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Polymorphism of Beta (β) Casein Gene and Their Association with Milk Production Traits in Malvi and Nimari Breeds of Cattle

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ABSTRACT

Research work was carried out on 50 Malvi and 50 Nimari breed of cattle in the Department of Animal Genetics and Breeding of College of Veterinary Science and A.H Jabalpur. In present research work the PCR amplified products of 121bp was digested by restriction endonuclease enzyme DdeI, which recognizes G^AATTC sites. Present study showed that the presence of no restriction sites for the enzyme DdeI and only one band of 121bp was observed on the gel and such genotype was designated as A2A2 type. The β- casein gene showed A2A2 genotypes were observed in Malvi and Nimari breeds of cattle. 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. So 100% frequency of A2 allele was observed in both the breeds of cattle under the study.

Key words: Malvi, Nimari, Polymorphism, Beta (β) Casein Gene.

INTRODUCTION

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). Polymorphism in one of the beta-casein gene codons – CCT'® CAT – causes a substitution of Proline (A2) by Histidine (A1, B) at the position of 67th in the amino acid sequence. Association study reported that increase both protein yield and milk content as well as to decrease milk fat content and yield (Nilsen *et al*. 2009). 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), the 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.

The percentage of the A1 and A2 beta-casein protein varies between herds of cattle, and also between countries and provinces. While African and Asian cattle continue to produce only A2 beta-casein, the A1 version of the protein is common among cattle in the western world (Truswell, A.S. 2005). On average, more than 70 percent of Guernsey cows produce milk with predominantly A2 protein, while among Holsteins and Ayrshires between 46 and 70 percent produce A1 milk.

Further, the most frequently observed forms of beta-casein (β-Cn) in dairy cattle are A1 and A2. During the last few decades presence of 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).

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MATERIALS AND METHODS

The present research work was conducted on 100 lactating cows comprising 50 each of Malvi and Nimari breeds. The data was collected from the animals maintained at the following cattle breeding farms or from home tract of respective breeds of cattle.

Identification number along with the various desired parameters like Parity, Lactation length and Lactation yield 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. Collected samples are maintained in cold chain during transportation and in laboratory. In first phase of research the milk samples are processed for Protein (%), Fat (%), Lactose (%), SNF (%) and Milk density (Kg/L) analysis and they were analyzed by Milk analyzer of the Department of

Table 1: Details of experimental samples collection.

Breed	No. of Animals	Cattle breeding farms
Malvi	50	Cattle Breeding Farm Aagar, M.P
Nimari	50	Cattle Breeding Farm Rodiya, Khargon, M.P

Table 2: Published Primer.

1	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 standardized reaction programme in thermo cycler. (Mastercycler gradient, Eppendorf) .

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

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In second phase of research blood samples are processed and genomic DNA was extracted by modified John *et al*. (1991) method and then good samples was processed for PCR RFLP and sequencing. The β-casein gene primers were used for the amplification of PCR product as described in Table 2.

Digested PCR products were analyzed on 2.50% 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 for Milk yield (MY), Daily milk yield (DMY), Protein (%), Fat (%), Lactose (%), SNF (%) and Milk density (Kg/L) data were subjected to least squares analysis of variance employing linear model:

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).

RESULTS AND DISCUSSION

The PCR product of β-casein gene (CSN2) (121 bp) was not digested by HindIII restriction enzyme (RE) and resulted single compact band of 121 bp. All the screened animals of Mavi and Nimari, cattle were found to be monomorphic for β-casein gene. The RFLP band patterns obtained for β-casein gene locus in Mavi and Nimari, breeds was digested by restriction endonuclease enzyme DdeI. The patterns evolved in the present study showed that the presence of no restriction sites for this enzyme, only one band of 121bp was observed on the gel such genotype was designated as A2A2 type.

Gene and Genotypic frequencies at β-casein (CSN2) gene locus in different breeds of cattle

The result of RFLP revealed that gene and genotypic frequencies of β-casein (CSN2) gene for A2A2 was 1.00 for both of the breed Malvi and Nimari breed of cattle. So 100%

Table 4: Name of the following enzyme was used In PCR-RFLP profile.

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

Table 5: Distribution of gene and genotypic frequency of β -Casein gene (CSN2)/ *DdeI* variants in different breeds of cattle.

Breeds	Genotype Frequencies			Chi - square	Gene Frequencies	
	A1A1	A1A2	A2A2	(x^2) value	A1	A2
Malvi	0.00	0.00	1.00	0.00^{NS}	0.00	1.00
Nimari	0.00	0.00	1.00	0.00^{NS}	0.00	1.00

NS-Non-significant, **Significant (P < 0.01).

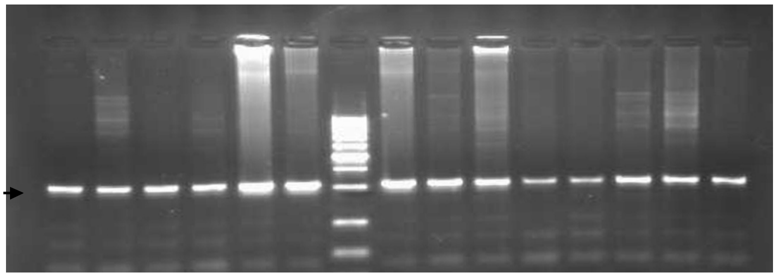

Plate 1: RFLP product of Beta (β) gene of Malvi cow, electrophoresed on 2.5% agarose gel, M : 100bp DNA ladder.

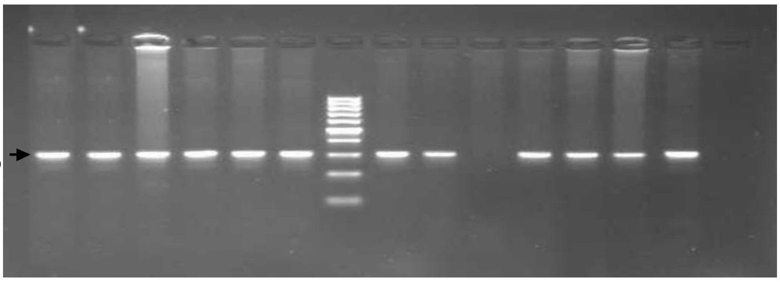

Plate 2: RFLP product of Beta (β) gene of Nimari, electrophoresed on 2.5% agarose gel, M: 100bp DNA ladder.

Table 6: Milk yield (L) of different variants at β-Casein (CSN2) gene locus in four breeds of cattle.

Variants	Breeds	
	Malvi	Nimari
A1A1	0.00±0.00(00)	0.00±0.00(00)
A1A2	0.00±0.0(00)	0.00±0.0(00)
A2A2	1035.10^{d}±36.40(50)	952.50^{d}±24.70(50)
Overall	1035.10^{d}±36.40(50)	952.50^{d}±24.70(50)

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

Table 7: Least squares means for DMY (L) of different variants at β-Casein (CSN2) gene locus in four breeds of cattle.

Strain	Breeds	
	Malvi	Nimari
A1A1	0.00±0.00(00)	0.00±0.00(00)
A1A2	0.00±0.00(00)	0.00±0.00(00)
A2A2	3.43^{d}±0.08(50)	4.77^{c}±0.14(50)
Overall	3.40^{d}±0.78(50)	4.77^{c}±0.14(50)

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

Table 8: Least squares means for LL (days) of different variants at β-Casein (CSN2) gene locus in Malvi and Nimari breeds of cattle.

Genotypes	Malvi	Nimari
A1A1	0.00±0.00(00)	0.00±0.00(00)
A1A2	0.00±0.00(00)	0.00±0.00(00)
A2A2	301.08^{b}±5.20(50)	202.42^{e}±3.67(50)
Overall	301.08^{b}±5.20(50)	202.42^{e}±3.67(50)

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

or highest frequency of A2 allele was observed in both the breeds of cattle under the study.

Hanusova *et al.* (2010) reported that the Slovakian bulls with frequencies of 0.20 and 0.80 for A1A1 and A1A2 genotypes, respectively while Malarmathi *et al*. (2014) reported three genotypes *viz*., A2A2: 0.37, A1A1: 0.17 and A1A2: 0.46 in Kangayam and HF crossbred cattle. The pure Kangayam (Bos indicus) cattle breed had only A2 gene and showed only A2A2 genotype, which produce safer A2 milk for the human consumption. The Holstein Friesian crossbred animals also showed mostly of A2 gene with the frequency 0.59. Ramesha *et al*. (2016) reported that the frequency of A1 allele was very low in Malnad Gidda (0.01), Kasargod variety (0.04) and Jersey (0.08), while the frequency of A1 allele in Holstein Friesian and HF crossbred male was 0.17 and 0.29, respectively.

Association of β -casein (CSN2)/*DdeI* gene polymorphic variants with Milk yield and milk composition traits

The polymorphic variants of β -casein gene (CSN2) in Malvi and Nimari breeds of cattle and their association with milk yield per lactation (L), daily milk yield (L), have been studied as below:

Milk yield (MY) of different variants at β -casein (CSN2)/ *DdeI* gene locus in two breeds of cattle

Only A2A2 genotype were observed in all the animals of Malvi and Nimari. The mean MY (L) for A2A2 genotype of Malvi and Nimari was 1035.10±36.40 and 952.50±24.70, respectively. As shown in Table 06, the A2A2 genotype of Malvi showed non- significantly higher MY than A2A2 genotype of Nimari.

Daily Milk yield (L) of different variants at β-Casein (CSN2) gene locus in two breeds of cattle

The mean DMY (L) for A2A2 genotype of Malvi and Nimari was 3.40±0.08 and 4.77±0.14, respectively as shown in Table 07, the DMY of A2A2 genotype of the Nimari was significantly higher than A2A2 genotype of Malvi..

Lactation length (days) of different variants at β-Casein (CSN2) gene locus in two breeds of cattle

The results of analysis of variance have been presented in Table 8. The effect of genotypes was found significant ($P<0.01$) for lactation length (LL) trait. The mean LL (days) in Malvi and Nimari breeds of cattle has been presented in Table 8.

The mean LL (days) for A2A2 genotype in Malvi and Nimari breed cattles were found to be 301.08±5.20 and 202.42±3.67days, respectively. As per Table 8, the significantly higher LL was noticed in A2A2 genotype of Malvi than Nimari breed of cattle.

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