

Association of Beta-lactoglobulin Polymorphism with Milk Production Traits in Cattle

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ABSTRACT : The study was carried out in Sahiwal, Holstein Friesian, Jersey and crossbred cattle to find out the effect of genotype of beta-lactoglobulin gene on milk production traits. The polymorphism at beta-lactoglobulin gene was identified by conducting PCR-RFLP studies. A 398 bp fragment of the gene was amplified and digested with *Hae* III restriction enzyme. The two alleles A and B and three genotypes AA, AB and BB were identified in all cattle breeds. The frequency of B allele was comparatively higher than that of A allele. The AA genotype produced significantly higher milk yield in Sahiwal cattle whereas BB genotype yielded higher milk in Holstein Friesian cattle. In other cattle breeds the genotypic effect was non-significant. In conclusion it may be stated that the genotype with significantly higher milk yield may be favoured in the farm along with other conventional selection criteria to enhance the milk production of animals. (*Asian-Aust. J. Anim. Sci.* 2003, Vol 16, No. 11 : 1560-1564)

Key Words : Association, Beta-lactoglobulin Gene, Milk Production Trait, Polymorphism

INTRODUCTION

Genetic improvement by selection based on breeding value provides enormous potential to enhance the performance of animals. But, it is a time consuming exercise as generation interval of cattle is very high and we have to wait for selection till the expression of desirable traits. Besides, for milk yield as sex limited traits, it is not possible to select male animal on the basis of its own performance. To minimize these problems, selection based on markers can play an immense role to carry out genetic improvement of animals before expressing actual target traits. Sometimes, before birth selection can also be made based on the marker value, which in turn is called as marker assisted selection (MAS). MAS gives a new hope to the animal breeders, which when used in conjunction with conventional selection methods can prove to be extremely advantageous (Bhattacharya and Gandhi, 1997). Hence, to search out such a marker, the possible way is to go for candidate gene approach.

Beta-lactoglobulin, a milk whey protein constitutes about 14% of the total milk protein and 53% of the total whey protein (Kuzdal-Savoie et al., 1980). It is the first protein in which polymorphism was detected at protein level (Aschaffenburg and Drewry, 1955). The polymorphism, which may be SNP and if detected at nucleotide level, could be exploited if associated with some economic traits. The possible significant association may be used as the basis to get genetic marker for the economic traits. For example, beta-lactoglobulin locus was reported to have significant influence on cheese yield indicating the higher yield by heterozygotes (Aleandri et al., 1990). Jairam

and Nair (1983) revealed that cows with beta-lactoglobulin AB genotype had lower age at first calving. Weights at birth to 12 months of age were also found to be influenced by beta-lactoglobulin loci (Singh et al., 1981). However, Ng-Kwai-Hang et al. (1990) found no association of milk protein types with days to attain first breeding, days open and number of service per conception. Keeping these reports about significant association of beta-lactoglobulin polymorphism with different economic traits in view, the investigation was undertaken to explore possible association of beta-lactoglobulin polymorphism with milk production traits in cattle.

MATERIALS AND METHODS

Experimental animals

The study was carried out on 164 cattle of three different categories namely *Bos taurus*, *Bos indicus* and crossbred. Taurine cattle included 39 Jersey and 32 Holstein Friesian maintained at Bull Mother Farm, Lucknow and Cattle and Buffalo farm, IVRI, Izatnagar, respectively. Indicine breed included 31 Sahiwal cattle maintained at LRC, Pantnagar and 62 crossbred animals maintained at from Cattle and Buffalo farm, IVRI, Izatnagar.

Collection of sample

About 10-15 ml venous blood was collected, under sterile conditions, from the jugular vein of the animals into a sterile 50 ml polypropylene vial containing 0.5 ml 0.5 M EDTA as anticoagulant. After collection of blood, tubes were capped and shaken gently to facilitate thorough mixing of blood with anticoagulant. Then, blood samples were kept immediately in ice-box containing gel cool packs and were transported to the laboratory without delay.

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Collection of data

The data on animal No.; date of birth; date of first, second and third calving; first, second and third lactation milk yield; first, second and third lactation length were collected from daily farm register maintained at the farms. These data were used for statistical analysis.

DNA isolation

Genomic DNA was isolated from blood samples following phenol-chloroform extraction method described by Sambrook et al. (1989). After isolation DNA pellet was dissolved in water and was kept in water bath at 60°C for 2 h to inhibit Dnase activity and to dissolve pellet properly in water. Then DNA was cooled and stored at -20°C for further use.

Quality of DNA was checked under spectrophotometry by taking ratio O.D. at 260 and 280 nm. The samples lying in the range of O.D. ratio between 1.7 and 1.9 were considered as good and used for further study. The samples beyond this range were re-extracted with phenol-chloroform extraction method. The quantity of DNA was estimated using the formula. DNA ($\mu\text{g/ml}$) = OD 260 \times 50 \times Dilution factor

PCR amplification

A 398 bp fragment of beta-lactoglobulin gene spanning over part of exon IV and intron IV (104 bases of exon IV and 294 bases of intron IV) was amplified using a pair of forward (5'-CGAGAACAAAGTCCTTGTGCT-3') and reverse (5'-CCGGT AACAAAGGCTGTTAGA-3') primers. A total of 25 μl reaction volume containing 100-200 ng DNA template, 20 pM of each primer, 100 μM each dNTP, 1.5 mM MgCl_2 , 1 U Taq DNA polymerase, 10 \times PCR assay buffer (Bangalore Genei Pvt. Ltd.) was set up for each individual animal. A negative control, containing all reaction components except DNA was also made to check any contamination of foreign DNA.

PCR reaction was carried out in PTC-200 programmable thermal cycler (MJ Research Inc., USA). The cycling conditions for DNA amplification were 34 cycles of denaturation at 95°C for 30 sec, annealing at 58°C for 90 sec and extension at 72°C for 2 min followed by final extension at 72°C for 5 min. The PCR product was kept at 4°C in the refrigerator till further use.

RE digestion

The 398 bp amplicon was treated with *Hae* III enzyme to identify RFLPs of beta-lactoglobulin gene. Ten μl PCR product was digested with 10 U *Hae* III enzyme and 10 X digestion buffer at 37°C for overnight. The reaction was stopped by adding 0.5 M EDTA.

Electrophoresis

The digested product was electrophoresed in 4% w/v agarose gel at 50 V for 3 h at 4°C temperature. The gel was stained with ethidium bromide (0.5 $\mu\text{g/ml}$), visualized under UV-transillumintor and finally documented in the gel doc system.

Statistical analysis

Gene and genotype frequency along with S.E. was estimated as per the method described by Falconer (1998). The effect of genotype on milk yield per day of first lactation length (MY/FLL), milk yield per day of first three lactation length (MY/TLL) was tested by analysis of variance using linear models (Snedecor and Cochran, 1967). For milk yield per day of first lactation length, the effect of genotype was checked with the linear model.

$$Y_{ijkl} = \mu + G_i + S_j + A_k + E_{ijkl}$$

where, Y_{ijkl} = l^{th} Observation of the target trait

μ = Overall mean

G_i = Fixed effect of i^{th} genotype

S_j = Fixed effect of j^{th} season of calving

A_k = Fixed effect of k^{th} age at first calving

E_{ijkl} = Random error with NID (0, σ^2_e)

Here, season was classified into three groups i.e. summer (March to July), rainy (August to September) and winter (October to February). The age at first calving for Sahiwal cattle was divided into three groups i.e. upto 1,300 days, 1,300 to 1,600 days and more than 1,600 days and for Jersey cattle into two groups i.e. upto 1,000 days and more than 1,000 days. For milk yield per day of first three lactation length, to see the effect of genotype the following linear model was used,

$$Y_{ij} = \mu + G_i + E_{ij}$$

where, Y_{ijkl} = l^{th} Observation of the target trait

μ = Overall mean

G_i = Fixed effect of i^{th} genotype

E_{ijkl} = Random error with NID (0, σ^2_e)

Besides, chi-square test was performed and all the animals were classified into two groups i.e. high producing and low producing.

RESULTS

Genotypes

The restriction digestion analysis of 398 bp fragment of beta-lactoglobulin gene indicated the presence of three types of restriction patterns. In the first pattern, two restriction sites resulting into three fragments 162, 137 and 99 bp were observed while in the second pattern four

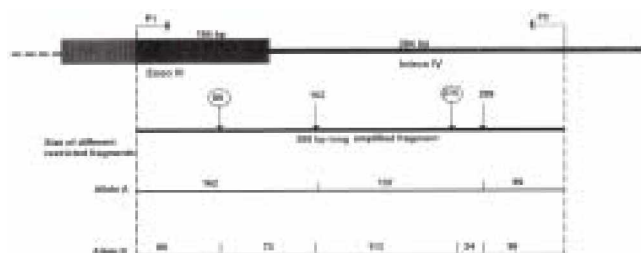


Figure 2. *Hae* III restriction map of 398 bp long β -Lg gene fragment in cattle.

restriction sites with five fragments 113, 99, 89, 73 and 24 bp were found. In the third pattern, six restriction sites, resulting into seven fragments 162, 137, 113, 99, 89, 73 and 24 bp was observed. The third pattern was in between the pattern first and second. Hence, the first pattern was assigned as genotype AA, second pattern as genotype BB and the third pattern as genotype AB (Figure 1). All the three genotypes were observed in each genetic group of cattle.

Gene and genotype frequency

Three types of genotypes AA, BB and AB were observed in cattle. The frequencies of AA, AB and BB genotype in Jersey cattle were 0.07, 0.23 and 0.70 while the magnitudes in Holstein friesian cattle were 0.12, 0.44 and 0.44. In Sahiwal cattle the genotype frequencies for AA, AB and BB were 0.13, 0.29 and 0.58 whereas in crossbred cattle these frequencies were 0.07, 0.29 and 0.64, respectively.

From the three groups of genotypes, two alleles A and B have been exerted. In Jersey, Holstein friesian, Sahiwal and crossbred cattle the frequencies of A allele were 0.19 ± 0.04 , 0.34 ± 0.06 , 0.27 ± 0.06 and 0.20 ± 0.04 whereas the frequencies of B allele were 0.81 ± 0.04 , 0.66 ± 0.09 , 0.73 ± 0.06 and 0.80 ± 0.04 , respectively.

Breedwise performance

Table 1 shows the performance of animals under different breed groups. The average milk yield per day of first lactation length varied from 4.32 ± 0.20 kg/day (Sahiwal) to 8.35 ± 0.18 kg/day (Holstein friesian) while the

Table 1. Breed-wise mean performance of animals for milk production

Breed	LY/FLL (kg/day)	LY/TLL (kg/day)
Jersey	6.52 ± 0.33	6.59 ± 0.31
Holstein	8.35 ± 0.18	9.30 ± 0.13
Friesian		
Sahiwal	4.32 ± 0.20	4.52 ± 0.16
Crossbred	7.35 ± 0.46	7.69 ± 0.48

value of MY/TLL ranged from 4.52 ± 0.16 kg/day (Sahiwal) to 9.30 ± 0.31 kg/day (Holstein friesian).

Effect of genotype on milk production traits

In Holstein friesian cattle, the effect of genotype on first lactation milk yield per day of first lactation length was non-significant. The trait values were ranging from 7.94 ± 0.27 (AB genotypes) to 8.99 ± 0.27 kg/day (BB). The MY/TLL was having a significant variation ($p \leq 0.01$) due to beta-lactoglobulin genotype in Holstein friesian cattle. The highest value was found for BB genotype (9.74 ± 0.20 kg/day) while AA genotype produced lowest yield (7.77 ± 0.18). In Sahiwal breed the genotypic effect on MY/FLL was significant ($p \leq 0.05$), where AA genotype produced highest yield of 4.70 ± 0.54 kg/day and BB genotype yielded the lowest (4.22 ± 0.25 kg/day). The MY/TLL in Sahiwal breed had got significant ($p \leq 0.05$) impact from the beta-lactoglobulin genotype. The AA genotype had produced the highest yield, 5.65 ± 0.43 kg/day while the genotype BB had the lowest performance, 4.24 ± 0.20 kg/day. In Jersey and crossbred animals, the genotype effects on MY/FLL and MY/TLL were non-significant (Table 2). In Jersey, the MY/FLL value was ranging from 5.01 ± 0.06 kg/day (AA genotype) to 6.80 ± 0.35 kg/day for BB genotype. In crossbred cattle, AB genotype possessed highest magnitude of milk yield (8.17 ± 0.85 kg/day) while lowest value of MY/FLL was observed for BB genotype. For MY/TLL in Jersey cattle, BB genotype yielded highest quantity of milk yield (6.82 ± 0.39 kg/day) while the least value was recorded for AA genotype. However, in crossbred cattle, the highest yield was observed for AB genotype and the lowest MY/TLL (7.43 ± 0.71 kg/day) was produced by BB genotype.

Table 2. Genotype-wise average performance of animals

Traits	Genotype	Breeds			
		Jersey	Holstein friesian	Sahiwal	Crossbred
MY/FLL (kg/day)	AA	5.01 ± 0.06	8.08 ± 0.50	$4.70 \pm 0.54^*$	7.52 ± 0.80
	AB	6.43 ± 0.61	7.94 ± 0.27	$4.61 \pm 0.36^*$	8.17 ± 0.85
	BB	6.80 ± 0.35	8.99 ± 0.27	$4.22 \pm 0.25^*$	7.02 ± 0.54
MY/TLL (kg/day)	AA	5.07 ± 0.17	$7.77 \pm 0.18^{**}$	$5.65 \pm 0.43^*$	7.77 ± 0.81
	AB	6.49 ± 0.68	$9.28 \pm 0.20^{**}$	$5.19 \pm 0.29^*$	8.38 ± 0.85
	BB	6.82 ± 0.39	$9.74 \pm 0.20^{**}$	$4.24 \pm 0.20^*$	7.43 ± 0.71

* Significance at $p \leq 0.05$. ** Significance at $p \leq 0.01$.

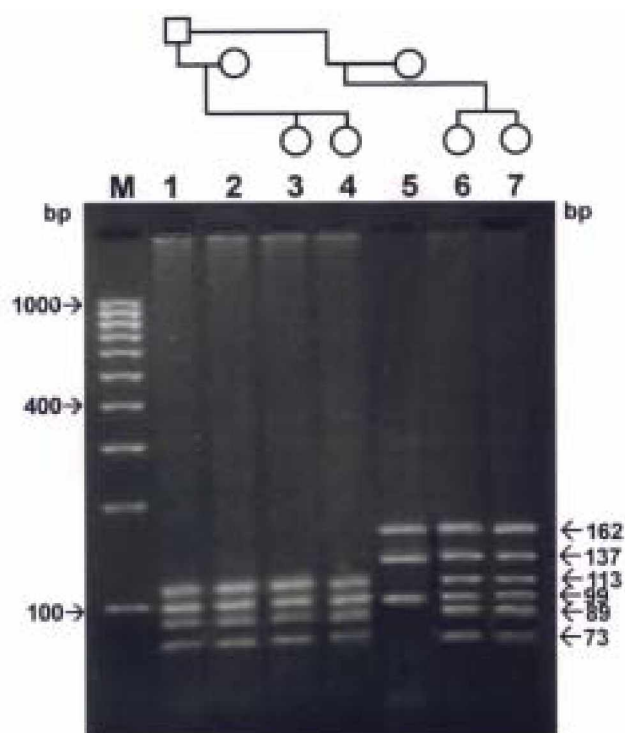


Figure 1. Genotyping of Shival half sib family. Lane M: Molecular size marker (100 bp DNA ladder), lane 1: Sire-S (BB genotype), lane 2: Dam-D1 (BB genotype), lane 3: Offspring 1 of S and D1 (BB genotype), lane 4: Offspring 2 of S and D1 (BB genotype), lane 5: Dam-D2 (AA genotype), lane 6: Offspring 1 of S and D2 (AB genotype), lane 7: Offspring 2 of S and D2 (AB genotype).

DISCUSSION

Hae III restriction digestion of 398 bp fragment revealed two restriction site at 162nd and 299th position producing three fragments. This enzyme has restriction-recognition sequence GG'CC. But, in allele B the restriction sites of *Hae* III were on 90th, 275th positions besides the restrictions sites for A alleles. In the whole 398 bp fragment one restriction site is present in the exon (first 104 bases) and others are in introns (Figure 2). The only B allele has enzyme-cutting site on exon (90th) where nucleotide T has been substituted by C. The presence of nucleotide T in the codon encodes the amino acid alanine. Thus, T/C substitution in the nucleotide has produced two variants of polypeptide differed by substitution of valine with alanine which is present at amino acid position 118 of beta-lactoglobulin polypeptide (Eigel et al., 1986, Alexander et al., 1989). The other nucleotide substitution in introns has no direct effect on polypeptide composition but it may have an impact on the expression of beta-lactoglobulin gene. Sometimes, intronic variation have effect on production of total quantity of mRNA which after

all translated into beta-lactoglobulin protein. Thus, intronic mutation, may be called as silent mutation, regulates the transcription of the whole gene.

The genotypic frequency was highest for genotype BB followed by AB and AA in all the cattle breeds except Holstein friesian where the ferquency of BB and AB were same. Out of all the cattle breeds, Holstein friesian and Sahiwal revealed comparatively higher AA genotype frequency. The gene frequency of allele B was highest in all the cattle population while the frequency of allele A was lowest. The frequency of allele B was comparatively higher than that of allele A. If we see breed-wise differences, the frequency of B allele was the highest in Jersey followed by crossbred, Sahiwal and Holstein friesian. Our results were in agreement with the findings reported by Zhou et al. (1996) in Holstein friesian cattle and Kim et al. (1997) in Hanwoo cattle.

Milk yield per day of first lactation length in Sahiwal cattle had significant association with genotype where AA genotype produced relatively much more milk than AB or BB genotypes. But, Jairam and Nair (1983) could not find any significant association between beta-lactoglobulin polymorphism and milk yield. However, the absence of *Hae* III cutting site at both the homologous chromosome indicating AA genotype may have certain role either during the process of transcription of the gene or the time of post-transcriptional modification. As this polymorphic site regulates the quality and quantity of milk production, nucleotide T/C substitution at the position 90th in the 398 bp fragment of beta-lactoglobulin may be used as genetic marker for milk yield in Sahiwal cattle. Earlier this type of candidate gene approach was also attempted to find the genetic marker for birth weight in cattle by Biswas et al. (2003). Thus, in Sahiwal cattle AA genotype may be favoured in the farm to get better first lactation milk yield.

As far as first three lactation total milk yield per day of total three lactation length is concerned, the significant effect of genotype was observed both in Holstein friesian and Sahiwal cattle. In Holstein friesian, BB genotype produced significantly higher milk than AA or AB genotype. In Sahiwal cattle, AA genotype yielded much more milk than AB or BB genotype. It is a fact that traits are mostly breed specific within a species. However, the presence or absence of *Hae* III cleavage site at 90th position in the 398 bp fragment of beta-lactoglobulin gene may play significant role during the process of transcription and thus, regulate the quality and quantity of beta-lactoglobulin protein present in milk. Hence, the particular genotype having significant association with milk yield in particular cattle may be favoured in the farm along with other selection criteria to enhance the productivity. This will be a kind of indirect selection, which can be made at the very early age to reduce the generation interval. Culling of animals can be

done on the basis of this marker study along with conventional selection criteria.

In conclusion, it may be stated that all the cattle breeds were polymorphic for beta-lactoglobulin gene producing two types of alleles and three types of genotypes. The frequency of B allele was comparatively higher in all the cattle breeds. The genotype AA produced significantly higher milk in Saliwal cattle whereas genotype BB yielded higher in Holstein Friesian cattle depicting the breed specific nature of the association. Hence, these genotypes may be favoured in the farm along with other conventional selection criteria to enhance the milk production of animals.

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