

A Comparison on Polymorphism of Beta-lactoglobulin Gene in *Bos indicus*, *Bos taurus* and *Indicine*×*Taurine* Crossbred Cattle

S. Badola, T. K. Bhattacharya*, T. K. Biswas, B. M. Shivakumar, Pushendra Kumar and Arjava Sharma

Animal Genetics Division, Indian Veterinary Research Institute, Izatnagar, Bareilly, U.P.-243 122, India

ABSTRACT : The study was carried out on eight *Bos indicus* cattle breeds namely, Sahiwal, Tharparkar, Nimari, Khilari, Deoni, Amritmahal, Haryana and Hilly cattle; two *Bos taurus* cattle breeds namely, Jersey and Holstein Friesian and *Indicine*×*Taurine* crossbred cattle to find out the polymorphic pattern of beta-lactoglobulin gene. The polymorphism at beta-lactoglobulin gene was detected by conducting PCR-RFLP studies on 398 bp fragment spanning over 104 bases of exon IV and 294 bases of intron IV. Two alleles A and B and three genotypes AA, AB and BB were observed in all the cattle breeds. The frequency of B allele was comparatively higher than that of A allele. The allelic frequency of A varied from 0.20 to 0.30 in *Bos indicus* cattle breeds and 0.19 to 0.34 in *Bos taurus* breeds while in crossbred cattle the frequency was estimated as 0.21. The weighted frequency of A allele was highest in Indian cattle and lowest in crossbred cattle while the frequency in taurine cattle was found to be in between indicus and crossbred cattle. The non-significant differences of allelic frequency amongst *Bos indicus*, *Bos taurus* and crossbred cattle was observed. The effect of genotype on fat percentage was also found to be non-significant in cattle. (*Asian-Aust. J. Anim. Sci.* 2004. Vol 17, No. 6 : 733-736)

Key Words : Beta-lactoglobulin, *Bos indicus*, *Bos taurus*, Cattle, Crossbred, Fat Percentage, Gene, Polymorphism

INTRODUCTION

An estimate of 5 to 10% of the whole genome constitutes structural loci in mammals. There is an extensive polymorphism present in the structural genes, where most of the polymorphisms have two alleles with codominant expression (Biswas et al., 2003). There is a wide range in allelic frequencies of different structural genes, but frequencies at extreme values help in detecting polymorphic markers. Besides, allelic frequencies in a particular breed are not varying too much. The possible applications of polymorphism are characterization of a population, estimation of genetic divergence between population, understanding of evolutionary relationship amongst different population, detection of genetic markers for quantitative trait loci (QTL) etc.

Beta-lactoglobulin is considered as an important milk protein because of its high biological value. A number of techniques were adopted to detect polymorphism at structural loci, of which polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) is the most preferred one because of its simplicity, quickness, economical, very high repeatability and non-use of hazardous radioactive material. A PCR-RFLP of beta-lactoglobulin gene has been reported by a number of workers (Medrano and Aguilar-Cordova, 1990; Wilkins and Kuys, 1992) in *Bos taurus* cattle. But no reports in *Bos indicus* and crossbred cattle have been found in the literature. It is a fact that *Bos indicus* cattle has very high

milk fat % and possesses very good adaptability in extreme environments. Keeping these facts in mind, the present study was undertaken to detect the allelic frequency of beta-lactoglobulin gene in *Bos indicus*, *Bos taurus* and crossbred cattle, compare those frequencies amongst these three bovine populations and to estimate the association of genotypes with economic traits like milk fat percentage in different breeds of cattle.

MATERIALS AND METHODS

Animals

The study was carried out on *Bos indicus*, *Bos taurus* and *Indicine*×*Taurine* crossbred cattle. *Bos indicus* cattle breeds included 31 Sahiwal (LRC, Pantnagar), 42 Tharparkar (Central cattle Breeding farm, Lakhimpur-Kheri, U.P.), 30 Nimari (State Govt's Farm, Khandwa, M.P.), 30 Khilari (Farmers' herds, Dharward District, Karnataka), 30 Deoni (Deoni cattle Breeding Farm, Bidar, Karnataka), 25 Amritmahal (Farmers' herds, Maharashtra), 40 Haryana (Cattle and Buffalo Farm, IVRI, Izatnagar) and 20 Hilly cattle (Farmers' herds, Kumaon District, Uttaranchal) while *Bos taurus* cattle used for the study were 39 Jersey and 32 Holstein Friesian maintained at Bull Mother farm, Lucknow; Cattle and Buffalo farm, IVRI, Izatnagar, respectively. A total of 62 crossbred animals from Cattle and Buffalo farm, IVRI, Izatnagar were included for our study. The animals were selected randomly to achieve the goals under the present study.

Collection of blood and semen

Nearly 10 ml blood was collected from the jugular vein

* Corresponding Author: T. K. Bhattacharya. Tel: +91-581-2303382, Fax: +91-581-2303284, E-mail: tarun@ivri.up.nic.in
Received July 15, 2003; Accepted January 6, 2004

Table 1. Allelic and genotype frequency of beta-lactoglobulin gene in Indian, exotic and crossbred cattle

Breed	Genotype frequency			Allelic frequency		Average milk fat percentage with SE		
	AA	AB	BB	A	B	AA	AB	BB
<i>Bos indicus</i>								
Sahiwal	0.13	0.29	0.58	0.27	0.73	5.1±0.2	5.3±0.1	5.6±0.1
Tharparkar	0.10	0.22	0.68	0.21	0.79	-	-	-
Amrtmahal	0.08	0.30	0.62	0.23	0.77	-	-	-
Khillari	0.13	0.31	0.56	0.29	0.71	-	-	-
Hilly cattle	0.12	0.35	0.53	0.30	0.70	-	-	-
Deoni	0.09	0.21	0.70	0.20	0.80	-	-	-
Haryana	0.13	0.32	0.55	0.29	0.71	-	-	-
Nimari	0.12	0.30	0.58	0.27	0.73	-	-	-
<i>Bos taurus</i>								
Jersey	0.07	0.23	0.70	0.19	0.81	-	-	-
Holstein	0.12	0.44	0.44	0.34	0.66	3.3±0.2	3.7±0.1	3.6±0.1
<i>Friesian</i>								
<i>Indicine×Taurine</i>								
Crossbred	0.07	0.29	0.64	0.21	0.79	4.6±0.2	4.3±0.1	4.4±0.1

of the animals into 50 ml sterile polypropylene vial containing 1 ml 0.5 M EDTA. The vial was, then, shaken gently for thorough mixing of blood with anticoagulant. The samples were kept immediately in the icebox containing gel cool packs. About 4-5 mini straw containing frozen semen of each male animals were collected from the farms.

Isolation of genomic DNA and estimation of fat %

Genomic DNA was isolated from the blood using phenol-chloroform extraction method described by Sambrook et al. (1989) and from semen following the method of Lien et al. (1990). Then DNA pellet was dissolved in sterile triple distilled water and was kept in water bath at 60°C for 2 h to inhibit Dnase activity and to dissolve pellet properly in water. Fat percentage of milk samples was estimated by conventional Gerber method.

Checking quality and quantity of DNA

Quality of genomic DNA was checked by taking ratio of O.D. at 260 and 280 nm in the spectrophotometer. The samples having O.D. ratio between 1.7 and 1.9 were considered as good and used for PCR study. The quantity of DNA was estimated by spectrophotometry taking O.D. value at 260 nm.

PCR-RFLP

A 398 bp fragment of beta-lactoglobulin gene spanning over 104 bases of exon IV and 294 bases of intron IV was amplified with a couple of primers (5'-CGAGAA CAAAGTCCTTGTGCT-3' and 5'-CCGGT AACAAAGGC TGTTAGA-3'). A total of 25 µl reaction mixture containing 100-200 ng DNA template, 20 pM of each primer, 100 µM each dNTP, 1.5 mM MgCl₂, 1 U Taq DNA polymerase, 10× PCR assay buffer was set up for amplifying DNA in a PTC-200 thermocycler (M J research Inc., USA). PCR conditions

applied for 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.

A total of 10 µl PCR product was digested with 10 U Hae III enzyme at 37°C for overnight. The reaction was stopped by adding 2 µl 0.5 M EDTA. The digested product was electrophoresed in 4% w/v agarose gel at 50 V for 3 h at 4°C temperature. Then, the gel was stained with ethidium bromide (0.5 µg/ml) and documented under the Gel Documentation system. The size of DNA bands were determined from the gel Documentation system while analyzing with 100 bp DNA ladder.

Statistical analysis

Gene and genotype frequency was estimated as per the method described by Falconer (1998). The weighted average of gene frequency under different category was calculated following the standard method of Snedecor and Cochran (1967). The gene frequency between different groups were compared by Z test (Snedecor and Cochran, 1967). The effect of genotype on milk fat percentage was tested by chi-square test. The genetic divergence amongst different groups was calculated following the method of Nguyen et al. (1972).

RESULTS AND DISCUSSION

Genotypic pattern

The restriction digestion analysis of 398 bp fragment of beta-lactoglobulin gene indicated the presence of three types of restriction patterns. In first pattern, three fragments 162, 137 and 99 bp were observed while in the second pattern five fragments 113, 99, 89, 73 and 24 bp were found. The third pattern produced seven fragments 162, 137, 113,

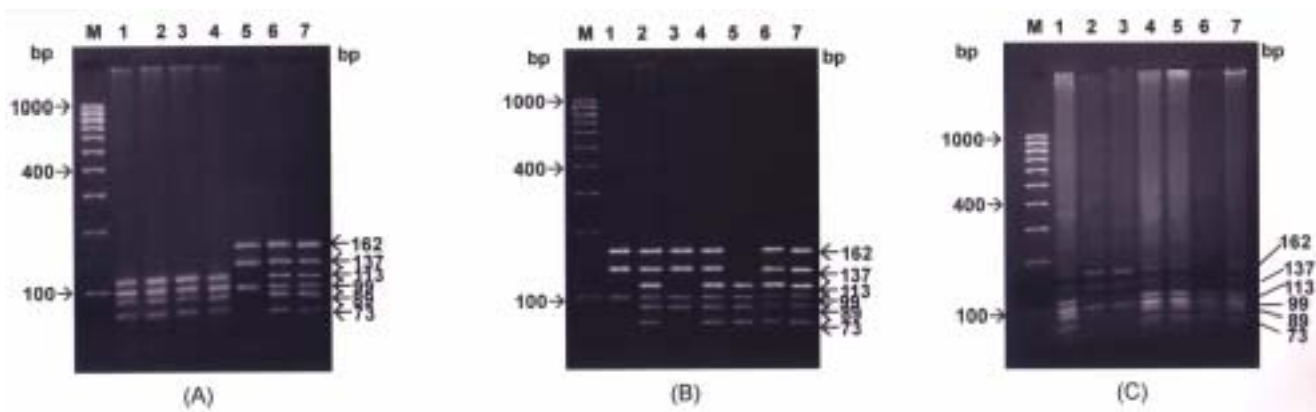


Figure 1. Genotypic patterns of beta-lactoglobulin gene in *Bos indicus* (A), *Bos taurus* (B) and crossbred cattle (C). In figure A, the lanes 1, 2, 3 and 4 were genotype BB, lane 5 was genotype AA and lanes 6 and 7 were genotype AB. In figure B, the lane 5 was genotype BB, lanes 1 and 3 were genotype AA and lanes 2, 4, 6 and 7 were genotype AB. In figure C, the lane 6 was genotype BB, lanes 2 and 3 were genotype AA and lanes 1, 4, 5 and 7 were genotype AB. Lane M in all the figures was 100 bp DNA ladder marker.

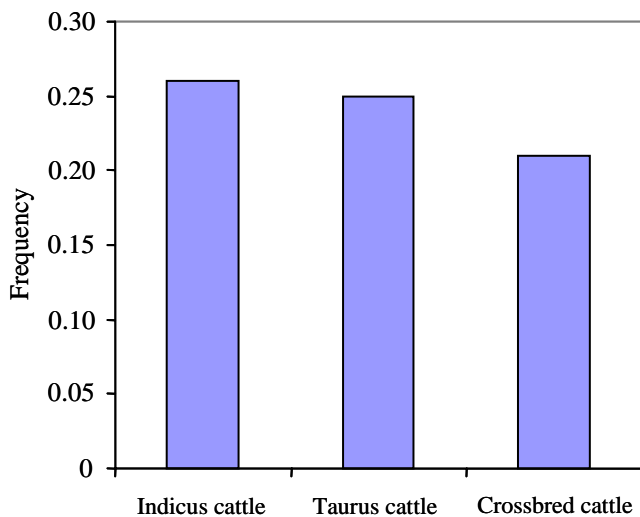


Figure 2. Mean frequency of 'A' allele in different groups of cattle.

99, 89, 73 and 24 bp, which was the coupling of first and second pattern, in other word it is a heterozygote (Figure 1). Hence, the first pattern was assigned as genotype AA, second pattern as genotype BB and the third as genotype AB. In genotype AA two restriction sites were found to be present at 162nd and 299th bases of the fragment while in genotype BB four restriction sites were observed at 89, 162, 275 and 299th bases of the beta-lactoglobulin gene fragment. However, in the whole 398 bp fragment, one restriction site was present in the exon (first 104 bases) and others were found to be in intron. Only B allele had cleavage site at exon (89th) where nucleotide 'T' has been substituted by 'C'. Literature says that the presence of nucleotide 'T' in the codon encodes the amino acid, alanine while the presence of nucleotide 'C' encodes the amino acid, valine. Thus, T/C substitution in the gene was predicted to produce two variants of polypeptide differed by substitution

of valine with alanine, which was confirmed by the protein sequence data reported by Alexander et al. (1989).

Gene and genotype frequency

Three types of genotypes, AA, BB and AB and two types of alleles, A and B, were observed in all categories of cattle breeds. Perusal of Table 1 shows allelic and genotype frequencies distributed over different breeds of cattle. Frequency of AA genotype was the lowest while that of BB genotype was highest in all breeds except Holstein Friesian where frequencies of BB and AB were similar. Consequently, the frequency of A allele was found to be relatively lower than that of B allele, which were in close agreement to the results of earlier workers in *Bos taurus* cattle (Chung et al., 1992; Schlee et al., 1992). The allelic frequency of A varied from 0.20 to 0.30 in *Bos indicus* cattle breeds and 0.19 to 0.34 in *Bos taurus* breeds while in crossbred cattle the frequency was estimated as 0.21 (Table 1). Kim et al. (1997) reported frequency of A allele as 0.21 in Hanwoo cattle.

The weighted frequency of A allele was highest in Indian cattle and lowest in crossbred cattle while the frequency in taurine cattle was found to be in between indicus and crossbred cattle (Figure 2). Further classification of indicus breeds depicted that the frequency of A allele in Indian milch variety cattle (Sahiwal and Tharparker) was 0.23, in draught variety (Amritmahal, Khillari and Hilly) was 0.27 and in dual variety (Deoni, Haryana and Nimari) was 0.26. The statistical test indicated the non-significant differences of allelic frequency amongst *Bos indicus*, *Bos taurus* and crossbred cattle and the trend was found to be similar in three varieties of indicus breeds. The highest Nguyen's genetic divergence was found between indicus and crossbred cattle (0.1506) while the lowest estimate was revealed between indicus and taurus

cattle. The distance between taurus and crossbred cattle was estimated as 0.0096. Although, these divergence data is not consistent with the overall phenotypes of three groups of cattle, it may be true with respect to beta-lactoglobulin gene polymorphism. The divergence estimate based on just only one or few structural gene's polymorphism may give the rough idea of diversity between population, but the accuracy of estimate will be increasing while analyzing with both structural genes as well as repeat DNA sequences.

The beta-lactoglobulin genotypes had non-significant effect on first lactation milk fat percentage in Sahiwal, Holstein Friesian and crossbred cattle. However, genotypic trends for the yield of fat percentage were different in indicine, taurine and crossbred cattle. The reports on association of beta-lactoglobulin genotypes on fat percentage in cattle were found to be scanty, on the otherhand, Badola et al. (2003) reported significant effect of beta-lactoglobulin genotype on first lactation milk yield in taurine and indicine cattle. Thus, in conclusion, it may be stated that the allelic frequencies differed non-significantly in *Bos indicus*, *Bos taurus* and crossbred cattle and the effect of genotype on fat percentage was non-significant in all the cattle population under study.

ACKNOWLEDGEMENT

Authors are thankful to the Director, IVRI for providing necessary facilities to conduct this research work. We also extend our gratitude to all the Incharges of different farms for giving blood samples. Financial assistance to the first author in the form of I.C.A.R. Junior Research Fellowship is highly acknowledged.

REFERENCES

- Alexander, L. J., G. Hayes, W. Bawden, A. F. Stewart and A. G. Mackinlay. 1993. Complete nucleotide sequence of the bovine beta-lactoglobulin gene. *Anim. Biotechnol.* 4:1.
- Badola, S., T. K. Bhattacharya, T. K. Biswas, P. Kumar and A. Sharma. 2003. Association of beta-lactoglobulin polymorphism with milk production traits in cattle. *Asian-Aust. J. Anim. Sci.* 16:1560-1564.
- Biswas, T. K., T. K. Bhattacharya, A. D. Narayan, S. Badola, Pushpendra Kumar and A. Sharma. 2003. Growth hormone gene polymorphism and its effect on birth weight in cattle and buffalo. *Asian-Aust. J. Anim. Sci.* 16:494-497.
- Chung, E. R., W. T. Kim and C. S. Lee. 1998. DNA polymorphism of k-casein, beta-lactoglobulin, growth hormone and prolactin genes in Korean cattle. *Asian-Aust. J. Anim. Sci.* 11:422-427.
- Falconer, D. S. 1998. *Introduction to Quantitative Genetics*. Ed 4th, Addison Wesley Longman Ltd. Essex, England.
- Kim, J. H., H. D. Shin, S. W. Han, B. C. Sang and Y. S. Won. 1997. Polymorphism of kappa-casein and beta-lactoglobulin genes using the polymerase chain reaction in Hanwoo (Korean native) cattle. *Kor. J. Anim. Sci.* 39:481-488.
- Lien, S., S. Rogne, M. J. Brovold and P. Alestrom. 1990. A method of isolation of DNA from frozen (A.I.) bull semen. *J. Anim. Breed. Genet.* 107:74.
- Sambrook, J., E. F. Fritsch and T. Maniatis. 1989. *Molecular Cloning: A laboratory Manual*. Ed 2nd, Vol 3. Cold Spring Harbour Laboratory Press, New York.
- Medrano, J. F. and E. Aguilar-Cordova. 1990. Polymerase chain reaction amplification of bovine beta-lactoglobulin genomic sequences and identification of genetic variants by restriction fragment length polymorphism analysis. *Anim. Biotechnol.* 1:73.
- Nguyen, T. C., L. Morera, D. Llanes and P. Leger. 1992. Sheep blood polymorphism and genetic divergence between French Rambouillet and Spanish Merino: Role of genetic drift. *Anim. Genet.* 23:325-332.
- Schlee, P., O. Rottmann and L. Alderson. 1992. Sheep beta-lactoglobulin determination of alleles A and B by PCR and RFLP analysis using plucked hair as a source of DNA. *Genet. Conserv. Livest.* 2:243-246.
- Snedecor, G. W. and W. G. Cochran. 1967. *Statistical Methods*. 6th edn. Oxford & IBH Publishing Co. India.
- Wilkins, R. J. and Y. M. Kuys. 1992. Rapid beta-lactoglobulin genotyping of cattle using polymerase chain reaction. *Anim. Genet.* 23:175-178.