

# Identification of the $\kappa$ -casein and $\beta$ -lactoglobulin genotypes in Brazilian *Bos indicus* and *Bubalus bubalis* populations

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## ABSTRACT

Blood samples from two *Bos indicus* breeds, Nelore and Gyr, and from one water buffalo herd, *Bubalus bubalis*, raised in Brazil, were genotyped for kappa-casein and beta-lactoglobulin loci. Variants A and B were identified at two loci by polymerase chain reaction and digestion with Hind III and Hph I. The lengths of the amplified and digested fragments from cattle and buffalo DNA were similar. Genetic variation was detected in the bovine breeds but no polymorphism was observed in the buffalo population, which presented  $\kappa$ -Cn B and  $\beta$ -Lg B alleles. The frequencies of B alleles at the  $\kappa$ -casein and  $\beta$ -lactoglobulin loci were 0.08 and 0.77 in Nelore, and 0.06 and 0.62 in Gyr. Compared to the original Indian breeds, the Brazilian bovine populations showed a significant decrease in the frequency of the  $\kappa$ -Cn B allele and non-significant differences in the frequency of  $\beta$ -Lg B. Genotyping of males and young animals is recommended for the planning of matings to increase the  $\kappa$ -Cn B allele frequency in Brazilian populations.

## INTRODUCTION

The bovine milk specific proteins include four caseins,  $\alpha$ s1,  $\alpha$ s2,  $\beta$  and  $\kappa$ -casein, and two whey proteins,  $\alpha$ -lactalbumin and  $\beta$ -lactoglobulin. Milk protein polymorphisms have been intensively studied because of their effect on the processing properties of milk and on the yield of milk products.

A single genetic locus with two common alleles, A and B, determines the type of  $\kappa$ -casein of cow's

milk. The  $\kappa$ -Cn B allele has a significant direct effect on protein content and on milk yield (Marzali and Ng-Kwai-Hang, 1986). Consistent reports, reviewed by Grosclaude (1988), indicate that the milk from BB genotypes results in shorter rennet coagulation time, formation of a firmer curd, and a greater cheese yield than milk from AA genotypes.

At the  $\beta$ -lactoglobulin locus, variants A and B influence milk composition and its processing properties. The  $\beta$ -Lg BB genotype is important because of its association with high fat percentage, which can improve cheese yield (Aleandri *et al.*, 1990). According to Bovenhuis *et al.* (1992)  $\beta$ -lactoglobulin genotypes affect milk production and protein yield. Cows carrying  $\beta$ -Lg AA genotype produce more milk than  $\beta$ -Lg BB cows, and  $\beta$ -Lg AA cows have higher protein yields than

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BB or AB cows. Contradictory results were reported by Graml *et al.* (1986) who did not find a significant effect of the  $\beta$ -Lg genotypes on protein yield.

Genotyping at the bovine  $\kappa$ -casein and  $\beta$ -lactoglobulin loci was initially done using Southern blotting technique (Rando *et al.*, 1988, Lién *et al.*, 1990). The test has been simplified with the introduction of polymerase chain reaction amplification, followed by digestion through a restriction enzyme (Medrano and Aguilar-Cordova, 1990, Denicourt *et al.*, 1990, Wilkins and Kuys, 1992). This PCR test is simple, rapid and effective for animals of any age or sex, thus permitting the inclusion of milk protein genotypes into selection programs.

Nelore and Gyr are the two most important Zebu breeds raised in Brazil. Nelore is the dominant breed and has been selected to provide meat. It originated from the Indian Ongole breed, which has a moderate capacity for milk production. Brazilian Gyr descends directly from the Indian Gyr breed, recognized for its high milk yield. Some Brazilian herds of this breed have been selected for more than fifty years to provide milk, and another group has been selected to provide meat (Santiago, 1985). Water buffaloes were first introduced into Brazil at the end of last century. The population expanded into the largest herd in America (Cockrill, 1984). Milk production by this herd has increased in the southeastern region of the country.

Brazil produces more than 15 million tons of bovine milk per year and, according to Madalena *et al.* (1990), most dairy farmers keep their herd intermediate between purebred Holstein-Friesian and purebred *Bos indicus*. The farmers follow different crossbreeding strategies to produce 1/2 to 5/8 European grades.

Direct selection of specific alleles that are associated with manufacturing properties of milk has never been tried in these tropical populations. To start a program of selection using data on milk proteins, it is necessary to determine the frequencies of the relevant alleles in the breeds which most contribute to Brazilian dairy production. Most of the studies on the frequencies of alleles at lactoprotein loci have been carried out on *Bos taurus* breeds, especially Holstein-Friesian, but data on *Bos indicus* breeds raised in Brazil are scarce and have been studied only at the protein level (Silva and Del Lama, 1993).

The aim of the present study was to provide genetic data on  $\kappa$ -casein and  $\beta$ -lactoglobulin loci by PCR technique for two *Bos indicus* breeds, Nelore and Gyr. Furthermore, we intended to standardize conditions for identification of these genotypes in buffaloes. Partial results have been presented in abstract form (Del Lama and Zago, 1995).

## MATERIAL AND METHODS

### Sampling

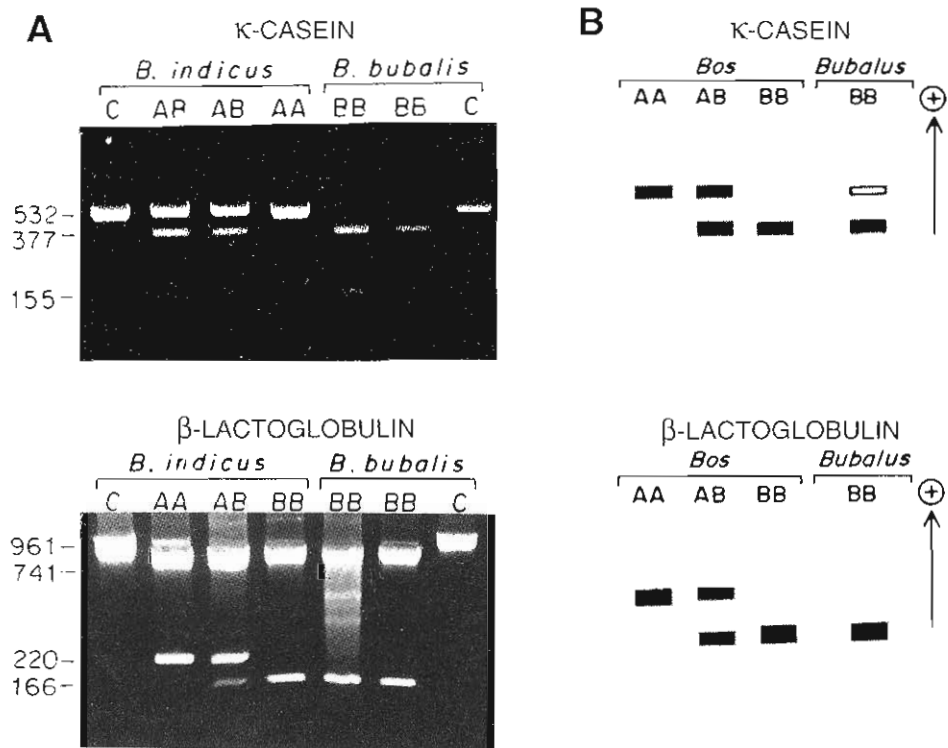
Genomic DNA was isolated from blood obtained from young males and females (six months to one year of age) of the Nelore and Gyr breeds (*Bos indicus*), and from 15 animals of a water buffalo herd (*Bubalus bubalis*), resulting from crosses between Murrah and Mediterranean breeds. The sampled Gyr herds have been highly selected for milk production. Sixteen animals were sampled per herd, and four herds for each bovine breed. These are from southeastern Brazil. The DNA was obtained by phenol and chloroform-isoamyl alcohol extractions and recovered by ethanol precipitation.

### Genotyping and data analysis

The amplified region of the kappa-casein gene contains the nucleotide substitutions for diagnosing the A and B variants (Denicourt *et al.*, 1990). One hundred ng DNA in each reaction (25  $\mu$ l) was amplified at an annealing temperature of 52°C. The product of each PCR reaction was digested with Hind III at 37°C for three hours. For the  $\beta$ -lactoglobulin region, we used primers designed by Wilkins and Kuys (1992) to detect A and B alleles. Two to four hundred ng DNA was used in each reaction at an annealing temperature of 61°C. The PCR product was digested by Hph I at 37°C for three hours. Allele frequencies were determined by gene counting. A chi-square test was carried out to test for Hardy-Weinberg equilibrium. Allele frequencies among populations were compared through a homogeneity test.

## RESULTS AND DISCUSSION

Figure 1A shows examples of  $\kappa$ -casein and  $\beta$ -lactoglobulin genotyping in cattle and in buffaloes. Mobility of the bands of undigested and digested DNA was similar for the two genera. The success of the amplification of DNA in buffalo with primers designed for cattle DNA, and the size identity of the amplified segments suggest that this region of the gene is conserved in *Bos* and *Bubalus*. The presence of a Hind III restriction site in both genera and in *Bison* (Cronim and Cockett, 1993) indicates that the  $\kappa$ -Cn B allele may be present in the common ancestors of these genera. The fact that the  $\kappa$ -Cn B allele was detected at a high average frequency in Podolic, Italian and Iberian breeds, a cattle group designated by Baker and Manwell (1980) as



**Figure 1** - Genotypes and phenotypes at the  $\kappa$ -casein and  $\beta$ -lactoglobulin loci in cattle and buffalo. A) Genotyping of the  $\kappa$ -casein A and B alleles (top) and of the  $\beta$ -lactoglobulin A and B alleles (bottom); undigested DNA was designated C. B) Phenotyping of the  $\kappa$ -casein A and B alleles (top) and of the  $\beta$ -lactoglobulin A and B alleles (bottom) (Sen and Sinha, 1961; Ganguli and Majumder, 1968).

primigenius cattle because they present some characteristics which existed in wild cattle (*Bos primigenius*), corroborates our proposition. Our results do not support the hypothesis that the  $\kappa$ -Cn A allele is the ancestral gene (Grosclaude, 1988).

The protein phenotype at the  $\kappa$ -Cn locus for buffalo milk samples, described by Ganguli and Majumder (1968), revealed two bands, one of greater intensity, and a more anodal band of lesser intensity (Figure 1B). In spite of the homozygous variants  $\kappa$ -Cn A and  $\kappa$ -Cn B not being found, the electrophoretic pattern was designated as  $\kappa$ -Cn AB. Genotyping of buffalo DNA in this study showed the characteristic homozygous BB pattern (Figure 1A). Considering that a correspondence exists between the restriction site polymorphism analyzed and the amino acid sequence responsible for the protein polymorphism, our results confirm that the more anodal band detected at the protein level is a secondary region.

The allele frequencies of the  $\kappa$ -casein and  $\beta$ -lactoglobulin loci in the Nelore and Gyr breeds and in the buffalo herd are in Table I. No genetic variation was detected at the two loci studied in the buffalo sample. The results obtained with this small sample of animals are in agreement with the monomorphism previously

described for Indian populations by Tandom (1982) using protein analysis, and also with the data on the  $\kappa$ -Cn region of DNA reported by Mitra *et al.* (1994) in Murrah and Nili-Ravi buffaloes.

A homogeneity test did not show significant differences among the  $\kappa$ -casein allele frequencies of the herds which contributed to the Nelore and Gyr samples. However, significant heterogeneity at the  $\beta$ -lactoglobulin locus was detected among the Nelore herds ( $\chi^2 = 7.89$ ,  $P < 0.05$ ), a result that may be explained by the high frequency of the  $\beta$ -Lg A allele observed for herd 4. Close similarities between the Nelore and Gyr allele frequencies observed for the two loci were demonstrated by non-significant  $\chi^2$  values obtained in the homogeneity test for all herds. Assuming Hardy-Weinberg equilibrium, there was close agreement between the observed and expected numbers of each genotype in both bovine breeds, as was revealed by the  $\chi^2$  values presented in Table I.

Low frequencies of the  $\kappa$ -Cn B allele were observed in Nelore and Gyr (Table I). A similar result has been observed at the protein level in the Gyr breed (Silva and Del Lama, 1993). The frequency of  $\kappa$ -Cn B in Indian breeds ranged from 0.15 to 0.33 (Juneja and Chaudhary, 1973; Jairam and Nair, 1983). The lower

**Table I** - Allele frequencies at the  $\kappa$ -casein and  $\beta$ -lactoglobulin loci in two Brazilian bovine breeds, Nelore and Gyr (*Bos indicus*) (N = 16 per herd), and in one water buffalo herd (*Bubalus bubalis*) (N = 15).  $\chi^2$  values were calculated to test Hardy-Weinberg equilibrium.

	$\kappa$ -Casein		$\beta$ -Lactoglobulin	
	$\kappa$ -Cn A	$\kappa$ -Cn B	$\beta$ -Lg A	$\beta$ -Lg B
<b>Gyr</b>				
herd 1	0.94	0.06	0.46	0.54
herd 2	0.91	0.09	0.35	0.65
herd 3	0.94	0.06	0.45	0.55
herd 4	0.94	0.06	0.25	0.75
Total	0.94	0.06	0.38	0.62
$\chi^2$	0.22		0.57	
<b>Nelore</b>				
herd 1	0.97	0.03	0.15	0.85
herd 2	0.94	0.06	0.25	0.75
herd 3	0.84	0.16	0.10	0.90
herd 4	0.94	0.06	0.45	0.55
Total	0.92	0.08	0.23	0.77
$\chi^2$	0.46		1.34	
<b>Buffalo</b>				
	0.00	1.00	0.00	1.00

frequencies of  $\kappa$ -Cn B allele in the Brazilian breeds when compared with the original Indian breeds could be explained as a result of a founder effect. However, the gene flow maintained by continuous introduction of Indian germ plasm into Brazilian cattle does not support this assumption. A second explanation might be that breeders could have indirectly selected in favor of some genetic milk variants which are associated with quantitative characteristics. The fact that Nelore and Gyr have been more intensively selected for milk and meat by Brazilian cattle breeders than Indian Ongole and Gyr populations supports this proposition.

Allele frequencies in Table I show that the  $\beta$ -Lg B variant is predominant in Gyr and Nelore breeds. These results are consistent with those determined in samples of milk from these two Zebu breeds by Silva and Del Lama (1993) (0.64 and 0.56, respectively), and also with the frequencies observed in Indian Gyr and Ongole, (0.60 and 0.77) by Singh and Bhat (1980). These data indicate that the process of artificial selection for the purpose of improving milk yield in Brazilian Gyr did not modify the frequency of the  $\beta$ -Lg A found in the original Indian breed.

Considering that the frequencies of  $\kappa$ -Cn B and  $\beta$ -Lg B alleles in Brazilian Holstein are 0.17 and 0.49,

respectively (Del Lama and Molfetta, 1991), and that European x Zebu crosses is the breeding strategy preferred by dairy farmers, the expected frequency of  $\kappa$ -Cn B allele in the resulting population will be very low and the  $\beta$ -Lg B allele will be in the medium range. Since the  $\beta$ -Lg and  $\kappa$ -Cn loci are found on different chromosomes, selection for the  $\kappa$ -Cn B allele and for the  $\beta$ -Lg A or B could be carried out independently. An increase in allele frequency of  $\kappa$ -Cn B would be feasible by using molecular genetic techniques that permit rapid identification of the genotypes of males and young animals. As many breeding programs are centered on a few bulls selected by progeny tests for yield and conformation, the approved bulls would be genotyped for these lactoprotein loci before they are entered into the breeding program.

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## RESUMO

Amostras de sangue de duas raças *Bos indicus*, Nelore e Gyr e de um rebanho de búfalos, *Bubalus bubalis*, criados no Brasil, foram genotipadas nos locos da  $\kappa$ -caseína e da  $\beta$ -lactoglobulina. Os variantes A e B foram identificados nos dois locos após amplificação do DNA pela reação em cadeia da polimerase, seguida da digestão pelas enzimas de restrição, Hind III e Hph I. Os fragmentos de DNA bovino e bubalino amplificados e digeridos apresentaram tamanhos correspondentes. Foi detectada variação genética nas raças bovinas mas nenhum polimorfismo foi observado na população bubalina, onde estão fixados os alelos  $\kappa$ -Cn B e  $\beta$ -Lg B. As populações bovinas brasileiras quando comparadas com as raças indianas originais apresentam um decréscimo significativo na frequência do alelo  $\kappa$ -Cn B e diferenças não significativas na frequência do alelo  $\beta$ -Lg B. A genotipagem rápida de machos e animais jovens é recomendada para o planejamento de acasalamentos, que resultem no aumento da frequência do alelo  $\kappa$ -Cn B nas populações brasileiras.

## REFERENCES

- Aleandri, R., Buttazzoni, L.G., Schneider, J.C., Caroli, A. and Davoli, R. (1990). The effects of milk protein polymorphisms on milk components and cheese-producing ability. *J. Dairy Sci.* 73: 241-255.

- Baker, A.C.M. and Manwell, C.** (1980). Chemical classification of cattle. I. Breed groups. *Anim. Blood Grps biochem. Genet.* 11: 127-150.
- Bovenhuis, H., Van Arendonk, J.A.M. and Korver, S.** (1992). Associations between milk protein polymorphisms and milk production traits. *J. Dairy Sci.* 75: 2549-2559.
- Cockrill, W.R.** (1984). Water buffalo. In: *Evolution of Domesticated Animals* (Mason, I.L., ed.). Longman, London, pp. 52-63.
- Cronim, M.A. and Cockett, N.** (1993). Kappa-casein polymorphisms among cattle breeds and bison herds. *Anim. Genet.* 24: 135-138.
- Del Lama, M.A. and Molfetta, J.B.** (1991). Estudo dos polimorfismos proteicos do leite na raça Holandesa. *Rev. Bras. Genet.* 14 (Suppl. 1): 127 (Abstract).
- Del Lama, S.N. and Zago M.A.** (1995). Genetic polymorphism at the  $\kappa$ -casein and  $\beta$ -lactoglobulin loci in Brazilian cattle and buffalo populations. Beltsville Symposium XX, Biotechnology's Role in the Genetic Improvement of Farm Animals, Beltsville, Maryland. *Program and Abstracts*, May 14-17, pp. 12.
- Denicourt, D., Sabour, M.P. and McAllister, A.J.** (1990). Detection of bovine  $\kappa$ -casein genomic variants by the polymerase chain reaction method. *Anim. Genet.* 21: 215-216.
- Ganguli, N.C. and Majumder, G.C.** (1968).  $\kappa$ -casein variants in buffalo milk. *J. Dairy Sci.* 51: 796-797.
- Graml, R., Buchberger, J., Klostermeyer, H. and Pirchner, F.** (1986). Pleiotrope Wirkungen von  $\beta$ -Lactoglobulin und Casein Genotypen auf Milchfett und Milchproteinmengen des Bayerischen Fleckviehs und Braunviehs. *Z. Tierz. Zuchtungsbiol.* 103: 33-45.
- Grosclaude, F.** (1988). Le polymorphisme génétique des principales lactoprotéines bovines: Relations avec la quantité, la composition et les aptitudes fromagères du lait. *INRA Prod. Anim.* 1: 5-17.
- Jairam, B.T. and Nair, P.G.** (1983). Genetic variants of milk proteins in different breeds of cattle. *Indian J. Dairy Sci.* 36: 5-11.
- Juneja, R.R. and Chaudhary, R.P.** (1973). Simultaneous phenotyping of milk proteins in Indian cattle. *Indian J. Dairy Sci.* 26: 104-106.
- Lién, S., Alestrom, P., Steine, T., Langsrud, T., Vegarud, G. and Rogne, S.** (1990). A method for  $\beta$ -lactoglobulin genotyping of cattle. *Livest. Prod. Sci.* 25: 173-176.
- Madalena, F.E., Teodoro, R.L., Lemos, A.M., Monteiro, J.B.N. and Barbosa, R.T.** (1990). Evaluation of strategies for crossbreeding of dairy cattle in Brazil. *J. Dairy Sci.* 73: 1887-1901.
- Marzali, A.S. and Ng-Kwai-Hang, K.F.** (1986). Relationships between milk protein polymorphisms and cheese yielding capacity. *J. Dairy Sci.* 69: 1193-1201.
- Medrano, J.F. and Aguilar-Cordova, E.** (1990). Genotyping of bovine kappa-casein loci following DNA sequence amplification. *Bio/Technology* 8: 144-146.
- Mitra, A., Schlee, P., Balakrishnan, C.R. and Pirchner, F.** (1994). Polymorphisms in growth hormone, prolactin and kappa-casein loci in Indian Sahiwal cattle, Murrah and Nili-Ravi buffaloes. *Anim. Genet.* 25 (Suppl. 1): 25 (Abstract).
- Rando, A., Di Gregorio, P. and Masina, P.** (1988). Identification of bovine  $\kappa$ -casein genotypes at the DNA level. *Anim. Genet.* 19: 51-54.
- Santiago, A.A.** (1985). *O Zebu na Índia, no Brasil e no Mundo*. Instituto Campineiro de Ensino Agrícola, Campinas, Brasil, pp. 191-334 and 407-463.
- Sen, A. and Sinha, N.K.** (1961). Comparison of the  $\beta$ -lactoglobulins of buffalo's milk and cow's milk. *Nature* 190: 343-344.
- Silva, I.T. and Del Lama, M.A.** (1993). Estudos dos polimorfismos proteicos do leite nas raças zebuínas criadas no Brasil. *Rev. Bras. Genet.* 16 (Suppl. 1): 308 (Abstract).
- Singh, H. and Bhat, P.N.** (1980).  $\beta$ -lactoglobulin polymorphism in indigenous cattle. *Indian J. Anim. Sci.* 50: 932-937.
- Tandom, S.N.** (1982). Genetic studies on biochemical polymorphism of milk proteins and blood enzymes in Indian buffaloes. Doctoral thesis, Rohilkhand University, Bareilly.
- Wilkins, R.J. and Kuys, Y.M.** (1992). Rapid  $\beta$ -lactoglobulin genotyping of cattle using the polymerase chain reaction. *Anim. Genet.* 23: 175-176.

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