# κ-CASEIN, β-LACTOGLOBULIN AND GROWTH HORMONE ALLELE FREQUENCIES AND GENETIC DISTANCES IN NELORE, GYR, GUZERÁ, CARACU, CHAROLAIS, CANCHIM AND SANTA GERTRUDIS CATTLE

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## ABSTRACT

The genotypes for  $\kappa$ -casein ( $\kappa$ -CN),  $\beta$ -lactoglobulin ( $\beta$ -LG) and growth hormone (GH) were determined by polymerase chain reaction (PCR) and restriction enzyme digestion in seven breeds of cattle (Nelore, Gyr, Guzerá, Caracu, Charolais, Canchim and Santa Gertrudis).  $\kappa$ -Casein had two alleles with the A allele occurring at a higher frequency in *Bos indicus* breeds (0.93, 0.92 and 0.91% for Gyr, Guzerá and Nelore, respectively). The  $\beta$ -lactoglobulin locus had two alleles in all of the breeds. European breeds had a higher frequency of the  $\beta$ -LG A allele than Zebu breeds. The GH locus had two alleles (L and V) in *Bos taurus* and was monomorphic (L allele only) in all of the *Bos indicus* breeds evaluated. The highest frequency for the V allele was observed in Charolais cattle. The markers used revealed a considerable similarity among breeds, with two main groups being discernible. One group consisted of Zebu and Santa Gertrudis breeds and the other consisted of European and Canchim breeds.

## INTRODUCTION

Milk protein polymorphisms have been studied intensively because of their effect on the yield and processing properties of milk and its products.  $\kappa$ -Casein constitutes about 25% of the casein fraction of milk and  $\beta$ lactoglobulin accounts for about 75% of the albumin fraction. Several polymorphisms have been found for each of these proteins.  $\kappa$ -Casein variants A and B differ by two amino acid substitutions, Thr136/Ile and Asp148/Ala (Lin *et al.*, 1992). The  $\beta$ -lactoglobulin variants A and B also differ by two amino acid substitutions, Asp64/Gly and Val118/Ala. The B variants of  $\kappa$ -casein and  $\beta$ -lactoglobulin are associated with an increase in milk protein and fat content as well as cheese production (Van Eenennaam and Medrano, 1991; Bovenhuis *et al.*, 1992).

Another gene intensively investigated in farm animals is that of growth hormone (GH). GH deserves special attention because of its major role in milk production and post-natal growth and development. Two GH alleles that differ by a valine (V)/leucine (L) substitution at position 127 have been identified and are easily detected by PCR amplification and *Alu*I digestion (Lucy *et al.*, 1991). According to Schlee *et al.* (1994a), Simmental cattle carrying the heterozygous LV genotype have a superior weight gain and carcass composition.

Of the three most common Bos indicus breeds rais-

ed in Brazil (Nelore, Gyr and Guzerá), the Nelore form the main basis of the Brazilian cattle population. Unlike in India, *Bos indicus* bred in Brazil are used mostly for meat production, an exception being the Gyr breed which is also bred for milk production. Caracu cattle represent a dual purpose breed produced in Brazil from cattle brought from Portugal during colonization. Canchim and Santa Gertrudis cattle are hybrids derived from a cross between *Bos indicus* and *Bos taurus:* Canchim is 5/8 Charolais and 3/8 Zebu, while Santa Gertrudis is 5/8 Shorthorn and 3/8 Brahman.

Despite their importance for the Brazilian cattle industry, little is known of the allelic frequencies or DNA polymorphisms of these breeds. The purpose of this study, therefore, was to estimate the allelic frequencies of  $\kappa$ -casein,  $\beta$ -lactoglobulin and growth hormone, as well as the genetic distances among Nelore, Gyr, Guzerá, Caracu, Charolais, Canchim and Santa Gertrudis cattle raised in Brazil.

## MATERIAL AND METHODS

Genomic DNA was obtained from unrelated Nelore (N = 63), Gyr (N = 20), Guzerá (N = 25), Caracu (N = 30), Charolais (N = 32), Canchim (N = 30) and Santa Gertrudis (N = 20) cattle. For the determination of  $\kappa$ -casein and  $\beta$ -lactoglobulin in Gyr cattle, an additional group of 63 animals of unknown relationships was also analyzed. DNA was obtained by simple digestion with proteinase K (Innis, 1992) or by digestion with proteinase K and salt extraction (Olerup and Zetterquist, 1992). PCR for  $\kappa$ -casein,  $\beta$ -lactoglobulin and growth hormone was performed as described by Medrano and Aguilar-Cordova (1990), Ron *et al.* (1994) and Schlee *et al.* (1994b), respectively. The PCR

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 $\begin{array}{l} \textbf{Table I} - Allele \ frequencies \ for \ \kappa-case in \ (\kappa-CN), \ \beta-lactoglobulin \ (\beta-LG) \ and \ growth \ hormone \ (GH) \ in \ Nelore, \ Gyr, \ Guzerá, \ Caracu, \ Charolais, \ Canchim \ and \ Santa \ Gertrudis \ cattle. \end{array}$ 

		κ-CN alleles			β-LG alleles			GH alleles		
Breed	Ν	A	В	SD	А	В	SD	L	v	SD
Nelore	63	0.91	0.09	0.03	0.40	0.60	0.04	1.00	0.00	0.00
Gyr	83	0.93	0.07	0.02	0.37	0.63	0.04	1.00	0.00	0.00
Guzerá	25	0.92	0.08	0.04	0.34	0.66	0.07	1.00	0.00	0.00
Caracu	30	0.68	0.32	0.06	0.57	0.43	0.06	0.80	0.20	0.05
Charolais	32	0.48	0.52	0.06	0.55	0.45	0.06	0.72	0.28	0.06
Canchim	30	0.63	0.37	0.06	0.40	0.60	0.06	0.90	0.10	0.04
St. Gertrudis	20	0.85	0.15	0.06	0.17	0.83	0.06	0.97	0.03	0.03

A, B and L, V = alleles; SD = standard deviation; N = number of animals.

products and digested fragments were visualized by ethidium bromide staining following electrophoresis in 3% low melting point agarose gels. The allelic frequencies and standard errors were calculated for each locus. Genetic distances (Nei, 1978) were determined and a dendrogram constructed by the unweighted pair group method with arithmetical averages (UPGMA) method using the computer program NTSYS-PC (Rohlf, 1992).

#### **RESULTS AND DISCUSSION**

Table I shows the alelle frequencies and standard errors for  $\kappa$ -casein,  $\beta$ -lactoglobulin and growth hormone in the breeds of cattle studied. The highest frequencies for the κ-casein A allele were observed in Gyr, Guzerá and Nelore cattle (0.93, 0.92 and 0.91, respectively). The results for the Nelore and Gyr breeds agree with those reported by Del Lama and Zago (1996). A similar study conducted in dairy breeds showed that Holstein cattle have a higher frequency of the A allele than Jersey, Guernsey and dual purpose breeds (Van Eenennaam and Medrano, 1991). Among European dairy breeds, the  $\kappa$ casein A allele is associated with a higher milk production. However, this association did not occur among the Bos indicus breeds investigated in this study since Nelore, Gyr and Guzerá cattle had a higher frequency than Holsteins, and in milk-producing Gyr the frequency of the A

 Table II - Allele frequencies for κ-casein (κ-CN) and β-lactoglobulin (β-LG) in Gyr animals from herds selected for meat (IZ Sertãozinho) and milk (IZ Ribeirão Preto) production.

		ĸ	-CN allel	es		β-LG alleles			
Herd	Ν	А	В	SD	А	В	SD		
Milk Meat Total	50 33 83	0.89 1.00 0.93	0.11 0.00 0.07	$0.03 \\ 0.00 \\ 0.02$	0.35 0.41 0.37	0.65 0.59 0.63	0.05 0.06 0.04		

A, B and L, V = alleles; SD = standard deviation; N = number of animals.

allele was not significantly greater than in Nelore or Guzerá cattle. The  $\kappa$ -casein A allele, however, could have an association within a specific breed and to further explore the association of this allele with milk production, we compared Gyr animals from a herd selected for meat (Instituto de Zootecnia (IZ), Sertãozinho) and one selected for milk production (IZ, Riberão Preto). As shown in Table II, the frequency of the A allele for  $\kappa$ -casein was similar in both herds.

The GH locus had two alleles in *Bos taurus* and was monomorphic (L allele only) in the *Bos indicus* breeds (Table I). The frequency of the V allele was small in the hybrid breeds and the highest frequency for the V allele was observed in Charolais. Based on a genetic composition of 5/8 Charolais, the expected frequency for the V allele in Canchim would be 0.17 whereas the observed frequency was 0.10. This result could represent a sampling artifact, but it is also possible that the gene frequencies of the Charolais population sampled differed from the population used nearly 50 years ago in the crosses to obtain Canchim cattle.

The genetic distances among the breeds are shown in Figure 1. Although the markers investigated were similar to each other among breeds, two main groups were discernible. One consisted of the Zebu and Santa Gertrudis breeds, and the other consisted of the European and Canchim breeds. In the first cluster, the hybrid breed Santa Gertrudis diverged from the Zebu breeds. In the second cluster, Caracu and Charolais were more similar to each other than to Canchim. Thus, the markers investigated did not provide the expected separation of the breeds. The Santa Gertrudis breed being 5/8 European was expected to be closer to European breeds. Although the finding for Santa Gertrudis cattle was somewhat unexpected, similar results were obtained in a genetic distance analysis based on biochemical and immunological markers (Del Lama, 1991). The higher-than-expected similarity with Zebu cattle may reflect selection for Zebu genes after formation of the breed. Such an event would be expected if environmental conditions were to favor animals better adapted to the tropics.

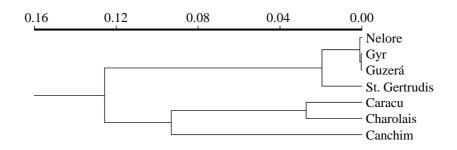


Figure 1 - Dendrogram of the genetic distances among Nelore, Gyr, Guzerá, Caracu, Charolais, Canchim and Santa Gertrudis cattle.

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#### RESUMO

Os genótipos de  $\kappa$ -caseína ( $\kappa$ -CN),  $\beta$ -lactoglobulina ( $\beta$ -LG) e hormônio de crescimento foram determinados por reação em cadeia de polimerase (PCR) e digestão com enzima de restrição em sete raças de bovinos (Nelore, Gir, Guzerá, Caracu, Charolesa, Canchim and Santa Gertrudis). A ĸ-caseína apresentou dois alelos e as freqüências mais elevadas para o alelo A foram observadas em Bos indicus (0,93, 0,92 e 0,91% para as raças Gir, Guzerá e Nelore, respectivamente). A β-lactoglobulina apresentou dois alelos em todas as raças estudadas, sendo a freqüência do alelo A mais elevada nas raças européias. O loco de hormônio de crescimento apresentou dois alelos em Bos taurus e foi monomórfico (alelo L) em todas as raças zebuínas. A maior freqüência para o alelo V foi observado na raça Charolesa. Os marcadores investigados revelaram alta similaridade entre as raças, com a formação de dois grupos principais: um composto de raças zebuínas e a raça Santa Gertrudis e outro composto das raças européias e a raça Canchim.

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