International Journal of Veterinary Sciences and Animal Husbandry

ISSN: 2456-2912
VET 2024; 9(1): 1077-1080

www.veterinarypaper.com
Received: 08-10-2023
Accepted: 13-11-2023

Akhilesh Pandey
Department of Animal Genetics and Breeding, College of Veterinary Science and Animal Husbandry, Mhow, Indore, Madhya Pradesh, India

DS Gonge
Department of Animal Genetics and Breeding, College of Veterinary Science and Animal Husbandry, Mhow, Indore, Madhya Pradesh, India

Yogita Pandey
Department of Veterinary Anatomy, College of Veterinary Science, Jabalpur, Madhya Pradesh, India

Corresponding Author:
Akhilesh Pandey
Department of Animal Genetics and Breeding, College of Veterinary Science and Animal Husbandry, Mhow, Indore, Madhya Pradesh, India

Allelic variants of the Kappa (K) casein gene and its association with milk fat (%) traits in Malvi and Nimari breed of cow

Akhilesh Pandey, DS Gonge and Yogita Pandey

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. 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 Fat (%) revealed that the mean value of Fat (%) in Malvi, Nimari cattle has been showed non-significant difference between AA and AB genotypes. Among the above genotypes, the significantly higher Fat (%) was noticed in AA genotype of Nimari compared to Malvi.

Keywords: Malvi, Nimari, κ -Casein, Fat %

1. Introduction
Apart from the Jersey breed the variant κ-CN A is the most common in dairy cattle and their crosses (Awad *et al*., 2016; Neamt *et al*., 2017; Houaga *et al*., 2020) [1, 14, 8]. Deb *et al*., (2014) [5] found that in Frieswal cattle (Friesian x Sahiwal) at the κCN locus, the A allele (0.58) was higher than B allele (0.42). Some researchers have confirmed positive association while others found non-association therefore, more studies are required for specific conditions (Neamt *et al*., 2017) [14]. For instance, studies have demonstrated that at the κ-casein gene locus, the A allele in both the homozygous (AA) and heterozygous (AB) genotypes is related with higher milk yield while the B allele, on the other hand, is associated with higher contents of fat and/or protein in milk which makes it a better material for cheese and yoghurt production (Neamt *et al*., 2017) [14]. Previous studies have shown that k-casein offers the best technological properties of milk and is considered as one of key markers in cattle selection and breeding. The Bunaji breed is the main indigenous cattle used for dairy production in Nigeria and makes up 37 percent of the national cattle population. Recent review has stated that the limitation of the Bunaji breed includes low milk production, long calving interval, delayed conception, late sexual maturity, and short lactation period one of the strategies that has been used to improve their productivity is via crossbreeding schemes with the exotic breed (mostly Friesian sires). In view that Friesian X Bunaji cattle is one of the main dairy cattle breeds in Nigeria and the role of kappa casein gene in milk related traits, this study was designed to identify alleles and genotype frequencies at the k-CN gene, determine the effects the genotypes on milk yield and composition traits in Friesian X Bunaji cows. In bovine milk, κ-CN exit in several forms with varied physicochemical properties due to the presence of missense mutations (genetic polymorphism), intermolecular disulphide linkages, glycosylation, and phosphorylation (Huppertz, 2013) [9]. 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) [3]. 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) [6, 7, 13]. 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) [21]. 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) [6, 2, 13, 1]. 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.

Material and Methods

Collection of milk samples with economic traits

About 100 ml milk samples were collected each from 50 cows of Malvi and 50 cows of Nimari. The milk samples brought to the laboratory, maintaining cold chain and then Fat (%) were determined.

Estimation of Fat (%)

The data of Fat (%) were analyzed and then collected from Milk analyzer.

Blood collection

5 ml blood sample was collected in EDTA coated vaccutainer aseptically from 50 animals of each of the four breeds *viz*. Malvi and Nimari brought to the laboratory, maintaining cold chain then processed for DNA isolation.

Genomic DNA isolation

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

Agarose gel electrophoresis

Quality of DNA was assessed through 0.80% horizontal submarine agarose gel electrophoresis Lee *et al.* (2012) [12].

Concentration, purity and quality check of DNA

The concentration, purity and quality of DNA were checked by Nanodrop spectrophotometer and agarose gel electrophoresis (Desjardins and Conklin, 2010) [4].

Spectrophotometry

The DNA concentration was determined and samples were diluted up to approximate 30 ng/µl for final concentration with sterile nuclease free water (MiliQ) for further use (Desjardins and Conklin, 2010) [4].

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) [18] was used for the amplification of PCR product.

Polymerase Chain Reaction (PCR) (Barnes, 1994) [24]

Setting of PCR Reaction

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 fi nal denaturation (1 minute) temperature was 94 ºC 60 ºC anniling temperature (1 min) was 60 ºC where extension (1 minute) and final extension (5 minutes) temperature was 72 ºC.

Agarose gel electrophoresis of PCR reaction product

To confirm the targeted PCR amplification the PCR products were analyzed on 2% 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

Restriction digestion: All the PCR products of k casein gene were digested by *HindIII* restriction enzymes Nwankwo *et al.* (1994) [15]. The reaction mixture was spanned for few seconds for uniform mixing and then incubated at 37°C for 3 hrs in the water bath.

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 (Matthew *et al*, 2004) [25].

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) [22] and analyzed by using MEGA 6 software Tamura *et al*. (2004) [20]. Aligned sequences were analyzed for group specifi c 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 (Yeh *et al*, 1999) [23].

Association study of various polymorphic variants of milk protein genes with Fat %

Association study of various polymorphic variants of K casein genes for Fat% data were subjected to least squares analysis of variance employing following linear model Pande*y et a.l* (2021) [21].

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

Where,
Yijkl - is the Observed value of Fat %
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

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 four breeds of cattle (Snedecor and Cochran, 1994) [19].

To find out the association between the polymorphic variants/ genotypes of, k -casein genes with milk production traits like Fat% in Malvi and Nimari breed cattle by linear regression model was employed.

Results

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 least square mean Fat (%) in Malvi, Nimari breed cattle has been presented in table 01. 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. Among all the genotypes, the highest fat (%) was noticed in AA genotype of Nimari (3.37±0.20) but lowest fat (%) was noticed in AB genotype of Nimari (2.39±0.13).

Discussion

The mean of Fat (%) in Malvi, Nimari breed cattle has been presented in table 01. 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. Among all the genotypes, the highest Fat (%) was noticed in AA genotype of Nimari but lowest Fat (%) was noticed in AB genotype of Nimari. Ikonen *et al.* (2001) [10] reported in Brown Swiss cattle that Fat (%) in the milk increases by presence of B allele in the population. Similar to above result Deb *et al* (2014) [5] reported the association of genotypes with certain milk production traits revealed that AB had significant ($p<0.05$) effect on total milk yield, peak yield, yield at 300 days. Pandey *et al. (*2018) [16] noticed that Malvi breed showed that the significant and positive correlation of daily milk yield (DMY) and lactation length with milk yield but Fat% showed negative correlation with milk yield in both Malvi and Nimari breed of cow.

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. Among all the genotypes significantly highest Fat (%) was noticed in AA genotype of Nimari whereas lowest Fat (%) was noticed in AB genotype of Nimari.

References

1. Awad A, El Araby IE, El-Bayomi KN, Zaglool AW. Association of polymorphisms in kappa casein gene with milk traits in Holstein Friesian cattle. Japan Journal of Veterinary Research. 2016;64:39-43.
2. Caroli AM, Chessa S, Erhardt GJ. Invited review: milk protein polymorphisms in cattle: effect on animal breeding and human nutrition. Journal of Dairy Science. 2009;92(11):5335-5352.
3. Contreras PVI, Lourenco Jaramillo DL, Parra Bracamonte GM, Martínez González JC, Sifuentes Rincón AM. Convenient genotyping of nine bovine K-casein variants. Electronic Journal of Biotechnology. 2011;14(4):1-6.
4. Desjardins P, Conklin D. NanoDrop microvolume quantitation of nucleic acids. Journal of Visualized Experiments. 2010;(45):1-6.
5. Deb R, Singh U, Kumar S, Singh R, Sengar G, Sharma A. Genetic polymorphism and association with milk production traits among Frieswal (HF x Sahiwal) crossbreed of Indian origin. Iran Journal of Veterinary Research. 2014;15(4):406-408.
6. Farrell Jr HM, Jimenez-Flores R, Bleck GT, Brown EM, Butler JE, Creamer LK. Nomenclature of the proteins of cows' milk-sixth revision. Journal of Dairy Science; c2004.
7. Gallinat JL, Qanbari S, Drögemüller C, Pimentel ECG, Thaller G, Tetens J. DNA-based identification of novel bovine casein gene variants. Journal of Dairy Science. 2013;96:699-709.
8. Houaga I, Asalifou CF, Missihoun AAM, Sedah P, Agbangla C, Muigai AWT, *et al*. Genetic diversity and relationships among indigenous Borgou and White Fulani cattle breeds based on milk protein loci: Implications for breed improvement and conservation in Benin. Livestock Research for Rural Development. 2020;32(1):1-12.
9. Huppertz T. Chemistry of the caseins. In: McSweeney PLH, Fox PF, editors. Advanced Dairy Chemistry: Volume 1A: Proteins: Basic Aspects; c2013. p. 135-160.
10. Ikonen T, Bovenhuis H, Ojala M, Ruottinen O, Georges M. Associations between casein haplotypes and first lactation milk production traits in Finnish Ayrshire cows. Journal of Dairy Science. 2001;84:507-514.
11. John SW, Weitzner G, Rozen R, Scriver CR. A rapid procedure for extracting genomic DNA from leukocytes. Nucleic Acid Research. 1991;19(2):408.
12. Lee PY, Costumbrado J, Hsu CY, Kim YH, Vis J. Agarose gel electrophoresis for the separation of DNA fragments. 2012;20(2):3923.
13. Martin P, Bianchi L, Cebo C, Miranda G. Genetic polymorphism of milk proteins. In: McSweeney PLH, Fox PF, editors. Advanced Chemistry; c2013. p. 463-514.
14. Neamt RI, Saplacan G, Acatincai S, Cziszter LTC, Gavojdian D, Ilie DE. The influence of CSN3 and LGB polymorphism on milk production and chemical composition in Romanian Simmental cattle. Acta Biochimica Polonica. 2017;64(3):493-497.
15. Nwankwo DO, Moran LS, Slatko BE, Waite Rees PA, Dorner LF, Benner JS, *et al*. Gene. 1994;150:75-80.
16. Pandey A, Thakur MS. Correlations between the milk production traits in different variants of k-casein gene in Malvi, Nimari, Sahiwal and HF crossbred cattle. Journal of Entomology and Zoology Studies. 2018;6(2):1390-1393.
17. Pandey A, Thakur MS, Pandey Y. Association Study of Polymorphic Variants of Beta (B) Casein Gene with Milk Production Traits (Lactose, SNF and Density); c2021.
18. Schlieben S, Erhardt G, Senft B. Genotyping of bovine kappa-casein following DNA sequence amplification and direct sequencing of kappa-CNE PCR product. Animal Genetics. 1991;22:333-342.
19. Snedecor GW, Cochran WG. Statistical Methods. 8th ed. The Iowa State College Press, Inc.; c1994.
20. Tamura K, Nei M, Kumar S. Prospects for inferring very large phylogenies by using the neighbor-joining method. Proceedings of the National Academy of Sciences of the United States of America. 2004;101:11030-11035.

21. Teneva A, Petrovic MP. Application of molecular markers in livestock improvement. Biotechnology of Animal Husbandry. 2010;6(34):135-155.
22. Thompson JD, Higgins DG, Gibson TJ. Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties, and weight matrix choice. Nucleic Acids Research. 1994;22:4673-4680.
23. Yeh FC, Yang RC, Boyle TBJ, Ye ZH, Mao JX. Popgene 32 version 1.32. Molecular Biology and Biotechnology Centre, University of Alberta, Canada; c1999.
24. Barnes WM. PCR amplification of up to 35-kb DNA with high fidelity and high yield from lambda bacteriophage templates. Proceedings of the National Academy of Sciences. 1994 Mar 15;91(6):2216-20.
25. Matthew HC, Harrison B, Long RJ. The Oxford dictionary of national biography. R. James Long (2004).; c2004.