International Journal of Veterinary Sciences and Animal Husbandry

ISSN: 2456-2912
VET 2024; 9(1): 1081-1083

www.veterinarypaper.com
Received: 12-10-2023
Accepted: 18-11-2023

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Relationship between Kappa (K) casein genetic varients and its association with lactation length (LL) of Malvi and Nimari breed of cow of M.P. India

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Abstract
Study revealed with polymorphic variants and their association with lactation length (LL) trait at κ-casein gene (CNS3) locus in Malvi and Nimari, cattle. The frequency of A allele was found to be highest 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 both Malvi and Nimari breeds of cattle, indicating that the populations of these animals were in Hardy-Weinberg equilibrium at this locus. Association of polymorphic variants of κ -Casein (CSN3) gene with lactation length (LL) revealed that the mean LL difference was found to be non-significant between AA and AB genotypes of Malvi, Nimari breed of cow.

Keywords: Malvi, nimari, lactation length, κ -casein

1. Introduction
The main objectives of dairy farming are to select animals with desired genotypes to improve milk yield and composition traits (Boro *et al*, 2016; Desyibelew and Wondifraw, 2019) [4, 7].

The compositional quality of milk depends not only on environmental factors, but also on genetic traits such as the Kappa Casein gene (CSN3), which has been widely studied in order to establish relationships between its polymorphisms with the percentage of total milk protein and industrial yield NaranjoJ *et al.* (2007) [1]. In bovine milk, κ-CN exhist in several forms with varied physicochemical properties due to the presence of missense mutations (genetic polymorphism), intermolecular disulphide linkages, glycosylation, and phosphorylation (Huppertz, 2013) [10]. K casein gene of Bovine showing codominance and situated on autosomal chromosome and also inherited according to Mendelian inheritance. The κ Casein (κ-CN) protein comprises about 12 to15 percent of the total casein fraction in bovine milk (Contreras *et al*, 2011) [6]. So as per various study 13 variants of the mature κ CN gene: A, B, B2 C, D, E, F1, F2, G1, G2, H, I and J are reported. Above varients are located on the fourth exon that is 517bp in length plus A1 which is synonymous (Farrell *et al*, 2004; Gallinat *et al*, 2013; Martin *et al*, 2013) [8, 9, 12]. Presently several techniques have been applied for genotyping of polymorphisms in major milk proteins genes and the iPlexmass ARRAY genotyping technique is one of the techniques that relies on polymorphism identified at the DNA level regardless of age, sex, and physiological status of cattle (Teneva and Petrović, 2010) [16]. In dairy cattle, the κ-CN variants A and B are the most common but E variant has been reported in rare frequency (Farrell Jr *et al*, 2004; Caroli *et al*, 2009; Martin *et al*, 2013; Awad *et al*, 2016) [8, 5, 12, 3]. In addition, at the κ-CN locus, the variants A, B and E are situated around the C-terminal part (the caseinomacropeptide; CMP); here, two polar residues Thr at position 136 and Asp at position 148 in variants A are substituted in Variant B by hydrophobic Ile and Ala respectively; while at position 155 of the E variant, hydrophobic Gly substitute polar Ser in the A and B variants.

2. Materials and Methods
2.1 Lactation length (LL): Lactation length data was collected from 50 Malvi and 50 Nimari breed of cows.

2.2 Genomic DNA isolation
Genomic DNA was extracted from venous blood as per the method described by John *et al*. (1991) [11] with minor modifications.

2.3 Agarose gel electrophoresis
Quality of DNA was assessed through 0.80% horizontal submarine agarose gel electrophoresis.

2.4 Concentration, purity and quality check of DNA
The concentration, purity and quality of DNA were checked by Nanodrop spectrophotometer and agarose gel electrophoresis.

2.5 Spectrophotometry
The concentration, purity of DNA was checked by Nanodrop Spectrophotometer. The Optical density (OD) value at 260 nm and 280 nm was measured using Nanodrop Spectrophotometer (Nanodrop 1000, Thermo Scientifi c). DNA samples with an OD 260/280 ratio of 1.70 to 1.90 were considered further subjected to agarose gel electrophoresis for quality check.

2.6 Casein gene primer sequence:
The K-casein gene primers (F): 5' -GCTGAGCAG GTATCCTAGTTAT- 3'
(R): 5' - CTTCTTTGATGTCTCCTTAGAG - 3' T- 3'443 bp Schlieben *et al*. (1991) [19] was used for the amplification of PCR product.

2.7 Polymerase Chain Reaction (PCR)
The PCR tubes were kept in a preprogrammed thermo cycler (Mastercycler gradient, Eppendorf) and set at the standardized reaction programme. Initial denaturation (5 minutes) and final denaturation (1 minute) temp. Was 940C 600C anniling temp. (1 min.) Was 600C where extension (1 minute) and fi nal extension(5 minutes) temp. was 720C.

2.8 PCR- RFLP Assay
2.8.1 Restriction digestion
All the PCR products of K casein gene were digested by *Hind III* restriction enzymes. The reaction mixture was spanned for few seconds for uniform mixing and then incubated at 37°C for 3 hrs in the water bath.

2.8.2 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). The mass ruler DNA ladder (100 bp- 1000 bp) as a molecular size marker was used for sizing of the DNA bands.

2.9 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) [17] and analyzed by using MEGA 6 software (Tamura *et al*., 2004) [15]. Aligned sequences were analyzed for group specific SNP marker.

2.10 Statistical analysis
2.10.1 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) [18].

2.10.2 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 lactation length
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

2.10.3 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 above two breeds of cattle (Snedecor and Cochran, 1994) [14].

3. Results
3.1 Association study 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 Pandey *et al.* (2021) [2]
The frequency of A allele was found to be highest 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 Malvi and Nimari breeds of cattle, indicating that the populations of these animals were in Hardy-Weinberg equilibrium at this locus.

Association of polymorphic variants of κ -Casein (CSN3) gene
Lactation length (LL), Lactation length (days) of different variants at κ-Casein (CSN3) gene locus in four breeds of cattle
The least square mean of LL in Malvi and Nimari breed of cattle have been presented in table 01. The mean LL of AA and AB genotype of Malvi was significantly higher than both AA and AB genotype of Nimari, while this difference was found to be non significant between AA and AB genotypes of Malvi, breed of cattle. The LL was recorded maximum in AB genotype of Malvi (301.50^{b}±10.80 days) compared to Nimari (196.53±4.34 days) for AB genotype (Table 01).

Table 1: Least squares means for LL (days) of κ-casein gene (CSN3) variants in Malvi and Nimari breeds of cattle

	LL (days)	LL (days)
Variants	Malvi	Nimari
AA	300.92^{b}±5.97 (36)	205.45^{a}±5.06 (33)
AB	301.50^{b}±10.80 (14)	196.53^{a}±4.34 (17)
BB	0.00±0.00 (0)	0.00±0.00 (0)
Overall	301.08^{b}±8.02 (50)	202.42^{a}±4.66 (50)

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

4. Discussion

The least square mean of LL in Malvi and Nimari breed of cattle have been presented in table 1. The mean LL of AA and AB genotype of Malvi was significantly higher than both AA and AB genotype of Nimari, while this difference was found to be non significant between AA and AB genotypes of Malvi, breed of cattle. The LL was recorded maximum in AB genotype of Malvi (301.50^{b}±10.80 days) and minimum in Nimari (196.53±4.34 days) for AB genotype (Table 01). As per Deb *et al* (2014) [13] Genotypic varients of k casein gene revealed that AB had significant ($P<0.05$) effect with 300 days and SNF% as compared to AA.

5. Conclusion

The frequency of A allele was found to be highest 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 Malvi and Nimari breeds of cattle, indicating that the populations of these animals were in Hardy-Weinberg equilibrium at this locus. The mean LL of AA and AB genotype of Malvi was significantly higher than both AA and AB genotype of Nimari, while this difference was found to be non-significant between AA and AB genotypes of Malvi, breed of cattle. There was a non-significant difference between AA and AB genotypes of Malvi and Nimari breed cattle, while the breed wise difference was found significant in both Malvi and Nimari.

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