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Association Study of Polymorphic Varients of Beta (B) Casein Gene with Milk Production Traits (Lactose, Snf and Density) in Malvi, Nimari, Sahiwal and H.F. Cross Breeds Cow

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ABSTRACT

Background: The most frequently observed forms of beta casein (β-Cn) in dairy cattle are A1 and A2. A2 β-Cn is recognized as the original β-Cn protein because it existed before a mutation caused the appearance of A1 β-Cn in European cattle (Bos taurus) a few thousand years ago. In cattle, beta-casein (CSN2) gene is highly polymorphic with at least 13 genetic variants known until now.

Methods: Research work was carried out on 50 Malvi and 50 Nimari, 50 Sahiwal and 50 H F Cross bred cow. In present research work the PCR amplicons of 121bp were digested by restriction endonuclease enzyme DdeI, which recognizes G^AATTC sites. Present association study of polymorphic variants' showed that the presence of no restriction sites for the enzyme DdeI in both Malvi and Nimari. So one band of 121bp was observed on the gel and such genotype was designated as A2A2 type. Whereas in Sahiwal and H F Crossbred showed two type of genotypes A1A2 and A2A2.

Result: The result of RFLP revealed that the gene and genotypic frequencies of β-casein (CSN2) gene for A2A2 was 1.00 for both Malvi and Nimari breed of cattle but 0.00, 0.30 and 0.70 in Sahiwal and 0.00, 0.64 and 0.36 in HF crossbred cattle, respectively. Association study showed that the lactose per cent was significantly higher in Nimari as compared to Malvi and Sahiwal. Among all the four breeds of cattle, significantly lower SNF (%) and density was noticed in Malvi breed of cattle for A2A2 genotype compared to remaining three breeds.

Key words: Beta (β) casein gene, H.F. Cross bred, Malvi, Nimari, Polymorphism, Sahiwal.

INTRODUCTION

The most frequently observed forms of β-Cn in dairy cattle are A1 and A2. A2 β-Cn is recognized as the original β-Cn protein because it existed before a mutation caused the appearance of A1 β-Cn in European cattle (Bos taurus) a few thousand years ago. In cattle, beta-casein (CSN2) gene is highly polymorphic with at least 13 genetic variants known until now (Farrel *et al.* 2004). Study of the β-casein polymorphism at the protein level showed that A1 and A2 variants' are most frequently noticed compared to others (e.g. B, A3, C). Genetic variants of the beta-casein milk protein differ by only one amino acid. The A1 beta-casein type is the most common type found in cow's milk in Europe (excluding France), USA, Australia and New Zealand. A genetic test, developed by the A2 Milk Company, determines whether a cow produces A2 or A1 type of protein in its milk.

A1 beta-casein (β-Cn) in milk has been found to be associated with range of illnesses in human being including type 1 diabetes mellitus, autism, cancer and other immune suppression activities (Bell *et al*. 2006 and Truswell, 2005). At the same time presence of A2 has been reported to be associated with reduced serum cholesterol and decrease concentration of low density lipoprotein which play an important role in prevention of a wide range of human vascular diseases (Ikonen *et al*. 2001). Variants of casein genes have also been reported to be associated with milk yield and composition (Cardak, 2005 and Mir *et al.* 2014). Sodhi *et al.* (2012) studied 91 exotic (51 Holstein Friesian, 40 Jersey) and 89 crossbred bulls approved for genetic improvement programme. They reported the presence of desirable A2 allele across all cattle types with a mean frequency of 0.65. The genotype, A1A2 was more common in Holstein Friesian and Jersey while A2A2 was at higher frequency in the crossbreds. The results of association studies of the different genotype with different milk trait provide the guideline to the breeder in selection. Shende *et al.* (2017) observed that the gene frequencies of A1 and A2

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genes are 0.64 and 0.36, respectively in HF cattle. The genotype frequency of A1A1 genotype was 0.28 and that of A1A2 genotype was 0.72. They reported that Milk yield (kg) 2719.24±41 in A1A1, 2769.12±35.51 in A1A2 whereas the Fat % 3.61+0.05 in A1A1 and 3.67+0.04 in A1A2 was noticed. So the findings of my work will helpful to the breeders for selection of high quality milk traits animals as per the need.

MATERIALS AND METHODS

The present research work was conducted on 200 lactating cows comprising 50 each of Malvi, Nimari, Sahiwal and H.F. cross bred cow. The data was collected from the animals maintained as per the following Table 1.

Identification number along with the various desired parameters of each animal under study, were recorded. About 100ml milk sample from each cow was collected in the sterilized tube and mixed with 0.8% formalin and then 5 ml blood sample was collected from same cow in EDTA coated test tube. The collected samples are maintained in cold chain during transportation and in laboratory. In first phase of research the milk samples are processed for estimation of Lactose, SNF and Density of the milk by Milk analyzer machine College of Veterinary Science and A.H., Jabalpur.

In second part of experiment the blood samples are processed for genomic DNA isolation by John *et al.* (1991) method. The β casein gene primers were used for the amplification of PCR product as described in following Table 2.

Digested PCR products were analyzed on 2.0% agarose gel (5 µl of PCR product mixed with 1 µl of gel loading dye). The electrophoresis at constant voltage of 90 volt for 50 minutes at 37°C using 0.5X TBE buffer was conducted. The mass ruler DNA ladder (100 bp- 1000 bp) as a molecular size marker was used for sizing of the DNA bands.

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). Association study of various polymorphic variants of milk protein genes Lactose (%), SNF (%) and Milk density (Kg/L) data were subjected to least squares analysis of variance employing linear model: to least squares analysis of variance employing following linear model:

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

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

Where,

Yijkl - is the observed value of milk yield
µ - is the population mean
Pi - is the 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.

The chi-square test (χ^2) was employed to test the status of Hardy-Weinberg equilibrium in the different population of four breeds of cattle (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

Allelic and genotypic frequencies were estimated using Popgene 32 (version1.32), microsoft Windows-based freeware for population genetic analysis and the population was tested for genetic equilibrium at this locus. The tested populations of Malvi (50), Nimari (50) are monomorphic, whereas Sahiwal (50) and H.F. cross bred (50) cattle at the β -casein gene (CSN2) locus were found to be polymorphic. Only A2A2 genotypes was found in all animals of Malvi and Nimari but Sahiwal and HF crossbred showed both A2A2 and A1A2 genotype. No A1A1 genotype was recorded in any of the four cattle breeds. Chi-square values 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 under study were in HWE at β-Casein (CSN2)/*DdeI* gene locus, while Chi-square value was found to be significant (P<0.01) for H.F. cross bred cattle revealed that Hardy Weinberg disequilibrium at the locus for this population under study.

Association studied of different genotypes with the following milk productive traits

Lactose (%) of different variants at β-Casein (CSN2)/ *DdeI* gene locus in four breeds of cattle

The results of analysis of variance have been presented in Table 5. The effect of genotypes was found significant (P<0.01) for lactose.

The mean lactose (%) in Malvi, Nimari, Sahiwal and HF crossbred cattle has been presented in Table 1. The mean milk lactose per cent for A2A2 genotype was ranged from 4.89±0.07 (Malvi) to 5.56±0.07 (Nimari) and for A1A2 genotype it was ranged from 5.19±0.13 (Sahiwal) to 5.33±0.09 (H.F. cross bred) among all the four breeds of cattle. Among A2A2 genotyped animals, the lactose per cent was significantly higher in Nimari (5.56±0.07) as compared to Malvi and Sahiwal; while this mean difference was found non-significant with A2A2 genotyped H.F. cross bred cattle (Table 6).

SNF (%) of different variants at β-Casein (CSN2)/ *DdeI* gene locus in four breeds of cattle

The results of analysis of variance have been presented in Table 7. The effect of genotypes was found significant (P<0.01) for SNF per cent trait. The mean SNF (%) in Malvi, Nimari, Sahiwal and HF crossbred cattle has been presented in Table 8.

Table 1: Details of experimental samples collection.

Breed	No. of Animals
Malvi	50
Nimari	50
Sahiwal	50
H.F. cross bred	50

Table 2: Published Primer.

β-casein	(F): 5′- CCT TCT TTC CAG GAT GAA CTCCAG G-3′ (R): 5′- GAG TAA GAG GAG GGA TGT TTTGTG GGAGGC TCT- 3′	121 bp	Miluchova *et al.* (2013)

Table 3: Duration of thermo cycler for following standardized reaction programme.

Initial denaturation	94	05 min
Final denaturation	94	01 min
Annealing	58.1	01 min
Extension	72	01 min
Repeat cycle 2 to 4 for 35 times		
Final Extension	72	10 min

Table 4: In PCR-RFLP profile the following enzymes were used.

Casein Gene	Restriction Enzyme	Restriction Site	Base Pair	RFL Pproduct
β	*DdeI*	5′….G ↓ AATTC….3′	121bp	121bp
		3′….CTT ↑ AA G….5′		86 bp
	Hind III	5′….A ↓ AGCTT….3′	121bp	35bp
		3′….TTC ↑ GA A…5′		121bp

Table 5: Least squares analysis of variance for lactose (%) at β-casein (CSN2) gene locus in Malvi, Nimari, Sahiwal and H.F. crossbred cattle.

Source of variance	DF	MS	F-value
Genotypes	5	2.5039	10.09**
Error	194	0.2480	
Total	199		

** Highly significant (p<0.01).

Table 6: Least squares means for lactose (%) of different variants at β-casein (CSN2) gene locus in four breeds of cattle.

Variants	Breeds			
	Malvi	Nimari	Sahiwal	H.F. crossbred
A1A1	0.00±0.00 (00)	0.00±0.00 (00)	0.00±0.00 (00)	0.00±0.00 (00)
A1A2	0.00±0.00 (00)	0.00±0.00 (00)	5.19^{b}±0.13 (16)	5.33^{b}±0.09 (32)
A2A2	4.89^{c}±0.07 (50)	5.56^{a}±0.07 (50)	5.27^{b}±0.08 (34)	5.49^{ab}±0.08 (18)
Overall	4.89^{c}±0.07 (50)	5.56^{a}±0.07 (50)	5.25^{b}±0.11 (50)	5.39^{ab}±0.08 (50)

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

As shown in Table 3, the mean SNF (%) in A2A2 genotype of Malvi and Nimari was 8.03±0.11 and 8.84±0.13, respectively. The corresponding mean SNF(%) for A1A2 and A2A2 genotypes was 8.69±0.23 and 8.76±0.14 in Sahiwal and 8.55±0.11 and 8.51±0.14 in H.F. crossbred cattle. The mean SNF(%) between A1A2 and A2A2 genotype of Sahiwal and H.F. cross bred cattle did not differ significantly. Among all the four breeds of cattle, significantly lower SNF (%) was noticed in Malvi breed of cattle for A2A2 genotype (Table 8).

Density (kg/L) of different variants at β-Casein (CSN2)/ *DdeI* gene locus in four breeds of cattle

The results of analysis of variance have been presented in Table 9. The effect of genotypes was found significant (P<0.01) for density (kg/L) trait. The mean density (kg/L) in Malvi, Nimari, Sahiwal and H.F. cross bred cattle has been presented in Table 10.

The mean milk density (kg/L) for A1A2 genotype was ranged from 1.03±0.08 (Sahiwal) to 1.04±0.05 (H.F. cross bred) and for A2A2 genotype it was ranged from 1.03±0.07 (Malvi) to 1.05±0.06 (Nimari) among all the four breeds of cattle. Among all the genotypes, the higher milk density (kg/L) was noticed in A2A2 genotype of Nimari (1.05±0.06) cattle; whereas, it was significantly lower in A2A2 genotype of Malvi (1.03±0.07) breed of cattle (Table 10).

Photograph of RFLP of following 04 breeds

As per the Hardy- Weinberg law the result of RFLP revealed that the gene and genotypic frequencies of β-casein (CSN2) gene for A2A2 was 1.00 for both Malvi and Nimari breed of cattle but 0.00, 0.30 and 0.70 in Sahiwal and 0.00, 0.64 and 0.36 in HF crossbred cattle, respectively.

The mean lactose per cent was significantly higher in Nimari (5.56±0.07) as compared to Malvi (5.89±0.06) and Sahiwal (5.25±0.07). However, the mean lactose per cent in milk of Sahiwal and H.F. cross bred cattle showed non-significant difference (Table 2). Available literature did not reveal any traceable information on association of polymorphic variants with lactose per cent in dairy cattle.

The mean SNF per cent in Malvi, Nimari, Sahiwal and H.F. cross bred cattle were found to be 8.03±0.11, 8.84±0.13, 8.74±0.12 and 8.54±0.08 per cent, respectively. The mean SNF per cent of Malvi was significantly lower than Nimari, Sahiwal and H.F. cross bred cattle. The maximum SNF per cent was noticed in Nimari breed of cattle (Table 4). Our findings agree with the results of Szymanowska *et al.* (2004) who reported higher lactose (%) and SNF (%) for AA genotyped Polish Black and White cattle.

The mean density (Kg/L) was significantly higher in Nimari (1.04±0.09) as compared to Sahiwal and Malvi, while it was non-significantly higher than H.F. cross bred cattle (Table 6). Available literature did not reveal any traceable information on association of polymorphic variants with milk density (Kg/L) in dairy cattle.

Table 7: Least squares analysis of variance for SNF (%) at β-casein (CSN2) gene locus in Malvi, Nimari, Sahiwal and H.F. crossbred cattle.

Source of variance	DF	MS	F-value
Genotypes	5	3.9361	6.30**
Error	194	0.6243	
Total	199		

**Highly significant (p<0.01).

Table 8: Least squares means for SNF (%) of different variants at β-casein (CSN2) gene locus in four breeds of cattle.

Variants	Breeds			
	Malvi	Nimari	Sahiwal	H.F. cross bred
A1A1	0.00±0.00 (00)	0.00±0.00 (00)	0.00±0.00 (00)	0.00±0.00 (00)
A1A2	0.00±0.00 (00)	0.00±0.00 (00)	8.69^{a}±0.23 (16)	8.55^{a}±0.11 (32)
A2A2	8.03^{b}±0.11 (50)	8.84^{a}±0.13 (50)	8.76^{a}±0.14 (34)	8.51^{a}±0.14 (18)
Overall	3.40^{d}±0.78 (50)	4.77^{c}±0.14 (50)	5.77^{b}±0.14 (50)	7.62^{a}±0.39 (50)

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

Table 9: Least squares analysis of variance for density (kg/L) at β-casein (CSN2) gene locus in Malvi, Nimari, Sahiwal and H.F. cross bred cattle.

Source of variance	DF	MS	F-Value
Genotypes	5	159.17	15.37**
Error	194	10.35	
Total	199		

**Highly significant (p<0.01).

Table 10: Least squares means for density (kg/L) of different variants at β-casein (CSN2) gene locus in four breeds of cattle.

Variants	Breeds			
	Malvi	Nimari	Sahiwal	H.F. cross bred
A1A1	0.00±0.00 (00)	0.00±0.00 (00)	0.00±0.00 (00)	0.00±0.00 (00)
A1A2	0.00±0.00 (00)	0.00±0.00 (00)	1.03^{bc}±0.08 (16)	1.04^{a}±0.05 (32)
A2A2	1.03^{c}±0.07 (50)	1.05^{a}±0.06 (50)	1.03^{a}±0.08 (34)	1.04^{a}±0.05 (18)
Overall	1.03^{c}±0.07 (50)	1.04^{a}±0.06 (50)	1.03^{b}±0.08 (50)	1.04^{a}±0.06 (50)

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

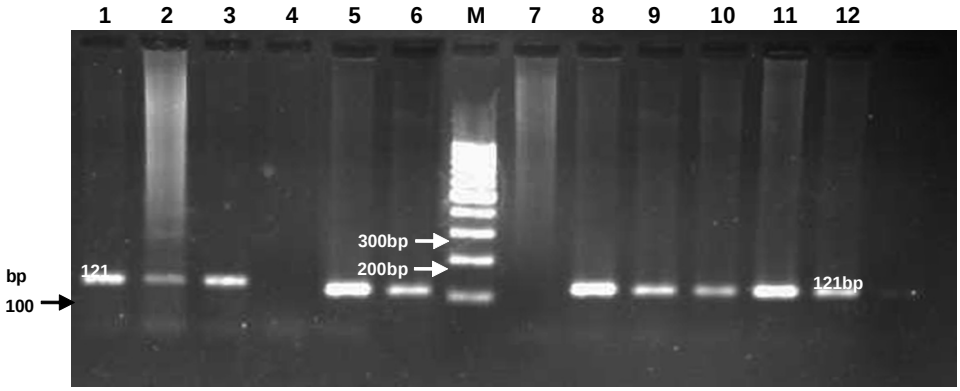


Plate 1: RFLP product of Beta (β) gene of Malvi cow, electrophoresed on 2.5% agarose gel, M : 100bp DNA ladder, Lanes : 1-12.

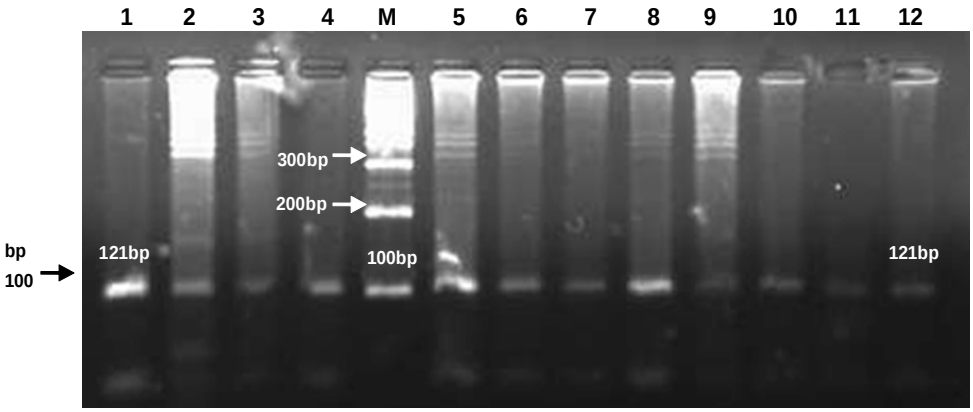


Plate 2: RFLP product of Beta (β) gene of Nimari cow, electrophoresed on 2.5% agarose gel, M : 100bp DNA ladder, Lanes : 1-12.

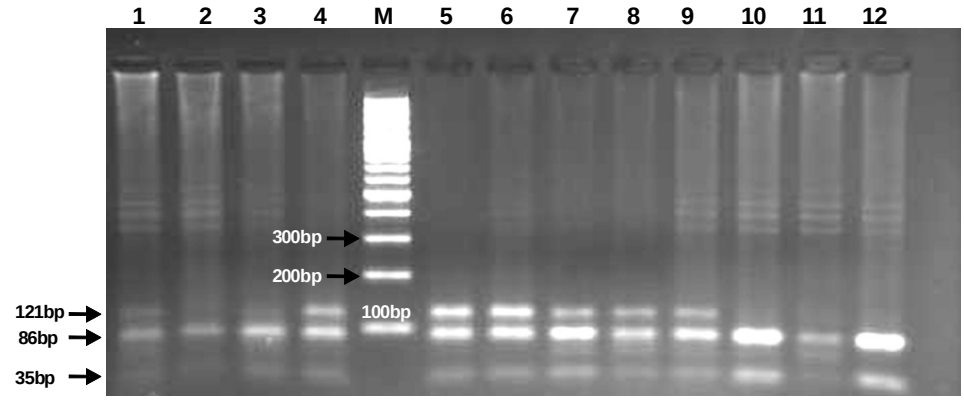


Plate 3: RFLP product of Beta (β) gene of H.F. cross bred cow, electrophoresed on 2.5% agarose gel, M : 100bp DNA ladder, Lanes : 1-12.

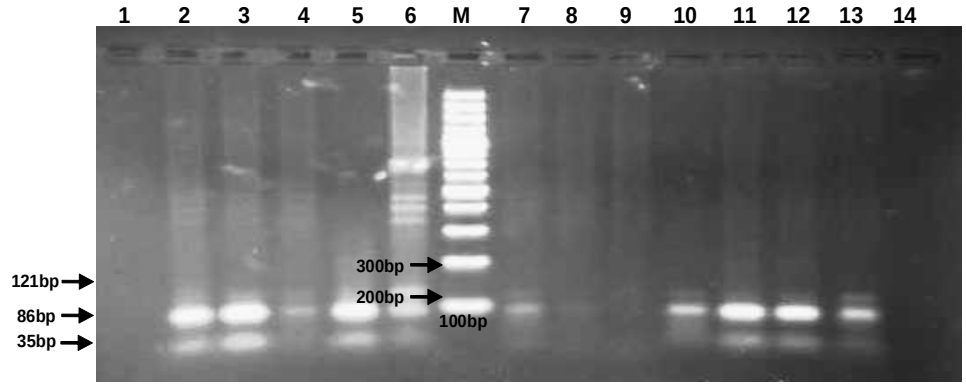


Plate 4: RFLP product of Beta (β) gene of Sahiwal cow, electrophoresed on 2.5% agarose gel, M : 100bp DNA ladder, Lanes : 1-14.

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