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CARBONIC ANHYDRASE POLYMORPHISMS IN NELLORE, CANCHIM AND IN DAIRY CROSSBRED CATTLE IN BRAZIL

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ABSTRACT

Carbonic anhydrase (CA) polymorphism was studied in 779 animals from three different breeding populations (Canchim, Nellore and European-Zebu crossbred dairy cattle). Six different phenotypes were detected, probably the result of three alleles: CAS, CAF and CAZ. The frequencies of the alleles for CAS, CAF and CAZ were 0.931, 0.055 and 0.014 in Canchim (5/8 Charolais + 3/8 Zebu), 0.750, 0.008 and 0.242 in Nellore and 0.852, 0.103 and 0.045 for the dairy group, respectively. The CA isozymes showed different thermostability when tested at 57 and 59°C. Phenotype CAZ had higher susceptibility than CAS. This suggests a functional difference that could be related to environmental factors.

INTRODUCTION

Polymorphism of carbonic anhydrase in cattle was first described by Sartore *et al.* (1969). The most frequent codominant alleles are those for CAS and CAF with phenoytpes SS, FS and FF resulting from these alleles. Several authors reported additional phenotypes for this enzyme. Stormont *et al.* (1972) described the phenotype CACS in three Aberdeen Angus animals where the isozyme C migrated more anodally than the F form. Crimella and Carenzi (1972) found another zone of activity localized between bands S and F. Dragnew (1973) described two different phenotypes in Bulgarian cattle and designated them FS1 and SS1. A new CAX zone which migrated more anodally than the FF form was reported by Han and Suzuki (1976) in Korean cattle. Sartore (1970) and Shanker *et al.* (1983) described other CA isozymes with the same electrophoretic mobility, slower than the S isozyme, which were called

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CAS Piedmont and CAS Sahiwal, respectively. Penedo *et al.* (1982) reported a new zone of activity in alcohol-chloroform extracts of an enzyme, called CA Z, which migrated much slower than the S isozyme at pH 7.3 in Zebu cattle (Gyr, Guzerat and Nellore). Graml *et al.* (1986) described a very slow migrating band which they called L.

This paper describes the electrophoretical identification of the carbonic anhydrase polymorphism after hemoglobin precipitation in cattle blood.

MATERIALS AND METHODS

Experimental animals

Blood samples were obtained from 448 dairy crossbred (1/2 to 7/8 European-Zebu) females and 120 Nellore and 211 Canchim (5/8 Charolais + 3/8 Zebu) animals. The dairy crossbred females, which were the daughters of crossbred bulls in a progeny test, were from the State of Minas Gerais. The Canchim animals from a closed herd, located in the State of São Paulo (EMBRAPA-UEPAE de São Carlos), can be considered as a good sample of the breed population in Brazil, because most of the private herds come from that one. The Nellore animals, most of them born on the above farm, came from four different herds in Brazil, and had all been controlled at birth by the Zebu Breeders Association.

Preparation of extracts and electrophoresis

Blood samples were taken from the jugular vein and kept refrigerated until arrival to the laboratory. Samples were centrifuged for 15 min at 3000 rpm. Plasma was saved for other purposes and erythrocytes were washed three times in a 0.9% NaCl solution. As it was suggested by Penedo *et al.* (1982), alcohol-chloroform extracts were used for typing carbonic anhydrase in all animals screened, in order to remove hemoglobin from the samples. When hemolysates are used as samples, the hemoglobin fraction overlaps with the CAZ band, masking it. Precipitation of hemoglobin was achieved as described by Roughton and Booth (1946): 0.5 ml thawed erythrocytes were mixed with 0.4 ml ethanol (40%) and 0.2 ml chloroform and shaken vigorously. After 30 min at 4°C the extracts were centrifuged at 3000 rpm for 15 min and the supernatant was used for electrophoresis. Electrophoresis was carried out in horizontal gels containing 13% (w/v) potato starch using 14 mM Tris, 4 mM citrate buffer, pH 7.3, in the bridge and 0.30 M boric acid, 0.10 M NaOH, pH 8.6, for the electrode buffer. A voltage of 9 V/cm was applied until the borate boundary reached 9 cm from the origin. After electrophoresis the starch gel was sliced lengthwise, the bottom slice was stained with amido black for 2 min and destained in methanol-

acetate with fast blue RR salt as coupler dye at 37°C. Acetazolamide (0.1 mM) was used as a specific inhibitor of carbonic anhydrase activity. To measure the effect of inactivation temperature on the activity of carbonic anhydrase isozymes, the samples were incubated at different temperatures (51, 54, 57 and 59 ± 0.5°C) for different periods of time and cooled on ice. The heated extracts were then electrophoresed. This experiment was repeated three times.

RESULTS

Carbonic anhydrase (CA) phenotypes are shown in Figure 1. As reported before, CAS has more esterase activity than CAF (Shanker *et al.*, 1983), while CAZ isozyme has an intermediate activity as compared to them (Figure 1). In amido black staining the CAZ band has almost the same intensity as the CAS band. The CAZ band migrates near the application point at pH 7.3. The specific inhibition of carbonic anhydrase activity with acetazolamide helped to identify the CA isozymes, especially in samples containing the CAZ isozyme.

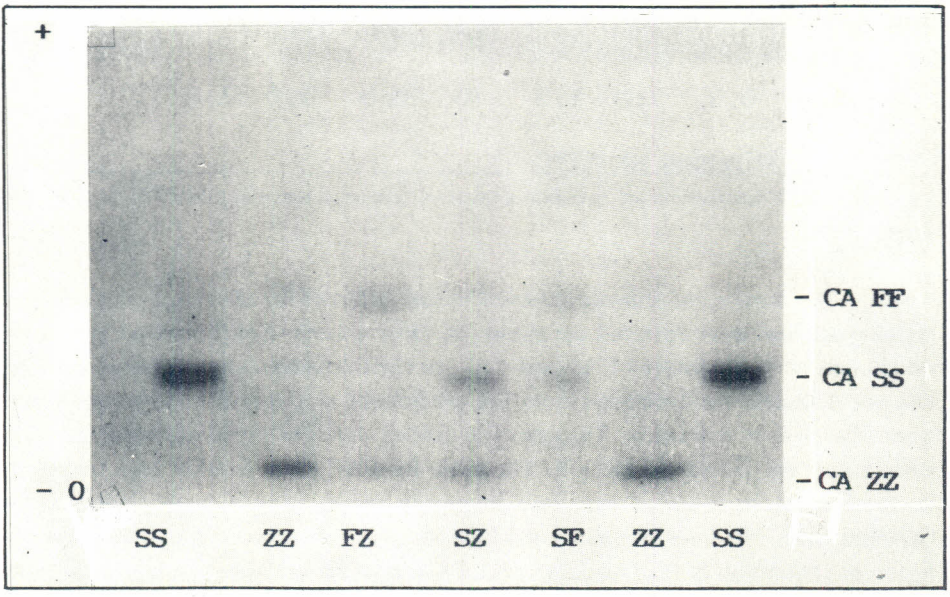

Figure 1 - Carbonic anhydrase phenotypes detected in starch gel electrophoresis.

The susceptibility of carbonic anhydrase isozymes to temperature was studied. The extracts heated at 51 and 54°C did not show inactivation of the different isozymes by temperature. The partial inactivation of isozyme CAS and CAZ when heated for 1

and 1.5 min at 57°C is shown in Figure 2. When the extracts were submitted for 1.5 min at 59°C, band CAZ was completely inactivated and band CAS was partially inactivated (Figure 3).

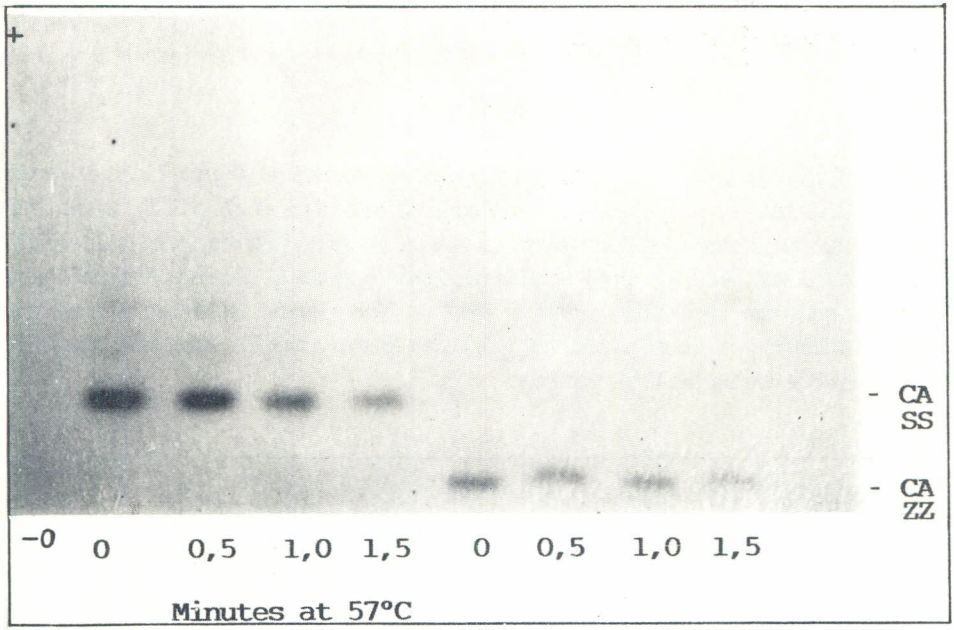

Figure 2 - Heat inactivation of carbonic anhydrase isozymes submitted to 57°C for different periods of time.

The number of animals screened in the different breeds and all the different phenotypes that were detected are shown in Table I. In the Canchin breed there were no animals with phenotypes CAF and CAZ. In the European-Zebu crossbred the CAZ was not detected. No deviations from the predictions of Hardy-Weinberg equilibrium were observed in this study. The gene frequencies detected in the different breeding populations are shown in Table II. The allele for CAZ has a notably high frequency in the Nellore herd and a very low frequency in the Dairy Crossbred and in the Canchim herd. The CAS Piedmont and Sahiwal types of carbonic anhydrase were not found in this study. In the Canchim breed it was possible to delineate gene frequencies between sexes, but the differences were not significant.

DISCUSSION

Carbonic anhydrase polymorphism has been widely studied. The allele for

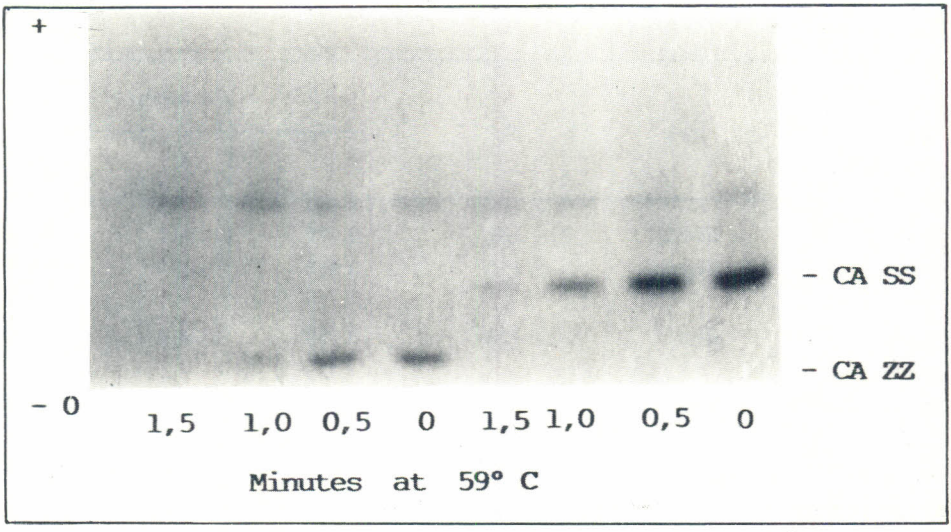

Figure 3 - Heat inactivation of carbonic anhydrase isozymes submitted to 59°C for different periods of time.

Table I - Number of animals with the different electrophoretic phenotypes detected for carbonic anhydrase, according to breeding populations.

Breeding populations		CA Phenotypes				χ^2
		SS	SF	SZ	(FZ, FF, ZZ)	
Canchim	Obs.	183	22	5	1	1.867
	Exp.	182.49	21.39	5.48	1.01	
Nellore	Obs.	67	1	46	6	0.564
	Exp.	67.50	1.44	43.56	7.47	
Dairy Crossbred	Obs.	324	83	32	9	0.474
	Exp.	325.20	78.62	34.36	9.8	

CAS is the predominant allele in all breeds and breeding populations, with the frequency ranging from 0.59 to 1.00 (Sartore *et al.*, 1969; Soos, 1970; Han and Suzuki, 1976; Efremov, *et al.*, 1980; Shanker *et al.*, 1983). Examples of these gene frequencies in cattle are shown in Table III. The lowest CAS frequencies were found in Hereford,

Table II - Frequencies of the different carbonic anhydrase alleles detected in the three breeding populations.

Breeding populations	Sex	No.	CA Alleles		
			S	F	Z
Canchim	Males	101	0.945	0.045	0.010
	Females	110	0.918	0.064	0.018
Nellore	Both	120	0.750	0.008	0.242
Dairy Crossbred	Both	448	0.852	0.103	0.045
All together		779	0.860	0.080	0.060

Jersey and Nellore breeds and, consequently, the highest CAF frequencies were found in Hereford and Jersey. The Nellore group of the present study showed a lower frequency for CAS than the one studied by Penedo *et al.* (1982). The same authors reported a frequency of 0.096 for the allele which controls CAZ, with 68 animals analyzed. Graml *et al.* (1986) detected in Egyptian Baladi cattle a slowly migranting band (frequency 0.03) which, probably, corresponds to the Z band described here. The value of 0.242 found in the present study using 120 animals of the Nellore breed, is much higher than in Baladi cattle. Larger populations of other breeds of Zebu cattle should be screened to determine the frequency of the allele for CAZ and possible adaptive values.

Except for the work of Penedo *et al.* (1982), the present study is, to the knowledge of the author, the only one with a large number of animals performed with the hemoglobin precipitation technique.

Thermostability tests showed differences among carbonic anhydrase isozymes. The CAZ isozyme is more susceptible to heat inactivation than isozyme CAS (Figure 3), suggesting a functional difference between these isozymes, that could be related to environmental factors.

It seems puzzling that the allele for CAZ was only described in Brazilian Zebu herds. This allele is probably present in all populations, but since it has a low frequency and is only detected with the hemoglobin precipitation technique, it probably passed undetected in the studies already conducted with the exception of the study of Baladi cattle by Graml *et al.* (1986).

Table III - Gene frequencies in the different breeds or breeding populations considering only CAS and CAF alleles.

Breed or Breeding populations	Number of animals	Frequency of CAS allele	Frequency of CAF allele	Author
Aberdeen Angus	114	.99	.09	1
Ayrshire	86	.86	.14	1
Brown Swiss	95	.93	.07	1
Guernsey	352	.96	.04	1
Hereford	408	.76	.24	1
Holstein-Friesian	102	.80	.20	1
Jersey	395	.59	.41	1
Polled Hereford	365	.89	.11	1
Korean	490	.91	.09	2
Deutsche schwartzbunte	550	.827	.173	3
Nellore	203	.998	.002	4
Guzerá	86	.994	.006	4
Gyr	100	1.000	.000	4
Sahiwal	61	.95	.05	5
Karan Swiss	370	.94	.06	5
Karan Fries	210	.95	.05	5
Canchim	211	.931*	.059	6
Nellore	120	.750*	.008	6
Dairy Crossbred	448	.852*	.103	6

*These values were calculated considering 3 alleles (S, F and Z) (see Table II).

1. Sartore *et al.* (1969); 2. Han and Suzuki (1976); 3. Thinnes *et al.* (1976); 4. Penedo *et al.* (1982); 5. Shanker *et al.* (1983); 6. Present study.

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RESUMO

O polimorfismo da anidrase carbônica (CA) foi estudado em 779 animals pertencentes a 3 grupos genéticos diferentes (Canchim, Nelore e Cruzamentos Europeu-

Zebu de gado leiteiro) pela técnica de precipitação da hemoglobina. Foram detectados seis fenótipos diferentes, provavelmente, resultado de três alelos: CAS, CAF e CAZ. As freqüências destes alelos nessa ordem foram 0,931, 0,055 e 0,014 para a raça Canchim, 0,750, 0,008 e 0,242 para a raça Nelore e 0,852, 0,103 e 0,045 para o gado leiteiro. As isozimas da CA mostraram termostabilidade diferencial quando testadas a 57 e 59°C. A isozima CAZ teve maior suscetibilidade que a isozima CAS. Isto sugere uma diferença funcional que poderia estar relacionada com fatores ambientais.

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