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Correlation and regression study of different variants of beta (β) case in gene with lactation length in Sahiwal and HF crossbred cattle

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Abstract
Background: β-casein is a primary protein in milk, and its variants have been associated with changes in the protein content of bovine milk. However, there has been little research focused on the effects of β-casein variants on milk. The major protein found in ruminant's milk is Beta-casein (CSN2), which is encoded by a CSN2 gene that was mapped on chromosome 6. 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 cattle had 11 allelic variants of the gene encoding it, A1, A2, and B alleles being the most frequent.
Method: Research work was carried out on 50 Sahiwal and 50 HF Crossbred cattle in the department of Animal Genetics and Breeding College of Veterinary Science & A.H. Jabalpur. During the research work the PCR amplified products of 121bp was digested by restriction endonuclease enzyme DdeI, which recognizes G^AATTC sites.
Result: The patterns showed that the presence of one restriction site on one alleles and absence of restriction site on other alleles resulted in the appearance of three bands of 121, 86 and 35bp. The name of above genotypic pattern was referred to as A1A2. In another case both strand had no restriction sites for the enzyme, so only one band of 121bp was observed on the gel and such genotype was designated as A2A2 type. So in β- casein gene both A2A2 and A1A2 genotypes were observed in Sahiwal and HF crossbred cattle. The genotypic frequencies of β-casein (CSN2) gene for A1A1, A1A2 and A2A2 are 0.00, 0.30 and 0.70 in Sahiwal and 0.00, 0.64 and 0.36 in HF crossbred cattle, respectively and the gene frequency A1 and A2 is 0.15 and 0.85 in Sahiwal and 0.32 and 0.68 in HF crossbred cattle. High frequency of A2 allele was observed in both the breeds of cattle under the study. Association studies with lactation length revealed that significantly higher LL was noticed in A2A2 genotype of HF crossbred among both breeds of cattle's. The higher LL was also noticed in A2A2 genotyped animals as compared to A1A2 genotyped animals of Sahiwal and HF crossbred cattle.

Keywords: Sahiwal, HF crossbred, polymorphism, beta (β) casein gene

Introduction
The major protein found in ruminant's milk is Beta-casein (CSN2), which is encoded by a CSN2 gene that was mapped on chromosome 6. CSN2 gene is highly polymorphic with at least 13 genetic variants known until now (Farrel *et al*. 2004) [3]. Study of the β-casein polymorphism at the protein level showed that cattle had 11 allelic variants of the gene encoding it, A1, A2, and B alleles being the most frequent.
Further, 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. During the last few decades presence of A1 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) [1, 10]. 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) [4]. Variants of casein genes have also been reported to be associated with milk yield and composition (Cardak, 2005 and Mir *et al*. 2014) [2, 5].

Materials and Methods

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) [9] and analyzed by using MEGA 6 software (Tamura *et al.*, 2004) [8]. Aligned sequences were analyzed for group specific SNP marker.

Statistical analysis

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) [11].

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 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

Testing Hardy-Weinberg (H-W) equilibrium

The chi-square test (x^2) was employed to test the status of Hardy-Weinberg equilibrium in the different population of four breeds of cattle (Snedecor and Cochran, 1994) [7].

Regression between various traits (Milk yield and Milk composition traits) for different genotypes, breeds wise.

To find out the association between the polymorphic variants/ genotypes of, β -casein genes with milk production traits like, Milk yield (MY), Daily milk yield (DMY), Protein (%), Fat (%), Lactose (%), SNF (%) and Milk density (Kg/L) in of Sahiwal and HF crossbred cattle by linear regression model was employed.

Results & Discussion

Lactation length (LL in days) of different variants at β-Casein (CSN2)/*DdeI* gene locus in Sahiwal and HF crossbred cattles

The results of analysis of variance raveled that the effect of genotypes was significant ($P<0.01$) for lactation length (LL) trait.

The mean LL (days) for A2A2 genotype in Sahiwal and HF crossbred cattle were found to be 281.03±5.65 and 342.80±19.10 days, respectively. The A1A2 genotype was observed only in Sahiwal and HF crossbred cattle with their respective LL of 239.90±13.90 and 322.31±8.89 days. As shown in table 01, the significantly higher LL was noticed in A2A2 genotype of HF crossbred among both breeds of cattles. The higher LL was also noticed in A2A2 genotyped animals as compared to A1A2 genotyped animals of Sahiwal and HF crossbred cattle. In agreement of above findings Ozdemir and Dogru (2005) [6], reported that the AB genotype of Brown Swiss and HF cattle had higher lactation length than AA genotype.

Table 1: Least squares means for LL (days) of different variants at β-Casein (CSN2) gene locus in four breeds of cattle

Variants	Sahiwal	HF crossbred
A1A1	0.00±0.00 (00)	0.00±0.00 (00)
A1A2	239.90^{d}±13.90 (16)	322.31^{a}±8.89 (32)
A2A2	281.03^{c}±5.65 (34)	342.80^{a}±19.10 (18)
Overall	267.86^{c}±6.41 (50)	329.70^{a}±8.93 (50)

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

Table 2: Regression analysis of Sahiwal breed of beta (β) Casein Gene

Parameters	Mean Value	Genotype A1A1	Genotype A1A2	R-sq	F-Value	P-Value
Lactation Yield (Ly)	1151.10	-78.1	78.1	7.12	3.68	0.05
Daily milk yield (DMY)	6.06	-0.82	0.82	61.52	76.73	0.00
Lactation Length (LL)	260.45	20.58	- 20.58	18.28	10.74	0.00
Fat	3.26	- 0.069	0.069	0.38	0.18	0.67
Protein	3.57	0.0287	-0.0287	0.62	0.30	0.58
Lactose	5.237	0.0367	0.0367	0.49	0.24	0.69
SNF	8.73	0.031	-0.031	0.12	0.06	0.83
Density	33.58	0.269	-0.269	0.61	0.30	0.589

Regression analysis revealed that significant but negative regression of A1A1 genotype with the lactation yield and Daily milk yield but Lactation Length showed positive and significant regression value with A1A1 genotype where as A1A2 genotype showed positive and significant regression value with Lactation Yield (Ly) and Daily milk yield (DMY) but significantly negative value with Lactation Length (LL).

Table 3: Regression analysis of hf-cross breed of beta (β) Casein Gene

Parameters	Mean Value	Genotype AA	Genotype AB	R-sq	F-Value	P-Value
Lactation Yield (Ly) Vs. β	2326.70	-503.80	503.80	34.76	25.58	0.000
Daily milk yield (DMY) Vs. β	7.110	-1.815	1.815	40.11	32.15	0.000
Lactation Length (LL) Vs. β	332.57	10.26	-10.26	2.49	1.22	0.274
Fat Vs. β	1.998	-0.128	0.128	1.22	0.59	0.445
Protein Vs. β	3.6870	-0.0114	0.0114	0.12	0.06	0.810
Lactose Vs. β	5.4118	0.0777	-0.0777	2.31	1.13	0.292
SNF Vs. β	8.5300	-0.0178	0.0178	0.08	0.04	0.842
Density Vs. β	35.807	0.019	-0.019	0.01	0.00	0.955

As per the above mentioned table regression analysis revealed that significant and positive regression of A1A1 genotype with the lactation yield and Daily milk yield but Lactation Length and other remaining parameters showed non-significant regression value with A1A1 genotype where as A1A2 genotype showed negative but significant regression value with Lactation Yield (Ly) and Daily milk yield (DMY) and other remaining parameters showed non-significant regression value.

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