I J R B A T, Issue (XII) Vol (I) Jan 2024: 33-36
A Double-Blind Peer Reviewed & Refereed Journal
OPEN ACCESS
e-ISSN 2347 – 517X
Original Article
INTERNATIONAL JOURNAL OF RESEARCHES IN BIOSCIENCES, AGRICULTURE AND TECHNOLOGY
 www.ijrbat.in

MOLICULAR CHRACTRISATION OF KAPPA (κ) CASEIN GENE AND ITS ASSOCIATION WITH MILK DENSITY IN MALVI AND NIMARI BREED OF COW OF M P, INDIA.

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Communicated :09.10.2023 | Revision: 26.10.2023 & 13.11.2023 Accepted: 22.11.2023 | Published: 30.01.2024

ABSTRACT:
Present study revealed that polymorphic variants and their association with milk production traits at κ-casein gene (CNS3) locus in Malvi and Nimari, cattle. Association of polymorphic variants of κ -Casein (CSN3) gene revealed that the Only AA and AB genotypes were found in all animals of Malvi and Nimari breed of cattle. No BB genotype was recorded in both cattle breeds from blood samples in this study. The frequency of A allele was found to be highest as compared to B allele in above both breeds of cattle under the study. The milk density between AA and AB genotypes of Malvi and Nimari breeds of cow showed non-significant difference. The higher mean milk density (Kg/L) was noticed in AA and AB genotypes of Nimari breed of cow.

Keywords: - K casein gene, Milk Density, Malvi, Nimari. .

INTRODUCTION :

Two κ-CN variants, A and B were confirmed using alkaline gel electrophoresis Mackinlay et al.(1966) and Swaisgood et al. (1977) the A variant had a greater mobility to the B variant as zero carbohydrate chain was associated to A, Whitney et al. (1976)and their primary structures were established by Jollès et al. (1972). In most European breeds, the A variant of κ-CN is more frequent than the B variant, Lien et al. (1999)Jakob et al.(1991) Eenennaam et al. (1991) while E is the least frequent Lien et al. (1999). The B variant is found to be associated with high milk quality in European cattle breeds Martin et al. (2002)and, in comparison to the A variant, B is found to be associated with shorter rennet coagulation time Lundén et al. (1997)while cheese formed using milk with BB variant has higher yield, higher protein content and better quality compared to AB variant Martin et al. (2002). High casein yield is positively associated with cheese yield, and a high content of κ-CN is favourable for its positive effect on milk coagulation Wedholm et al. (2006). The mean milk density (kg/L) was significantly higher in the HF Crossbred milk breed compared to Sahiwal milk., Pandey et al .(2023)

MATERIAL AND METHODS :

Collection of milk samples with economic traits: After the collection of milk samples was brought to the laboratory, maintaining cold chain and then Density(Kg/L in the milk were determined.

Estimation of Lactose, SNF and milk density: The data of then Density(Kg/L were analyzed by Milk analyzer.

Blood Collection

5 ml blood sample was collected in EDTA coated vaccutainer aseptically from 50 animals of each of Malvi and Nimari breeds and maintaining cold chain then processed for DNA isolation.

Genomic DNA isolation:

As per the method described by John et al. (1991) with minor modifications Genomic DNA was extracted from blood.

http://doi.org/10.29369/ijrbat.2024.010.1.0006

Agarose gel electrophoresis:

0.80% horizontal submarine agarose gel electrophoresis performed for Quality check of DNA.

Concentration, purity and quality check of DNA :

Taken the help of Nanodrop, spectrophotometer and agarose gel electrophoresis .

Casein gene primer sequence:

The κ -casein gene primers (F): 5'-GCTGAGCAG GTATCCTAGTTAT- 3' (R): 5' - CTTCTTTGATGTCTCCTTAGAG - 3', 443 bp Schlieben et al. (1991) was used for the amplification of PCR product.

Polymerase Chain Reaction (PCR)

Setting of PCR Reaction:

The PCR tubes were kept in a pre-programmed thermo cycler where the Initial denaturation (5 minutes) and final denaturation (1 minute) temp. was 940C and 600C anniling temp.(1 min.) Was 600C where extension(1 minute) and final extension(5 minutes) temp. was 720C

Agarose gel electrophoresis of PCR reaction product :

To confirm the targeted PCR amplification the PCR products were analyzed on 2.00 % agarose gel. The mass ruler DNA ladder (100 bp - 1000 bp) as a molecular size marker was used for sizing of the DNA bands.

PCR- RFLP Assay:

All the PCR products of k casein gene were digested by HindIII restriction enzymes in water bath at 37°C for 3 hrs.

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).

Sequencing:

The sequences obtained from genotype were aligned using Clustal W. (Thompson et al., 1994) and analyzed by using MEGA 6 software (Tamura et al., 2004). Aligned sequences were analyzed for group specific SNP marker.

Association of various polymorphic variants of milk protein genes with Density(Kg/L:

Association study of various polymorphic variants of milk protein genes for Density(Kg/L data were subjected to least squares analysis of variance employing following linear model:

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

Testing Hardy-Weinberg (H-W) equilibrium:

Hardy-Weinberg equilibrium tested by the chi-square test (x2) in above mentioned both breeds of cattle (Snedecor and Cochran, 1994).

Association study conducted between the polymorphic variants/ genotypes of, k -casein genes with milk Density(Kg/L of Malvi and Nimari breeds of cattle by linear regression model .

RESULTS :

Density(Kg/L: The results of least squares analysis of variance presented in table 01, showed that the effect of breed genotype, parity, breed x genotype and breed x parity interactions were non-significant for density trait.

Density (kg/L)in milk of different variants at κ-Casein (CSN3) gene locus in Malvi and Nimari breeds of cattle.

The least square means for density in Malvi and Nimari breeds cattle have been presented in table 02. The milk density between AA and AB genotypes of Malvi and Nimari breeds of cow showed non-significant difference. The higher mean milk density (Kg/L) was noticed for AA and AB genotypes of Nimari .

DISCUSSION:

The tested populations of Malvi (50), Nimari (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 Malvi and Nimari breed of cattle. No BB genotype was recorded in both cattle breeds from blood samples in this study. The frequency of A allele

http://doi.org/10.29369/ijrbat.2024.010.1.0006

was found to be highest as compared to B allele in above both breeds of cattle under the study. The milk density between AA and AB genotypes of Malvi and Nimari breeds of cow showed non-significant difference. The higher mean milk density (Kg/L) was noticed for AA and AB genotypes of Nimar. As per Pandey et al.(2023) The frequency of A allele was found to be highest as compared to B allele in above both breeds of cattle and they also noticed the mean milk density (kg/L) was significantly higher in Nimari breed compared to Malvi.

CONCLUSION:

It is concluded that the frequency of A allele was found to be highest as compared to B allele in above both breeds of cattle under the study. The milk density between AA and AB genotypes of Malvi and Nimari breeds of cow showed non-significant difference. Comparatively higher mean milk density (Kg/L) was noticed for AA and AB genotypes of Nimari breed of cow of Madhya Pradesh, India.

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Table 01: Least squares analysis of variance for density (Kg/L) at κ -Casein (CSN3) gene locus in Malvi and Nimari breeds of cattle

	DF	MS	F-value
Breeds	3	120.33	11.68**
Genotype (Variants)	1	11.20	1.09^{NS}
Parity	4	10.18	0.99^{NS}
Breed x Genotype	3	10.17	0.99^{NS}
Breed x Parity	12	6.71	0.65^{NS}
Genotype x Parity	4	14.36	1.39^{NS}
Error	172	10.31	-

**Highly significant (p<0.01)

Table 02: Least squares means for SNF (%) in different breeds at κ-Casein (CSN3) gene locus.

Variants	Malvi	Nimari
AA	1. 03^{a}±0.07 (36)	1.04^{a}±0.07 (33)
AB	1.03^{a}±0.09 (14)	1.04^{a}±0.08 (17)
BB	0.00±0.00 (0)	0.00±0.00 (0)
Overall	1.03^{c}±0.08 (50)	1.04^{a}±0.07 (50)

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

http://doi.org/10.29369/ijrbat.2024.010.1.0006