

Milk protein polymorphisms in Brazilian Zebu cattle*

Ivana Tramontina da Silva and Marco Antonio Del Lama

ABSTRACT

Five bovine milk protein polymorphisms were studied in Zebuine cattle raised in Brazil, through horizontal electrophoresis on starch gel containing urea and 2-mercaptoethanol, using basic and acidic buffer systems. Allelic frequencies for α -La, β -Lg, α_{S1} -Cn, β -Cn and κ -Cn loci were estimated in six Gyr herds (N = 283), six Guzerat herds (N = 205), one Nelore herd (N = 17) and one Sindi herd (N = 22), all from São Paulo or Minas Gerais State, Brazil. Genotypic frequencies observed for each locus and breed studied are in accordance with the assumption of genetic equilibrium, demonstrating absence of high inbreeding levels for the breeds tested. The F_{ST} value found indicated significant genetic differentiation among breeds; however, the Gyr and Guzerat herds showed significantly different gene frequencies. Genetic distance estimates among zebuine breeds studied and the Holstein breed, taken as a reference for a taurine breed, showed strong differences between these two racial groups.

INTRODUCTION

The first specimens of European cattle introduced by settlers in Brazil did not adapt to the prevailing climatic conditions, especially in the North and Northeast regions (Lopes and Resende, 1984). Occasional introductions of Zebu cattle led to the onset of halfbred animals with greater rusticity, fecundity, ease of acclimation, and weight. This fact motivated subsequent large-scale importations of Zebu cattle into the country.

Next to India, Brazil is today the most important center of Zebu selection. The importance of Zebu cattle in the country is demonstrated by the 60 million head, possessing different proportions of genes from cattle originating from India. Four "pure" Zebu breeds are raised in Brasil today: Nelore, Gyr, Guzerat and Sindi.

Since the first importations into Brazil, Zebu cattle were regarded as a meat breed. After observing that some herds had reasonable milk yields, breeders became interested in selection for milk production. As a result of this process, milk yield was significantly improved (Villares, 1979; Santiago, 1985).

Milk can be divided into two fractions: serum and caseins (Chianese *et al.*, 1988). The major protein components of serum are α -lactalbumin (α -La) and β -lactoglobulin (β -Lg). Genetic studies have reported the existence of two electrophoretic variants of α -La and four of β -Lg (see Jakob, 1994).

In the casein fraction, four proteins have been intensively studied: α_{S1} -casein (α_{S1} -Cn), α_{S2} -casein (α_{S2} -Cn), β -casein (β -Cn) and κ -casein (κ -Cn). Electrophoretic analyses have demonstrated the existence of different variants for these proteins (see Jakob, 1994), all of them determined by codominant alleles of the closely linked autosomal genes (Hines *et al.*, 1969).

Several studies have reported that some of these bovine protein variants, particularly certain β -Lg and κ -Cn variants, are associated with lactation performance and have a major influence on the composi-

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Departamento de Genética e Evolução, Universidade Federal de São Carlos, Rodovia Washington Luiz, km 235, 13565-905 São Carlos, SP, Brasil. Send correspondence to M.A.D.L.

tion of milk and on its processing properties, including cheese yield (Marziali and Ng-Kwai-Hang, 1986; Grosclaude, 1988; Aleandri *et al.*, 1990). From these studies, it is known that the AA β -Lg genotype is associated with high milk yield, while the BB genotype is related to high fat and casein contents and is, therefore, more desirable for cheese making. The B allele of κ -Cn is associated with high total milk protein yield, first lactation milk yield, herd life, and multiple lactation milk yield (see Sabour *et al.*, 1993).

The first studies on biochemical markers of *Bos indicus* raised in Brazil were performed by Mortari (1989) and Del Lama (1992), who studied several blood group systems and serum and erythrocytic protein polymorphisms. Data about milk protein polymorphisms in these animals were not available. The present study was conducted in order to estimate the allele frequencies at the loci that code milk proteins in Brazilian Gyr, Guzerat, Nelore and Sindi breeds.

MATERIAL AND METHODS

A total of 527 casual milk samples from six Gyr herds (N = 283), six Guzerat herds (N = 205), a Sindi herd (N = 22), and a Nelore herd (N = 17) was electrophoretically analyzed. All these samples came from São Paulo or Minas Gerais State, Brazil. Specific preservatives (Grosclaude *et al.*, 1987) were added to the samples at the time of collection and the material was transported and stored under refrigeration until processing. Milk was titrated at pH 4.6 with 1 N HCl for casein precipitation, and serum was separated and stored at -20°C . Casein was lyophilized and resuspended before use at 25 mg/ml in a 6.6 M urea solution.

Serum and casein milk fractions were electrophoretically analyzed on starch gels containing urea and 2-mercaptoethanol, using a basic buffer (Aschaffenburg and Michalak, 1968). Additionally, an acidic buffer (Peterson and Kopfler, 1966) was employed for β -Cn A fractionation into A¹, A² and A³ variants.

Gene frequencies and their standard errors were determined by direct phenotype counts for five gene loci: α -La, β -Lg, α_{S1} -Cn, β -Cn and κ -Cn. Genetic equilibrium and homogeneity of gene frequencies were determined using the chi-square test, with a 5% significance level.

Intralocus heterozygosity and its mean value (\bar{H}) were estimated as

described in Weir (1990). Mean inbreeding coefficients (f) were estimated according to the Alpha program of the software GENIOC (developed by P. Cabello and H. Krieger, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil). The components of the F-statistics were estimated according to Weir and Cockerham (1984) and the F_{IS} and F_{ST} significances were tested with a chi-square following Nei (1977) and Workman and Niswander (1970), respectively. Genetic distances were determined according to the method of Nei (1978) and Cavalli-Sforza and Edwards (1967), and the corresponding dendrograms were constructed with BIOSYS computer software (Swofford and Selander, 1981).

RESULTS AND DISCUSSION

Figure 1 shows some α -La, β -Lg, α_{S1} -Cn, β -Cn and κ -Cn electrophoretic variants observed. The frequencies of these variants were used to estimate the gene frequencies for the loci sampled by direct counting (Table I).

The observed higher frequency of the α -La B allele was consistent with results reported previously (Bhattacharya *et al.*, 1963). Aschaffenburg (1963) first suggested that the α -La A allele was a Zebu marker, since this allele is usually absent in *Bos taurus*. However, the presence of α -La A has been observed in different herds of the Italian Podolic breeds and has been explained as the result of the introgression of *Bos indicus* in this region, possibly having been brought to Rome as a tribute to Cesar (Baker and Manwell, 1980). Recently, Del Lama *et al.* (1992) demonstrated the presence of a Pep B variant, characteristic of zebuine cattle, in animals of Marchigiana and Chianina breeds, giving additional support to Baker and Manwell's hypothesis and, consequently, to the assumption that the α -La A variant is a reliable zebuine racial marker.

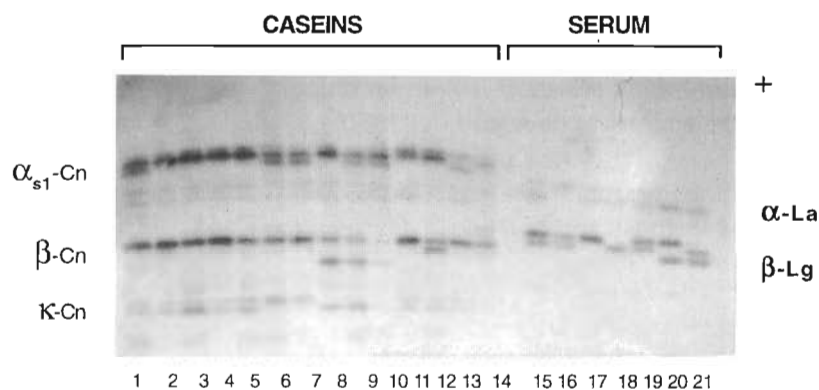


Figure 1 - Electrophoretic profile of α_{S1} -, β - and κ -caseins upon starch gel containing urea and 2-mercaptoethanol utilizing Tris-glycine, pH 8.5, buffer system. Sample 7, phenotype α_{S1} -Cn BC, β -Cn AA, κ -Cn AA; sample 12, α_{S1} -Cn BB, β -Cn AB, κ -Cn BB; sample 9, α_{S1} -Cn BC, β -Cn AD, κ -Cn BB.

Table I - Allelic frequencies at α -La, β -Lg, α_{S1} -Cn, β -Cn and κ -Cn loci, chi-square values for the determination of genetic equilibrium, heterozygosity (\bar{H}) and mean inbreeding coefficient (f) for the Zebu breeds raised in Brazil: Gyr (N = 283), Guzerat (N = 205), Sindi (N = 22) and Nelore (N = 17).

		Gyr	Guzerat	Sindi	Nelore
α -La	A	0.331 \pm 0.019	0.297 \pm 0.022	0.386 \pm 0.073	0.176 \pm 0.065
	B	0.669 \pm 0.019	0.703 \pm 0.022	0.614 \pm 0.073	0.824 \pm 0.065
	χ^2	3.538	0.148	1.334	0.781
	Ho(He)	0.492 (0.443)	0.429 (0.418)	0.591 (0.474)	0.353 (0.291)
β -Lg	A	0.362 \pm 0.020	0.136 \pm 0.017	0.045 \pm 0.085	0.441 \pm 0.085
	B	0.638 \pm 0.020	0.864 \pm 0.017	0.955 \pm 0.085	0.559 \pm 0.085
	χ^2	2.480	6.116	0.049	1.658
	Ho(He)	0.418 (0.462)	0.195 (0.236)	0.091 (0.087)	0.647 (0.493)
α_{S1} -Cn	B	0.108 \pm 0.013	0.060 \pm 0.012	0.136 \pm 0.052	0.000
	C	0.892 \pm 0.013	0.940 \pm 0.012	0.864 \pm 0.052	1.000
	χ^2	0.194	0.123	0.548	
	Ho(He)	0.187 (0.193)	0.111 (0.113)	0.273 (0.235)	---
β -Cn	A ¹	0.011 \pm 0.011	0.022 \pm 0.007	0.000	0.029 \pm 0.030
	A ²	0.935 \pm 0.010	0.928 \pm 0.013	0.864 \pm 0.052	0.971 \pm 0.030
	B	0.052 \pm 0.009	0.045 \pm 0.009	0.136 \pm 0.052	0.000
	D	0.002 \pm 0.002	0.005 \pm 0.003	0.000	0.000
	χ^2	1.436	6.982	0.548	0.016
	Ho	0.131 (0.123)	0.125 (0.117)	0.273 (0.235)	0.059 (0.057)
κ -Cn	A	0.945 \pm 0.009	0.802 \pm 0.019	0.659 \pm 0.071	0.971 \pm 0.029
	B	0.055 \pm 0.009	0.198 \pm 0.019	0.341 \pm 0.071	0.029 \pm 0.029
	χ^2	0.030	2.037	2.182	0.016
	Ho(He)	0.102 (0.104)	0.285 (0.316)	0.591 (0.449)	0.059 (0.057)
	\bar{H}	0.266 \pm 0.079	0.229 \pm 0.059	0.364 \pm 0.098	0.224 \pm 0.112
	f	0.007 \pm 0.029	0.045 \pm 0.035	-0.362 \pm 0.105	0.054 \pm 0.089

β -Lg is the major serum protein in bovine milk and its polymorphism was the first to be described in cow's milk (Aschaffenburg and Drewry, 1957). Nine genetic variants have been identified, but only one in particular, the B variant, is reported to be associated with superior milk production and cheese-making characteristics (Marziali and Ng-Kwai-Hang, 1986; Grosclaude, 1988; Aleandri *et al.*, 1990). This variant was found to be predominant in all Brazilian Zebu breeds, confirming results reported previously by Singh and Bhat (1980) in Indian Zebu cattle, although the frequency of this variant in Brazilian breeds was higher than that observed in corresponding Indian breeds. Low frequencies of the β -Lg C allele were reported among Zebus from Africa and India by Aschaffenburg *et al.* (1968) and among indigenous cattle by Singh and Khanna (1972). However, Singh and Bath (1980) report relatively high gene frequencies of β -Lg C among certain groups of Indian cattle. We did not observe this variant in our samples. These discrepancies are difficult to

explain and require further confirmation through additional studies.

The predominance of the α_{S1} -Cn C allele in the Zebu populations contrasts with the high frequency of the α_{S1} -Cn B allele in *Bos taurus* breeds (Aschaffenburg, 1968; Aschaffenburg *et al.*, 1968). A frequency close to 0.9 was reported for the C variant in *Bos indicus* while in *Bos taurus* its frequency ranges from 0.2 to 0.4 (Baker and Manwell, 1980). This asymmetric distribution in Zebu and European cattle has been explained by the different processes of domestication to which these animals were submitted (Grosclaude *et al.*, 1974). Our data agree with these previous reports, except for the findings in the Nelore breed. The exclusive presence of the α_{S1} -Cn C variant found in this breed could be due to the small number of animals analyzed from a single herd.

The β -Cn A allele is the most frequent at the β -Cn locus in *Bos indicus* and *Bos taurus* breeds. When the samples were analyzed on an acidic gel, with the consequent fractionation of the A variant, we found that the β -Cn A² allele presented higher frequencies in *Bos indicus* than in *Bos taurus*, confirming findings reported by Singh and Bhat (1981a) in Indian Zebu cattle.

The β -Cn A³ allele, detected in *Bos taurus* herds (Grosclaude *et al.*, 1974) and in the Indian Ongole breed (Singh and Bhat, 1981a), was not found in our samples. Besides A¹, A² and B variants, a fourth variant was found in a few Gyr and Guzerat animals, with an electrophoretic mobility which corresponds to the mobility assigned to the β -Cn D variant (Aschaffenburg *et al.*, 1968; Grosclaude *et al.*, 1974).

Of the four κ -Cn variants described, the most frequent are κ -Cn A and κ -Cn B. In the Zebu herds studied here there was a higher frequency of the κ -Cn A allele, corroborating the findings of Baker and Manwell (1980), who observed that, like British herds, Zebu cattle present high frequencies for this allele. Because of the effect of κ -casein genetic variants on renneting time, interest in selecting animals with the favorable κ -Cn B allele is considerable. The low frequency of this allele in Zebu and Holstein breeds raised in Brazil suggests that a selection program directed by molecular markers would help increase milk production.

As there are large differences in gene frequency, particularly at the α -*La*, α_{ST} -*Cn* and β -*Cn* loci, between European and Zebu breeds, these markers could be used for monitoring the genetic changes taking place among the Zebu-European cross-bred populations. Because of the increasing importance of the Girolando (Gyr x Holstein) and Guzolando (Guzerat x Holstein) breeds in Brazil, as a result of their milk production, these molecular markers could be used effectively to compare expected mixture values with present values to ascertain the effects of selective forces.

Similar \bar{H} values were observed in Zebu breeds, except for Sindi (Table I). Our Sindi \bar{H} estimate was similar to a value previously reported for Indian Red Sindi by Singh and Bhat (1981b). Del Lama (1992) and Singh and Bhat (1981b) obtained similar \bar{H} estimates for Indian and Brazilian Nelore, Guzerat and Gyr breeds; however, our present estimates were lower, probably due to differences among the samples analyzed and the loci studied.

With few exceptions, the genotypic distribution of all herds and breeds did not deviate significantly from the value expected by assuming genetic equilibrium (Table I). According to the Hardy-Weinberg law, these populations approximate genetic equilibrium for these loci; we can therefore assume that the inbreeding values do not deviate significantly from zero. However, this f value was calculated in order to determine the differences between the observed and expected values only for the heterozygote fraction (Table II). The herds presented negative f values for most loci, especially α -*La* and β -*Cn* and, as a consequence, negative f values were observed for the Gyr, Sindi and Nelore breeds.

The components of the F-statistics were estimated (Table II) to verify the combined effects of inbreeding (F_{IS}) and genetic drift (F_{ST}) in the subpopulations (breeds) studied. The F_{IS} value, as expected, was negative and non-significant, confirming the absence of consanguineous matings between individuals in the subpopulations. The F_{ST} value indicates that there is a marked reduction in heterozygosity within these breeds in relation to that of the population (*Bos indicus*). This decrease may be attributed to the fact that these breeds function as true genetic isolates.

A dendrogram was constructed on the basis of Cavalli-Sforza and Edwards (1967) chord distance values of genetic distance (Figure 2). Data from our laboratory from Brazilian Holstein samples were included for comparison between *Bos indicus* and *Bos taurus* cattle. Two distinct branches can be observed in the figure: the first consists of the four zebu breeds and the second of the *Bos taurus* breed.

The dendrograms constructed on the basis of the genetic distances of Nei (1978) and Cavalli-Sforza and Edwards (1967) showed essentially similar clusters. Smaller differences detected between the two methods may be attributed to the fact that, in certain breeds, a given allele was practically fixed. In this particular situation, according to Vienne and Demerval (1985), the method of Cavalli-Sforza and Edwards (1967) is more appropriate for estimates of genetic distance, and this is the reason why only results obtained by this method were presented.

The F_{ST} value indicates a highly significant level of genetic differentiation among Zebu breeds. Homogeneity tests and genetic distances estimates demonstrated also that the Gyr and Guzerat herds studied do not constitute a homogeneous group. These differences among herds must have been minimized by pooling unrelated individuals from the different Gyr and Guzerat herds when the F-statistics were estimated. However, a clear differentiation between *Bos taurus* and *Bos indicus* can be achieved on the basis of these milk protein polymorphisms (Figure 2).

The rather limited number of Sindi and Nelore samples examined may have had an effect on the conclusions presented here. Since Sindi and Nelore are not traditionally dairy herds, only a limited sample was available. This difficulty could be overcome by a genetic

Table II - Components of the F-statistics (F_{IS} , F_{IT} and F_{ST}) for the Gyr, Guzerat, Sindi and Nelore breeds sampled considered as part of a larger population.

Locus	F_{IS}	χ^2	F_{ST}	χ^2	d.f.	F_{IT}
α - <i>La</i>	-0.148	11.54*	0.028	29.51**	3	-0.115
β - <i>Lg</i>	-0.058	1.77	0.140	147.56**	3	0.090
α_{ST} - <i>Cn</i>	-0.054	1.54	0.038	40.05**	3	-0.014
β - <i>Cn</i>	-0.061	1.96	0.028	88.54**	9	-0.031
κ - <i>Cn</i>	-0.119	7.46	0.118	124.37**	3	0.130
Mean	-0.099	24.27	0.078	430.03**	21	-0.013

*Significant at the 1% level.

**Significant at the 0.1% level.



Figure 2 - Dendrogram illustrating the genetic relationships among Zebu breeds and one *Bos taurus* breed (Holstein) determined by Cavalli-Sforza and Edwards chord distance values (1967).

analysis based on DNA markers (Rando *et al.*, 1988; Rogne *et al.*, 1989; Wilkins and Kuys, 1992). Genotyping of bulls and embryos at this level is faster and less expensive than milk protein analysis of multiple dam-daughter pairs (Del Lama and Zago, 1996).

A more definite understanding of the genetic structure of Brazilian Zebu breeds requires an analysis of markers other than serum, erythrocyte and milk polymorphisms, and it will emerge when studies based on a large number of randomly chosen mitochondrial and nuclear DNA marker loci are undertaken.

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RESUMO

Cinco polimorfismos protéicos do leite bovino foram estudados em raças zebuínas criadas no Brasil através de eletroforese horizontal em gel de amido contendo uréia e 2-mercaptoetanol, utilizando tampão básico e ácido. As frequências alélicas nos locos α -La, β -Lg, α S1-Cn, β -Cn e κ -Cn foram estimadas em seis rebanhos da raça Gir (N = 283), seis da raça Guzera (N = 205), um da raça Nelore (N = 17) e um da raça Sindi (N = 22), todos provenientes dos Estados de São Paulo e Minas Gerais, Brasil. As frequências genotípicas observadas para cada loco em cada raça estudada encontram-se próximas às esperadas supondo equilíbrio genético, evidenciando ausência de coeficientes elevados de endogamia dentro de raças. O valor estimado de F_{ST} indica significativa diferenciação genética entre as raças. Entretanto, os rebanhos analisados das raças Gir e Guzera não constituem grupos geneticamente homogêneos. Estimativas de distância genética entre as raças zebuínas estudadas e a raça Holandesa, tomada como referência de raça taurina, mostram uma nítida diferenciação entre estes dois grupos raciais.

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