NEW ALLELE FOR *PIT 1 LOCUS* IN NELLORE CATTLE BREED (*Bos indicus*)

F.H. Biase[1], L. Martelli[1], A.J.M. Rosa[1], F.M.F. Nunes[1], A. del V. Garnero[1], R.J. Gunski[2], L.A.F. Bezerra[1] and R.B. Lôbo[1]

[1] FMRP-USP - Depto. de Genética - Bl. C - Av Bandeirantes, 3900 - Ribeirão Preto-SP CEP14040-030, Brasil
[2] Fac. Cs. Ex. Qcas. y Nat. – UNaM - Depto. de Genética – Félix de Azara 1552 - Posadas - Mnes. CP3300, Argentina

INTRODUCTION

Polymorphisms in candidate genes markers, known as type I markers, are good source of investigation for improving production, since they are involved in metabolic paths of important characteristics (Rotschild and Morris, 1999). PCR-RFLP is the easiest kind of marker for genotyping animals including commercial production farms.

Growth Hormone (GH) and its regulation are important for studies as polymorphic markers influencing growth traits in cattle. Polymorphisms in GH are related to increased milk production in Holstein-Friesian Bulls (Falaki *et al*., 1996), milk fat (Høj *et al*., 1993) and protein production (Lagziel *et al*., 1996). Schelle *et al.* (1994) have showed that Simmental bulls with LV type have higher breeding values for growth than the homozygotes and the carcass yeld was also influenced by the genotypes. Lechniak *et al*. (1999) demonstrated that some variants of GH might affect the pattern of sperm production in bulls.

Pituitary-Specific Transcription Factor (*PIT 1*) is important in somatotropin synthesis once it regulates the growth hormone liberation (Tuggle and Trenkle, 1996). Although its participation on growth hormone synthesis, there is no report of polymorphisms influencing growth traits in cattle.

Woollard *et al*. (1994) developed a RFLP-PCR based test evidencing polymorphism in *PIT 1* bovine gene, considering it as a candidate for improving growth efficiency in cattle. Moody *et al*. (1995) assigned the *locus* to chromosome 1 and designed primers to amplify ~1355bp, which was polymorphic for *Hinf* I enzyme restriction, distinguishing two allele (A and B) in Angus, Holstein, Hereford, Gelbviech and Brahman breeds, with an overall frequency of 0.25 for A allele. In this paper we describe a new allele in Nellore cattle breed (*Bos indicus*) and analyzed if the genotype has influenced the production.

MATERIAL AND METHODS

The present study was carried using 241 animals of Nellore breed (*Bos indicus*). The animals have the breeding value evaluated by Nellore Catlle Breeding Program (NCBP) – University of São Paulo. Analysis was performed in the "Laboratório de Micromanipulação de Embriões e Genética Molecular", stated at School of Medicine of Ribeirão Preto, SP, Brazil.

Genotyping animals. Blood samples were collected, in sterile tubes containing EDTA as anticoagulant. DNA extraction was performed according Sambrook *et al.* (1989). Primers described by Moody *et al*. (1995) were used for Polymerase Chain Reaction (PCR) to amplify 1355bp. The PCR reaction consisted of 1x PCR buffer, 2.5 mM of $MgCl_2$, 200μM of each dNTP, 0.2μM of each primer, 1U of Taq DNA Polymerase and 100-150ηg of genomic DNA, at a final volume of 50μL. The cycles were 5 min 95°C, once; thirty five times 50s 95°C, 30s 55°C, 50s 72°C, and a final extension of 5min 72°C. An aliquot of 5μL of each amplification was restricted with 1 unit of *Hinf*I for 15 hours at 37°C. The fragments were visualized by electrophoresis in 12% polyacrylamide gel and silver stained.

Statistical Analysis. The records of 241 animals were analyzed by mixed models methodology and Best Linear Unbiased Prediction (BLUP), according to the animal model, using the MTDFREML software (Boldman *et al*., 1995). Expected Progeny Difference (EPD) was predicted for standardized weight at 210 and 450 days (W210, W450) and for scrotal circumference at 365 and 455 days (SC365, SC450).

To analyze the effect of genotype on these traits, the following linear model was used:

$$Y_{ijk}=\mu + a_i + g_j + e_{ij}$$

Where:
Y_{ijk}: Expected Progeny Difference of trade k for individual ij;
μ: population mean;
a_i: random effect of sire (I=1,2,…,59);
g_j: fixed effect of genotype (j = BB, BC);
e_{ij}: random residual effect, assuming $N \sim (0, \sigma^2)$.

For the General Linear Model we considered the BB and BC genotypes, due to the reduced number of the other two types. To execute the program, the Software Statistical Analysis System (SAS Institute, 1995) was used.

RESULTS AND DISCUSSION

The genotypes patterns are demonstrated in Figure 1. Nellore animals typed for *PIT 1* RFLP did not presented homozygote type of A allele. A and B alleles were seen as the standard in Moody *et al*. (1995). Differing the two alleles, a new pattern of fragments in gel was observed, which was considered as a new polymorphic allele *C* for Nellore cattle. Due to proposed evolutive divergence at 575,000 – 1,150,000 years of *Bos indicus* from *Bos taurus* (Loftus, 1994), it is quite possible that mutations are specific in this species. As explained by Moody *et al*. (1995), the amplified fragment is expected to contain the exon 5, intron 5 and exon 6. Mutation rate is higher in introns enhancing the possibility of having new restrictions sites for enzyme action.

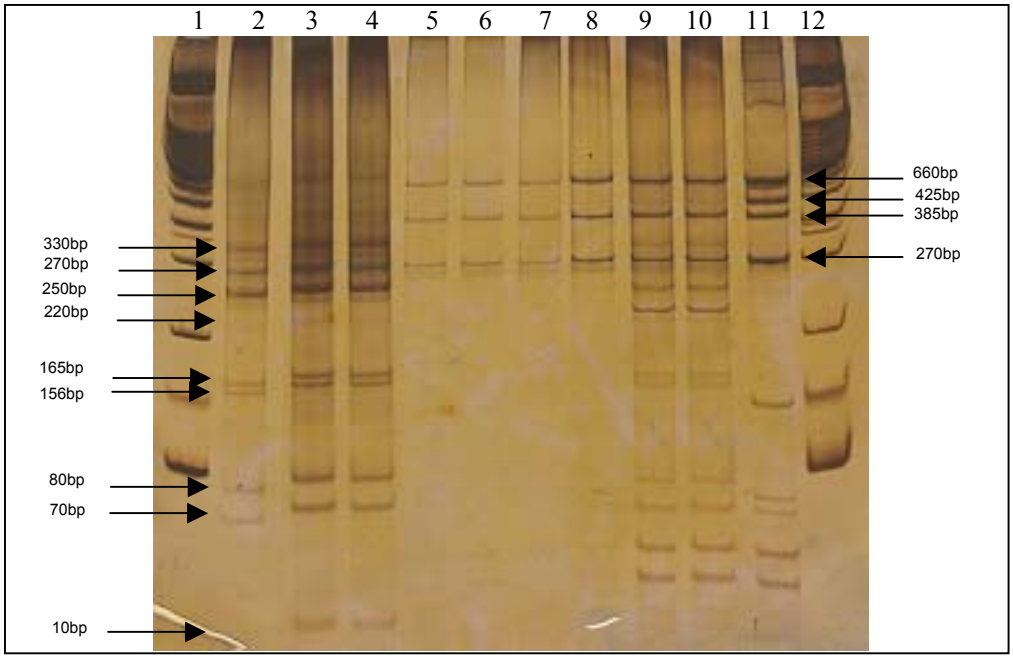

Figure 1. Hinf I PCR/RFLP at the *PIT 1 locus*. Lanes 1 and 12, 100 bp Ladder (Gibbco); 2, 3 and 4, CC genotype; 5, 6, 7 and 8, BB genotypes; 9 and 10, BC genotypes; 11, AB genotype

The frequencies were 0.00825, 0.7841 and 0.2072 for A, B and C, respectively. The C allele frequency of 0.2072 of supports the possibility of being a new polymorphism (Morton *et al.*, 1966). χ^2 test revealed Hardy-Weinberg disequilibrium at level of P = 0,05. The low frequency of A allele and the AA and AC genotypes absence suggest that selection for meat production is favoring the BB and BC animals. Otherwise we must consider the limited size of the sample.

Means, maximum and minimum values and number of observations of traits for BB and BC genotypes are seen in Table 1. There was no significance (P=0,05) between EPD values of BB and BC for traits considered (EPD W210, P=0.49; EPD W450, P=0.36; EPD SC365, P=0.11; EPD SC450, P=0.20). Several genes are responsible for the growth and animal development. Despite of the Pituitary-Specific Transcription Factor importance on GH synthesis and the existence of polymorphisms, the absence of genotype's influence on these EPD can be considered.

This gene may act in dominance relation among alleles, being necessary to analyze all possible genotypes to consider this hypothesis. Even more, once of the amplified region by PCR contains an intron, there is a possibility that the mutations do not modify the structure of protein product and the rate of expression. Then, these polymorphic alleles would be selectively neutral.

Table 1. Number (N) of observations and values of Expected Progeny Difference (EPD) of traits[A] by genotype.

	BB					BC				
Traits[A]	Mean	Max	Min	S	N	Mean	Max	Min	S	N
EPD W210	4.37	11.42	-1.71	2.65	154	3.75	9.72	-2.31	2.56	84
EPD W450	6.01	18.22	-4.88	4.32	145	5.85	15.89	-4.64	4.21	84
EPD SC365	-0.01	0.063	-0.50	0.23	100	0.05	0.55	-0.39	0.21	69
EPD SC450	-0.15	0.77	-0.27	0.40	102	0.06	0.89	-0.9	0.40	69

[A] Weight in Kilograms, Scrotal Circumference in Centimeters. Max: Maximum value; Min: Minimum value; S: Standard deviation.

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