ISSN: 2456-2912
VET 2024; 9(1): 1294-1296
© 2024 VET
www.veterinarypaper.com
Received: 08-11-2023
Accepted: 20-12-2023

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

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

Y Pandey
College of Veterinary Science and Animal Husbandry, Mhow, Indore, 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

DNA-based identification of novel bovine Kappa (K) casein genetic variants and its association with milk protein of Malvi and Nimari breed cow of Madhya Pradesh India

Akhilesh Pandey, DS Gonge and Y 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 rotein (%) revealed that the mean protein per cent in the milk of AB genotype (4.02±0.15) of Nimari was significantly higher among the both AA and AB genotypes of Malvi.

Keywords: Malvi, Nimari, genotype, κ -casein

1. Introduction
Present study was conducted in Department of Animal Genetics and Breeding of NDVSU Jabalpur, MP India. The *CSN3* gene is highly polymorphic and still 35 variants are known that yield 24 protein variants and 11 synonymous mutations which are detectable only at the DNA level. In this review we follow the new nomenclatures of the k-casein variants recently described by Gautam *et al.* (2019) [4]. The DNA (NC_030813, goat genome reference version LWLT01 the *CSN3* gene is located on chromosome number 6 between 86.197 and 86.211 Mb Bickhart *et al.* (2017)) [3] and protein reference sequences of *CSN3* (NP_001272516, NCBI database) represent the protein variant *CSN3**B as the reference Yahyaoui *et al.* (2001) [14] Jann *et al.* (2004) [6]. The gene spans 14,114 bp and comprises five exons Martin *et al*. (2002) [8]. The protein contains 192 amino acids which include 21 amino acids of the signal peptide and 171 amino acids of the mature protein. The mature protein is encoded in only two exons, exons 3 (9 amino acids) and exon 4 (162 amino acids) Yahyaoui *et al.* (2003) [15]. The κ-CN A and B allele can be present as homozygous (AA or BB) and heterozygous (AB) where the homozygous κ-CN BB genotype is less frequent in native dairy cattle breed than crossbreed dairy cattle. As an example, Sodhi *et al.* (2010) [11] investigated allelic variants of κ-CN across 744 animals representing 17 Indian native cattle breeds (Tharparkar, Rathi, Mewati, Nagori, Kankrej, Gir, Sahiwal, Hariana, Deoni, Red Kandhari, Dangi, Gaolao, Khillar, Ongole, Umblachery, Amritmahal and Kangyam breeds) and found the frequency of κ-CN A to be 90.8%. The same trend was observed for native cattle breeds in Brazil. The most important economic effects of the milk casein proteins come from the relationship between protein quality, amount, coagulation, and techno-functional properties to produce yoghurts and cheese. Therefore, casein polymorphisms play a significant role both in milk coagulation performance and cheese quality. Since the identification of the impact of milk protein polymorphisms on milk quality and its technological properties Aschaffenburg and Drewry, (1955) [1].

2. Material and Methods

2.1 Milk Protein: Data of milk protein was collected was collected from the milk analyser machine of the milk of the 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) [7] 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

DNA samples with an Optical density (OD0 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) [9] 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 60 °C anniling temp. (1 min.) Was 60 °C where extension (1 minute) and final extension (5 minutes) temp. was 72 °C.

2.10 PCR- RFLP Assay

2.10.1 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.

2.10.2 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.

2.11 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) [13] and analyzed by using MEGA 6 software (Tamura *et al.*, 2004) [12]. Aligned sequences were analyzed for group specific SNP marker.

2.12 Statistical analysis

2.12.1 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) [16].

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

Association study of various polymorphic variants of milk protein genes for milk protein data were subjected to least squares analysis of variance employing following linear model Pandey *et al* (2021) [2].

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

Where,
Yijkl - is the Observed value of milk protein
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

3. 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.

Association of polymorphic variants of κ -Casein (CSN3) gene

Protein (%) in milk of different variants at κ-Casein (CSN3) in Malvi and Nimari breeds of cattle

The least square means for protein (%) in Malvi and Nimari breed cattle have been presented in table 01 and both breed showed non-significant difference between AA and its own AB genotype. The mean protein per cent in the milk of AB genotype (4.02±0.15) of Nimari was significantly higher among the AA and AB genotypes of Malvi cattle whereas AA genotype of Malvi and Nimari cow showed non-significant difference but AB genotype of Nimari cow showed higher protein than its own AA genotype. So the lowest protein (%) was observed in AB genotype of Malvi (3.29±0.07).

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

Variants	Protein %	Protein %
	Malvi	Nimari
AA	3.38^{A}±0.06 (36)	3.59^{Aa} ±0.05 (33)
AB	3.29^{A}±0.07 (14)	4.02^{Ba}±0.15 (17)
BB	0.00±0.00 (0)	0.00±0.00 (0)
Overall	3.36^{b}±0.05 (50)	3.74^{a}±0.06 (50)

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

4. Discussion

The least square means for protein (%) in Malvi and Nimari breed cattle have been presented in table 01 and both breed showed non-significant difference between AA and its own AB genotype. The mean protein per cent in the milk of AB genotype (4.02±0.15) of Nimari was significantly higher among the AA and AB genotypes of Malvi cattle whereas AA genotype of Malvi and Nimari cow showed non-significant difference with each other but AB genotype of Nimari cow showed higher protein% than its own AA genotype. So the lowest protein (%) was observed in AB genotype of Malvi (3.29±0.07). Contrary to above findings Ikonen *et al* (2001) [5] reported the positive association of B allele with milk protein (%) in Brown Swiss cattle.

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 protein per cent in the milk of AB genotype of Nimari was significantly higher than the AA and AB genotypes of Malvi breed cattle whereas the lowest protein (%) was observed in AB genotype of Malvi.

6. References

1. Aschaffenburg R, Drewry J. Occurrence of different beta-lactoglobulins in cow's milk. Nature. 1955;176:218-219. doi:10.1038/176218b0.
2. Pandey A, Thakur MS, Pandey Y. Association Study of Polymorphic Variants of Beta (B) Casein Gene with Milk Production Traits (Lactose, Snf and Density) in Malvi, Nimari, Sahiwal, and H.F. Cross Breeds Cow. Indian J Anim Res. 2021;55(1):1-5.
3. Bickhart DM, Rosen BD, Koren S, Sayre BL, Hastie AR, Chan S, *et al*. Single-molecule sequencing and chromatin conformation capture enable de novo reference assembly of the domestic goat genome. Nat Genet. 2017;49:643-650. doi:10.1038/ng.3802
4. Gautam D, Vats A, Verma M, Rout PK, Meena AS, Ali M, *et al*. Genetic variation in CSN3 exon 4 region of Indian goats and a new nomenclature of CSN3 variants. Anim Genet. 2019;50:191-192. doi:10.1111/age.12767
5. Ikonen T, Bovenhuis H, Ojala M, Ruottinen O, Georges M. Associations between casein haplotypes and first lactation milk production traits in Finnish Ayrshire cows. J Dairy Sci. 2001;84:507-514.
6. Jann OC, Prinzenberg EM, Luikart G, Caroli A, Erhardt G. High polymorphism in the kappa-casein (CSN3) gene from wild and domestic caprine species revealed by DNA sequencing. J Dairy Res. 2004;71:188-195. doi:10.1017/s0022029904000093
7. John SW, Weitzner G, Rozen R, Scriver CR. A rapid procedure for extracting genomic DNA from leukocytes. Nucleic Acids Res. 1991;19(2):408.
8. Martin P, Szymanowska M, Zwierzchowski L, Leroux C. The impact of genetic polymorphisms on the protein composition of ruminant milks. Reprod Nutr Dev. 2002;42:433-459. doi:10.1051/rnd:2002036
9. Schlieben S, Erhardt G, Senft B. Genotyping of bovine kappa - casein following DNA sequence amplification and direct sequencing of kappa-CNE PCR product. Anim Genet. 1991;22:333-342.
10. Snedecor GW, Cochran WG. Statistical Methods. 8th ed. Ames, IA, USA: Iowa State College Press; c1994.
11. Sodhi M, Mishra BP, Prakash B, Kaushik R, Singh KP, Mukesh M. Distribution of major allelic variants at exon-IV of kappa casein gene in Indian native cattle. J Appl Anim Res. 2010;38:117-121.
12. Tamura K, Nei M, Kumar S. Prospects for inferring very large phylogenies by using the Neighbor-joining method. In: Proc Natl Acad Sci U S A. 2004;101:11030-11035.
13. 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 Res. 1994;22:4673-4680.
14. Yahyaoui MH, Coll A, Sanchez A, Folch JM. Genetic polymorphism of the caprine kappa casein gene. J Dairy Res. 2001;68:209-216. doi:10.1017/s0022029901004733
15. Yahyaoui MH, Angiolillo A, Pilla F, Sanchez A, Folch JM. Characterization and genotyping of the caprine kappa-casein variants. J Dairy Sci. 2003;86:2715-2720. doi:10.3168/jds.S0022-0302(03)73867-3
16. Yeh FC, Yang RC, Boyle TBJ, Ye ZH, Mao JX. Popgene 32 version 1.32, the user-friendly shareware for population genetic analysis. Mol Biol Biotechnol Centre, Univ Alberta, Canada; c1999.