The Pharma Innovation Journal 2023; SP-12(7): 107-109

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; SP-12(7): 107-109

www.thepharmajournal.com
Received: 24-05-2023
Accepted: 30-06-2023

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

DS Gonge
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

Relationship of different genetic variants of αS2-casein gene (CSN1S2) with milk production traits (lactose, SNF & density) in Sahiwal and HF crossbred cattle of Madhya Pradesh, India

Akhilesh Pandey, MS Thakur, DS Gonge and Y Pandey

Abstract
αs2-casein is a primary milk protein. However, there has been little research focused on the effects of αs2- casein variants on milk. The αs2-casein (CSN1S2) play a major protein found in ruminant's milk, which is encoded by a highly polymorphic CSN1S2 gene present on chromosome 6. Research work was carried out on 50 animals each of Sahiwal and HF Crossbred cow at College of Veterinary Science Jabalpur, Madhya Pradesh, India. The αS2 casein gene (1267 bp) was digested by EcoRV restriction enzyme yielding two genotypes *viz*., AA (1267 bp) and BB (1267/1150/117 bp) in HF crossbred cattle, while only one genotype (AA) in Sahiwal. The restriction site was absent in Sahiwal resulting in only single compact band of 1267 bp. The tested animals of Sahiwal were found monomorphic at this locus. Association study of different genotypes with milk composition traits revealed that the mean lactose % showed non- significant difference in A1A2 and A2A2 genotype of Sahiwal and HF Crossbred whereas Sahiwal showed non- significantly higher mean value of SNF % compared to HF Crossbred cow milk.The mean milk density (kg/L) was significantly higher in the HF Crossbred milk breed compared to Sahiwal milk. 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. breed of cow. The frequency of A2 allele was found to be highest(1.00) as compared to A1 allele(0.00) in above breeds of cattle under the study.

Keywords: αS2, casein gene, Sahiwal, Hf cross breds

1. Introduction
In recent years, the genetic polymorphism of casein has raised considerable research interest because of casein polymorphisms are related to milk quality, milk composition and technological properties. Several alleles, showing different synthesis levels, have been identified so far. In ruminants, several studies have shown the association of β-casein gene polymorphism with economic important traits such as yield and composition of milk in cattle (Huang *et al*., 2012; Viale *et al*., 2017; Soyudal *et al*., 2018) [3, 13, 8], in buffalo (Singh *et al*., 2007) [7], in sheep (Corral *et al*., 2010) [5], and in goats (Cosenza *et al*., 2007) [2]. India, with nearly millions of sahiwal and HF crosssbred, is one of largest producers of milk. Sahiwal breed is the best-known, not only due to their tolerance of crushed feed and the local fluctuating harsh weather, but also to their high milk yield. While as for above breeds we found few reports about studying DNA polymorphism and their association with economic traits. The aim of the present study was to investigate the genetic structure of CSN1S2 locus. Association study as per Pandey *et al.* (2018) [6] the higher mean fat (%) was noticed for AA genotype than AB genotype of Malvi and Nimari, however, in HF crossbred higher fat (%) was observed for AB genotype. Therefore, the genetic variations analysis of the CSN1S2 gene in Sahiwal and HF Crossbred may provide useful information related to the understanding of their genetic characteristics. The genetic associations related to cow milk traits like Lactose, SNF & Density in above breeds will possibly contribute to improving the quality of high milk yielder breeds in India.

2. Material and Methods
2.1 Collection of milk samples
Collection of milk samples with economic traits about 100ml milk sample was collected from each of the above 100 cattle. The milk samples brought to the laboratory, maintaining cold chain and then Lactose (%), SNF (%) and Milk density (Kg/L) were determined.

2.2 Estimation of Lactose (%), SNF (%) and Milk density (Kg/L): The Lactose (%), SNF (%) and Milk density (Kg/L) were analyzed by Milk analyzer of the Department of Veterinary Medicine, College of Veterinary Science & A.H., Jabalpur.

2.3 Blood Collection: 5 ml blood sample was collected in EDTA coated vaccutainer aseptically from 50 animals of each of the four breeds *viz*. Malvi, Nimari, Sahiwal and HF crossbred cattle and 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) [5] 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 Nano drop spectrophotometer and agarose gel electrophoresis.

2.7 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. 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 αS2-casein gene primers (F): 5'-TATGACATGTCGAGAAATGAG-3'
(R): 5'-TTGGAACAATGCTATTAGGT T- 3'1267 bp (Szymanowska *et al*. 2004) [10] was used for the amplification of PCR product.

2.9 Polymerase Chain Reaction (PCR)

2.9.1. 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) temp. Was 940C 600C anniling temp. (1 min.) Was 600C where extension (1 minute) and fi NAL extension (5 minutes) temp. was 720C

2.9.2 Agarose gel electrophoresis of PCR reaction product

To confirm the targeted PCR amplifi cation 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 αs2 casein gene were digested by EcoRV 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) [12] and analyzed by using MEGA 6 software (Tamura *et al*., 2004) [11]. Aligned sequences were analyzed for group specifi c 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) [14].

2.12.2 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 milk yield
µ - is the population mean
Pi - is the fi xed effect of parity
Bj - is the fi xed effect of breed
Gk - is fi xed 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

2.12.3 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) [9].
To find out the association between the polymorphic variants/ genotypes of, αs2 -casein genes with milk production traits like, Lactose (%), SNF (%) and Milk density (Kg/L) in of Sahiwal and HF crossbred cattle by linear regression model was employed.

3. Results and Discussion

Table 1: Least squares means for Lactose (%) in the milk of different breeds of cattle at αS2-Casein (CSN1S2) gene locus

Variants	Breeds	
	Sahiwal	HF crossbred
AA	5.25^{b}±0.07 (50)	5.35^{b}±0.10 (29)
AB	0.00±0.00 (00)	5.44^{ab}±0.09 (21)
BB	0.00±0.00 (00)	0.00±0.00 (00)
Overall	5.25^{b}±0.07 (50)	5.39^{ab}±0.07 (50)

Means bearing the different superscript differ significantly (p<0.01), Numbers in the parentheses denotes number of animals

As shown in table 01, the mean lactose per cent showed non-significantly higher mean value in HF crossbred for both AA and AB genotyped animals as compared to AA genotype of Sahiwal.

Table 2: Least squares means for SNF (%) in the milk of different breeds of cattle at αS2-Casein (CSN1S2) gene

Variants	Breeds	
	Sahiwal	HF crossbred
AA	8.74^{a}±0.12 (50)	8.47^{ab}±0.10 (29)
AB	0.00±00 (00)	8.63^{a}±0.14 (21)
BB	0.00±0.00 (00)	0.00±0.00 (00)
Overall	8.74^{a}±0.12 (50)	8.54^{a}±0.08 (50)

Means bearing the different superscript differ significantly (p<0.01), Numbers in the parentheses denotes number of animals

The mean SNF per cent between AA and AB was found non significantly higher in AA genotype of Sahiwal (8.74^{a}±0.12) compared to AA (8.47^{ab}±0.10) and AB (8.63^{a}±0.14) genotype of HF crossbred cow milk (Table 02). In accordance to the above findings, Szymanowska *et al.* (2004) [10] showed that the AA genotype determine higher lactose and SNF per cent in Polish Black and White cattle.

Table 3: Least squares means for milk density (kg/L) of different breeds of cattle at αS2-Casein (CSN1S2) gene

Variants	Breeds	
	Sahiwal	HF crossbred
AA	1.03^{c}±0.09 (50)	1.04^{b}±0.11 (29)
AB	00±00 (50)	1.04^{b}±0.06 (21)
BB	0.00±0.00 (00)	0.00±0.00 (00)
Overall	1.03^{b}±0.09 (50)	1.04^{a}±0.08 (50)

Means bearing the different superscript differ significantly (p<0.01), Numbers in the parentheses denotes number of animals

The mean milk density (kg/L) of both AA (1.04^{b}±0.11) and AB (1.04^{b}±0.06) genotype of HF Crossbred was significantly higher than AA genotype (1.03^{c}±0.09) of Sahiwal but AA genotype of HF Crossbred showed non significant difference with its own AB genotype.(Table 03).

4. Conclusion

In last to conclude PCR-RFLP analysis of αs2-Cn gene (1267bp) with EcoRV RE revealed two genotypes *viz*., AA (1267/1267bp) and AB (1267/1150/117bp) in HF crossbred animals, whereas, only AA (1267/1267 bp) genotype was observed Sahiwal cattle. All the screened animals of Sahiwal were found monomorphic at αs2-Cn/EcoRV gene locus. The genotypic and gene frequencies of αS2-casein gene (CSN1S2)/EcoRV locus. 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 study of different genotypes with milk composition traits revealed that the mean lactose per cent showed non- significantly higher mean value in HF crossbred for both AA and AB genotyped animals as compared to AA genotype of Sahiwal. The mean SNF per cent between AA and AB was found non significantly higher in AA genotype of Sahiwal than HF crossbred of cattle. The mean milk density (kg/L) of both AA (1.and AB genotype of HF Crossbred was significantly higher than AA genotype of Sahiwal but AA genotype of HF Crossbred showed non significant difference with its own AB genotype.

5. References

1. Corral J, Padilla J, Izquierdo M. Associations between milk protein genetic polymorphisms and milk production traits in Merino sheep breed. Livest Sci. 2010;129:73-79. [Google Scholar].
2. Cosenza G, Pauciullo A, Colimoro L, Mancusi A, Rando A, Di Berardino D, *et al*. An SNP in the goat CSN2 promoter region is associated with the absence of β-casein in milk. Anim Genet. 2007;38:655-658. [PubMed] [Google Scholar]
3. Huang W, Peñagaricano F, Ahmad K, Lucey J, Weigel K, Khatib H. Association between milk protein gene variants and protein composition traits in dairy cattle. J Dairy Sci. 2012;95:440-449. [PubMed] [Google Scholar]
4. Hayes H, Petit E, Bouniol C, Popescu P. Localization of the alpha-s2-casein gene (CASAS2) to the homologous cattle, sheep and goat chromosome 4 by in situ hybridization. Cytogenetics Cell Genetics. 1993;64:281-285.
5. John SW, Weitzner G, Rozen R, Scriver CR. A rapid procedure for extracting genomic DNA from leukocytes. Nucleic Acid Research. 1991;19(2):408.
6. Pandey, Akhilesh, Thakur MS. Association of Different Genetic Variants of Alpha S2-Casein Gene (CSN1S2) with Milk Production Traits in Cattle. Journal of Animal Research. 2018 June;8(3):463-466.
7. Singh S, Kumar P, Bhattacharya T, Bhushan B, Sharma A. Association of ss-casein gene polymorphism with milk production traits in buffalo (Bubalus bubalis) Indian J Anim Sci. 2007;77:752-754. [Google Scholar]
8. Soyudal B, Ardicli S, Samli H, Dincel D, Balci F. Association of polymorphisms in the CSN2, CSN3, LGB and LALBA genes with milk production traits in Holstein cows raised in Turkey. J Hellenic Vet Med Soc. 2018;69:1271-1282. [Google Scholar]
9. Snedecor GW, Cochran WG. Statistical method. 8th edn. The lowa State College Press, Inc. Amer. lowa USA; c1994. p. 950.
10. Szymanowsky M, Eulalia S, Marek L, Lech Z. Association of nucleotide-sequence polymorphism in the 5'-flanking regions of bovine casein genes with casein content in cow's milk. INRAEDP Sciences. 2004;84:579-590.
11. Tamura K, Nei M, Kumar S. Prospects for inferring very large phylogenies by using the neighbor-joining method. In: Proceedings of the National Academy of sciences of the United States of America. 2004;101:11030-11035.
12. Thompson JD, Higgins DG, Gibson TJ, Clustal W. Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specifi c gap penalties and weight matrix choice. Nucleic Acids Research. 1994;22:4673-4680.
13. Viale E, Tiezzi F, Maretto F, De Marchi M, Penasa M, Cassandro M. Association of candidate gene polymorphisms with milk technological traits, yield, composition, and somatic cell score in Italian Holstein-Friesian sires. J Dairy Sci. 2017;100:7271–7281. [PubMed] [Google Scholar]
14. 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.