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Alpha-S1 Casein Gene Polymorphism and Association with Milk Production Traits in Sahiwal and Holstein Frisian Crossbred Cattle

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ABSTRACT

The present research work was conducted on 100 lactating cows comprising 50 each of Sahiwal and HF Crossbred cattle. All the screened in Sahiwal (n = 50) and HF Crossbred (n = 50) cows revealed monomorphic pattern, only, AA genotype (310 bp) was found at αs1-casein gene (CSN1S1)/*HindIII* locus. The allelic frequency for allele A in the screened animals of Sahiwal and HF Crossbred cattle was 1.00 and for allele B was 0.00. The population was found under HWE at this locus (χ^2=0.00^{NS}). Milk yield and Daily milk yield was noticed significantly higher in HF Crossbred as compared to Sahiwal cattle.

Keywords: Casein gene, lactating cows, milk yield, PCR- RFLP

The αS1-casein (CSN1S1) is localized in bovine chromosome 6. It is a loose flexible medium sized protein consists of two hydrophobic regions connected by a hydrophilic region. It has phosphate groups located in the hydrophilic region and most of the calcium associated with is exists at the phosphate groups (Ferranti *et al.*, 1998). Nucleic acid sequences have been reported for αS1 like casein mRNAs from cow, sheep, rat and guinea pigs. They are found to be divergent sequences with a large number of point mutations Ferranti *et al.* (1998). It has an important role in the capacity of milk to transport calcium phosphate and is organized at 5′-terminus of casein cluster. Till now, 9 variants (A-I) have been reported in the coding region of αS1-CN (Kishore *et al*., 2013).

MATERIALS AND METHODS

Animals and DNA isolation

The present research work was conducted on 100 lactating cows comprising 50 each of Sahiwal and HF Crossbred cattle. The blood samples were collected randomly from a total of 50 animals of Sahiwal cattle breed maintained in Livestock Farm Anjora (Durg) Chhattisgarh and 50 HF Crossbred cattle maintained at Livestock Farm, College of Veterinary Science and A.H., Jabalpur and Private Dairy Farm at Pariyat, Jabalpur.

Genomic DNA was extracted by John *et al.* (1991) method. The concentration and purity of DNA was checked by Nanodrop Spectrophotometer (ND-1000). The integrity of the DNA was examined by agarose gel (0.8%) electrophoresis and visualized the gel under UV transilluminator using Gel documentation system (Gel-doc Bio-Rad, USA).

Gene and genotype frequencies were estimated using Popgene 32 (version 1.32), Microsoft Windows-based freeware for population genetic analysis (Yeh *et al.,* 1999).

The data related to milk production was recorded from 50 each of Sahiwal and HF Crossbred cattle (i.e., identification number, parity, lactation length, milk yield and daily milk yield etc.). The data was subjected to least squares analysis of variance (Snedecor and Cochran, 1994).

Amplification of αs1-casein gene (CSN1S1)

The αS1-casein (CSN1S1) gene of 310 bp was amplified

using a specific primer pairs (CSN1S1/start F: 5′-TGCATGTTCTCATAATAACC -3′ and CSN1S1/stop R: 5′- GAAGAAGCAGCAAGCTGG -3′; Mir *et al*., 2014). PCR amplification was carried out in a total volume of 25 µl that contained 3.0 µl of genomic DNA (90 nmoles), 12.5 µl 2X PCR mastermix (Fermentas, USA), 1.0 µl of each primer (forward and reverse) and 7.5 µl of DNAase free water. The cycle conditions included an initial period of denaturation at 94ºC for 5 min, followed by 35 cycles of denaturation at 94ºC for 1 min, annealing at 54.5ºC for 1 min and extension at 72ºC for 1 min, and a final extension at 72ºC for 10 min. The PCR product was checked by agarose gel (2.0%) electrophoresis in 0.5X TAE buffer at constant voltage of 90 volt for 50 minutes after staining with EtBr. The mass ruler DNA ladder (100 bp- 1000 bp) as a molecular size marker was used for sizing of the DNA bands. The amplified product in the gel was visualized by UV transilluminator and photographed using Gel documentation system (Gel-doc Bio-Rad, USA).

Restriction digestion of amplified PCR product

The amplified PCR products of all the samples were digested by restriction enzyme (RE) *HindIII* at 37°C for overnight in water bath. The RE digested PCR products were analyzed on 2.5% agarose gel in 0.5 × TBE buffer. The 10µl of RE digested PCR product was mixed with 2 µl of 6x gel loading dye (Bromophenol blue) and loaded along with 100 bp DNA ladder as a molecular size marker in a separate lane. The electrophoresis was performed at constant voltage of 90 volt for 50 min at 37°C using 0.5 × TBE buffer. After gel electrophoresis the digested PCR product was visualized by UV transilluminator and photographed using Gel documentation system (Geldoc Bio-Rad, USA) to detect the banding pattern/ genotype of CSN1S1 gene of each individual sample.

RESULTS AND DISCUSSION

Gene and Genotypic frequencies at αs1-casein (CSN1S1) gene locus

The genotypic and allelic frequencies of αs1-casein gene (CSN1S1) in Sahiwal and HF Crossbred cattle are presented in table 1. In the present study, all the screened populations of Sahiwal (n=50) and HF Crossbred (n=50) revealed only the one type of genotype (AA) with 100 per cent frequency. The allelic frequency for allele A in Sahiwal and HF Crossbred cattle was 1.00 and for allele B was 0.00. Contrary to above findings, Silva and Lama (1997) and Caroli *et al*. (2008) reported predominance of B allele over C allele in HF, Brazilian Zebu cattle and Carora cattle, respectively.

Table 1: Distribution of gene and genotypic frequency of αs1-casein (CSN1S1) variants in different breeds of cattle

Breeds	Genotype Freq			χ^2 value	Gene Freq	
	AA	AB	BB		A	B
Sahiwal	1.00 (50)	0.00 (0)	0.00 (0)	0.00^{NS}	1.00	0.00
HFC	1.00 (50)	0.00 (0)	0.00 (0)	0.00^{NS}	1.00	0.00

NS-Non-significant, Freq- frequencies, HFC- HF Crossbreds

Chi-square values for testing correspondence between observed and expected genotypic frequencies at this locus were found to be non- significant in Sahiwal, and HF Crossbred cattle, indicating that the populations of these two cattle breeds under study were in Hardy-Weinberg equilibrium (Table 1). In accordance to above findings, Golijow *et al.* (1999) reported that the populations of Argentine Creole and Argentine Holstein were found in HWE for CSN1S1 loci. These facts indicate that the artificial selection is not disturbing the equilibrium of gene frequencies in the milk production related loci in these breeds of cattle.

Association of αs1-Casein (CSN1S1) gene polymorphic variants with milk yield

All the screened animals of Sahiwal and HF crossbred cattle were found monomorphic for αs1-Casein (CSN1S1) gene/*HindIII* locus (Fig. 1 and 2). Therefore, the association study of different CSN1S1 gene variants with milk production traits could not perform.

The effect of breed was found significant ($P<0.01$) for daily milk yield (DMY) and milk yield per lactation (MY) trait. The mean DMY and MY in Sahiwal and HF Crossbred cattle have been presented in table 2.

Only AA genotype was observed in all the animals of Sahiwal and HF Crossbred cattle. The mean MY of AA

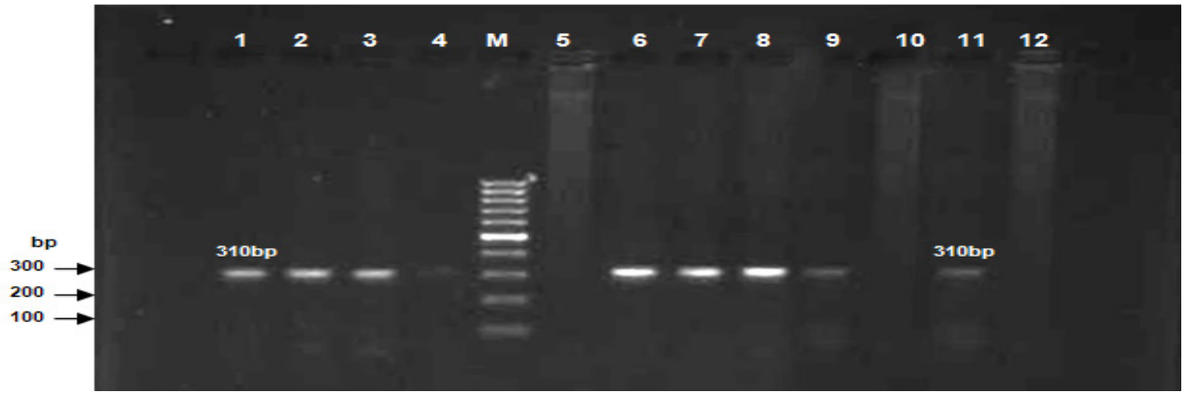

Fig. 1: PCR-RFLP/*HindIII* assay of αS1 gene showing genotype pattern in 2.5% agarose gel of Sahiwal cattle. M: 100bp DNA ladder, Lanes: 1-12

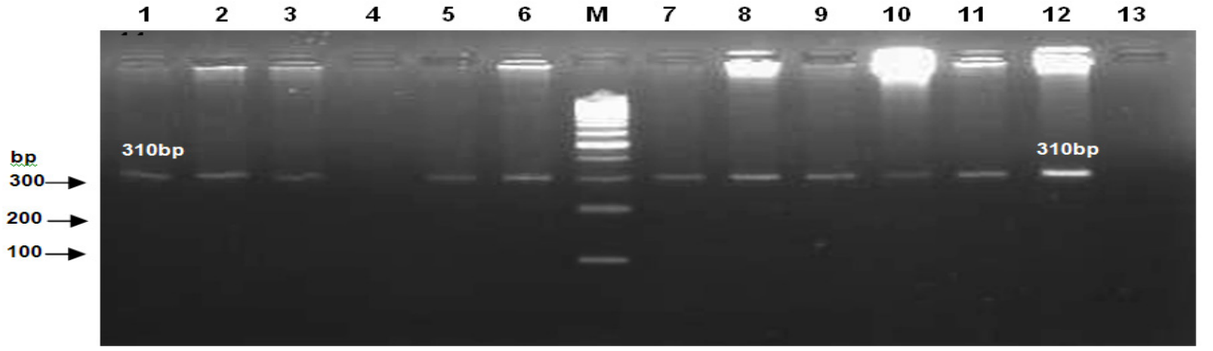

Fig. 2: PCR-RFLP/*HindIII* assay of αS1 gene showing genotype pattern in 2.5% agarose gel of HF Crossbred cattle. M: 100bp DNA ladder, Lanes: 1-13

Table 2: Mean Milk Yield per lactation (L) and daily milk yield in different breeds of cattle at αs1-Casein (CSN1S1) gene locus

Variants	Milk yield/lactation (litres)		Daily milk yield (litres)	
	Sahiwal	HF crossbred	Sahiwal	HF crossbred
AA	1523.00^{b} ± 39.00 (50)	2468.00^{a} ± 111.00 (50)	5.77^{b} ± 0.14 (50)	7.62^{a} ± 0.39 (50)
AB	0.00 ± 0.00 (0)	0.00 ± 0.00 (0)	0.00 ± 00 (0)	0.00 ± 00 (0)
BB	0.00 ± 0.00 (0)	0.00 ± 0.00 (0)	0.00 ± 00 (0)	0.00 ± 00 (0)
Overall	1523.00^{b} ± 39.00 (50)	2468.00^{a} ± 111.00 (50)	5.77^{b} ± 0.14 (50)	7.62^{a} ± 0.39 (50)

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

genotyped animals was 1523.0±39.0 and 2468.0±111.0, respectively in Sahiwal and HF Crossbred cattle. However, DMY was 5.77±0.14 and 7.62±0.39 liters, respectively, in Sahiwal and HF Crossbred cattle. Among the both breeds of cattle, significantly higher mean DMY and MY was recorded in HF Crossbred as compared to Sahiwal breed of cattle (Table 2).

The fixation of favourable allele (A) might be due to high milk producing nature of HF crossbred and Sahiwal cattle. Consequently, we could not establish any association of the observed polymorphism with milk production traits. Contrary to these findings, Hristov *et al.* (2014) showed that the BB genotype determines higher milk production. The results revealed in the present study are in accordance

as reported by Szymanowska *et al.* (2004) in Polish Black and White cattle.

CONCLUSION

In the present study, we could not establish any association between genotype and milk production trait because these cattle were found homozygous for αs1-Casein (CSN1S1) gene/*HindIII* locus. The population of HF Crossbred and Sahiwal cattle was in HWE at this locus. The DMY and MY was noticed higher in HF Crossbred compared to Sahiwal cattle.

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REFERENCES

Caroli, S., Chessa, F., Chiatti, D., Rignanese, B., Melendez, R. and Rizzi, G.C. 2008. Carora cattle show high variability in αs1-casein. *J. Dairy Sci.,* **91**(1): 354–359.

Ferranti, P., Scaloni, A., Caira, S., Chianese, L., Malorni, A., and Addeo, F. 1998. The primary structure of water buffalo alpha (s1) and beta- casein identification of phosphorylation sites and characterization of a novel beta- casein variant. *J. Protein Chem*., **17**: 835–844.

Golijow, C.D., Giovambattista, G., Rípoli, M.V., Dulout, F.N. and Lojo, M.M. 1999. Genetic variability and population structure in loci related to milk production traits in native Argentine Creole and commercial Argentine Holstein cattle. *Genet. Mol. Biol*., **22**(3): 395-398.

Hristov, P., Neov, B., Sbirkova, H., Teofanova, D., Radoslavov, G., Shivachev, B. 2014. Genetic polymorphism of kappa casein and casein micelle size in the Bulgarian Rhodopean cattle breed. *Biotechnol. Anim. Husb*., **30**(4): 561-570.

John, S.W., Weitzner, G., Rozen, R. and Scriver, C.R. 1991. A rapid procedure for extracting genomic DNA from leukocytes. *Nucleic Acid Res.*, **19**(2): 408.

Kishore, A., Mukesh, M., Sobti, R.C., Mishra, B.P. and Sodhi, M. 2013. Variations in the regulatory region of alpha S1-Casein milk protein gene among tropically adapted Indian native (*Bos Indicus*) Cattle. *ISRN Biotechnol*., 926025: http://doi.org/10.5402/2013/926025.

Mir, N.S., Ullah, O. and Sheikh, R. 2014. Genetic polymorphism of milk protein variants and their association studies with milk yield in Sahiwal cattle. *Afri. J. Biotechnol*., **13**(4): 555-565.

Silva, I.T.D. and Lama, M.A.D. 1997. Milk protein polymorphisms in Brazilian Zebu cattle. *Braz. J. Genet*., **20**(4): 103-106.

Snedecor, G.W. and Cochran, W.G. 1994. Statistical method. 8th edn. The lowa State College Press, Inc. Amer. lowa USA. 950 p.

Szymanowsky, M., Eulalia, S., Marek, L. and Lech, Z. 2004. Association of nucleotide-sequence polymorphism in the 5'-flanking regions of bovine casein genes with casein content in cow's milk. *INRAEDP Sci*., **84**: 579–590.

Yeh, F.C., Yang, R.C., Boyle, T.B.J., Ye, Z.H. and Mao, J.X. 1999. Popgene 32 version 1.32, the user-friendly shareware for population genetic analysis. Molecular Biology and Biotechnology Centre, University of Alberta, Canada.