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SSCP typing of alpha-lactalbumin and beta-lactoglobulin gene and its association with milk production and constituent traits in Indian riverine buffalo

VIKAS VOHRA[1], SHANKAR DAYAL[2] and TARUN KUMAR BHATTACHARYA[3]

Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh 243 122 India

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ABSTRACT

A study was conducted to explore genetic polymorphism in alpha-lactalbumin and beta-lactoglobulin gene and to elucidate the effect of polymorphism on milk production and constituent traits in Indian riverine buffaloes namely, Murrah, Bhadawari, Mehsana and Surti. Single strand conformation polymorphism (SSCP) was conducted on exon II of alpha-lactalbumin and exon I of beta-lactoglobulin gene. The study on alpha-lactalbumin gene revealed the presence of a number of genotypes namely, AB, AC, BB, BC, BD and BE in Mehsana; AC, BB, BC, BD, BE and CC in Surti; AC, BB, BC, BE and CC in Bhadawari and BC, BE, CC and CD in Murrah buffalo. In beta-lactoglobulin gene, genotypes like AA, BB, CC, AB and AC were observed in Murrah buffalo; AA, BB, AB and AC in Mehsana; AA, BB, CC, AB and AC in Surti and BB, CC, AB and AC in Bhadawari buffalo. All the alleles were sequenced and found to be differed at different locations. The alpha-lactalbumin genotypes had significant effect ($P<0.05$) on fat%, daily fat yield, total fat yield, SNF%, daily solid not fat and total solid not fat yield in Murrah buffalo; whereas, in Surti breed total milk yield, daily solid yield, total solid, daily solid not fat, total solid not fat yield, daily and total protein yield were significantly associated with the genotypes. The beta-lactoglobulin genotypes showed significant effect ($P<0.05$) on daily milk yield in Murrah, Bhadawari and Mehsana buffalo. In all the breeds, BB genotypes produced highest daily milk yield. In Murrah, Bhadawari and Mehsana breed, genotypes AA, CC and AC were associated with lowest production.

Key words: Alpha-Lactalbumin, Beta-Lactoglobulin, Buffalo, Milk Constituent, Milk Yield, Polymorphism, SSCP

In cattle, genetic variants of alpha-lactalbumin such as A and B were reported earlier (Blumberg and Tombs 1958, Osterhoff and Pretorious 1966, Chianese *et al.* 1988) of which B variant is predominant in *Bos taurus* (Blumberg and Tombs 1958). Variant A is reported in South African cows (Osterhoff and Pretorious 1966). In *Bos indicus* and the drought master (*B. indicus* × *B. taurus*) both B and A variant were reported (Blumberg and Tombs 1958, Bhattacharya *et al.* 1963, Bell *et al.* 1970) whereas C, a third variant, was reported in Bali cattle (Bell *et al.* 1981). DNA study on alpha-lactalbumin gene depicted presence of polymorphic sites or SNPs at this gene in cattle (Mao 1994, Cosenza *et al.* 2003, Kazmer *et al*. 2001). A variant was found associated with greater milk, protein and fat yields while alpha-lactalbumin (+15) B allele with higher proteins and fat% in dairy cow (Bleck and Bermel 1993). Heifers having alpha-lactalbumin genotype BB were usually found associated with lower age at first calving than genotype AA (Jairam and Nair 1983).

Medrano *et al. (*1990) and Wilkins and Kuys (1992) reported A *Hae III* PCR-RFLP on partial exon IV and intron IV region of beta-lactoglobulin gene. Polymorphism in partial exon IV and intron IV indicated a valine/alanine substitution in amino acid residue 118 (Wilkins and Kuys 1992) which influences total milk protein% and fat% in taurine cattle (Bovenhuis *et al.* 1992). Badola *et al.* (2004) reported beta-lactoglobulin gene polymorphism in Indian cattle. Beta-lactoglobulin locus was reported to have significant influence on cheese yield (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 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. Thus, the present study was confined to detect polymorphism at alpha-lactalbumin and beta-lactoglobulin gene by SSCP typing and to estimate the effect of polymorphism on different milk production and constituent traits in riverine buffalo.

Present address: [1]Senior Scientist, (vohravikas@gmail.com), National Bureau of Animal Genetic Resources, Karnal, Haryana.

[2]Senior Scientist, ICAR Research Complex for Eastern Region, Patna.

[3]National Fellow, Project Directorate on Poultry, Rajendra Nagar, Hyderabad, Andhra Pradesh.

MATERIALS AND METHODS

Collection of sample: The study was carried out on 196 Indian riverine buffaloes comprising 50 Murrah, 48 Bhadawari, 48 Mehsana and 50 Surti lactating buffaloes maintained at different organized herds located in different parts of India (LPM Section, IVRI, Uttar Pradesh; Bhadawari farm, Etawah (Uttar Pradesh); S.K. Nagar, GAU, Gujarat; Surti farm, Anand, GAU; Gujarat). Sample was taken randomly from each herd for the present endeavor. Genomic DNA was prepared from 5 ml blood by phenol/ chloroform extraction method (Sambrook and Russel 2001).

Milk samples and analysis: Approximately 50 ml milk was collected twice each, first during 30 to 50 days and next during 180 to 200 days from each animal. The total protein (TP) percentage and whey protein (WP) percentage were estimated by formal titration method. Fat percentage was estimated by Gerber method (ISI, 1977). Lactometer reading was taken from all milk samples. Total solid (TS) and solid not fat (SNF) percentage were calculated as per Wehr and Frank (2004). The average milk constituent percentages were estimated from which, daily and total constituent yield was calculated. Total milk production record was collected from animal register maintained at the farm and further daily milk production was calculated. All the animals, which were in their first parity, were included in the present study.

PCR-single strand conformation polymorphism (PCR-SSCP): A 159 bp fragment spanning whole exon 2 of alpha-lactalbumin gene and 119 bp fragment spanning exon 1 of beta-lactoglobulin gene was amplified for polymorphism study. Primers used for amplification, were designed on the basis of cattle and sheep whole gene sequences with *DNASIS MAX* software. For alpha-lactalbumin gene, the primers used were, Forward *5'-GGG TCT GTA CCG CGT TT-3'* and reverse *5'-TGTCAC AGG AGA TGT TAC AG-3'* and for beta-lactoglobulin gene the primers were forward *5'- TGC AGA GCT CAG AAG C-3'* and the reverse *5'- GGA TAT CCA GGC CCT TCA-3'*. The annealing temperature for amplification of alpha-lactalbumin and beta-lactoglobulin gene fragment were 49°C for 1 min and 50°C for 1 min, respectively.

For SSCP, 12% native PAGE (50:1, acrylamide and bis-acrylamide) with 5% glycerol was used. A total volume of 3 ml of PCR product was properly mixed with 15 ml 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 loaded in poly acrylamide gel and electrophoresis was performed at 4°C temperature for 12 h at 200 v. After electrophoresis, silver nitrate staining was performed to visualize banding patterns associated with various polymorphisms present at this locus (Hayashi 1991, Bassam *et al.* 1991).

Sequencing: PCR products belonging to different genotypes were eluted from the 1% low melting agarose gel using gel elution kit for purification. The purified PCR-products were sequenced following the automated dye-terminator cycle sequencing method with Ampli *Taq* DNA polymerase in ABI PRIZM 377 DNA sequencer.

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. A general linear model (Harvey 1987) incorporating sire, genotype and season of calving as fixed effect was employed to estimate the effect of genotype on milk production and composition traits in buffaloes.

Model: $Y_{ikl} = \mu + G_i + A_j + S_k + e_{ikl}$

where, $Y_{ikl,}$ l^{th} milk yield for k^{th} season, for jth sire, i^{th} genotype; μ, overall mean; $G_{i,}$ effect of i^{th} genotype milk yield; $A_{j,}$ effect of j^{th} sire on milk yield; S_k, effect of k^{th} season on milk yield; $e_{ikl,}$ random error associated with each observation assumed to be NID (0, σ_e^2)

RESULTS AND DISCUSSION

Genotype frequency: The genotype frequency estimated in 4 different buffalo breeds for α-lactalbumin and β lactoglobulin are presented in Table 1. In Mehsana and Surti breeds 6 genotypes, Bhadawari 5 genotypes, and Murrah 4 genotypes were observed for α-lactalbumin allele. Whereas, for β-lactoglobulin allele 5 genotypes were observed in Murrah and Surti buffaloes while 4 genotypes were obtained for Mehsana and Bhadawari breeed.

Sequence analysis of alpha-lactalbumin: All the alleles detected through SSCP were sequenced and submitted to the NCBI from where the accession numbers such as AY726613 (A allele), AY726614 (B allele), AY726615 (C allele), AY726616 (D allele) and AY726617 (E allele) have been obtained. Sequence analysis of various alleles revealed that a silent mutation was present in A allele, which leads to

Table 1. Genotype frequency of α lactoalbumin and β lactoglobulin

Genotype	Mehsana	Surti	Bhadawari	Murrah
Alpha lactalbumin				
AB	0.021	-	-	-
AC	0.104	0.02	0.042	-
BB	0.333	0.18	0.271	-
BC	0.479	0.44	0.437	0.54
BD	0.042	0.28	-	-
BE	0.021	0.04	0.125	0.16
CC	-	0.04	0.125	0.22
CD	-	-	-	0.08
Beta lactoglobulin				
AA	0.13	0.14	-	0.10
BB	0.06	0.22	0.09	0.24
CC	-	0.28	0.09	0.20
AB	0.48	0.16	0.48	0.26
AC	0.33	0.20	0.34	0.20

Table 2. Nucleotide and amino acid changes in different alleles (alpha-lactalbumin) of buffalo

Allele	Position of nucleotide variability						Position of amino acid variability				
	71	113	121	122	146	153	23	37	40	48	51
A	A	T	A	C	C	T	glu	asp	asn	asn	cys
B	A	C	A	C	C	T	glu	asp	asn	asn	cys
C	T	C	T	G	G	G	asp	asp	met	lys	gly
D	A	C	A	C	C	G	glu	asp	asn	asn	gly
E	A	C	A	G	C	G	glu	asp	lys	asn	gly

Table 3. Nucleotide and amino acid changes in different alleles (β-lactoglobulin) of buffalo

Allele	Position of nucleotide variability				Position of amino acid variability	
	18	25	28	43	9	10
A	C	A	C	T	ser	arg
B	T	G	T	C	ser	arg
C	C	G	T	C	gly	cys

change in polypeptide sequence (Table 2). Addeo *et al.* (1976) reported that Italian water-buffalo alpha- lactalbumin differs from its cow B counterpart by the presence of 2 substitutions one at 17th position of mature peptide from asparagine to glycine and one at unknown position from either glutamic acid to glutamine or aspartic acid to asparagine. However, silent mutation observed in 37th codon of exon 2 is important since this mutated site may be relatively more prone to future mutation or indirectly affect the process of transcription and translation during the expression of protein/polypeptide. Chianese *et al.* (2004) detected two α-lactalbumin protein variants i.e. A and B in Italian water buffalo. The variant A was reported to be different from variant B with the presence of a substitution of aparagine to aspartic acid at 45th position of mature peptide. They also inferred that amino acid substitution altered the N-glycosylation of a consensus sequence of asn^{45}-×-ser^{46}. They deduced that the protein glycosylation level of α -lactalbumin A would decrease.

Sequence analysis of β-lactoglobulin: All the alleles were sequenced and the data were submitted to the Genbank from where accession numbers have been obtained as Genbank accession No. AY775796 for A allele; AY775797 for B allele and AY775798 for C allele. The A allele while compared with B allele, had cytosine in place of thymine at 18th position (Table 3). At 25th position, allele A had adenine whereas B allele had guanine at the same location. The B allele also showed difference at 28th position where thymine was replaced by cytosine of A allele. At 43rd position, the B allele had cytosine in place of thymine of A allele. The serine and arginine of A and B alleles were mutated into glycine and cysteine in C allele. Naturally, the change in amino acid sequence may play enormous role in deciding the structure of polypeptide. In the present study, serine, a polar amino acid and arginine, a basic amino acid has been replaced by non-polar amino acids, glycine and cysteine. Structural differences in polypeptides ultimately lead to differences in the biological/physiological functions of the protein.

Effect of α-lactalbumin genotypes on milk production and constituent's traits: Genotypes showed nonsignificant effect on daily milk yield for all four breeds of riverine buffalo. But, they showed significant effect (P<0.05) on total milk yield in Surti buffalo. In remaining 3 breeds viz. Murrah, Mehsana, and Bhadawari, nonsignificant effect of genotype on total milk yield was observed (Table 4). However, in Surti, animals with AC, BB and BC genotype had more or less

Table 4. Genotype wise least-square means of total milk yield (kg) of different buffalo breeds

Breed	AC	BB	BC	BD	BE	CC	CD
			Alpha-lactalbumin				
Bhadawari	952±193	860±166	1047±141	-	890±140	957±164	-
Mehsana	1320±187	1446±142	1419±139	1391±266	-	-	-
Surti	903±120^{a}	962±101^{a}	1063±81^{a}	1115±89^{b}	-	1239±197^{b}	-
Murrah	-	-	2036±177	-	1947±83	1403±261	2020±264
			Beta lactoglobulin				
Murrah	1228±202 ^{a}	1653±117 ^{b}	1108±122 ^{a}	1994±139 ^{b}	1802±156 ^{b}	-	
Bhadawari	885±96 ^{b}	897±104 ^{b}	118±213 ^{a}	990±129 ^{b}	361±265 ^{a}	-	
Mehsana	1346±128 ^{a}	1420±97 ^{a}	1927±208 ^{b}	1343±127 ^{a}	-	-	
Surti	1079±62	998±65	1060±69	1149±62	1095±57	-	

Different superscripts indicate significant difference at 5% level.

Table 5. Genotype wise (β lactoglobulin) least-square means of total milk yield (kg) of different buffalo breeds

Breed	A/A	A/B	B/B	A/C	C/C	C/D
Murrah	1228 ±202^{A}	1653±117^{B}	1108±122^{A}	1994±139^{B}	1802±156^{B}	–
Bhadawari	885 ± 96^{B}	897±104^{B}	118±213^{A}	990±129^{B}	361±265^{A}	–
Mehsana	1346±128^{A}	1420±97^{A}	1927±208^{B}	1343±127^{A}	–	–
Surti	1079±62	998±65	1060±69	1149±62	1095±57	–

Different superscripts indicate significant difference at 5% level.

similar milk yield which is quite lower than animals having BD and CC genotype.

Genotypes were found to have significant effect on SNF%, TSNF and DSNF yields in Murrah buffalo. Their order of performance for SNF% amongst genotypes were CD > BC, CC, BE whereas, for TSNF and DSNF yield it was CD, BC >BE >CC and CD, BC > BE > CC respectively. In Surti, genotypic variants had showed significant effect (P<0.05) on TSNF and DSNF yield. Order of performance observed amongst different genotypes were CC > BC, BD, BE > AC, BB and BC, BD, BE > AC, BB, CC, respectively. Genotypic variants had significant effect on daily and total solid yield. Their order of performance for TS yield was CC>BB, BE, BC, BD>AC and for DS yield, it was BC, BD>BB, BE, CC>AC. Genotypic variants had significant effect on TP and DP yield in Mehsana and Surti buffalo. Their order of performance in this breed for TP yield was CC, BC, BD, BE>AC, BB whereas, for DP yield it was BC, BD, BE>AC, BB, CC.

In Surti buffalo, genotype had significant effect on TWP and DWP yield. For TWP yield the order of performance was BC, CC, CD> AC, BB, BE while for DWP yield it was BC, CD>AC, BB, BE, CC.

Genotypes had significant effect on fat%, daily fat yield during first lactation and total first lactation fat yield at P<0.05 in Murrah buffalo. Animals of BC genotype produced highest fat production and the lowest performance of fat production was observed with CC genotype.

Effect of β-lactoglobulin genotypes on milk production traits: Least square analysis indicated significant effect (P<0.05) of genotype on total milk yield in all the four breeds studied (Table 5) but on daily milk yield in Murrah, Bhadawari and Mehsana buffalo whereas in Surti buffalo, genotype showed non-significant effect. In Murrah buffalo, animals of BB genotype produced highest daily milk yield whereas AA animals showed lowest production. However, the production performances of BB, AB, AC and CC genotypes were not differed significantly. In Bhadawari buffalo, BB genotype yielded highest performances whereas CC genotype produced lowest yield of daily milk. However, genotypes like AA, AB, BB and AC showed non-significant differences of production demonstrating superior performances to CC genotype. In Mehsana breed, BB genotype produced highest daily milk and AC genotype yielded lowest production. The performances of BB and AA were not differed significantly but significantly differed from the performances of AB and AC genotypes, which showed non-significant differences of production amongst each other. Badola *et al.* (2003) also reported significant association of genotype with milk production traits in cattle. But, Jairam and Nair (1983) could not find any significant association between beta-lactoglobulin polymorphism and milk yield in cattle. However, such kind of genotype and traits relationship may be verified both biochemically as well as physiologically before inferring a strong conclusion in this regard. In conclusion, it may be stated that genotypes having significantly higher milk and constituent yield in specific breed may be favoured in the farm for augmenting the productivity of animals.

The buffalo is an important dairy animal in the country and association of different polymorphic variants of milk protein genes will help in as an aid in developing the marker assisted selection of superior animals.

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