Genotype Profiles for the Quantitative Trait Related to Milk Composition in Bulls Used for Artificial Insemination in India

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ABSTRACT : A population of exotic Holstein Friesian, Jersey, their crossbreds and the indigenous Murrah breed of buffalo bulls (n=486), used in artificial insemination breeding program were screened for the allelic distribution of the κ-casein and β-lactoglobulin genotypes. The preferred "B" allele frequency was highest in Murrah buffalo bulls followed by Jersey and Holstein Friesian. The increase in this particular allele frequency in the Holstein Friesian crossbred bulls was more when compared to their Jersey counterparts. Hardy-Weinberg's equilibrium was maintained albeit with some deviations, which was higher in crossbreds than in purebreds. The feasibility of using such large-scale molecular diagnostic tools in the field and their significance with regards to the dairy economy is discussed. ***(Asian-Aust. J. Anim. Sci. 2002. Vol 15, No. 3 : 326-329)***

Key Words : κ-Casein, β-Lactoglobulin, Quantitative Trait, Artificial Insemination, Genotyping

INTRODUCTION

Six major proteins in bovine milk, *viz*., αs_1-, αs_2-, κ- and β-casein, β-lactoglobulin and α-lactalbumin are known to exhibit polymorphism (Grosclaude, 1988). Variants of genes of some of these proteins correlate with certain milk properties (Damiani et al., 1992). It has been documented that the polymorphic forms of the gene for κ-casein, β-casein and β-lactoglobulin are associated with increased protein content in milk and its better cheese-making properties (Marziali and Ng-Kwai-Hang, 1986). In the modern method of animal breeding, selection of favorable haplotype can be significantly augmented by identification of the genotypes of the bulls as well as the embryos. Genotyping of an animal at the DNA level is much faster, rapid and less expensive than analysis of the milk proteins of multiple dam-daughter pairs (Damiani et al., 1992). Restriction analysis of PCR amplicons (Saiki et al., 1988) has been developed for genotyping farm animals to identify polymorphic forms of the genes for κ-casein (Damiani et al., 1989; Denicourt et al., 1990; Medrano and Aguilar-Cordova, 1990a; Pinder et al., 1991; Schleiben et al., 1991) and β-lactoglobulin (Medrano and Aguilar-Cordova, 1990b). Milk composition is acknowledged to be an important quantitative trait and its genetic improvement is technically as well as economically feasible by such rapid techniques of genotyping (Medrano and Aguilar-Cordovo, 1990a).

Artificial insemination (AI) based animal breeding is known to transmit superior genetic resources horizontally into the population within a short period of time and the organized dairy sector in India is using this technique. The present study deals with field screening of AI bulls for κ-casein and β-lactoglobulin gene polymorphs to ascertain the distribution of the preferred alleles in the population. This could, in turn, help in the selection of genetically superior bulls for organized animal breeding programmes.

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MATERIALS AND METHODS

Analytical techniques

Stored DNA samples isolated from the blood of bulls kept at the various AI farms in India were used as samples for PCR-RFLP analysis. The screened AI bull population comprised of Holstein Friesian (HF) breed (n=156) followed by Jersey (n=105) and Murrah breed of buffalo (n=51). 91 HF and 83 Jersey crossbreds, generated through cross breeding with indigenous cattle breeds were also analyzed in this study as a single cluster of "Crossbred" bulls.

Oligonucleotide primers were synthesized by the automated phosphoramidate method (Oligo Inc., USA). Approximately 100 ng of DNA were used for PCR amplification using an automated thermal cycler (Perkin Elmer Geneamp PCR system - Model No. 9600). The PCR mix contained 2.5 µl of 10×PCR buffer (100 mM Tris- pH-9.0, 500 mM KCl, 15 mM $MgCl_2$ and 0.1% Gelatin), 200 µM dNTPs, 1 unit of *Ta*q DNA polymerase (Bangalore Genie Pvt. Ltd., Bangalore, India), 20 pM of each primer (forward and reverse) and sterile water. Samples were amplified for 30 cycles; each cycle comprised of 97°C hold for 2 minutes (denaturation), 55°C for 1 minute (primer annealing) and 72°C for 1 minute (primer extension). Fifteen µl of the PCR product solution were digested with 5 units of restriction endonuclease (*Hin*fI for κ- casein and *Hae*III for β-lactoglobulin amplicons) for 3 h at 37°C and analyzed by electrophoresis in 4% TBE/ETBr agarose gels (2% NuSieve GTG and 2% Ultrapure BioRad agarose).

DNA bands were visualized under UV light and documented using a Polaroid MP-4+Camera System.

The primer pairs employed in the study for κ-casein genotyping were 5'-ATC ATT TAT GGC CAT TCC ACC AAA G-3' (forward) and 5'-GGC CAT TTC GCC TTC TCT GTA ACA GA-3' (reverse) while that for β-lactoglobulin genotyping were 5'- TGT GCT GGA CAC CGA CTA CAA AAA G-3' (forward) and 5'-GCT CCC GGT ATA TGA CCA CCC TCT -3' (reverse).

Mathematical analysis

The Hardy-Weinberg (HW) equation (Kirby, 1990) was employed assuming that in a two-allele system (*A* and *B*) with the frequency of allele "*A*" equals to *p* and that of "*B*" equals to *q*, the genotypic proportion frequencies of *AA*: 2*AB*:*BB* or p^2:2*pq*:q^2 are valid. Allele frequencies were calculated according to the formula of Nei and Li, 1979. Graphs were generated using the MS-Excel 2000 software.

RESULTS AND DISCUSSION

Thermal amplification of genomic DNA with κ-casein gene specific primers generated a 350 bp amplicon, which had an asymmetrically positioned non-polymorphic *Hin*fI recognition site generating an 84 bp product that served as a positive control for complete restriction digestion. However, the remaining larger 266 bp fragments in allele "A" had a functional *Hin*fI site at a near-symmetrical position bisecting the fragment into 132 and 134 bp respectively, which appeared as an intense doublet DNA band on agarose gel. This recognition site was non-functional in allele "B" thereby retaining the intact 266 bp fragment. Therefore, a single band at 132/134 bp represented "A" allele while that in the range of 266 bp represented allele "B".

Primers specific for the β- lactoglobulin gene amplified a 247 bp amplicon that had an asymmetrically placed *Hae*III recognition site bisecting the fragment into 99 bp and 148 bp sizes. In allele "A", an inactive *Hae*III site, differing from the functional one by a single base, existed exactly in the midpoint, which was functional in allele "B" thereby bisecting the fragment into a doublet of two 74 bp bands. Allele "A" was therefore represented by a 148 bp band while allele "B", by an intense band of size 74 bp.

The allele frequency of bulls for the preferred allele "B" of κ-casein gene was 0.23, 0.60 and 0.94 for purebred cattle (HF and Jersey) and Murrah breed of buffalo, while the same for β-lactoglobulin gene was 0.61, 0.65 and 0.83, respectively (figure 1).

The results show that prevalence of the preferred "B" allele for the κ-casein gene was higher in Jersey bulls (0.60) than in HF (0.23). However, this difference was marginal for the two breeds of bulls for the β-lactoglobulin gene (0.65 and 0.61, respectively). For the buffalo population represented by the Murrah breed, the frequency of the preferred allele was a high of 0.94 and 0.83 for the κ-casein

Breed	B (Ca)	A (Ca)	B (La)	A (La)
HF[C]	0.23	0.77	0.61	0.39
Je[C]	0.6	0.4	0.65	0.35
Mu[B]	0.94	0.06	0.83	0.17
HFCB[C]	0.34	0.66	0.63	0.37
JeCB[C]	0.42	0.58	0.58	0.42

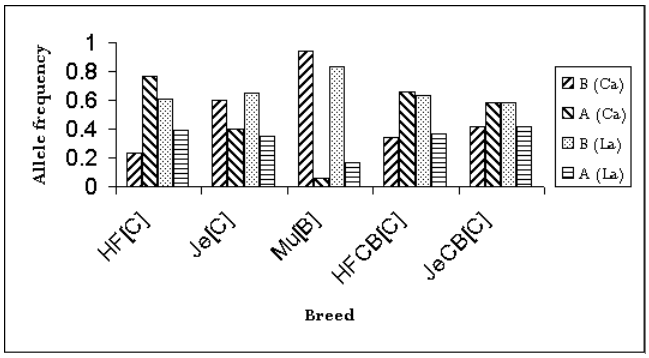

Figure 1. "A" and "B" Allele frequency for κ-casein and β-lactoglobulin genes in different breeds of bulls screened in the A.I. population.
HF, Holstein Friesian; Je, Jersey; CB, Crossbred; Mu, Murrah; [C], Cattle; [B], Buffalo; Ca, κ-casein; La, β-lactoglobulin; A, Allele A; B, Allele B.

Table 1. Genotype frequency of κ-casein and β-lactoglobulin genes in different breeds of A. I. bulls screened in this study

Breed	N	Genotype frequency κ-casein						Genotype frequency β-lactoglobulin					
		AA		AB		BB		AA		AB		BB	
		Ob	Pr	Ob	Pr	Ob	Pr	Ob	Pr	Ob	Pr	Ob	Pr
HF[C]	156	0.65	0.60	0.23	0.25	0.12	0.05	0.14	0.15	0.50	0.48	0.36	0.37
Je[C]	105	0.18	0.16	0.44	0.48	0.38	0.36	0.10	0.12	0.51	0.46	0.39	0.42
Mu[B]	51	0.00	0.00	0.12	0.11	0.88	0.88	0.06	0.03	0.23	0.28	0.71	0.69
HFCB[C]	91	0.46	0.43	0.41	0.49	0.14	0.11	0.06	0.14	0.62	0.47	0.32	0.40
JeCB[C]	83	0.25	0.34	0.65	0.49	0.10	0.18	0.08	0.18	0.66	0.49	0.25	0.34

HF, Holstein Friesian; Je, Jersey; Mu, Murrah; [C], Cattle; [B], Buffalo; CB, Crossbred; Ob, Observed; Pr, Predicted after implying Hardy-Weinberg equation; N, number.

and β-lactoglobulin genes, respectively (figure 1). For both the genes, comparison of the observed frequency for the three possible genotypes with that derived in accordance with the HW principle revealed some degree of variation, but an overall agreement for both cattle (HF and Jersey) and buffalo (Murrah) breed of bulls (table 1).

Crossbreds of HF and Jersey are born out of selective breeding with indigenous cattle breeds not only to propagate the exotic gene pool but also to incorporate economically important genetic traits into our indigenous animal population. The presence of a significant number of HF and Jersey crossbred AI bulls provided an opportunity to probe into the variation within various κ-casein and β-lactoglobulin genotypes as well as the preferred alleles' frequencies that prevailed after programmed crossbreeding.

Genotype analysis of a population of 91 HF crossbred AI bulls shows that the "B" allele frequency for the κ-casein and the β-lactoglobulin gene was 0.34 and 0.63 respectively. A comparison revealed that there is an increase of 47.83% in this value for the κ-casein gene when compared to that of its purebred counterparts. This rise was marginal for the β-lactoglobulin "B" allele frequency, which was placed at a low of 5%. This picture was different in Jersey crossbred bulls, where a drop of 30% was recorded in the "B" allele frequency for κ-casein and 10.8% for the β-lactoglobulin gene (figure 1). It appears that in the HF population there has been a positive genotypic enrichment with regard to these particular alleles, in the process of planned breeding with native cattle population. However, deviations in some of the genotype frequencies from those predicted employing the HW equation appeared to be higher in the crossbred population when compared to that of their purebred counterparts (table 1).

The segregation of the allelic forms of these genes follows simple Mendelian rules. This was confirmed by routine analysis of nine different families of cattle, each comprising of a dam, a sire and a calf (data not shown). Figure 2 shows the most conservative case where the dam is homozygous for allele A ("AA"). Under such circumstances, an AI bull, with identical genotype for κ-casein and/or β-lactoglobulin gene (i.e., "AA") can never introduce a "B" allele into the next generation. However, if the bull is heterozygous for the gene ("AB"), it can generate calf with genotype identical to itself at a frequency of 0.50. In the case of the bull being homozygous for the "B" allele ("BB"), 100% of the calf born will bear the preferred allele in heterozygous form. This picture will improve further if the mother is heterozygous or homozygous for the allele "B".

The present techniques for ascertaining the genetic value of a bull involve analysis of information on the production of milk and milk components by his daughters. A minimum time period of 2 years is required before the daughter of a bull enters the milking herd. However,

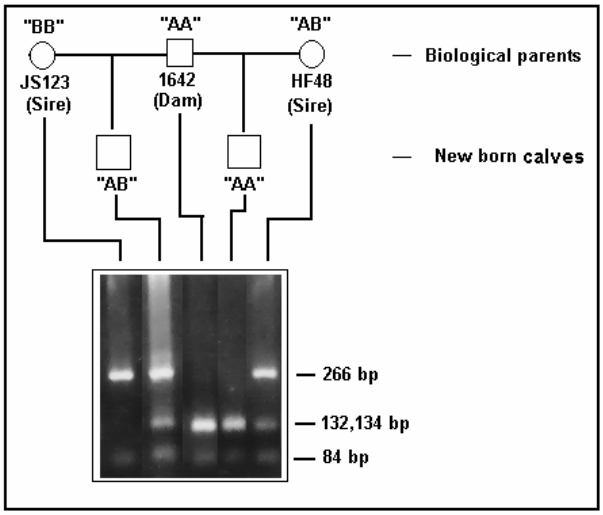

Figure 2. Typical Mendelian allelic segregation of κ-casein gene alleles in a cattle family. JS123 and HF48 are code name/number of A.I. bulls stationed at Sabarmati Ashram Gaushala (SAG) farm, Bidaj (Gujarat, India). Animal No. 1642 is the female cattle used in the breeding program.

utilizing such molecular technology, the desirable milk protein genetic make up, right at the birth of an offspring can be identified, thereby greatly shortening the period needed to identify superior individuals. Furthermore, such a study has the potential for assisting in establishment of a breeding procedure in milch herd that would increase the total amount of milk proteins essential for the production of important milk products such as cheese. Through such genotype selection, dairy producers can provide cheese manufacturers with the best combination of milk proteins and solids that can increase the total amount of cheese up to 5-8 per cent over current production levels.

From 1961 to 1978 India had imported 1,470 bulls and 6,165 females of exotic breeds of which 275 bulls and 1,825 females were of Friesian and HF strains while most of the others were Jersey. More than 23 farms of HF breed together contribute to the semen-freezing network, the majority of which have been established under the Operation Flood program in cooperation with the National Dairy Development Board and with assistance from the Governments of Australia, Canada, Denmark, New Zealand, Switzerland, UK and USA (Jasiorowski et al., 1988). The present work is of importance being a field study specific to breeding bulls meant for AI and for providing genotype profile for an economically important trait.

Rapid genotyping of bulls participating in AI is feasible since they are limited in number and stationed in organized farms. On the contrary, it is not possible to screen all the dams participating in AI due to their large number and dispersed location. Furthermore, milk composition can only be estimated in cow/she-buffalo although the male counterpart contributes 50% of the total genetic pool of a

calf. Therefore selection through the use of molecular markers (Marker Assisted Selection) provides the means for assessing the contribution of the male partner for such economically important phenotypes.

A knowledge of the trend of genotypes existing in the AI bull population in general and that of specific breeds in particular, can therefore help in fulfilling the goal of generating better quality genetic resources in the dairy animal population through judicious exploitation of the existing ones. This study reaffirms the feasibility of large-scale implementation of a molecular technique for achieving the goal.

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