ASSOCIATION OF *PIT1* GENOTYPES WITH GROWTH TRAITS IN CANCHIM CATTLE

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ABSTRACT: Use of genetic markers can aid on the identification of animals with highest breeding values in beef cattle. The *PIT1* gene codes for the Pit-1 transcription factor is essential for the activation of prolactin, growth hormone and *PIT1* genes. This research is an investigation of the effect of *PIT1*-*Hinf*I polymorphism on growth traits of 509 Canchim animals, from two lineages, GG1 and GG2. *PIT1* genotypes were identified through PCR-RFLP. Genotype effect on phenotypic values for birth weight (BW), standardized weaning weight (W240), weight at 12 months of age (W365), and the average daily weight gain from birth to weaning (AGBW), and from weaning to 12 months of age (AGW12) were analyzed by the least squares method. Effects of the interaction between the animal's genetic group and *PIT1* genotype for W240, AGBW and AGW12 were observed ($P < 0.05$). Differences between means of *Hinf*I (–/–), *Hinf*I (+/+) and *Hinf*I (+/–) genotypes for W240 and AGBW were observed in GG2 ($P < 0.05$), revealing superiority of (–/–) genotype for those traits. Means for genotypes (+/+) and (+/–) for W240 and AGBW, did not differ from one another, suggesting a dominance effect of the *Hinf*I (+) allele. The *Hinf*I (–) allele had a favorable effect on W240 and AGBW in GG2, when present in homozygosis. The difference between *PIT1* behavior observed in the two genetic groups may suggest the action of a quantitative trait *locus* linked to *PIT1*, segregating only in GG2 population.
Key words: RFLP, bovine, pituitary factor 1, candidate gene

ASSOCIAÇÃO DE GENÓTIPOS *PIT1* COM CARACTERÍSTICAS DE CRESCIMENTO EM GADO CANCHIM

RESUMO: O uso de marcadores genéticos pode favorecer a identificação de animais geneticamente superiores em bovino de corte. O gene *PIT1* codifica para o fator de transcrição Pit-1, crítico para ativar a expressão dos genes da prolactina, hormônio de crescimento e *PIT1*. Esta pesquisa investiga o efeito do polimorfismo *Hinf*I-*PIT1* sobre características de crescimento em 509 animais da raça Canchim, pertencentes à duas linhagens, GG1 e GG2. Os genótipos do gene *PIT1* foram identificados pela técnica PCR-RFLP. Os efeitos dos genótipos sobre valores fenotípicos para pesos ao nascimento (PN), pesos padronizados à desmama (P240) e ao ano (P365), ganhos médios diários de peso do nascimento à desmama (GMND), e da desmama aos 12 meses de idade (GMD12) foram analisados pelo método dos quadrados mínimos. Foram observados efeitos de interação entre grupo genético do animal e genótipo de *PIT1* sobre P240, GMND e GMD12 ($P < 0.05$). Diferenças entre médias dos genótipos *Hinf*I (–/–), *Hinf*I (+/+) e *Hinf*I (+/–) para P240 e GMND foram observadas em GG2 ($P < 0,05$), revelando superioridade do genótipo *Hinf*I (–/–). As médias dos genótipos (+/+) e (+/–) para P240 e GMND, não diferiram, sugerindo efeito de dominância do alelo *Hinf*I (+). O alelo *Hinf*I (–) mostrou-se favorável sobre P240 e GMND em GG2, quando em homozigose. A diferença de comportamento de *PIT1* observada nos dois grupos genéticos pode sugerir a ação de loco de característica quantitativa ligado ao gene *PIT1*, segregando apenas em GG2.
Palavras-chave: RFLP, bovinos, fator pituitário 1, gene candidato

INTRODUCTION

Most economic traits are conditioned by poly-genes and subject to environmental variations, which hamper the identification of the best genotypes. Thus, genetic progress in populations depends on statistical methodologies to reveal how the properties observed in populations are influenced by properties of the genes involved and by non-genetic circumstances that might affect a metric trait (Falconer & Mackay, 1996).

Polymorphism detection in genes related to production traits and the identification of the allele which results in a phenotype of interest can allow for marker assisted selection (MAS). Among the genes involved in mammalian growth, *PIT1* is considered as a candidate. *PIT1* was mapped to *Bos taurus* chromosome 1 (Moody et al., 1995), and belongs to a group of genes that code for proteins involved in animal development. The specific pituitary transcription factor Pit-1, coded by the *PIT1* gene, is required for the initiation of the expression of the growth hormone releasing factor gene (Lin et al., 1992) and is involved in the maintenance of the expression of the gene coding for the β subunit of thyroid stimulating hormone (Lin et al., 1994). It is a critical factor for the activation of the expression of prolactin and growth hormone genes, participating in the activation of *PIT1* itself (Parmentier et al., 1999).

A *Hinf*I polymorphism is located within exon 6 and characterized by a silent mutation on *Hinf*I (–) allele (Dierkes et al., 1998), and their effects were associated with growth traits in cattle (Conde et al., 2001; Silva et al., 2006). The objective of this study was to analyze the distribution of the *Restriction Fragment Length Polymorphism* (RFLP) *PIT1-Hinf*I and to investigate the influence of allelic variants on traits related to growth in a herd of Canchim cattle.

MATERIAL AND METHODS

Experimental animals

The sample included 509 Canchim animals, resulted from crosses between *Bos taurus* and *Bos indicus* (Zebu) breeds, born between 1998 and 2000, fed on pasture with mineral supplements. Animals were separated in two genetic groups. Genetic group 1 (GG1), consisting of 232 Canchim animals with average of 5/8 Charolais and 3/8 of Zebu (Nelore, Guzerá and Indubrasil) (Alencar, 1988). Genetic group 2 (GG2), consisting of 277 animals, with an average of 21/32 Charolais and 11/32 Nelore breeds (Barbosa, 2000). The phenotypic traits considered in the analyses were birth weight (BW), weaning weight (WW), weight at one year (W12), the average daily weight gain from birth to weaning (AGBW), and from weaning to 12 months of age (AGW12).

DNA isolation

DNA samples belonged to the bovine DNA collection, obtained from leukocytes by a salting out procedure (Regitano, 2001).

PCR-RFLP analysis

Polimerase Chain Reaction (PCR) with the *PIT1* specific primers 5'-CAATGAGAAAGTTGGTGC-3' and 5'-TCTGCATTCGAGATGCTC-3' was analyzed according to Moody et al. (1995). Amplified products were digested with the restriction enzyme *Hinf*I and fragments were separated in 3% agarose gel, stained with 0.45 μg mL^{-1} ethidium bromide. The DNA size standard ϕX174/*Hae*III was used for fragment size analysis.

Statistical analysis

The allelic frequencies of the candidate gene *PIT1* were estimated in each genetic group by direct count and consisted of the ratio between the number of alleles detected in the population and the number of chromosomes analyzed. The chi-square test was used to compare their proportions in the two genetic groups (GG1 and GG2) according to Snedecor & Cochran (1967).

The effect of the candidate gene *PIT1* was analyzed for BW, WW and W12, and for the average daily weight gain from birth to weaning (AGBW) and from weaning to 12 months of age (AGW12). WW and W12 values were standardized for 240 (W240) and 365 (W365) days of age, respectively. Before the analyses, BW, WW and W12 data for the total sample were investigated in comparison with normal distribution by the Shapiro-Wilk's statistics using the SAS univariate procedure, and for homogeneity of variance using Levene's test, using the GLM procedure (SAS, 2000).

The mean phenotypic values attributed to each genotype class were compared by variance analysis using the least squares method, following the GLM procedure (SAS, 2000).

The linear model used was:

$$Y_{ijkl} = \mu + GG_i + GF_j + GP_k + GG*PIT1_l + e_{ijkl}$$

where Y_{ijkl} represents the phenotypic value of the individual for the trait; μ, the overall population mean; GG_i, the effect of the i^{th} genetic group; GF_j, the effect of the j^{th} group of fixed effects (contemporary group, sex and maternal age as a linear and quadratic effect); GP_k, the fixed effect of the k^{th} genotype of the candidate gene *PIT1*; $GG*PIT1_l$, the effect of genetic group x *PIT1* genotype interaction; and e_{ijkl}, the residual random effect.

Scheffé's test was used to determine the differences between the mean values of the three genotypes at the *PIT1 locus* (Chew, 1976). To investigate the type of gene action at this *locus*, the F test was used to determine the contrast between the mean of the two homozygous and the mean of the heterozygous genotypes. Average effect of allele substitution, genetic values, genotypic values and dominance deviations were estimated according to Falconer & Mackay (1996).

RESULTS AND DISCUSSION

Allele frequencies

The amplification of the *PIT1* gene fragment resulted in a single product of 1.301 kb. Treatment of this product with the restriction enzyme *Hinf*I revealed the *Hinf*I (+) allele with fragments of 260, 617, 379 and 45 base pairs (bp) and the *Hinf*I (–) allele with fragments of 260, 617 and 424 bp. The *Hinf*I (–) allele was the less frequent in the two genetic groups (Table 1). The predominance of the *Hinf*I (+) allele was also detected in different European cattle breeds (Moody et al., 1995; Sabour et al., 1996; Klauzinska et al., 2000; Oprzadek et al., 2003), in Gir and crossbred dairy cattle (Mattos, 2000), and in Gir × Holstein crosses (Silva et al., 2006).

Test for the comparison of allele proportions in GG1 and GG2, revealed that the *Hinf*I (–) allele frequency was higher in GG2 than in GG1 ($P < 0.01$). Distribution differences of allele frequencies between different populations may indicate genetic differences in the base populations. Genetic group GG2 originated from genetically superior animals in terms of production traits, since animals from the Nelore, Charolais and Canchim breeds used to produce this population were approximately 40 years of selection apart from the ones used to produce GG1. Another factor that may be considered as having contributed to allelic frequency differences between the two populations is genetic drift.

PIT1 effects on production traits

Shapiro-Wilk's statistics indicated that the total population represents a population with normal distribution. Levene's tests for the assessment of homogeneity of variance indicated that the variances observed for the different treatments did not differ from one another. Variance analysis for the total population revealed interaction ($P < 0.05$) between genetic group and *PIT1* genotype for W240, AGBW and AGW12, demonstrating a different *PIT1* behavior in the two genetic groups (Table 2).

PIT1 genotype means were different for W240 and AGBW only in GG2 (Table 3). Genotype *Hinf*I (–/–) was superior to *Hinf*I (+/+) and *Hinf*I (+/–) by 24.37 and 24.26 kg, respectively. The *Hinf*I (–/–) was the most favorable genotype in terms of productivity, since it was related to higher weaning weight values without a concomitant increase in birth weight, which is a desirable condition in beef cattle breeding. Homozygous animals *Hinf*I (–/–), gained 90.8 and 89.3 g day^{-1} more than *Hinf*I (+/+) and *Hinf*I (+/–), respectively, from birth to weaning.

Table 1 - Allele frequencies with respective standard errors and test for the comparison of allele proportions for *PIT1 locus* in the two genetic groups (GG1 and GG2).

	GG1	GG2
Alleles	Frequency ± Standard error	Frequency ± Standard error
*Hinf*I (+)	0.87 ± 0.0156	0.73 ± 0.0188
*Hinf*I (-)	0.13 ± 0.0156	0.27 ± 0.0188
χ^2 (1 GL)	29.539**	

$^{**}P < 0.01$

Table 2 - Summary of variance analysis for weight at birth (BW), standardized weight at 240 (W240) and at 365 (W365) days of age, average daily weight gain from birth to weaning (AGBW) and from weaning to 12 months of age (AGW12).

		Mean Squares				
Source of Variation	DF[a]	BW (N=509)	W240 (N=478)	W365 (N=430)	AGBW (N=478)	AGW12 (N=430)
Genetic Group	1	68.20^{ns}	9673.44**	8516.69**	0.1388**	0.0075^{ns}
Contemporary Group	11	108.46**	13301.45**	21640.95**	0.2171**	0.3335**
Sex	1	548.41**	33666.38**	11155.76**	0.4525**	0.0960*
Cows'age (Linear)	1	215.11**	14073.46**	3021.81^{ns}	0.1955**	0.1013*
Cows'age (Quadratic)	1	140.82*	10648.49**	2022.92^{ns}	0.1505**	0.0680^{ns}
PIT1	2	16.94^{ns}	1026.31^{ns}	761.44^{ns}	0.0145^{ns}	0.0105^{ns}
GG**PIT1*	2	36.54^{ns}	2438.06*	1372.85^{ns}	0.0325*	0.0677*
R^2		0.15	0.40	0.40	0.41	0.33

[a]Degrees of freedom; $^{*}P < 0.05$; $^{**}P < 0.01$; ^{ns} not significant

Table 3 - Number of observations (N) and estimated standardized weight at 240 (W240) days of age and average daily weight gain from birth to weaning (AGBW) means, and respective standard errors (in parentheses) for *PIT1* genotypes in the genetic groups GG1 and GG2.

PIT1 Genotype	Standardized weight at 240 (W240) days of age (kg)				Average daily weight gain from birth to weaning (AGBW) (kg)			
	N	GG1 Means	N	GG2 Means	N	GG1 Means	N	GG2 Means
*Hinf*I (+/+)	169	212.87 (2.34)[a]	142	217.46 (2.73)[a]	169	0.74 (0.009)[a]	142	0.76 (0.010)[a]
*Hinf*I (+/-)	42	209.72 (4.27)[a]	96	217.57 (3.14)[a]	42	0.73 (0.016)[a]	96	0.76 (0.012)[a]
*Hinf*I (-/-)	8	206.37 (9.64)[a]	21	241.83 (5.99)[b]	8	0.72 (0.037)[a]	21	0.85 (0.023)[b]

Means followed by different letters in a column differed according to Scheffé's test ($P < 0.05$).

The effect of *PIT1* on W240 and AGBW as detected in GG2 may be the result of its influence on the expression of growth-hormone (GH) and prolactin (PRL) genes, which are equally important for growth from birth to weaning. Studies with swine showed correlation between pituitary GH and *PIT1*β mRNA levels and effect of *PIT1* genotypes on the plasma levels of GH and PRL (Sun et al., 1998, 2002). Therefore, the effects of *PIT1* on the W240 and AGBW traits, observed in GG2, ought to be carefully analyzed since, at this stage, calf development is subject to major influences of their mothers' genetic values concerning the maternal abilities, particularly in relation to milk capacity, which was not possible to take into consideration in these analyses. *Hinf*I (–/–) genotype at the *PIT1* gene was associated with higher milk, fat and protein production and with fat percentage in crossbred dairy cattle (Mattos, 2000).

The polymorphism at this *locus* lies at exon 6 of the *PIT1* gene, in the coding regions for the Pit-1 pituitary transcription factor. However, the mutation does not alter the amino acid in the peptide chain of Pit-1 protein. Thus, the differences in W240 and AGBW means for the genotypes of *PIT1* in GG2 could be related to other functional mutations in the same gene that would be in linkage disequilibrium with the *Hinf*I-*PIT1* polymorphism in GG2 but not in GG1 or would not be present in GG1.

PIT1 encodes proteins that bind to regulatory elements. Forms resulting from alternative splicing can produce proteins with altered properties. In mouse cell cultures, the Pit-1β protein activates the GH promoter more strongly than the promoters of the PRL (Konzak & Moore, 1992), *PIT1* (Theill et al., 1992) or β-subunit of thyroid stimulating hormone (TSHβ) gene (Haugen et al., 1994), but is an unstable protein (Konzak & Moore, 1992). Results regarding GH activation in swine, revealed a higher influence of Pit-1α mRNA than of *PIT1*-β on GH expression (Sun et al., 2002). Pit-1α and Pit-1T proteins interact to activate TSHβ transcription (Haugen et al., 1993), and this interaction is necessary and specific for the TSHβ promoter (Haugen et al., 1994).

Alternative forms of Pit-1, such as Pit-1Δ4, observed in swine, appear at times of highest PRL expression (Day & Day, 1994), and the Pit-1Δ3 form expressed in bacteria, was unable to recognize the GH-promoting elements, whereas Pit-1α was active (Yu et al., 2001). There is a strong similarity between the *PIT1* genes of swine, cattle and rodents, with approximately 90 to 95% identity between *PIT1*cDNA and the sequence of its protein deduced in swine, humans, cattle, sheep and rodents (Day & Day, 1994).

The different results observed in GG1 and GG2, concerning the effects of *PIT1* on the productive performance of animals, hint at the presence of a quantitative trait *locus* (QTL) strongly linked to *PIT1* in linkage disequilibrium only in GG2, and not the direct effect of this polymorphism on production. This hypothesis, however, could not be tested because of existing constraints due to sample size. Quantitative trait *loci* may present different effect among populations due to the epistatic interactions between the QTL and the genome (Pomp, 1994). The different behavior of *PIT1* in the two genetic groups in this study could be the interaction between the effects of the *PIT1* gene and the effects of the target regulatory sequences.

The absence of differences between *Hinf*I (+/+) and *Hinf*I (+/–) genotypes, for W240 and AGBW, suggest that the *Hinf*I (+) allele is expressed in a dominant manner in relation to the *Hinf*I (–) allele. Recessive nature of the allele *Hinf*I (–) may suggest that the product of this allele has less affinity for the region promoting the growth hormone gene or other genes of the somatotrophic axis on which *PIT1* may act, but results in a better pattern of expression. Studies on the affinity of allele specific forms of Pit-1 with the promoters involved in animal development might further elucidate this question.

Higher frequencies of the *Hinf*I (+) allele and of the (+/+) genotype of the *PIT1* gene, despite having no positive effect on production, suggest that *PIT1* could be linked to a *locus* with some adaptive advantage located at a distance that is small but enough to generate recombination between them. Alternatively,

they may reflect the evolutionary history of this mutation if the *Hinf*I (+) allele is considered as the ancestral allele that underwent a rare case of mutation. This hypothesis seems more appropriate, since the *Hinf*I (+) allele is prevalent in most bovine breeds (Moody et al., 1995; Mattos, 2000).

Allele substitution effect

The estimated population mean for W240 trait in GG2, as a function of allele frequencies, was 219.27 kg. The estimated mean effects of the *Hinf*I (–) and *Hinf*I (+) alleles were 4.84 and –1.79 kg, respectively, and the mean effect of the allele substitution was 6.63 kg. *Hinf*I (–) allele was responsible for 2.21% of the mean W240 value, and that the replacement of a *Hinf*I (+) allele with a *Hinf*I (–) allele may lead to an increase of 6.63 kg on W240 in the animals in this population.

Estimated population mean as a function of allele frequencies for AGBW in GG2, was 771 g day^{-1}. Estimated means of the effects of *Hinf*I (–) and *Hinf*I (+) alleles were 18.42 and – 6.81 g day^{-1}, respectively, and the mean for the effect of allele substitution was 25.23 g day^{-1}. In terms of traits mean, the *Hinf*I (–) allele may contribute 2.38% of the AGBW mean and that the replacement of one allele *Hinf*I (+) with a *Hinf*I (–) may lead to an increase of 25.23 g day^{-1} from birth to weaning in this group of animals.

*PIT1 Hinf*I (–) allele was related to higher body weight at 7 months of age in double-muscled Belgian Blue bulls (Renaville et al., 1997) and higher weights at 60, 250, 365 days of age and on weight gain from born to 60 days of age in Gir × Holstein crosses (Silva et al., 2006). This polymorphism was associated with weaning weight in Nelore cattle (Conde et al., 2001). On the other hand, no associations of the *PIT1* genotypes with meat production traits were found in Piemontese cattle (Di Stasio et al., 2002) or with growth and carcass traits in Angus beef cattle (Zhao et al., 2004). This genetic marker seems to have different effects in different populations, which is in agreement with the results presented here.

Concerning to W240, the genetic values for W240 of homozygous *Hinf*I (–/–) animals were superior by 6.64 kg compared to heterozygous and by 13.27 kg compared to *Hinf*I (+/+) animals. As for the AGBW trait, *Hinf*I (–/–) homozygous individuals presented genetic values 25.2 and 50.4 g day^{-1} greater than the values of heterozygous and of (+/+), respectively (Table 4). *Hinf*I (–) allele was favorably expressed when present in homozygosis and may be responsible for the superiority of the phenotypic values of weaning weight and of average daily weight gains from birth to weaning observed in this group of animals.

Genotypic value of the *Hinf*I (–/–) genotype, 22.55 kg, proved to be superior to the remaining genotypes for the W240 trait in GG2. Part of this value is attributed to dominance deviation, whose value was equal to 12.86 kg (Table 4). Similarly, for the AGBW trait, the portion of the genotypic value, 83.5 g day^{-1}, attributed to dominance deviation was 46.7 g day^{-1}. Deviations due to dominance proved to be more important than the genetic values for *Hinf*I (–/–) and *Hinf*I (+/–), implying in an advantage in the use of selection in favor of the *Hinf*I (–) allele to increase weaning weight and average daily weight gain from birth to weaning. Since the favorable *Hinf*I (–) allele is recessive to *Hinf*I (+), crossing in order to increase weaning weight and average daily weight gain from birth to weaning would be disadvantageous with respect to this *locus*.

CONCLUSIONS

Selection in favor of the *Hinf*I (–) allele of the *PIT1* gene may lead to increased weaning weight, without increasing birth weight. However, the difference in *PIT1* behavior observed between the two genetic groups denotes the need to test the effects of this polymorphism on different populations before using it in marker-assisted selection. It also indicates the need for a better understanding of the genetic mechanisms involved in the physiology of this pituitary transcription factor in the process of growth in cattle.

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Table 4 - Genotypic values (G), genetic values (A) and dominance deviation (D) for standardized weight at 240 days of age (W240) and average daily weight gain from birth to weaning (AGBW), in relation to *PIT1*, in the GG2 genetic group.

PIT1 Genotypes	W240			AGBW		
	G	A	D	G	A	D
	kg			kg day^{-1}		
*Hinf*I (-/-)	22.55	9.69	12.86	0.0835	0.0368	0.0467
*Hinf*I (+/-)	-1.70	3.05	-4.75	-0.0057	0.0116	-0.0173
*Hinf*I (+/+)	-1.82	-3.58	1.75	-0.0072	-0.0136	0.0064

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