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BIOCHEMICAL BLOOD POLYMORPHISMS IN PITANGUEIRAS CATTLE AND THEIR EFFECTS ON REPRODUCTIVE, PRODUCTIVE AND HEAT TOLERANCE TRAITS

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ABSTRACT

The objective of the present study was to evaluate the effects of hemoglobin and carbonic anhydrase phenotype on reproductive, productive and heat tolerance traits in a herd of Pitangueiras dairy cattle. Electrophoresis showed the occurrence of four hemoglobin phenotypes, i.e., A, AB, B and AC. The alelle frequencies for this system were 91.85%, 7.89% and 0.26% for alleles HB^A, HB^B and HB^C, respectively. The phenotypes observed for the carbonic anhydrase system were S, FS, F, SZ and FZ, with allele frequencies of 81.83%, 12.84% and 5.33% for CA^S, CA^F and CA^Z, respectively. The chi-square test showed that this herd is in Castle-Hardt-Weinberg equilibrium for the hemoglobin and carbonic anhydrase systems. The different phenotypes had no significant effect on reproduction, production or heat tolerance.

INTRODUCTION

Electrophoretic methods can be used to detect polymorphisms of major bovine blood proteins and protein variants determined by codominant alleles. Hb A is the most common hemoglobin type and is more frequently detected in European breeds, whereas Hb B is generally more common in Zebus, in which it occurs at intermediate frequency. Variants Hb C, Hb Khil, Hb D and Hb I are observed in Zebus and their crosses, though at low frequencies (Braend, 1972; Abreu Filho *et al.*, 1982). Carbonic anhydrase polymorphism involving a pair of codominant autosomal alleles,

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CA^F and CA^S, was described by Sartore *et al.* (1969). Two rare variants, CA $S_{Piedmont}$ and CAC, were later described by Sartore (1970) and Stormont *et al.* (1970). Penedo *et al.* (1982) were the first to describe the occurrence of the CA^Z allele in Zebus.

The genetic markers of cattle blood, including the blood groups, can be used for individual identification, for the solution of kinship problems, the identification of monozygotic and dizygotic twins, the diagnosis of freemartins, and the study of breed relationships (Stormont *et al.*, 1970; Penedo, 1981). Attempts have also been made to find associations between these markers and economic traits. The use of genetic markers for predicting performance would permit early decision making, decreasing the interval between generations and increasing annual genetic gain. Several investigators have studied the association of performance traits with different hemoglobin and carbonic anhydrase phenotypes, obtaining contradictory results (Singh *et al.*, 1978; Solanki and Shukla, 1979, 1980; Ronda and Granado, 1983; Fernandez and Perez-Beato, 1985; Haenlein *et al.*, 1987).

The objectives of the present study were 1) to estimate the frequencies of different hemoglobin and carbonic anhydrase genotypes in a population of Pitangueiras cattle, 2) determine whether this population is in genetic equilibrium for these loci, and 3) evaluate the possible association of the polymorphisms detected with reproductive, productive and heat tolerance traits.

MATERIAL AND METHODS

The Pitangueiras breed was synthesized by crossing pure Red Poll (RP) males with 1/4 RP 3/4 Zebu females in the "Três Barras" ranch, located in the municipality of Pitangueiras, State of São Paulo, Brazil. The ranch is located at 503 m altitude, 21°00'S latitude and 48°11'W longitude. The climate is humid tropical, of the Aw type in the classification of Koeppen, with a warm rainy season from October to March and a cooler and drier season from April to September. Mean annual temperature was 23.86°C and rainfall 1,652.43 mm from 1977 to 1984. The ranch covers a pasture area of 2381 ha in which "colonião" grass (*Panicum maximum*, Jacq) predominates and is combined with perennial soy (*Neotonia wightii*, Verdc) and "siratro" (*Macroptilium atropurpureum*, DC). For more details about the origin management and distribution of the Pitangueiras breed see Lôbo and Reis (1989).

The cows were typed for two red cell protein systems, i.e. hemoglobin and carbonic anhydrase. Typing was performed in the Immunogenetics Laboratory of the Federal University of São Carlos by horizontal starch gel electrophoresis (Smithies, 1955). This procedure permits the identification of variants of the same protein arising by mutation with amino acid substitutions in the peptide chain. When an electric field is established the variants migrate at different rates in the starch gel owing to differences in electric charge, size and conformation. After separation, the protein bands are visualized by staining the gel with amino black. Gel preparation and separation and staining methods were those described by Penedo (1981). The CA Z variant of carbonic anhydrase was identified by the procedure used by Penedo *et al.* (1982). Data were analyzed by the chi-square test to determine whether this herd is in genetic Castle-Hardy-Weinberg equilibrium (Hogben, 1946; Levene, 1949; Li, 1978).

Reproductive and productive performance was studied using data from 3008 calvings by 893 cows born from 1970 to 1981. To determine heat tolerance, the rectal temperature of the animals was taken twice a day, i.e. from 4:00 to 9:00 a.m. and from 12:00 to 6:00 p.m., twice in March 1983 and three times in January 1984. The herd under study consisted of different generations, each of which was considered as a separate genetic group.

The following reproductive traits were studied: age at first calving (AFC), number of services per conception (NSC), days open (DO) and calving interval (CI). The productive traits considered were lactation length (LL), milk yield (MY), fat percentage (F%) and fat yield (FY). The heat tolerance traits studied were initial rectal temperature (IRT), final rectal temperature (FRT) and increase in rectal temperature (IncRT).

Data were analyzed statistically using the type 2 model (MTY = 02) of the LSML 76 program (Mixed Model Least-Squares and Maximum Likelihood Program) of Harvey (1977) based on the following linear equation:

$$Y_{ijk} = \mu + a_i + F_j + E_{ijk}$$

where:

Y_{ijk} = response for each dependent variable
μ = overall mean
a_i = sire effect (random)
F_j = fixed effects
E_{ijk} = random error.

The model was initially used to obtain the adjustment constant relative to fixed effects. The fixed effects (F_j) included in the model were genetic group, month and year of calving and parity (except for AFC). The following fixed effects were considered for rectal temperature (IRT and FRT): time and year when temperature was taken, genetic group and calving order. Non-significant fixed effects ($P > 0.05$) were removed from the model and a new analysis was done to obtain adjustment constants. After adjustment of the reproduction and production dependent variables, the means for all calvings and the most probable ability (MPA) of each cow were calculated by the method of Lush (1945). MPA was obtained with the repeatability values estimated by Lôbo (1980) and Reis (1983). After IRT abd FRT were adjusted for the significant fixed effects, the increase in rectal temperature (IncRT) was calculated as a third heat tolerance trait. Mean rectal temperatures taken over consecutive years (1983 and 1984) were calculated for each animal. After obtaining the MPC of each cow for reproductive and productive traits and the mean heat tolerance values, a new statistical analysis was performed using the MTY = 02 model in order to test the possible effect of hemoglobin and carbonic anhydrase phenotype on the dependent variables, using the biochemical polymorphisms and the five different coat colors of the animals as fixed effects.

RESULTS AND DISCUSSION

The electrophoretic tests showed the presence of four hemoglobin phenotypes, i.e., A, AB, B and AC. The phenotype and allele frequencies for this system are shown in Table I. The frequency for the Hb^A allele was 0.92, a value similar to those detected in several studied on 5/8 European 3/8 Zebu animals, i.e. 0.94 for 5/8 Holstein 3/8 Guzerá cows (Penedo, 1981) and 0.92 for Santa Gertrudes (5/8 Shorthorn 3/8 Zebu) cows (Hod and Herz, 1969). Other investigators, however, detected lower HB^A frequencies than reported in the present study, i.e., 0.82 for Canchim (5/8 Charolais + 3/8 Zebu) animals (Silva, 1973) and 0.83 for 5/8 Holstein 3/8 Guzerá cows (Penedo, 1981). A higher frequency of the HB^A alleles has been observed in almost all cattle breeds, though the highest values occur in European breeds. The Hb^B allele is detected at intermediate frequency in Zebus, and Hb^C is rarer and occurs in Zebus and their crosses. Thus, the present results show the effect of the European

Table I - Observed and expected phenotype frequencies and alelle frequencies in the hemoglobin system.

Phenotype	Observed number	Expected number[a]	Allele frequency
A	490	491.843	f (Hb^A) = 0.9185
B	2	3.629	f (Hb^B) = 0.0789
C	0	0.004	f (Hb^C) = 0.0026
AB	88	85.5	
AC	3	2.786	
BC	0	0.239	
Total	583	583.000b	

[a]According to the Castle-Hardy-Weinberg theorem (Li, 1978).
[b]df = 2, p = 0.78.

Red Poll breed on the frequency of the Hb^A allele. Analysis of the differences between observed and expected genotype frequencies showed that the deviation was not significant. The chi-square test yielded $\chi^2 = 1.11$, a nonsignificant value ($P > 0.05$) for 3 degrees of freedom (d.f.) (n-k-1), where n is the number of genotypes involved and k the number of alelles to be estimated. Thus, we may consider this herd to be in Castle-Hardy-Weinberg equilibrium for the hemoglobin locus.

The electrophoretic tests showed the occurrence of the S, FS, F, SZ and FZ phenotypes for the carbonic anhydrase system. Table II shows the phenotype and allele frequencies for carbonic anhydrase in the herd under study. The most frequent allele was CA^{S}, with a value of 0.82. This result is slightly different from that obtained by Penedo (1981) who detected 1.00 and 0.94 for 5/8 Holstein 3/8 Guzerá cows and heifers, respectively. The lower frequency observed in Pitangueiras cows may have been due to the effect of the European breed involved in their formation. The differences between observed and expected genotype frequencies were not statistically significant. The chi-square test yielded $\chi^2 = 4.01$, a nonsignificant ($P > 0.05$) value for 3 d.f. (n-k-1). Thus, this herd is also in Castle-Hardy-Weinberg equilibrium for the carbonic anhydrase locus. This means that random mating occurred for the systems under study (Hb and CA).

Table II - Observed and expected phenotype frequencies and alelle frequencies in the carbonic anhydrase system.

Phenotype	Observed number	Expected number[a]	Allele frequency
S	374	370.297	$f(CA^S) = 0.8183$
F	11	9.117	$f(CA^F) = 0.1284$
Z	0	1.571	$f(CA^Z) = 0.0533$
FS	109	116.207	
SZ	48	48.239	
FZ	11	7.569	
Total	553	553.00[b]	

[a]According to the Castle-Hardy-Weinberg theorem (Li, 1978).
[b]df = 2, p = 0.32.

Least squares analysis of variance was used to determine the effect of the different hemoglobin (A and AB) and carbonic anhydrase (S, F, SZ and FZ) phenotypes on reproductive, productive and heat tolerance traits (Tables II, IV and V). The two hemoglobin and five carbonic anhydrase phenotypes had no significant effect ($P > 0.05$) on AFC, NSC, DO or CI. In studies carried out in India, hemoglobin

Table III - Least squares analysis of variance for reproductive traits carried out to test the effects of hemoglobin and carbonic anhydrase phenotype.

Trait	Source of variation	d.f.	Mean squares	P > F
Age at first calving	Sire	46	62.039	0.000
	Coat	4	14.556	0.434
	Hemoglobin	1	25.265	0.200
	Carbonic anhydrase	4	5.049	0.856
	Error	274	15.291	
	Total	330		
Number of services per conception	Sire	48	0.051	0.221
	Coat	4	0.093	0.081
	Hemoglobin	1	0.001	0.897
	Carbonic anhydrase	4	0.093	0.083
	Error	281	0.444	
	Total	339		
Days open	Sire	45	225.930	0.074
	Coat	4	137.157	0.509
	Hemoglobin	1	0.172	0.999
	Carbonic anhydrase	4	329.969	0.097
	Error	245	165.709	
	Total	300		
Calving interval	Sire	42	175.426	0.084
	Coat	4	144.935	0.348
	Hemoglobin	1	33.562	0.611
	Carbonic anhydrase	4	233.348	0.129
	Error	230	129.399	
	Total	282		

phenotype also had no effect on reproductive performance (Balakrishnan and Nair, 1966; Singh *et al.,* 1978; Solanki and Shukla, 1979). In contrast, Sengupta (1975) detected a significant effect of hemoglobin phenotype on AFC, the animals with the A phenotype being the most precocious, Velhankar *et al.* (1977) reported that Gir heifers with the AB phenotype reached puberty at a younger age; Berovides (1978) showed that crossbred cows with the A phenotype had shorter calving intervals and Shrimal and Parek (1982) reported that AB animals had the lowest AFC. Even though

Table IV - Least squares analysis of variance of productive traits carried out to test the effects of hemoglobin and carbonic anhydrase phenotypes.

Trait	Source of variation	d.f.	Mean squares	P > F
Lactation length	Sire	44	500.221	0.243
	Coat	4	250.306	0.678
	Hemoglobin	1	523.654	0.272
	Carbonic anhydrase	4	106.165	0.912
	Error	250	432.219	
	Total	304		
Milk yield	Sire	44	491743.956	0.000
	Coat	4	20845.043	0.974
	Hemoglobin	1	140178.841	0.365
	Carbonic anhydrase	4	136991.497	0.523
	Error	250	170056.119	
	Total	304		
Fat percentage	Sire	42	0.001	0.268
	Coat	4	0.001	0.563
	Hemoglobin	1	0.000	0.806
	Carbonic anhydrase	4	0.001	0.661
	Error	232	0.001	
	Total	284		
Fat yield	Sire	42	496.368	0.000
	Coat	4	56.454	0.902
	Hemoglobin	1	321.554	0.222
	Carbonic anhydrase	4	168.452	0.536
	Error	232	214.755	
	Total	284		

the literature does not report the same results with respect to the most precocious phenotype in relation to AFC, we noted that animals with the Hb^B allele always reached puberty at a later time. This suggests that the Hb^A allele may be related to sexual precocity in cattle. In the present study it was not possible to test the effect of the B phenotype on reproductive traits owing to its reduced frequency in Pitangueiras cattle. This fact reduces the possibility of detecting the effect of different hemoglobin phenotypes on the traits under study since both phenotypes tested had the Hb^A al-

Table V - Least squares analysis of variance of heat tolerance traits carried out to test the effects of hemoglobin and carbonic anhydrase phenotype.

Trait	Source of variation	d.f.	Mean squares	P > F
Initial rectal temperature	Sire	48	0.116	0.583
	Coat	4	0.211	0.146
	Hemoglobin	1	0.004	0.858
	Carbonic anhydrase	4	0.056	0.768
	Error	291	0.123	
	Total	349		
Final rectal temperature	Sire	48	0.183	0.215
	Coat	4	0.341	0.072
	Hemoglobin	1	0.017	0.740
	Carbonic anhydrase	4	0.074	0.754
	Error	291	0.156	
	Total	349		
Increase in rectal temperature	Sire	48	0.248	0.475
	Coat	4	0.765	0.016
	Hemoglobin	1	0.034	0.711
	Carbonic anhydrase	4	0.088	0.841
	Error	291	0.247	
	Total	349		

lele. More studies on this topic have been conducted on Zebus in India owing to the higher frequency of animals with the B phenotype among Indian breeds.

The hemoglobin and carbonic anhydrase phenotypes also had no significant effect on productive traits. As was the case in the present study, many studies published in the literature report no association of blood genetic markers with reproductive and productive traits, and the results obtained have often been conflicting. One of the possible causes of this is that genetic markers are qualitative genetic traits, whereas most of the economically important traits are quantitative. The results obtained for the hemoglobin phenotypes with respect to milk yield are similar to those obtained by Balakrishnan and Nair (1966), Fernandez *et al.* (1976), Berovides (1978), Singh *et al.* (1978), Solanki and Shukla (1980) and Zaragaza *et al.* (1983). Singh *et al.* (1978) and Solanki and Shukla (1979) also found no effect of hemoglobin phenotype on lactation length. The different carbonic anhydrase phenotypes had no effect on milk yield, in agreement with data reported by Novy (1976) and Zarazaga *et al.* (1983).

Analysis of variance showed that the different hemoglobin and carbonic anhydrase phenotypes also had no effect on initial rectal temperature or increase in rectal temperature. A similar result was obtained by Silva (1973) who found no effect of hemoglobin phenotype on heat tolerance traits (respiratory rhythm and rectal temperature) in Canchim animals. This investigator commented on the possibility that polymorphism for the hemoglobin locus may be more related to breed phylogeny regardless of whether or not this breed tolerates heat.

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RESUMO

O presente trabalho objetivou testar o efeito dos fenótipos da hemoglobina e da anidrase carbônica sobre características de reprodução, produção e tolerância ao calor em um rebanho de bovinos leiteiros da raça Pitangueiras. Através dos testes eletroforéticos foi observada a ocorrência de quatro fenótipos para o sistema de hemoglobina: A, AB, B e AC. As frequências alélicas para este sistema foram de 91,85%, 7,89% e 0,26%, para os alelos Hb^A, Hb^B e Hb^C, respectivamente. Para o sistema da anidrase carbônica, foram observados os seguintes fenótipos: S, FS, F, SZ e FZ, sendo as frequências alélicas de 81,83%, 12,84% e 5,33% para CA^S, CA^F e CA^Z, respectivamente. Pelo teste do qui-quadrado, verificou-se que este rebanho se encontra em equilíbrio genético de Castle-Hardy-Weinberg, para os locos da hemoglobina e da anidrase carbônica. No estudo dos efeitos destes sistemas sobre as características de reprodução, produção e tolerância ao calor, os diferentes fenótipos não apresentaram efeito significativo.

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