

Effect of Alpha-lactalbumin Gene Polymorphism on Milk Production Traits in Water Buffalo

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ABSTRACT : A genetic study was conducted to elucidate the effect of alpha-Lactalbumin (α -LA) gene polymorphism on milk production traits involving total milk yield and daily milk yield during first lactation in two breeds of water buffaloes namely, Murrah and Bhadawari. Single strand conformation polymorphism (SSCP) was carried out to explore genetic polymorphism present at this locus. For this study, exon 1 region of α -LA was analyzed. Finally, polymorphism data was associated with milk production traits by employing least square analysis. In Murrah buffalo, five genotypes such as AB, BB, BC, CC and CD and four alleles A, B, C and D were detected whereas in Bhadawari buffalo two genotypes namely, AB and BC and three alleles namely, A, B and C were found. Genotypes showed significant effects ($p \leq 0.05$) on total milk yield and daily milk yield in Bhadawari buffalo but had non-significant effects on these traits in Murrah buffalo. (*Asian-Aust. J. Anim. Sci.* 2006. Vol 19, No. 3 : 305-308)

Key Words : Alpha-lactalbumin, Milk Production Traits, Polymorphism, Water Buffalo

INTRODUCTION

Alpha-lactalbumin is major whey protein present in buffalo milk constituting 18% of total whey protein, which comprises approximately 3.5% of the total milk protein. Chemically, α -LA is a primary constituent of lactose synthase enzyme, which is mainly responsible for the synthesis of milk lactose (Kuhn et al., 1980). Lactose has been the principal osmole present in milk and finally, it has been hypothesized that the amount of lactose synthesized is directly proportional to the milk volume (Kuhn et al., 1980). McFadden et al. (1989) reported that mammary explant in Holstein Friesian produced concentration of α -lactalbumin 30 fold higher than those of Angus explant when cultured in presence of insulin, cortisol and prolactin. α -lactalbumin has also reported to have a role in induction of cell growth inhibition or apoptosis in tumor cells and immature cells (Hakansson et al., 1995).

α -Lactalbumin gene is poorly studied at molecular level, in buffalo. Few works are available in cattle, goat, sheep etc., but very few studies are reported in buffaloes. However, polymorphism of alpha-lactalbumin protein has been studied by Blumberg and Tombs as early as in 1958. Polymorphism specifically denotes the variation present at particular loci. The implication of polymorphism study in farm livestock is enormous. The most important one is exploration of genetic marker for specific trait. Besides, other applications of such study are characterization of population, evolutionary study, estimation of genetic diversity and finally, clustering of population with regard to genetic similarity. Two genetic variants of α -LA such as A

and B have been reported in cattle by several workers (Blumberg and Tomb, 1958; Osterhoff and Pretorius, 1966; Chianese et al., 1988). In European cattle, mostly B variant was reported to be predominant (Blumberg and Tomb, 1958). However, scientists has reported the presence of the A variant in South African cows of European descent (Osterhoff and Pretorius, 1966). Both B and A variant have been found in *Bos indicus* and the droughtmaser (*B. indicus* \times *B. Taurus*) (Blumberg and Tomb, 1958; Bhattacharya et al., 1963; Bell et al., 1970). A third variant namely, C, which has lower mobility than A and B, was found in Bali cattle (*B. javanicus*) (Bell et al., 1981). Until now, there is no evidence of this third allele neither in *B. taurus* nor in *B. indicus*.

In cattle, alpha-lactalbumin polymorphism has been reported to be genetically associated with some economic traits. Bleck and Bermel (1993) revealed that α -lactalbumin (+15) A variant was associated with greater milk, protein and fat yields while α -lactalbumin (+15) B allele is related to higher proteins and fat percentage in dairy cow. It was found that heifers having α -lactalbumin genotype BB were usually lower age at first calving than genotype AA (Jairam et al., 1983). The present investigation was undertaken to detect polymorphism at alpha-lactalbumin gene and to estimate the effect of polymorphism on milk production traits.

MATERIALS AND METHODS

Sample

Blood samples were collected randomly from 50 Murrah and 48 Bhadawari buffaloes maintained at Cattle and Buffalo farm, IVRI, Izatnagar and Bhadawari Buffalo farm, Etawah, U.P., India. Genomic DNA was extracted

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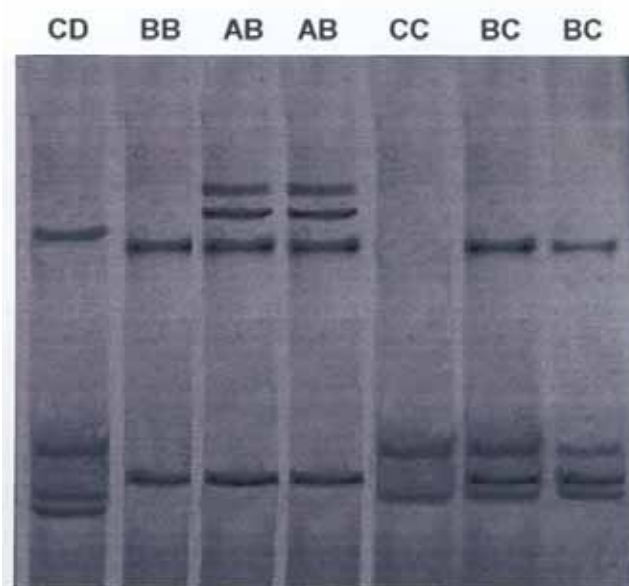


Figure 1. SSCP genotypes of alpha-lactalbumin gene in Murrah buffalo.

from 5 ml blood by phenol/chloroform extraction method (Bhattacharya et al., 2003).

PCR amplification

A 133 bp fragment of alpha-lactalbumin gene encompassing whole exon 1 region was analyzed for detection of polymorphism present at this locus. For amplification of this fragment, primers were designed by aligning cattle and sheep sequences with DNASIS Max software (Hitachi Genetic System, Miraibio Inc., USA) and the primers designed for this study was forward, 5'-ATGATGTCCTTTGTCTTCCT-3' and reverse 5'-ATTCAGGCAAACCTGACACCT-3'. The optimum PCR condition used was 100 ng of genomic DNA, 40 ng of each primer, 1.5 mM of MgCl₂, 100 μM of each dNTP, 1×PCR reaction buffer and 1 U of *Taq* DNA polymerase (MBI Fermentus) in a total volume of 25 μl. The PCR programme followed for amplification was initial denaturation for 2 min at 95°C, then 34 cycles of denaturation for 30 s at 95°C, annealing for 60 s at 60°C and extension for 30 s at 72°C followed by final extension for 5 min at 72°C.

Single strand conformation polymorphism (SSCP)

A total volume of 3 μl of PCR product was properly mixed with 15 μl formamide dye (95% Formamide, 0.025% Xylene cyanol, 0.025% Bromophenol blue, 0.5 M EDTA). The product was denatured at 95°C for 5 min and snapped cool on ice for 15 min. Finally, mixture was run on 12% native PAGE (50:1, Acrylamide and Bis-acrylamide) with 5% glycerol. The electrophoresis was performed at 4°C temperature for 14 h at 200 V. Finally, gel was stained with

silver nitrate to visualize the banding patterns (Basam et al., 1991).

Sequencing

PCR products belonging to different genotypes were run on 1% low melting agarose gel and the desired product was eluted from the gel using gel elution kit (GIBCO BRL) for purification. The purified PCR-products were sequenced following the automated dye-terminator cycle sequencing method with Ampli *Taq* DNA polymerase in ABI PRISM 377 DNA sequencer (Perkin-Elmer).

Statistical analysis

Gene and genotype frequencies were calculated by gene counting method described by Falconer and Mackay (1998). Sequence comparison was performed with "DNASIS MAX" software (Hitachi Genetic System, Miraibio Inc., USA). A general linear model (LSML91 Harvey programme) incorporating factors like season, sire and genotype as fixed effects was employed to estimate the effect of genotypes on milk production traits.

RESULTS

Polymorphism

In Murrah buffalo, five genotypes namely, AB, BB, BC, CC and CD and four alleles A, B, C and D were observed (Figure 1). The frequencies were estimated as 0.10 for AB, 0.04 for BB, 0.68 for BC, 0.12 for CC and 0.06 for CD genotype. The frequencies of A, B, C and D allele were observed to be 0.05, 0.43, 0.49 and 0.03, respectively. SSCP study in Bhadawari buffalo revealed the presence of two genotypes namely, AB and BC and three alleles namely, A, B and C at this locus. The frequencies of AB and BC genotypes and A, B and C alleles in this breed of buffalo were estimated as 0.917 and 0.08, and 0.46, 0.50 and 0.04, respectively.

Nucleotide sequence

The alleles, A (Accession no. AY726609) and C (Accession no. AY726611) were found to be differed from B (Accession no. AY726610) and D (Accession no. AY726612) by having the presence of adenine residue instead of guanine at 95th position of nucleotide sequence. As a result, amino acid, lysine (AAA) was observed to be present in A and C allele instead of arginine (AGA). B allele had guanine and cytosine residue instead of two adenine, which was present in alleles A, C and D at 103rd and 104th position of nucleotide sequence. It leads to change in polypeptide sequence by changing lysine (AAG) to alanine (GCG). Thymine was present instead of guanine at 115th position of nucleotide sequence in C allele due to which glycine (GGU) was replaced by cysteine (UGU) in the

Table 1. Least square means of milk yield traits in different buffalo breeds (TY = Total milk yield; DY = Daily milk yield; different superscripts indicate significance at 5% level)

Genotype	Bhadawari		Murrah	
	TY (kg)	DY (kg/day)	TY (kg)	DY (kg/day)
AB	785±67 ^a	2.62±0.18 ^a	1,806±237	5.72±0.67
BB	-	-	2,143±483	5.98±1.36
BC	1,098±217 ^b	3.38±0.57 ^b	1,979±203	5.64±0.57
CC	-	-	1,655±252	4.73±0.77
CD	-	-	2,077±353	4.10±0.99

mature alpha-lactalbumin polypeptide. D allele had guanine instead of thymine at 35th position of nucleotide sequence. Consequently, conversion of isoleucine (AUC) residue to serine (AGC) was determined to be present at the polypeptide sequence.

Effect of genotype

Genotypes had non-significant effect on total milk yield and daily milk yield in Murrah buffalo whereas, in Bhadawari they showed significant effect ($p \leq 0.05$) on total milk yield and daily milk yield. Animal having BC genotypes had a higher average milk yield than the animals with AB genotypes in Bhadawari breed (Table 1).

Discussion

Present study revealed that BC genotype was found to be predominant in Murrah buffalo whereas AB genotype was predominant in Bhadawari buffalo. In consequence, it has been estimated that B allele was predominant in Bhadawari buffalo whereas C allele was predominant in Murrah breed. D allele which was present only in Murrah buffalo was observed to be absent in Bhadawari buffalo. So presence of D allele may be the population characteristics of Murrah buffalo. However, in yak monomorphic pattern of alpha-lactalbumin protein was observed by Mao et al. (2004). While considering other milk protein polymorphism like beta-lactoglobulin in cattle, Badola et al. (2004) revealed the polymorphic nature of allelic distribution depicting genetic variability of the milk protein locus. Thus, this kind of information can be used for characterization of breed and estimation of genetic divergence amongst different breeds.

Various alleles have mutation at different position of nucleotide sequence. Some mutation was silent where as other expressed themselves by changing the amino acid sequence. The change in amino acid sequence is the principal factor involved in changing the structure and function of polypeptides/proteins. In case of exon 1 fragment, allele A, B, C and D had amino acid variability at codon 12th, 32nd, 35th and 39th. It has been observed that 12th as well as 39th codon consist of neutral amino acids while 32nd codon encodes basic amino acid. But, 35th codon contained both neutral as well as basic amino acid where

allele B had neutral amino acid, alanine and allele A, C and D showed the presence of basic amino acid, lysine. As there is a lot of variability present in the constitution of polypeptide in different alleles with various groups of amino acids, it may be predicted that there may be every possibility of changing in secondary/tertiary structure of polypeptides/proteins. Consequently, functions of proteins/polypeptides may be expected to be differed from allele to allele. If mutation occurs in exon, it may influence the biological activity through changing amino acid composition in the polypeptides and ultimately affect various traits.

In our study, it was found that in Bhadawari breed genotypes showed significant effect ($p \leq 0.05$) on milk yield whereas in Murrah breed it was observed to be non-significant. In Bhadawari buffalo BC genotype gave 40% more total lactation milk yield than the animals with AB genotype. In case of daily milk yield, superiority of BC genotype was 29% over AB genotype. However, in both the traits BC showed superior performance to AB genotype. Bleck and Bermel (1993) also revealed that α -lactalbumin (+15) A variant was significantly associated with greater milk yield in dairy cow. Finally, it may be concluded that bubaline alpha-lactalbumin gene was found to be polymorphic and having significant effect on milk production trait in Bhadawari breed of water buffalo.

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