tested for genetic equilibrium at this locus using chi-square test. The estimated allelic and genotypic frequencies are presented in table 01. The tested populations of Sahiwal (50) and HF crossbred (50) cattle at the κ-casein gene (CNS3) locus were found to be polymorphic. Only AA and AB genotypes were found in all animals of Sahiwal and HF crossbred cattle. No BB genotype was recorded in any of the cattle breeds from blood samples in this study.

**3.2 Association of polymorphic variants of κ -Casein (CSN3) gene with Milk yield and milk composition traits**

The polymorphic variants of κ-Casein (CSN3) gene in different breeds of cattle and their association with Lactation length (days), Fat (%), Protein (%), have been studied and discussed below:

**3.2.1 Lactation length (LL)**

The results of least squares analysis of variance presented in table 01, revealed that the effects of only breed was found significant, however the effect of genotype, parity, breed x genotype, breed x parity and genotype x parity interactions were found to be non-significant for lactation length trait.

**Table 1:** Least squares analysis of variance for LL (days) at κ - Casein (CSN3) gene locus in Sahiwal and Crossbred cattle.

	MS	F-value
Breeds	106682	57.32**
Genotype (Variants)	4448	2.39 <sup>NS</sup>
Parity	3098	1.66 <sup>NS</sup>
Breed x Genotype	1878	1.01 <sup>NS</sup>
Breed x Parity	2923	1.57 <sup>NS</sup>
Genotype x Parity	2444	1.31 <sup>NS</sup>
Error	1861	-

\*\*Highly significant (p<0.01), NS- Non-significant

**3.1.2 Lactation length (days) of different variants at κ-Casein (CSN3) gene locus in Sahiwal and Crossbred cattle**

The least square mean of LL in Sahiwal and HF crossbred cattle have been presented in table 2. The mean LL of AA genotype of Sahiwal was significantly higher than AB genotype, while this difference was found to be non

significant between AA and AB genotypes of HF crossbred cattle. The LL was recorded maximum in AA genotype of HF crossbred (332±14.40 days) compared to both AA and AB genotypes of Sahiwal (Table 2). Ozdemir and Dogru (2005) [11], who reported the higher lactation length in Brown Swiss, Holstein and East Anatolian Red cows for AB genotype.

**Table 2:** Least squares means for LL (days) of κ-Casein gene (CSN3) variants in Sahiwal and Crossbred cattle.

Variants	Sahiwal	HF crossbred
AA	277.94 <sup>d</sup> ±5.88 (35)	332 <sup>a</sup> ±14.40 (27)
AB	244.30 <sup>c</sup> ±15.10 (15)	326.96 <sup>a</sup> ±9.81 (23)
BB	0.00±0.00 (0)	0.00±0.00 (0)
Overall	267.86 <sup>c</sup> ±9.15 (50)	329.70 <sup>a</sup> ±10.80 (50)

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

### 3.1.3 Fat (%) in milk

The results of least squares analysis of variance has been presented in table 3. As shown in following table, the effects of breed and breed x genotype interaction were significant; however the genotype, parity and breed x parity interactions were non-significant for fat (%) trait.

**Table 3:** Least squares analysis of variance for Fat (%) at κ-Casein (CSN3) gene locus in milk of Sahiwal and HF Crossbred cattle

	MS	F-value
Breeds	8.38	7.74**
Genotype (Variants)	0.02	0.01NS
Parity	0.27	0.25NS
Breed x Genotype	3.99	3.69**
Breed x Parity	0.69	0.63NS
Genotype x Parity	0.41	0.38NS
Error	1.08	-

\*\*Highly significant ( $p < 0.01$ ), NS- Non-significant

### 3.1.4 Fat (%) in milk of different variants at κ-Casein (CSN3) gene locus in Sahiwal and HF Crossbred cattle

The least square mean Fat (%) in Sahiwal and HF crossbred cattle has been presented in table 4. There was a non significant difference between AA and AB genotypes of Sahiwal and HF crossbred, while the breed wise difference was found significant in HF crossbred with Sahiwal. Among both the genotypes, the highest Fat (%) was noticed in AB genotype of Sahiwal (3.37±0.20) but lowest Fat (%) was noticed in AA genotype of HF crossbred (1.71±0.13). The present findings are supported by Botaro (2009) who noticed that the Cows of AB and BB genotypes showed a higher milk fat content compared to the AA genotype in Holstein and Girolando cows. Contrary to these findings, Ng-Kwai-Hang *et al.* (1986) [13] showed that the BB genotypes determines the higher milk fat yield in Holstein Friesian herds.

**Table 4:** Least squares means for Fat (%) of milk in different breeds for κ-Casein (CSN3) gene locus

Variants	Sahiwal	HF crossbred
AA	3.18 <sup>ab</sup> ±0.19 (35)	1.71 <sup>c</sup> ±0.13 (27)
AB	3.36 <sup>ab</sup> ±0.22 (15)	2.41 <sup>c</sup> ±0.29 (23)
BB	0.00±0.00 (0)	0.00±0.00 (0)
Overall	3.23 <sup>a</sup> ±0.20 (50)	2.03 <sup>b</sup> ±0.23 (50)

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

### 3.1.5 Protein (%)

The least squares analysis of variance was conducted to study

the effect of κ-Casein (CSN3) gene polymorphism on protein (%) in the Sahiwal and HF Crossbred cattle along with the breed, genotype, parity and their interactions. The results of least squares analysis of variance presented in table 5 revealed that the effects of breed and breed x genotype interaction were significant, while the effects of genotype, parity, genotype x parity and breed x parity interaction were non-significant for protein (%) trait.

**Table 5:** Least squares analysis of variance for Protein (%) at κ-Casein (CSN3) gene locus in Sahiwal and HF Crossbred cattle

	MS	F-value
Breeds	0.64	4.80**
Genotype (Variants)	0.43	3.20 <sup>NS</sup>
Parity	0.19	1.40 <sup>NS</sup>
Breed x Genotype	0.47	3.51*
Breed x Parity	0.06	0.48 <sup>NS</sup>
Genotype x Parity	0.15	1.11 <sup>NS</sup>
Error	0.13	-

\*\*Highly significant ( $p < 0.01$ ), \*significant ( $p < 0.05$ ), NS- Non-significant

### 3.1.6 Protein (%) in milk of different variants at κ-Casein (CSN3) gene locus in Sahiwal and HF Crossbred cattle

The least square means for protein (%) in Sahiwal and HF crossbred cattle have been presented in table 6 and both breed showed non-significant difference between AA and its own AB genotype. The mean protein percent in the milk of AA genotype (3.70<sup>b</sup>±0.05) of HF Crossbred cattle was non-significantly higher among the AA and AB genotypes of Sahiwal and its own AB genotype. Present finding was in agreement with the finding of Heck *et al.* (2009) [5] in Holstein cows. As per Kucerova *et al.* (2006) [9] and Tsiaras *et al.* (2005) [22] who suggested the positive effect of B allele on protein and fat contents in milk.

**Table 6:** Least squares mean for Protein (%) in following breeds at κ-Casein (CSN3) gene locus.

Variants	Sahiwal	HF crossbred
AA	3.59 <sup>b</sup> ±0.06 (35)	3.70 <sup>b</sup> ±0.05 (27)
AB	3.57 <sup>bc</sup> ±0.09 (15)	3.67 <sup>b</sup> ±0.07 (23)
BB	0.00±0.00 (0)	0.00±0.00 (0)
Overall	3.59 <sup>a</sup> ±0.07 (50)	3.69 <sup>a</sup> ±0.05 (50)

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

## 4. Conclusion

It is concluded that The frequency of A allele was found to be higher as compared to B allele and genotypic frequency of AA genotype was also higher than AB and BB genotypes 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 Sahiwal breeds of cattle, indicating that the populations of these animals were in Hardy-Weinberg equilibrium at this locus, while the Chi-square value was found to be significant ( $p < 0.05$ ) for HF crossbred cattle revealing the Hardy Weinberg disequilibrium at this κ-casein (CSN3) gene locus for this population under study. The mean LL of AA genotype of Sahiwal was significantly higher than AB genotype, while this difference was found to be non significant between AA and AB genotypes of HF crossbred cattle. There was a non significant difference between AA and AB genotypes of Sahiwal and HF crossbred, while the breed wise difference was found



significant in HF crossbred with Sahiwal. Among both the genotypes, the higher Fat (%) was noticed in AB genotype of Sahiwal but lower Fat (%) was noticed in AA genotype of HF crossbred. both breed showed non-significant difference between AA and its own AB genotype. The mean protein percent in the milk of AA genotype of HF Crossbred cattle was non-significantly higher among the AA and AB genotypes of Sahiwal and its own AB genotype.

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