



Association of *GH* and *IGF-1* polymorphisms with growth traits in a synthetic beef cattle breed

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Abstract

The Canchim beef cattle (5/8 Charolais + 3/8 Zebu) has been selected for meat production in Brazil since late 1950. In the present work the effects of growth hormone (*GH*) and insulin-like growth factor 1 (*IGF-1*) polymorphisms were investigated in 688 animals born between 1998 and 2000. These animals belonged to two genetic groups, *i.e.*, traditional and new lineages. Genotype effects on expected breeding values for birth weight (BW), weaning weight (WW) and yearling weight (YW) were investigated by the least square method. Significant effects were found for *GH* genotype on YW ($p \leq 0.05$), with positive effects associated with the LV (leucine/valine) genotype. For *IGF-1* genotypes, significant effects were found on BW ($p \leq 0.01$) and YW ($p \leq 0.01$). Average substitution effects for *IGF-1* alleles estimated by regression analysis suggested a positive effect of the *IGF-1* 225 bp allele on BW and of the 229 bp allele on YW.

Key words: beef cattle, genetic association, genotype, growth hormone, insulin-like growth factor 1.

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Introduction

Crosses among breeds allow the formation of new breeds, selected for favorable traits including adaptation to the environment. The Canchim beef cattle breed was produced in Brazil by crosses between Charolais (*Bos taurus*) and several Zebu (*Bos indicus*) breeds such as Guzerat, Nelore and Indubrazil, with a higher contribution of the latter (Alencar, 1988), to combine the early maturity and productivity associated with European beef cattle and the rusticity attributed to Zebu breeds. Canchim animals, with a 5/8 Charolais + 3/8 Zebu genetic composition, have been selected for meat production traits since 1950.

Quantitative traits are often controlled by a great number of genes. Localization of QTLs (Quantitative Trait Loci) can be done by linkage disequilibrium analysis or by candidate gene approach.

Candidate genes have known biological functions related to the development or physiology of an important trait (Rothschild *et al.*, 1997). Such genes can encode structural

proteins or a member in a regulatory or biochemical pathway affecting the expression of the trait (Bryne and McMullen, 1996) and can be tested as putative QTLs (Yao *et al.*, 1996).

Growth in animals is controlled by a complex system, in which the somatotrophic axis plays a key role. The genes that operate in the somatotrophic axis are responsible for the postnatal growth, mainly GH that acts on the growth of bones and muscles mediated by IGF-1 (Sellier, 2000). The growth hormone (*GH*) and insulin-like growth factor 1 (*IGF-1*) genes are candidates for growth in bovine, since they play a key role in growth regulation and development (Breier, 1999; Hossner *et al.*, 1997; Tuggle and Trenkle, 1996). Effects of GH on growth are observed in several tissues, including bone, muscle and adipose tissue. These effects result from both direct action of GH on the partition of nutrients and cellular multiplication and IGF-1-mediated action stimulating cell proliferation and metabolic processes associated to protein deposition (Boyd and Bauman, 1989). IGF-1 stimulates the protein metabolism and is important for the function of some organs, being considered a factor of cellular proliferation and differentiation.

The bovine *GH* gene has a genetic variant characterized by the substitution of one amino acid (leucine) for another (valine) at position 127, that can be identified by *AluI* restriction digestion of polymerase chain reaction products (PCR-RFLP) (Lucy *et al.*, 1991).

The *IGF-1* gene is extremely conserved among species and few polymorphisms are described. The presence of a microsatellite at the promoter region of this gene in bovine, human and horse allows to analyze genetic variations related to this locus (Kirkpatrick, 1992; Caetano and Bowling, 1998; Vaessen *et al.*, 2001).

Evidence of selection effects on allele frequencies at these two loci was found in the traditional lineage of Canchim (Regitano *et al.*, 1999). The objective of the present study was to evaluate the influence of the *GH* and *IGF-1* genotypes on growth traits in Canchim cattle.

Material and Methods

Animals

Blood or semen samples were collected from 688 Canchim animals born between 1998 and 2000. Among these, 329 belonged to the traditional Canchim population that started to be selected in 1953 and was kept closed ever since it reached a reasonable number of individuals, in 1979. The genetic composition of this group is 62.5% Charolais + 37.5% Zebu, and in the present work it was named genetic group 1 (*GG1*). This sample was composed of 187 males and 142 females. The remaining animals, referred to as genetic group 2 (*GG2*), belonged to a new Canchim population, obtained by recent crosses between Charolais, Nelore and Canchim intended to introduce new variability to the breed. The Canchim animals of this group are approximately 65.7% Charolais + 34.3% Zebu. Of the 359 animals in this group, 183 were males and 176 were females. All animals were born at Embrapa Pecuária Sudeste and raised on pastures, with mineral supplementation.

DNA extraction and genotyping

DNA was extracted from leukocytes or semen using a standard salting-out procedure, as described in Regitano (2001). PCR conditions were: 200 ng of genomic DNA in a 25 μ L reaction containing 50 mM KCl, 1.5 mM MgCl₂, 10 mM Tris-HCl pH 8.4, 0.2 μ M of each dNTP, 0.5 units of Taq DNA polymerase, and 0.4 μ M of each primer. Thermocycling conditions included an initial denaturation cycle at 94 °C for 2 min, followed by 30 cycles of denaturation at 94 °C for 1 min, primer annealing at 58 °C for 30 s for the two loci, and primer extension at 72 °C for 1 min. Then, the amplified product was subjected to a final extension cycle at 72 °C for 4 min.

The primers for *GH* were those described in Schlee *et al.* (1994). *GH* alleles L and V were determined by *AluI* digestion of PCR products, according to Lucy *et al.* (1991).

The primers for the *IGF-1* microsatellite were those described in Bishop *et al.* (1994). The amplified products were separated by electrophoresis in 8% non-denaturing polyacrylamide gels for 4 h and 30 min at 45 W, and silver-stained for visualization. Allele sizes were estimated by comparison to a 10 bp ladder molecular weight marker.

Statistical analysis

Allele frequencies were calculated for the whole population and within each genetic group, using the method described by Weir (1996). For comparison of proportions in the two genetic groups a chi-square test was used (Snedecor and Cochran, 1967).

Phenotypic data regarding birth weight (BW), weaning weight (WW) and yearling weight (YW) were provided by the Canchim Breeding Program of Embrapa Pecuária Sudeste. The average weaning age was 210 days. Expected breeding values for BW, WW and YW were estimated in an animal model by the Best Linear Unbiased Method (BLUP) using Restricted Maximum Likelihood (REML), considering animal, sex, year and month of birth, and the age of the dam (in the model).

Genotype effects on expected breeding values for BW, WW and YW were investigated by the least square method using the GLM procedure (SAS, 1999). The statistical model included the effects of genetic group (*GG*), *GH* and *IGF-1* genotypes, and the interactions *GG*GH* and *GG*IGF-1*. Homozygotes with low frequencies (≤ 0.01) were not considered in this analysis.

Average allele substitution effects for *IGF-1* were estimated by regression analysis as deviations from the 229 bp allele, as described in Stear *et al.* (1989), according to the model:

$$Y_{ij} = \mu + GG_i + g_1 m_{ij} + g_2 n_{ij} + g_3 o_{ij} + e_{ij}$$

where Y_{ij} = individual breeding value for the trait; μ = the population mean; GG_i = the fixed effect of the i^{th} genetic group; m_{ij} , n_{ij} , o_{ij} = the scores for the proportion of each *IGF-1* allele in the genotype; g_1 to g_3 = the corresponding partial regression coefficients, which are the gene substitution effects as deviations from the 229 bp allele; and e_{ij} = the random residual effect. In this analysis, all genotypic classes were considered, even the rare homozygotes for alleles 231 and 225 bp.

For those traits in which a significant *GG*IGF-1* interaction was found in the least square analysis, the model was modified by nesting the allele substitution effect within *GG*.

Results

Allele frequencies

Two alleles for the *GH* locus, leucine (L) and valine (V), and four *IGF-1* microsatellite alleles were observed.

Table 1 - Allele frequencies for *GH* and *IGF-1*, standard error and results of χ^2 test for comparison of proportions.

	Alleles	WP ^a	<i>GG1</i> ^b	<i>GG2</i> ^c	p
<i>GH</i>	L	0.87 ± 0.009	0.90 ± 0.012	0.85 ± 0.013	0.001
	V	0.13 ± 0.009	0.10 ± 0.012	0.15 ± 0.013	0.001
<i>IGF-1</i>	231 bp	0.08 ± 0.007	0.06 ± 0.009	0.09 ± 0.010	0.091
	229 bp	0.55 ± 0.013	0.44 ± 0.020	0.64 ± 0.017	0.000
	227 bp	0.26 ± 0.012	0.37 ± 0.019	0.18 ± 0.014	0.045
	225 bp	0.11 ± 0.008	0.13 ± 0.014	0.09 ± 0.010	0.045

^aWP = whole population; ^b*GG1* = genetic group 1; ^c*GG2* = genetic group 2; p = probability associated to the χ^2 test for proportions.

Allele sizes for the microsatellite ranged from 225 bp to 231 bp. Allele frequencies for both loci are shown in Table 1. For *GH*, the L allele was the most frequent in both genetic groups, but its frequency was significantly higher in *GG1* ($p < 0.01$). Differences between the two groups were also found regarding the 229 bp ($p < 0.01$), 227 bp and 225 bp ($p < 0.05$) *IGF-1* alleles.

Genotype effects on expected breeding values

The genetic group had significant effects ($p < 0.01$) on WW and YW (Table 2). The expected breeding value means for WW and YW were higher in *GG2* than in *GG1* (Table 3). The *GH* genotype influenced the expected breeding value for YW, but the *GG*GH* interaction was also significant. This interaction can be clearly seen in Table 4, where the YW mean associated to the LV genotype is almost twice the estimated mean of the LL genotype in *GG1*, whereas for *GG2* the difference between these two genotypes is 3.6% favoring the latter, and not significant. For WW, the *GG*GH* effects were significant, but no effect of *GH* alone was observed (Table 2).

IGF-1 had significant effects on BW and YW (Table 2). The 229/225 genotype was associated with increased BW (Table 3), while for YW the 229/229 genotype had the highest mean value. Regarding YW, the interaction between genetic group and *IGF-1* (*GG*IGF-1*) showed a significant effect. Genotype 229/225 was the most favor-

able for this trait in *GG2*, while in *GG1* the most favorable genotype was the homozygote for the 229 bp allele (Table 4).

Since *IGF-1* had no significant effects on WW, regression analysis was done only for BW and YW. The results of this analysis are shown in Table 5. Genetic group had significant effects on BW and YW. This difference regarding the significance of *GG* for BW when compared by the ANOVA test shown in Table 2 may be the result of an increased number of individuals in the regression analysis, once the rare homozygotes were considered, since there was an approximation of significance in the first analysis ($p < 0.07$).

Average substitution effects were estimated regarding the 229 bp allele (Figure 1). For BW, substitution of this allele by the 231 bp allele was significant ($p < 0.01$) and

Table 3 - Least square means of direct effects on expected breeding values of BW, WW and YW, according to the main effects (genetic group and genotype for *GH* and *IGF-1*).

<i>GG</i> ^a	Genotype	n	Mean ± SE ^b (kg)		
			BW ^c	WW ^d	YW ^e
1	321	321	0.32 ± 0.21 ^A	4.45 ± 0.72 ^A	6.10 ± 0.88 ^A
			2	353	0.89 ± 0.23 ^A
<i>GH</i>					
	LL	511	0.65 ± 0.16 ^A	6.04 ± 0.52 ^A	7.27 ± 0.64 ^A
	LV	163	0.57 ± 0.21 ^A	6.79 ± 0.72 ^A	9.08 ± 0.88 ^B
<i>IGF-1</i>					
	231/229	53	-0.27 ± 0.34 ^A	6.34 ± 1.16 ^A	6.86 ± 1.43 ^{ABC}
	231/227	29	-0.10 ± 0.41 ^A	5.56 ± 1.40 ^A	6.61 ± 1.72 ^{AC}
	231/225	12	1.18 ± 0.66 ^{AB}	5.61 ± 2.23 ^A	5.49 ± 2.74 ^{ABC}
	229/229	202	0.65 ± 0.17 ^{AB}	8.25 ± 0.58 ^A	11.56 ± 0.7 ^B
	229/227	216	0.71 ± 0.15 ^{AB}	6.83 ± 0.53 ^A	10.04 ± 0.64 ^{ABC}
	229/225	79	1.31 ± 0.25 ^B	8.13 ± 0.85 ^A	10.12 ± 1.04 ^A
	227/227	30	0.48 ± 0.65 ^{AB}	5.65 ± 2.21 ^A	8.20 ± 2.71 ^{ABC}
	227/225	53	0.89 ± 0.36 ^{AB}	4.94 ± 1.21 ^A	6.52 ± 1.48 ^{AC}

^aGenetic group; ^bStandard error; ^cBirth weight; ^dWeaning weight; ^eYearling weight. Means followed by the same letter within columns for each treatment (*GG*, *GH*, *IGF-1*) are not significantly different ($p > 0.05$).

Table 2 - ANOVA test summary for BW, WW and YW expected breeding values.

Source	DF ^a	Mean square		
		BW ^b	WW ^c	YW ^d
<i>GG</i> ^e	1	15.31	722.51**	809.08**
<i>GH</i>	1	0.64	66.75	378.15*
<i>IGF-1</i>	7	12.44**	91.22	260.90**
<i>GG*GH</i>	1	6.26	342.54**	543.49**
<i>GG*IGF-1</i>	7	1.85	84.13	165.11*
Error	658	4.54	52.43	78.80

^aDF = degrees of freedom; ^bBW = birth weight; ^cWW = weaning weight; ^dYY = yearling weight; ^e*GG* = genetic group; * $p < 0.05$; ** $p < 0.01$.

Table 4 - Least square means of interaction effects on expected breeding values of BW, WW and YW, according to genetic group X genotype.

<i>GG</i> ^a	Genotype	<i>n</i>	Mean ± SE ^b (kg)	
			BW ^c	YW ^d
<i>GG*GH</i>	1 LL	261	0.24 ± 0.20	4.11 ± 0.82
	1 LV	60	0.40 ± 0.31	8.09 ± 1.30
	2 LL	250	1.04 ± 0.24	10.43 ± 0.98
	2 LV	103	0.74 ± 0.28	10.07 ± 1.18
<i>GG*IGF-1</i>	1 231/229	13	-0.79 ± 0.59	2.20 ± 2.47
	1 231/227	18	-0.32 ± 0.51	6.31 ± 2.13
	1 231/225	4	1.24 ± 1.08	0.79 ± 4.48
	1 229/229	68	0.51 ± 0.28	10.24 ± 1.16
	1 229/227	119	0.51 ± 0.21	7.20 ± 0.90
	1 229/225	31	0.93 ± 0.39	6.01 ± 1.63
	1 227/227	27	0.19 ± 0.42	9.65 ± 1.75
	1 227/225	41	0.27 ± 0.34	6.31 ± 1.43
	2 231/229	40	0.25 ± 0.31	11.52 ± 1.42
	2 231/227	11	0.11 ± 0.65	6.92 ± 2.70
	2 231/225	8	1.11 ± 0.75	10.19 ± 3.14
	2 229/229	134	0.78 ± 0.19	12.89 ± 0.81
	2 229/227	97	0.92 ± 0.22	12.78 ± 0.92
	2 229/225	48	1.69 ± 0.31	14.24 ± 1.29
	2 227/227	3	0.76 ± 1.23	6.75 ± 5.13
	2 227/225	12	1.50 ± 0.62	6.73 ± 2.60

^aGenetic group; ^bStandard error; ^cBirth weight; ^dYearling weight.

negative, while substitution by the 225 bp allele was significant ($p < 0.05$) and positive.

For YW, only the substitution of the 229 bp allele by the 231 bp allele had a significant and negative effect in both genetic groups ($p < 0.01$). For this trait, the substitution of the 229 bp allele by the 225 bp allele had different effects in the two genetic groups, as predicted by the significant interaction between genetic group and *IGF-1*. In *GG1*, this substitution had a significant ($p < 0.05$) and negative effect, while in *GG2* no significant effect was observed. In the latter group, the substitution of the 229 bp allele by the 227 bp allele approached significance ($p < 0.07$) and was also negative (Figure 2). Considering these results, the 229 bp allele appeared to be the most favorable one for YW in both genetic groups.

Discussion

The higher expected breeding value means found for *GG2* were expected, since *GG1* was obtained by crosses between Charolais and *Bos indicus* breeds in the early 1950s, and the first crosses for the obtention of *GG2* were started only in 1984. Thus, the Charolais stock used in the crosses for obtaining *GG2* was at least 30 years of selection apart from the original one. Moreover, the only Zebu breed

Table 5 - ANOVA test and average substitution effects of *IGF-1* alleles on expected breeding values for BW and YW.

Source	DF ^a	MS ^b	F value	Pr > F
BW ^c				
<i>GG</i>	1	59.99	13.36	0.00**
<i>GI</i> ^e	1	34.35	7.65	0.01**
<i>G2</i> ^f	1	1.32	0.29	0.59
<i>G3</i> ^g	1	22.97	5.12	0.02*
Error	677	4.49		
YW ^h				
<i>GG</i>	1	2196.28	27.48	0.00**
<i>g1</i> ^e (<i>GG</i>)	2	569.95	7.13	0.00**
<i>g2</i> ^f (<i>GG</i>)	2	140.11	1.75	0.17
<i>g3</i> ^g (<i>GG</i>)	2	182.04	2.28	0.10
Error	677	79.96		

^aDegrees of freedom; ^bMean square; ^cBirth weight; ^dGenetic group; ^eSubstitution effect of the 231 bp allele relative to the 229 bp allele; ^fSubstitution effect of the 227 bp allele relative to the 229 bp allele; ^gSubstitution effect of the 225 bp allele relative to the 229 bp allele; ^hYearling weight; * $p < 0.05$; ** $p < 0.01$.

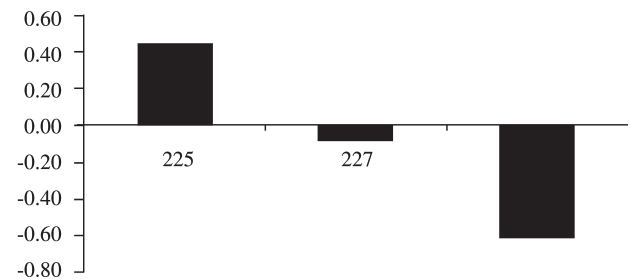


Figure 1 - Average substitution effects of *IGF-1* alleles on expected breeding values for BW.

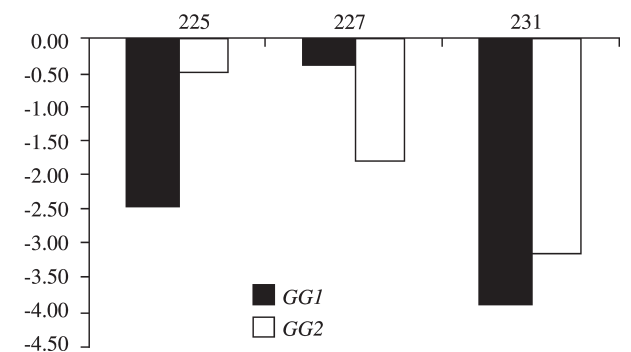


Figure 2 - Average substitution effects of *IGF-1* alleles on expected breeding values for YW in each genetic group.

that contributed to the formation of *GG2* was Nelore, which has one of the highest beef performances among Brazilian Zebu cattle.

The superiority of the LV growth hormone genotype compared to LL with regard to YW is in agreement with earlier suggestions of selection favoring the V allele over

generations of the traditional Canchim population (Regitano *et al.*, 1999). Tambasco *et al.* (2003) observed a positive association between genotype LV and daily body weight gain (BWG) from weaning to yearling in *Bos taurus* X *Bos indicus* crosses. It is also in agreement with the results observed in a Simmental population, where the LV genotype was associated with a higher BWG than LL and VV (Schlee *et al.*, 1994). Unfortunately, the number of VV homozygotes in the present study was too small and could not be included in the statistical analysis. The present data show that the effect of *GH* on YW was greater in the traditional Canchim population (*GG1*) than in the new one (*GG2*), which reinforces the need for testing the effects of candidate genes in the population prior to their application in marker-assisted selection. It may also indicate that the effect on YW attributed to this candidate gene reflects a segregating QTL or even a linked polymorphism in this gene rather than a direct effect of a *GH* *AluI* polymorphism. Bovine *GH* has been mapped to position 65.7 cM on chromosome 19 (Ihara *et al.*, 2004). The hypothesis of a segregating QTL close to the bovine *GH* is not supported by the results reported in the literature, since the QTLs for growth or meat production traits were mapped to intervals that do not include *bGH*, as discussed in Casas *et al.* (2003). The hypothesis that the effects of *GH* observed in the present study might result from a different polymorphism in this gene appears to be more acceptable, since other polymorphisms of this gene have been associated to growth traits in cattle (Taylor *et al.*, 1998).

IGF-1 had different effects on each trait studied, with positive substitution effects associated with the 225 bp allele on BW and with the 229 bp allele on YW. Using the same animals as in the present study in a chromosome scan approach, Machado *et al.* (2003) found evidence indicating the presence of a QTL controlling birth weight and another QTL influencing breeding value for yearling weight in the *IGF-1* chromosome region. The most likely positions of those QTLs were at 82.8 cM and 72.9 cM, respectively. Since the confidence interval for the position of the QTL included the *IGF-1* locus, located at 73 cM in the map used by the authors, the hypothesis that the QTL could be attributed to *IGF-1* could not be discarded. The 225 bp allele has been reported to be positively correlated to WW in Nelore cattle (Conde *et al.*, 2000). In Hereford cattle, the substitution of a B allele (128 bp) by an A allele (130 bp) of *IGF-1* had significant positive effects on direct and maternal EPDs for birth weight and 180-days gain from birth to weaning (Moody *et al.*, 1996). In the same study, direct EPDs for gain from weaning to yearling were shown to be negatively affected by the same allele. Since in that study different primers were used, it was not possible to establish a relationship between the alleles described by those authors and the ones found in the present study.

The fact that the 225 bp allele increased BW, had no effect on WW and had a negative effect on YW indicates that the genetic correlation between birth weight and postnatal growth should not be attributed to the action of this locus. Selection against this allele has potential to increase the adult weight mean without increasing the incidence of dystocia.

The hypothesis of a QTL linkage effect rather than a direct effect of *IGF-1* remains to be cleared. An influence of microsatellites within the promoter region of genes on gene regulation has been reported (Vaessen *et al.*, 2001). Prolactin expression and growth of salt-challenged tilapia are associated with the presence of a microsatellite in the promoter region of that gene (Streelman and Kocher, 2002). Microsatellites in promoter regions are also associated with the development of cancer (Deng *et al.*, 2002; Sakata *et al.*, 2002; Stefansson *et al.*, 2002), with gene expression in bacteria (Liu *et al.*, 2002) and with germination control in plants (Carrari *et al.*, 2001). On the other hand, there are several reports of evidence that QTLs in this region of BTA 5 affect rib bone and dressing percentage in Brahman X Hereford sire progenies (Stone *et al.*, 1999). QTLs for fat depth, retail product yield, and USDA yield grade, were reported between 62 and 72 cM, interval that includes the *IGF-1* locus, which is positioned at 74 cM (Ihara *et al.*, 2004). QTLs for birth weight were described in crosses between *Bos taurus* X *Bos indicus* at approximately 90 cM of BTA 5 (Davis *et al.*, 1998). Investigating growth and beef carcass fatness traits in an Angus x Brahman crossbreed, Kim *et al.* (2003) found a QTL in chromosome 5 for BW (48 cM) and YW (50cM). However, the results from SNP analysis of the *IGF-1* gene in Beefbooster lines M1 and M3 did not confirm *IGF-1* as the locus controlling these growth traits (Li *et al.*, 2004).

The two candidate genes studied here revealed significant effects on weight at different ages in Canchim cattle. From the breeding standpoint, the effect of *GH* on the traditional Canchim population and of the 229 bp allele of the *IGF-1* locus seem to be the most favorable ones, since both result in an increase of YW without jeopardizing calving ease due to increase in birth weight.

The differences in genotype effects between populations may suggest the effect of linked QTLs rather than of a direct effect in both loci.

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