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Association of Different Genetic Variants of Alpha S2-Casein Gene (CSN1S2) with Milk Production Traits in Cattle

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ABSTRACT

The present experiment has been planned to study the association of different genetic variants of αS2-Casein gene (CSN1S2) with lactation length (days), fat (%) and protein (%) in Malvi, Nimari, Sahiwal and HF crossbred cattle. The analysis of variance for different genotypes of αS2-casein gene in four breeds of cattle was found significant (P<0.01) for lactation length (LL) trait. Significantly higher LL was recorded in AA genotype of HF crossbred, while, the lowest LL was noticed in AB genotype of Nimari. The mean LL between AA and AB genotype of Malvi, Sahiwal and HF crossbred cattle showed non-significant difference. 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. The mean fat (%) between AA and AB genotypes showed non-significant difference in Malvi, Sahiwal and HF crossbred cattle but the mean protein per cent was significantly higher in Malvi and Nimari and non-significantly higher in HF crossbred for AB genotype as compared to AA genotype. Among the different genotypes of all four breeds of cattle, significantly higher protein per cent was recorded in Nimari for AB genotyped animals.

Keywords: Genotypes, Protein, Fat, Malvi, Nimari, Sahiwal, HF crossbred

The αs2-caseins are a highly phosphorylated peptide that occurs as different isoforms. So far, eight alleles have been identified, which are associated with different expression level of αs2-Cn (CSN1S2) in milk of dairy cattle. Out of these eight alleles, A, B, C, E and F are strongly associated with a normal content of the protein in milk. Intermediate D allele is associated with non-detectable amount of CSN1S2 milk (Ramunno *et al*., 2001). Milk protein genotypes have been correlated with many milk production traits (Winkelman and Wickham, 1996; Lunden *et al*., 1997; Kaygisiz and Dogan, 1999) and economic characteristics such as milk composition, cheese making traits (Savic *et al.*, 1996).

MATERIALS AND METHODS

Animals and milk production traits

The research work was carried out on 200 lactating cows comprising 50 each of Malvi Nimari, Sahiwal and HF crossbred cattle. The data and sample of Malvi collected from the Government Cattle Breeding Farm, Agar, (MP) and Nimari from the Government Cattle Breeding Farm, Agar, (MP), Sahiwal from Livestock Farm, College of Veterinary Science and A.H., Anjora (Durg) and Govt. Sahiwal Breeding Farm, Anjora (Durg) and HF crossbred from Livestock Farm, College of Veterinary Science and A.H., Jabalpur and Private Dairy Farm at Pariyat, Jabalpur.

Identification number, Parity, Lactation length and Lactation yield of each animal under study, were recorded. About 100ml milk sample from each cow are 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. The genotyping was done after DNA isolation using PCR-RFLP technique as per standard protocol (Sambrook and Russell, 2001). Gene and genotype frequencies for different casein genes under study were estimated using Popgene 32 (version 1.32), microsoft Windows-based freeware for population genetic analysis, Yeh *et al. (*1999).

Association study of various polymorphic variants of αS2-Casein gene for lactation length (days), Protein (%) and Fat (%) data were subjected to least squares analysis of variance employing following linear model:

$$Yijk = \mu + Bi + Gj + (BXG)\,ij + eijk$$

Where,

Yijk - is the observed value of milk production trait

μ - is the population mean

Bi - is the fixed effect of breed

Gj - is fixed effect of genotypes (k = 1, 2….)

(*BXG*)*ij* - is interaction effect of Breed and genotypes

eijk - is random error effect

RESULTS AND DISCUSSION

Amplification and restriction digestion of αs2-casein gene (CSN1S2)

The αS2-casein (CSN1S2) gene of 1267bp was amplified using a specific primer pairs (CSN1S2/start F: 5′-TATGACATGTCGAGAAATGAG -3′ and CSN1S2/ stop R: 5′- TTGGAACAATGCTATTAGGTT -3′; Szymanowska *et al*., 2003). PCR amplification was carried out in a total volume of 25 µl as per the standard protocol (Sambrook and Russell, 2001). 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 60.0ºC for 1 min and extension at 72ºC for 1 min, and a final extension at 72ºC for 10 min. PCR-RFLP/ *EcoRV* assay was carried out as per standard protocol and electrophoresed in 2.5% agarose gel (Sambrook and Russell, 2001). 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 of CSN1S2 gene of each individual sample.

PCR-RFLP's of αs2 casein gene using EcoRV

The PCR amplified products of 1267 base pair length were digested by *EcoRV*, which recognizes GAT^ATC sites. The 1267bp product was cut into two fragments of sizes 1150bp and 117bp (Fig. 1). Absence of restriction site at both the alleles that resulted in the appearance of single compact bands of size 1267bp was referred to as genotype AA. The samples exhibiting three fragments (1267bp/ 1150bp/ 117bp) were denoted as genotype AB. The RFLP analysis carried out in all the four breeds of cattle revealed dissimilar genotypic patterns (Fig. 1). AA and AB genotypes were observed in Malvi, Nimari and HF crossbred animals, whereas, only AA genotype was observed in all the tested Sahiwal cattle.

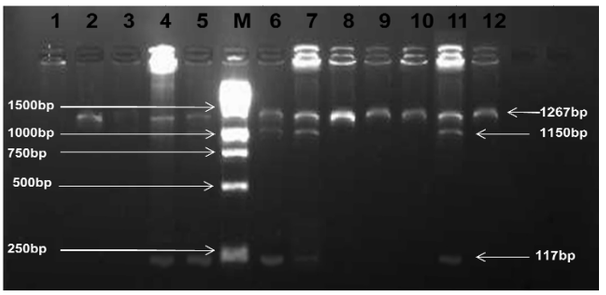

Fig. 1: PCR-RFLP/ *EcoRV* assay of αS2 gene showing genotype pattern in 2.5% agarose gel. M: 1000bp DNA ladder, Lanes: 2,3,8,9,10,12 (AA genotype, 1267 bp); Lanes: 4,5,6,7,11 (AB genotype, 1267, 1150, 117 bp)

Lactation length (days) of different variants at αS2-casein gene (CSN1S2) locus in four breeds of cattle

The effect of genotypes was found significant (P<0.01) for lactation length, fat (%) and protein (%) trait. The mean lactation length (LL), fat (%) and protein (%) trait in Malvi, Nimari, Sahiwal and HF crossbred cattle has been presented in table 1.

The significantly higher LL (Days) was recorded in AA genotype of HF crossbred (337.70±13.6), while the lowest LL was noticed in AB genotype of Nimari (193.05±4.19). The mean LL between AA and AB genotype of Malvi, Sahiwal and HF crossbred cattle showed non-significant difference, however AA genotype showed slightly higher LL in these breeds of cattle (Table 1). As shown in table 1, the higher mean fat (%) was noticed for AA genotype

Table 1: Least squares means for lactation length (days), fat (%) and protein (%) in four breeds at αS2-Casein (CSN1S2) gene locus

Traits	Breed	Genotypes		
		AA	AB	Overall Mean (50)
LL (days)	Malvi	$302.18^{b} \pm 9.00$ (22)	$300.21^{b} \pm 6.16$ (28)	$301.08^{b} \pm 5.20$
	Nimari	$208.16^{d} \pm 5.12$ (31)	$193.05^{d} \pm 4.19$ (19)	$202.42^{d} \pm 3.67$
	Sahiwal	$267.86^{c} \pm 6.41$ (50)	0.00 ± 0.00 (00)	$267.86^{c} \pm 8.89$
	HFC	$337.70^{a} \pm 13.60$ (29)	$318.62^{ab} \pm 9.76$ (21)	$329.70^{a} \pm 5.65$
Fat (%)	Malvi	$2.95^{a} \pm 0.19$ (22)	$2.77^{ab} \pm 0.18$ (28)	$2.85^{a} \pm 0.13$
	Nimari	$3.21^{a} \pm 0.21$ (31)	$2.77^{ab} \pm 0.21$ (19)	$3.04^{a} \pm 0.16$
	Sahiwal	$3.23^{a} \pm 0.15$ (50)	0.00 ± 0.00 (00)	$3.23^{a} \pm 0.15$
	HFC	$1.84^{c} \pm 0.12$ (29)	$2.29^{bc} \pm 0.34$ (21)	$2.03^{b} \pm 0.16$
Protein (%)	Malvi	$3.23^{d} \pm 0.06$ (22)	$3.45^{c} \pm 0.06$ (28)	$3.36^{b} \pm 0.05$
	Nimari	$3.60^{bc} \pm 0.05$ (31)	$3.95^{a} \pm 0.15$ (19)	$3.74^{a} \pm 0.07$
	Sahiwal	$3.59^{bc} \pm 0.05$ (50)	0.00 ± 0.00 (00)	$3.59^{a} \pm 0.05$
	HFC	$3.68^{b} \pm 0.06$ (29)	$3.70^{b} \pm 0.07$ (21)	$3.69^{a} \pm 0.04$

Means bearing the different superscript differ significantly ($p<0.01$), Numbers in the parentheses denotes number of animals, LL- lactation length, HFC-Holstein Friesian crossbred.

than AB genotype of Malvi and Nimari; however in HF crossbred higher fat (%) was observed for AB genotype. The mean fat per cent between AA and AB genotypes showed non-significant difference in Malvi, Sahiwal and HF crossbred cattle.

The mean protein per cent was significantly higher in Malvi and Nimari and non-significantly higher in HF crossbred for AB genotype as compared to AA genotype (Table 1). Among the different genotypes of all four breeds of cattle, significantly higher protein per cent was recorded in Nimari (3.95±0.15) for AB genotyped animals (Table 1). The present findings are in agreement with results of Szymanowska *et al.* (2004) who reported that the heterozygous genotype showed higher protein (%) in Polish Black and White cattle.

CONCLUSION

At αs2 gene locus, AA genotype was found superior for LL in HF crossbreds. AA genotype of Sahiwal and Nimari was found superior for fat (%), while higher protein per cent were recorded for AA genotype of Nimari.

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