International Journal of Veterinary Sciences and Animal Husbandry

ISSN: 2456-2912
VET 2024; SP-9(2): 399-401

www.veterinarypaper.com
Received: 12-12-2023
Accepted: 16-01-2024

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

MS Thakur
Department of Animal Genetics and Breeding, College of Veterinary Science, Mhow, Madhya Pradesh, India

Y Pandey
Department of Animal Genetics and Breeding, College of Veterinary Science, Mhow, Madhya Pradesh, India

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

Kappa (κ) casein haplotypes and its association with milk lactose traits in Malvi and Nimari breed of cow of M.P, India

Akhilesh Pandey, MS Thakur and Y Pandey

Abstract
Present study revealed that polymorphic variants and their association with milk production trait, Lactose % 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. Association of polymorphic variants of κ -Casein (CSN3) gene with revealed that the frequencies of AA is higher as compared to AB and BB genotypes. The frequency of A allele was found to be highest as compared to B allele in all the four breeds of cattle under the study. Association study revealed that the frequencies of AA genotype is higher as compared to AB and BB genotypes. The frequency of A allele was found to be highest as compared to B allele in above both breeds of cattle. The analysis of variance showed significant (p<0.01) effect of breed on Lactose (%) trait. The mean Lactose (%) of both AA and AB genotypes of Nimari showed significantly higher Lactose (%) in milk than both AA and AB genotypes of Malvi breed of cow whereas both Malvi and Nimari breed of cow showed non-significant difference among its own AA and AB genotypes.

Keywords: K casein gene, lactose, Malvi, Nimari

1. Introduction
Polymorphisms in casein genes have been shown to affect milk protein and fat contents, milk yield, the organoleptic traits of milk and cheese, coagulation properties, and cheese yield Leroux *et al.* (1992) [8]. The casein cluster is composed of four genes; αs1-, β-, αs2- and κ-casein (CSN1S1, CSN2, CSN1S2 and CSN3, respectively) producing approximately 80 percent of the protein content of cow's milk. The four casein genes have been mapped in the order CSN1S1-CSN2-CSN1S2-CSN3 to bovine chromosome 6 (BTA6) at q31-33 by in situ hybridisation Ferretti *et al.* & Threadgill *et al.* (1990) [6].This protein is encoded by a trait locus at chromosome 6 and comprises a sequence of 162 amino acids Rijnkels (2002) [10] and this protein represents up to 12% of the total casein in bovine milk .The two main k-casein variants, A and B, differ in the amino acids at positions 136 (Thr for Ile) and 148 (Asp for Ala) Alexander *et al.* (1988) [2]. Several studies have also reported the existence of QTL affecting milk production traits on bovine chromosome 6(BTA6) [1, 2] (http://genomes.sapac.edu.au/bovineqtl/ and http://www.vetsci.usyd.edu.au/reprogen/QTL_Map/). Several polymorphisms have been detected in the open reading frame reviewed by Caroli *et al.* (2006) [3] and in non-coding regions such as the 5'-flanking region of the casein genes Cosenza *et al.* (2007, 2008) [4, 5] Reports on the association of k-casein genetic variants and milk composition have somewhat conflicting results Ng-Kwai-Hang *et al.* (1990) [9] reported changes in milk protein concentrations due to the k-casein genotypes A and B. However found no effect of k-casein variants on milk crude protein, while others reported higher contents of protein Stevanovic *et al.* (2000) [13] and caseins in allele B k-casein cow milk. pH-related behaviour of the casein component in milk allows for a concentration of the casein and elimination of the other components in the milk (lactose, whey proteins and soluble minerals) Since all the other components of skimmed milk are soluble at this pH (lactose, whey proteins and minerals, including the colloidal calcium phosphate), it is an efficient method of producing high casein powders. The high protein, low lactose ratio makes MPC suitable for protein-fortified

beverages and low-carbohydrate foods.

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 Malvi and 50 cows of Nimari. The milk samples brought to the laboratory, maintaining cold chain and then Lactose% were determined.

2.2 Estimation of Lactose: The data of then Lactose % were analyzed by Milk analyzer of the Department of Veterinary Medicine, College of Veterinary Science & A.H., Jabalpur.MP, India

2.3 Blood Collection

5 ml blood sample was collected in EDTA coated vaccutainer aseptically from 50 animals of each of Malvi and Nimari 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 Agarose gel electrophoresis

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

2.6 Concentration, purity and quality check of DNA

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

2.7 Spectrophotometery

The purity of DNA was checked by Spectrophotometer and after that 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. 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.

2.8 Casein gene primer sequence

The K-casein gene primers (F): 5' -GCTGAGCAG GTATCCTAGTTAT- 3'
(R): 5' - CTTCTTTGATGTCTCCTTAGAG - 3', 443 bp Schlieben *et al.* (1991)) [11] was used for the amplification of PCR product.

2.9 Polymerase Chain Reaction (PCR)

2.9.1. Setting of PCR Reaction

In PCR Initial denaturation (5 minutes) and final denaturation (1 minute) temp. Was 94^{0}C and anniling temp.(1 min.) Was 60 ^{0}C where extension(1 minute) and final extension(5 minutes) temp. was 72^{0}C.

2.9.2 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.

2.10 PCR- RFLP Assay

2.10.1 Restriction digestion

All the PCR products of k casein gene were digested by HindIII 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

The sequences obtained from genotype were aligned using Clustal W. (Thompson *et al.*, 1994) [15] and analyzed by using MEGA 6 software. 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].

2.12.2 Association of various polymorphic variants of milk protein genes with Lactose%: Association study of various polymorphic variants of milk protein genes for Lactose % data were subjected to least squares analysis of variance employing following linear model:

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

2.12.3 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 four breeds of cattle (Snedecor and Cochran, 1994) [12].
To find out the association between the polymorphic variants/ genotypes of, k -casein genes with milk production traits like, Lactose (%) in Malvi and Nimari cattle by linear regression model was employed.

3. Results

3.1. Lactose (%)

The results of least square analysis of variance presented in table 01, showed that the effect of breed was significant, however the genotype, parity, breed x genotype and breed x parity interactions were non-significant for lactose (%) trait.

Table 1: Least squares analysis of variance for lactose (%) at κ - Casein (CSN3) gene locus in the milk of Malvi, Nimari, cow

	MS	F-value
Breeds	1.79	7.32**
Genotype (Variants)	0.31	1.26NS
Parity	0.12	0.50 NS
Breed x Genotype	0.24	0.99 NS
Breed x Parity	0.33	1.34 NS
Genotype x Parity	0.18	0.73 NS
Error	0.24	-

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

3.1.1 Lactose (%) in milk of different variants at κ-Casein (CSN3) gene locus in Malvi and Nimari breeds of cattle

Least square means for Lactose (%) in Malvi and Nimari cow have been presented in table 02. Both AA and AB genotype of Nimari (5.48^{b}±0.04) (5.71^{b}±0.20) showed significantly higher Lactose (%) in milk than Malvi breed of cow (4.92^{a}±0.08) (4.80^{a}±0.11) whereas both Malvi and Nimari breed of cow showed non-significant difference among its own AA (4.92^{a}±0.08) (4.80^{a}±0.11) and AB genotypes (5.48^{b}±0.04) (5.71^{b}±0.20).

Table 2: Least squares means of Lactose (%) in milk of Malvi and Nimari breed of cow at κ-Casein (CSN3) gene locus

Variants	Malvi	Nimari
AA	4.92a±0.08 (36)	5.48b±0.04 (33)
AB	4.80a±0.11 (14)	5.71b±0.20 (17)
BB	0.00±0.00 (0)	0.00±0.00 (0)
Overall	4.89c±0.09 (50)	5.56a±0.09 (50)

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

4. Discussion

The frequency of A allele was found to be highest as compared to B allele in all the four 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, Nimari and Sahiwal breeds of cattle, indicating that the populations of these animals were in Hardy-Weinberg equilibrium at this locus.

The analysis of variance showed significant (p<0.01) effect of breed on Lactose (%). Least square means for Lactose (%) in Malvi and Nimari cow have been presented in table 02. Both AA and AB genotype of Nimari (5.48b±0.04) (5.71b±0.20) showed significantly higher Lactose (%) in milk than Malvi breed of cow (4.92a±0.08) (4.80a±0.11) whereas both Malvi and Nimari breed of cow showed non-significant difference among its own AA (4.92a±0.08) (4.80a±0.11) and AB genotypes (5.48b±0.04) (5.71b±0.20). As per Tsiaras *et al.* (2005) [1] AB genotype showed more Lactose (%) in milk of HF compared to AA genotype.

5. Conclusion

It is concluded that the frequencies of AA genotype is higher as compared to AB and BB genotypes. The frequency of A allele was found to be highest as compared to B allele in above both breeds of cattle. The analysis of variance showed significant (p<0.01) effect of breed on Lactose (%) trait. Both AA and AB genotype of Nimari showed significantly higher Lactose (%) in milk than Malvi breed of cow whereas both Malvi and Nimari breed of cow showed non-significant difference among its own AA and AB genotypes.

6. References

1. Tsiaras AM, Bargouli GG, Banos G, Boscos CM. Effect of kappa-casein and beta-lactoglobulin loci on milk production traits and reproductive performance of Holstein cows. J Dairy Sci. 2005;88(1):327-334.
2. Alexander LJ, Stewart AF, Mackinlay AG, Kapelinskaya TV, Tkach TM, Gorodetsky SI. Isolation and characterization of the bovine κ-casein gene. Eur J Biochem. 1988;178:395-401.
3. Caroli A, Chiatti F, Chessa S, Rignanese D, Bolla P, Pagnacco G. Focusing on the goat casein complex. J Dairy Sci. 2006;89(8):3178-3187.
4. Cosenza G, Pauciullo A, Colimoro L, Mancusi A, Rando A, Di Berardino D, *et al*. A SNP in the goat CSN2 promoter region is associated with the absence of β-casein in milk. Anim Genet. 2007;38(6):655-658.
5. Cosenza G, Pauciullo A, Gallo D, Colimoro L, D'avino A, Mancusi A, *et al*. Genotyping at the CSN1S1 locus by PCR-RFLP and AS-PCR in a Neapolitan goat population. Small Ruminant Res. 2008;74(1-3):84-90.
6. Ferretti L, Leone P, Sgaramella V. Long-range restriction analysis of the bovine casein genes. Nucleic Acids Res. 1990;18:6829-6833.
7. John SW, Weitzner G, Rozen R, Scriver CR. A rapid procedure for extracting genomic DNA from leukocytes. Nucleic Acid Res. 1991;19(2):408.
8. Leroux C, Mazure N, Martine P. Mutations away from splice site recognition sequences might cis-modulate alternative splicing of goat aS1-casein transcripts. J Biol Chem. 1992;267:6147-6157.
9. Ng-Kwai-Hang KF, Monardes HG, Hayes JF. Association between genetic polymorphism of milk proteins and production traits during three lactations. J Dairy Sci. 1990;73:3414-3420.
10. Rijnkels M. Multispecies comparison of the casein gene loci and evolution of the casein gene family. J Mammary Gland Biol Neoplasia. 2002;7(3):327-345.
11. 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.
12. Snedecor GW, Cochran WG. Statistical methods. 8th edn. Iowa State College Press, Inc. Ames, Iowa, USA; c1994. p. 950.
13. Stevanovic M, Stanojcic S, Djurovic J. Molecular marker technologies and selection for the traits of economic interest. Biotechnol Anim Husbandry. 2000;16(1-2):25-34.
14. Threadgill DW, Womack JE. Genomic analysis of the major bovine milk protein genes. Nucleic Acids Res. 1990;18:6935-6942.
15. 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.
16. Yeh FC, Yang RC, Boyle TBJ, Ye ZH, Mao JX. Popgene 32 version 1.32, the user-friendly shareware for population genetic analysis. Molecular Biology and Biotechnology Centre, University of Alberta, Canada; c1999.