# Association of an *insulin-like growth factor 1* gene microsatellite with phenotypic variation and estimated breeding values of growth traits in Canchim cattle

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#### Summary

A population of 1398 Canchim (CA) cattle was genotyped to assess the association of an insulin-like growth factor 1 (IGF1) gene microsatellite with phenotypic variation and estimated breeding values of pre-weaning, weaning and post-weaning growth traits. After an initial analysis, the *IGF1* genotype only had a significant effect (P < 0.05) on birth weight (BW) and weaning weight adjusted to 240 days (WW240). For these two traits, direct and maternal breeding values were estimated using the restricted maximum likelihood (REML). Two analyses were carried out. In the first (Model I), all fixed effects were fitted. In the second (Model II), the fixed effect of the IGF1 genotype was omitted. The estimated genetic and phenotypic components of variance were similar for every trait in both models. For Model I, estimated direct and maternal heritabilities were 0.26 and 0.16 for BW and 0.23 and 0.14 for WW240 respectively. The genetic and phenotypic correlations between BW and WW240 were 0.38 and 0.38 (Model I) and 0.19 and 0.38 (Model II) respectively. Fifty animals were classified according to their direct and maternal breeding values for both traits. Spearman rank-order correlation between animal rankings in the two models was used to assess the effect of including the IGF1 genotype in the model. Non-significant values from this correlation were indicative of a difference in breeding value rankings between the two approaches. The IGF1 gene was found to be associated with phenotypic variation and breeding values in the early phase of growth.

**Keywords** body weight, cattle, *insulin-like growth factor* 1, microsatellite, restricted maximum likelihood.

# Introduction

Animal genetic improvement programmes have been aided by marker-assisted selection (MAS), which allows earlier and more accurate selection. The efficiency of MAS depends on the use of markers that can be strongly linked to economically important traits, as is the case with the *insulin-like growth factor* 1 (*IGF1*) gene (Moody *et al.* 1996). The main function of *IGF1* is in regulating growth, metabolism and development in vertebrates (Daughaday & Rotwein 1989). The *IGF1* gene product is a polypeptide of 70 amino acids that are highly conserved. The gene is regulated by growth

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hormone (GH) and insulin and contains six exons that can be spliced in alternative ways to give rise to four different mRNAs. These mRNAs have either exon 1 or exon 2 at the 5' end and, in both cases, there are many transcription initiation sites (Adamo *et al.* 1991), affording yet another means of controlling the complex expression of this gene (Kim *et al.* 1991).

Because the *IGF1* gene is the main mediator of GH effects, it can be treated as a candidate marker that is potentially associated with growth traits. The *IGF1* gene is capable of producing a great variety of effects on various tissues, resulting in body growth (Breier & Gluckman 1991). The expression of the *IGF1* gene may have a direct effect on growth traits and may be associated with blood serum IGF1 concentration (Ge *et al.* 2001). High heritability (0.48 ± 0.13) of mean serum *IGF1* concentration during the postweaning period in Angus cattle was found to indicate additive genetic variability for this growth factor (Davis & Simmen 1997). Moody *et al.* (1996) demonstrated that, apart from influencing the weaning weight of cattle, polymorphisms in the *IGF1* gene affected the weight at 365 days. This reinforces the view that this gene would make a useful marker in MAS programmes aimed at accelerated development and greater body growth in the first year of life.

Regitano *et al.* (1999) suggested that the region of chromosome 5 containing the *IGF1* gene was associated with growth in the Canchim (CA) cattle breed. In that study, four alleles (225, 227, 229 and 231 bp) were found. The CA is a synthetic beef cattle breed, produced by crossing the Charolais (*Bos taurus*) and Nelore (*Bos indicus*) breeds. Depending on the mating scheme, the genetic composition can either be 62.5% Charolais and 37.5% Nelore or 65.6% Charolais and 34.4% Nelore. An association between *IGF1* gene polymorphism and weight in a CA herd, at birth and at 1 year, was detected by Pereira *et al.* (2005). However, Curi *et al.* (2004) did not find significant effects from the *IGF1* gene on the growth and carcass traits of 30 cattle of the CA breed.

In the CA breed, several studies have estimated heritabilities for different growth traits and, in general, these traits exhibit sufficient direct additive genetic variation to respond to the selection process. Mello et al. (2002) estimated direct and maternal heritabilities of 0.39 and 0.04 for birth weight (BW). Direct heritability estimates for weaning weight, ranging from 0.29 to 0.78, were reported by Alencar et al. (1998), Freitas (2000), Mello et al. (2002) and Talhari et al. (2003), whereas the maternal heritability estimates for the same trait were 0.10 (Alencar et al. 1998) and 0.03 (Mello et al. 2002). The genetic correlations obtained by Talhari et al. (2003) for the weaning weight and weights at 12 and 18 months of age were positive (0.21 and 0.46), thus indicating that selection applied to weaning weight can favour body weights at other ages. These studies ignored the effect of the IGF1 gene microsatellite on estimates of genetic and phenotypic parameters for growth traits.

Estimates of genetic and phenotypic parameters and breeding values for economically important growth traits that take into consideration the effect of *IGF1* gene microsatellites are needed in order to determine which of them can be genetically improved by MAS. The objective of this study was to determine the association of an *IGF1* gene microsatellite with phenotypic variation and estimated breeding values of growth traits in CA cattle.

# Materials and methods

#### Animals

Data were obtained from 1398 CA cattle born between 1988 and 2003 at the cattle-breeding station Embrapa Pecuária Sudeste. This is located in the São Carlos munici-

pality. São Paulo state. Brazil. at 47°53' west by 22°01' south and at 856 m above sea level. In the genetic improvement programme for the CA breed, parent groups are used. MA are the offspring from matings between Charolais bulls and cows that resulted from previous matings between CA bulls and Nelore cows. The offspring of the MA × MA mating scheme are CA with genetic composition 65.6% Charolais and 34.4% Nelore, whereas those of the  $CA \times CA$  mating scheme have a genetic composition of 62.5% Charolais and 37.5% Nelore. The animals used in this study were the offspring from 49 bulls and 596 cows. Of these, 11 MA bulls were mated with 230 MA cows and 38 CA bulls with 366 CA cows. Both mating schemes resulted in animals that are accepted as members of the CA breed (Barbosa 2000), although with different ratios of each parental group involved. This herd had been kept on pastures and had been subjected to phenotypic selection for weaning weight, yearling weight and fertility (Alencar et al. 1981).

#### DNA extraction and analysis of microsatellite markers

DNA samples from the animals were extracted from white blood cells or from semen, following a protocol involving salting-out of proteins as described by Regitano (2001). These samples formed part of the cattle DNA bank at Embrapa Pecuária Sudeste. PCR primers flanking the *IGF1* gene microsatellite (Kirkpatrick 1992; Bishop *et al.* 1994) were used to amplify this sequence. The primer that was complementary to the antisense strand was marked at the 5' end with fluorescein, for subsequent detection of the amplified product by laser. The reaction was conducted in a Mastercycler gradient thermal cycler (Eppendorf) in an aqueous solution (total volume 12 µl) of 100 ng DNA sample, 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, 10 mM Tris–HCl (pH 8.4), each dNTP at 0.2 µM, 0.5 U *Taq* polymerase and each primer at 0.1 µM.

The amplification reactions included initial denaturation at 94 °C for 2 min, followed by 25 cycles of denaturation at 94 °C for 1 min, annealing at 58 °C for 30 s and elongation at 72 °C for 1 min and a final elongation of the amplicon for 4 min. This product was then subjected to DNA fragment size analysis by capillary gel electrophoresis in an automatic 16-capillary ABI Prism 3100-Avant Genetic Analyzer (Applied Biosystems) equipped with a laser detector of the fluorescein label, which reported the number of base pairs in the amplicon from each animal.

## Genetic and statistical analyses

The number of occurrences of each allele (DNA fragment size) among the cattle was counted to obtain the allele frequencies at the *IGF1* locus. The polymorphism information content (PIC) (Botstein *et al.* 1980) and the observed and expected heterozygosities were calculated to assess the variability of the population at this locus. The chi-squared test was used to check that the genotyped population was in Hardy–Weinberg equilibrium.

The traits analysed in this study included birth weight (BW), weaning weight (WW), WW adjusted to 240 days of age (WW240), weight at 1 year (W12), weight at 18 months (W18), mean daily gain from birth to weaning (MDG) and scrotal circumference at weaning (SCW), 1 year (SC12) and 18 months (SC18).

The data were subjected to an analysis model that included the fixed effects of contemporary group (CG), parent group (CA or MA) and IGF1 genotype (10 genotypes) and the linear and quadratic effects of the age of the dam at parturition, which was fitted as a covariable. One rare genotype class (231/231) was excluded from this analysis. The animals' ages were included as linear covariables in the WW, W12 and W18 analyses. The CG consisted of animals of the same sex, born in the same year and breeding season. Those born up to 1988 constituted a single class of year of birth. Two breeding seasons were defined, one from August to December (rainy season) and the other from May to July (dry season). Residual normality was verified for each variable and observations exhibiting a standardized residual 3.5-fold above or below the standard deviation were excluded. Table 2 shows the final number of observations for each trait included in the analysis of variance (ANOVA).

The analyses showed that BW and WW240 were significantly (P < 0.05) affected by *IGF1* genotype. Therefore, the direct and maternal additive genetic, environmental and phenotypic components of variance and heritability were estimated in a bi-trait animal model using restricted maximum likelihood (REML), only for BW and WW240. The multiple-trait derivative-free restricted maximum likelihood (MTDFREML) program described by Boldman *et al.* (1995) was used. Two analyses were carried out. In the first (Model I), all the fixed effects were fitted. In the second (Model II), the fixed effect of the *IGF1* genotype was omitted. The total number of animals in the numerator relationship matrix, including base animals, was 10 814. In matrix notation, the models can be represented as:

$$y = X\beta + Za + Mm + \varepsilon_{2}$$

where *y* is the observation vector;  $\beta$  is the vector of fixed effects; *a* and *m* are vectors of direct and maternal random additive genetic effects;  $\varepsilon$  is the vector of random residual effects and *X*, *Z* and *M* are the corresponding incidence matrices. The maternal permanent environmental effect was not considered in this model because most dams had only one offspring each. The top 50 animals were classified in terms of their calculated direct and maternal breeding values for BW and WW240. The Spearman rank-order correlation between the rankings obtained for these animals in Models I and II was used to assess the effect of including the *IGF1* genotype on the ranking of breeding values for both traits.

**Table 1** Number of animals (*N*), observed heterozygosity ( $H_O$ ), expected heterozygosity ( $H_E$ ) and polymorphism information content (PIC) of the *IGF1* locus in the progeny, grouped by year of birth.

Year	Ν	H <sub>O</sub>	H <sub>E</sub>	PIC
1998	196	0.689	0.648	0.584
1999	244	0.607	0.591	0.538
2000	302	0.672	0.604	0.548
2001	199	0.608	0.632	0.582
2002	219	0.566	0.592	0.546
2003	238	0.504	0.631	0.572
1998–2003	1398	0.609	0.619	0.567

## **Results and discussion**

Table 1 shows the number of genotyped animals, observed and expected heterozygosity (*H*) and PIC of the *IGF1* locus in the progeny by year of birth. The locus was considered polymorphic if *H* exceeded 0.1 and highly polymorphic if H > 0.7 (Ott *et al.* 1992). In this study, the observed value of *H* among the progeny was >0.1 in all years, thus indicating that the *IGF1* locus was appreciably polymorphic. These results were consistent with those of Machado *et al.* (2003), who reported an *H* value of 0.648 for the *IGF1* marker in CA. In the present study, the average PIC value was 57%, and the *IGF1* marker can thus be regarded as relatively informative, according to Liu (1998).

Figure 1 shows the allelic frequencies of the *IGF1* gene microsatellite for each year of birth. The mean frequencies over the whole period were 0.105 (225-bp allele), 0.261 (227 bp), 0.564 (229 bp) and 0.070 (231 bp). The allele detected most frequently was the 229-bp fragment, as a result of the high percentage of genes from the Charolais breed in the CA breed. A similar frequency of the 229-bp allele (0.583) was found by Curi *et al.* (2004) in CA cattle. In an earlier study by Regitano *et al.* (1999), the most frequent allele in the CA population was the 227-bp fragment (43.9%), but the 225-bp allele exhibited a significant, non-linear rise over successive generations. There was some degree of gene oscillation that may possibly be attributed to



**Figure 1** Allelic frequencies of the *IGF1* gene microsatellite among the genotyped Canchim cattle for each year of birth.

**Table 2** Number of observations (*N*), number of contemporary groups (CG), mean value, standard deviation (SD) and coefficient of variation (CV) of birth weight (BW), weaning weight (WW), WW adjusted to 240 days (WW240), weight at 12 months (W12) and 18 months (W18) of age, mean daily weight gain from birth to weaning (MDG), scrotal circumference at weaning (SCW), at 12 months (SC12) and at 18 months (SC18).

Trait	Ν	CG	Mean	SD	CV (%)
BW (kg)	1297	24	33.6	2.5	7.8
WW (kg)	575	12	205.2	24.8	12.1
WW240 (kg)	577	12	200.1	24.7	12.3
W12 (kg)	480	12	227.4	30.0	13.2
W18 (kg)	350	12	307.6	58.0	18.8
MDG (kg)	577	12	0.7	0.1	14.0
SCW (cm)	278	6	18.4	1.6	8.8
SC12 (cm)	212	6	21.7	0.8	3.6
SC18 (cm)	138	6	28.6	2.6	9.9

a strong founder effect caused by the small number of bulls used in the breeding seasons year by year. The result from the chi-squared test ( $\chi^2 = 5.30$ ; 6 d.f.; P > 0.05) showed conclusively that the differences between the observed and expected genotype proportions were due to chance and that the sample population was in Hardy–Weinberg equilibrium.

The numbers of observations, number of CG, mean value and the respective standard deviations and coefficients of variation for each trait are shown in Table 2. It can be seen that most of the data on the performance of the genotyped animals relate to their early life, up to weaning. The parent group significantly (P < 0.05) affected all traits except W18, SC12 and SC18 (P > 0.05). Effects from the genetic composition of parental groups were taken into consideration in the analysis because a gene that influences a trait in one group may have a different effect in another group, if there are epistatic interactions between the gene studied and the genetic background. The CG had a very significant (P < 0.0001) influence on all the traits. The age at the time of measurement significantly (P < 0.0003) influenced WW, W12, W18, SCW and SC12. The quadratic effect of age of the dam at parturition was a significant (P < 0.0003)source of variation for WW, W12, W18 and SCW.

As mentioned earlier, only BW and WW240 were significantly (P < 0.05) associated with the *IGF1* microsatellite genotype, and the mean values and their standard deviations for these traits for each genotype class are presented in Table 3. For both the traits, *IGF1* microsatellite genotypes that included the 231- and 225-bp alleles were associated with low and high body weights respectively.

Table 4 shows the estimates of genetic and phenotypic parameters for BW and WW240, which were similar for Models I and II, thus indicating that inclusion of information on *IGF1* marker genotyping in the model did not account for any extra variation in the data. The estimated direct heritabilities for BW (0.26 for both models) and for WW240 (0.23 for Model I and 0.27 for Model II) in the

**Table 3** Number of observations (*N*), mean value and standard deviation (SD) for birth weight (BW) and weaning weight adjusted to 240 days (WW240) for genotypic classes.

Genotype at	BW			WW240			
IGF1 gene microsatellite	N	Mean	SD	N	Mean	SD	
225/225	26	36.67 <sup>a</sup>	6.10	23	219.37 <sup>a</sup>	37.66	
225/227	83	34.77 <sup>a,b,c,d</sup>	5.26	27	197.15 <sup>b,c</sup>	37.52	
225/229	203	35.28 <sup>a,b</sup>	5.76	115	211.51 <sup>a,b</sup>	30.65	
225/231	26	35.04 <sup>a,b,c</sup>	6.94	16	217.48 <sup>a</sup>	26.65	
227/227	71	34.28 <sup>a,b,c,d</sup>	5.08	40	196.92 <sup>b,c</sup>	27.16	
227/229	320	33.61 <sup>b,c,d</sup>	5.73	105	194.65 <sup>b,c</sup>	32.78	
227/231	53	32.05 <sup>d</sup>	4.45	21	185.66 <sup>c</sup>	41.43	
229/229	407	33.30 <sup>b,c,d</sup>	5.64	186	195.79 <sup>b,c</sup>	28.79	
229/231	97	32.18 <sup>c,d</sup>	5.12	37	194.16 <sup>b,c</sup>	32.56	

One rare genotype class (231/231) was excluded from this analysis. Different superscript letters within column represent significant differences between mean values for every trait (P < 0.05, Tukey comparisons test following analysis of variance).

 Table 4
 Genetic and phenotypic parameters, estimated by two-trait analysis, for birth weight (BW) and weaning weight adjusted to 240 days of age (WW240), for Model I and Model II (in parentheses).

	Variance	Heritability estimates <sup>2</sup>				
Trait	$\sigma_a^2$	$\sigma_{\rm m}^2$	$\sigma_{\rm e}^2$	$\sigma_{\rm p}^2$	h <sup>2</sup>	$h_m^2$
BW	7.4	4.6	16.7	28.31	0.26	0.16
	(7.4)	(5.7)	(16.8)	(28.8)	(0.26)	(0.20)
WW240	140.0	85.9	337.0	603.4	0.23	0.14
	(169.4)	(121.6)	(333.2)	(619.9)	(0.27)	(0.20)

<sup>1</sup>Direct additive genetic,  $\sigma_{a}^2$  maternal additive genetic,  $\sigma_{m}^2$ 

environmental,  $\sigma_{\rm e}^2$ ; phenotypic,  $\sigma_{\rm p}^2$ .

<sup>2</sup>Direct additive genetic,  $h_{a}^{2}$ ; maternal additive genetic,  $h_{m}^{2}$ .

In Model I, all fixed effects of the general linear model were taken into account; in Model II, the fixed effect of the *IGF1* genotype class was excluded.

present study were lower than reported by Mello *et al.* (2002) for BW and by Alencar *et al.* (1998), Freitas (2000), Mello *et al.* (2002) and Talhari *et al.* (2003) for WW240 in CA. These heritability estimates for both traits were indicative of sufficient genetic additive variance for individual selection, considering that the aim of the selection process in this CA breeding programme included maintenance of BW and improvement of WW240. BW and WW240 were heavily influenced by maternal genetic effects. However, these effects could be considered fragile because the number of dams was limited. Nevertheless, maternal genetic effects were taken into consideration in the model because the direct breeding value and heritability estimates of both traits would be more accurate.

The phenotypic correlations were equal in both models (0.38), but the genetic and environmental correlations were

different (0.38 and 0.37 for Model I vs. 0.19 and 0.44 for Model II respectively). Thus, a stronger genetic association between BW and WW240 was indicated when the *IGF1* genotype class was included in the model because this gene was associated with the traits studied.

Non-significant values of the Spearman rank-order correlation (*r*) for direct (r = 0.223, P = 0.1098 for BW and r = -0.25, P = 0.0799 for WW240) and maternal (r = 0.063, P = 0.6641 for BW and r = -0.059, P = 0.683 for WW240) breeding values of the top 50 animals in the two models indicated that including the *IGF1* genotype effect (Model I) would modify the ranking of the animals on the basis of their breeding values and would consequently influence the set of animals to be selected.

Regarding the top 10 direct breeding values for both traits, the 227- and 229-bp alleles were involved in BW and the 229-bp allele in WW240 for the animals whose *IGF1* genotype was known. For BW, it was found that the ranking of the first three animals was unaffected by the choice of model, while four of the other breeding values were not present in the top 10 in both models. For WW240, it was found that the first four animals maintained the same positions in each model and that just one was not present in both models.

In conclusion, a microsatellite in the *IGF1* gene was found to be associated with phenotypic variation and breeding values of traits during the early growth phase of the CA cattle breed, although the proportions of variance components were not changed. The inclusion of *IGF1* gene microsatellite genotyping and the evaluation of performance traits measured up to weaning may be useful in the selection of CA animals for breeding programmes.

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