



# Allele substitution effects of IGF1, GH and PIT1 markers on estimated breeding values for weight and reproduction traits in Canchim beef cattle

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## ABSTRACT

The aim of this study was to evaluate the allele substitution effects of IGF1, GH and PIT1 markers on the estimated breeding values (EBVs) for birth (BW) and weaning weights (WW), weight at 12 (W12) and 18 (W18) months of age, weight (WFC) and age (AFC) at first calving, and scrotal circumference measured at 12 (SC12) and 18 (SC18) months of age in Canchim beef cattle. Maternal effects were considered for birth (BW<sub>maternal</sub>) and weaning (WW<sub>maternal</sub>) weight. Regression analyses were carried out considering the EBVs, obtained from multi-trait analyses, and the deviations for each allele studied (four alleles for the IGF1 markers and two for GH and PIT1 markers, respectively). According to the results obtained for IGF1, the substitution effect of the “225” allele was significant ( $P \leq 0.05$ ) and presented higher EBVs for BW, BW<sub>maternal</sub>, WW, W12, W18, and SC18; and lower EBVs for WW<sub>maternal</sub>. The allele substitution effect observed for GH gene was significant ( $P \leq 0.05$ ) on EBVs for AFC and WFC. The “Valine” allele was responsible for lower EBVs for AFC and higher EBVs for WFC. For the PIT1 gene, the substitution effect of the “Hinf–” allele was significant ( $P \leq 0.05$ ) on the EBVs for WW, WW<sub>maternal</sub>, AFC, and WFC, respectively. Important allele substitution effects were found for weight and reproduction traits in Canchim cattle. In general, markers on the IGF1 gene are related to higher EBVs for weight, while markers on the GH and PIT1 showed greater influence on the EBVs for age and weight at first calving. Adding molecular markers information on the selection process could result in increased genetic gains in Canchim cattle. Future studies using high-density genotyping platforms may contribute to our understanding of the effects of these genes on traits of economic importance in the Canchim breed.

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## 1. Introduction

Molecular markers have been used to identify loci or chromosome regions that might be responsible for variations in quantitative traits. Because of the difficulty to identify quantitative trait loci (QTL), markers presenting linkage disequilibrium with a QTL may be used to aid on inferences about QTL effects. These markers can be identified through certain candidate genes or regions.

The insulin-like growth factor type 1 (IGF1), growth hormone (GH) and pituitary transcription factor (PIT1) are candidate genes for growth and reproduction traits, because they participate on the

hormonal system with a fundamental role in regulating animal's development. The IGF1 and GH form the somatotrophic axis and are involved in such hormonal system (Renaville et al., 2002), while PIT1 is a regulatory factor for synthesis of GH, prolactin and thyrotropin (Oprzadek et al., 2003).

In Canchim breed, Pereira et al. (2005), Andrade et al. (2008), and Carrijo et al. (2008) reported that IGF1, GH, and PIT1 markers had significant effects on weight traits. Moody et al. (1996) observed significant effects on body weight traits when studying GH, IGF1, and PIT1 markers in Hereford cattle. According to Ishida et al. (2010), single nucleotide polymorphisms in the GH gene were responsible for lighter calves at various ages in Japanese Black breed. Rogberg-Muñoz et al. (2011) observed association of weaning weight with IGF1 markers in commercial and experimental Hereford herds. Association of PIT1 markers with

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intramuscular fat deposition was found by [Thomas et al. \(2007\)](#) in Brangus breed. These authors also found association of GH makers with weight gain, scrotal circumference, and fat thickness, respectively.

Most of the previously cited studies were conducted considering a genotypic model, in which the contribution of each genotype to the phenotypic variation of the trait was estimated. However, using a statistical model based on allele substitution effects would be the most fitting from the point of view of genetic improvement, because the additive effect of each allele could be estimated independently of the animal's genotype. The estimated breeding values (EBVs) and variations, such as deregressed EBVs ([Garrick et al., 2009](#)), are indicated to study genomic associations, mainly when traits are observed in only one sex (i.e. scrotal circumference) as well as when phenotypes are obtained at older ages (i.e. stayability). Normally, EBVs are more accurate than raw phenotypes, however the EBVs depend on the correct removal of environmental effects, pedigree structure and heritability estimate to present a desirable accuracy.

Thus, the aim of our study was to evaluate allele substitution effects for growth and reproduction traits of the Canchim breed on the genetic markers for IGF1, GH, and PIT1 genes.

## 2. Material and methods

### 2.1. Animals and data

Data on birth (BW) and weaning weights (WW), weights at 12 (W12) and 18 (W18) months of age, weight (WFC) and age at the first calving (AFC), and scrotal circumference at 12 (SC12) and 18 (SC18) months were used in our study. Animals were from a herd from the Embrapa Southeast Livestock, a Brazilian research facility which is located in the municipality of São Carlos, state of São Paulo (22°01' latitude south, 47°53' longitude west and 856 m in altitude). The animals were reared exclusively in a pasture regime, while receiving mineral supplementation throughout the year. Details on herd management are described by [Mello et al. \(2002\)](#).

In the Canchim breed, different crossing schemes are used to create genetic variability ([Andrade et al., 2008](#)). In our study, eight different genetic groups (GG) were classified according to the genetic composition of the animals, sires and dams. Heterotic effects are present in crossbred populations and are an important source of variation. In our study, the GG was included in the models, for genetic parameter estimates, in order to account for part of these effects.

The statistical analyses were conducted using performance data on Canchim cattle and on different genetic groups. Given that Canchim animals are 5/8 Charolais and 3/8 Zebu, the genetic groups were as follows:

- GG1: offspring from the mating of Charolais with F1 (½ Canchim ½ Zebu).
- GG2: Canchim, offspring from the mating of GG1 with GC1.
- GG3: Canchim, offspring from the mating of GG1 with Canchim.
- GG4: Canchim, offspring from the mating of F1 (½ Zebu ½ Charolais) with F1 (½ Zebu ½ Charolais).
- GG5: Canchim, offspring from the mating of F1 (½ Zebu ½ Charolais) with Canchim.
- GG6: Canchim, offspring of Canchim and grand-offspring of Canchim.
- GG7: Canchim, offspring from the mating of GG2 with GG2; and
- GG8: Canchim, offspring from the mating of GG2 with GG6.

**Table 1**

Number of animals (N), means with respective standard deviations (SD), minimum (Min) and maximum values (Max) for birth weight (BW), weaning weight (WW), weight at 12 (W12) and 18 (W18) months of age, age (AFC) and weight (WFC) at first calving and scrotal circumference at 12 (SC12) and 18 (SC18) months of age in Canchim cattle.

Trait	N	Mean	SD	Min	Max
BW (kg)	11,401	35.03	5.68	18.00	52.00
WW (kg)	9035	204.90	40.69	82.00	327.00
W12 (kg)	8889	229.00	47.10	90.00	370.00
W18 (kg)	7863	302.30	60.23	125.00	480.00
AFC (days)	2148	1206.00	178.10	697.00	1736.00
WFC (kg)	1963	439.50	54.94	300.00	600.00
SC12 (cm)	1844	22.66	3.19	13.30	32.00
SC18 (cm)	1519	28.96	3.19	19.00	38.00

### 2.2. Genetic parameter estimates

Contemporary groups (CGs) were defined as animals from the same genetic group (GG1–GG8) that were born in the same year (from 1949 to 2008) and season (spring, summer, fall or winter). CGs comprising only one animal and standardized observations (that were higher than +3 or lower than –3 standard deviations in relation to the phenotypic mean) were excluded. The structure of the data used in the multi-trait analyses is presented in [Table 1](#).

The effects considered in the analysis for each trait are presented as follows:

- BW: CG as a random effect, sex as a fixed effect, and age of the dam at calving as a covariate (linear and quadratic effect).
- WW: CG as a random effect, sex as a fixed effect, and age of the animal at weaning (linear effect) and age of the dam at calving (linear and quadratic effect) as covariates.
- W12 and W18: CG as a random effect, sex as a fixed effect, and age of the animal as a covariate (linear effect).
- AFC: CG as a random effect and age of the dam at calving as a covariate (linear and quadratic effect).
- WFC: CG as a random effect.
- SC12 and SC18: CG as a random effect and age (linear effect) and weight (linear effect) of the animal as covariates.

The EBVs were estimated in two different analyses. The first considered the traits measured in both sexes (BW, WW, W12, and W18) and the second considered the traits measured in only one of the sexes (AFC, WFC, SC12, and SC18). We conducted all analyses using the restricted maximum likelihood method, under an animal model, by means of the ASREML software ([Gilmour et al., 2009](#)). For the multi-trait analysis for AFC, WFC, SC12, and SC18, the covariances between traits measured on opposite sex were set to zero.

For BW and WW, the random maternal genetic effect was taken into consideration, along with the random effects of animal, CG and residual. For W12, W18, AFC, WFC, SC12, and SC18 the model included the random effects of animal, CG and residual. The random permanent environment effect was not included in the analyses because the structure of the data, given that around 50% or more of the dams had only one offspring.

### 2.3. DNA extraction and genotyping

The genotype information of the animals are part of the database provided by Embrapa Southeast Livestock ([Pereira et al., 2005](#); [Andrade et al., 2008](#); [Carrijo et al., 2008](#)). A total of 1397, 755, and 517 animals were genotyped using molecular markers on IGF1 (chromosome 5, GenBank ID [X64400](#)), GH (chromosome 19, GenBank ID [M57764](#)), and PIT1 (chromosome 1, GenBank ID

**Table 2**

Allelic frequencies observed for the genetic markers on IGF1, GH and PIT1 genes in Canchim cattle.

Marker	Allele	Observed frequency
IGF1	225	0.14
	227	0.24
	229	0.55
	231	0.07
GH	L	0.87
	V	0.13
PIT1	"Hinf+"	0.79
	"Hinf–"	0.21

Y15995.1) genes, respectively. The IGF1 marker used in this study was a microsatellite with four alleles (225, 227, 229, and 231) at the promoter region of this gene (Kirkpatrick, 1992). The GH marker had two alleles and was defined by the substitution of the amino acid leucine (L) to valine (V). This polymorphism is due to a nucleotide substitution (C/G) at exon 5 and was identified by AluI restriction digestion of polymerase chain reaction products (PCR–RFLP) (Lucy et al., 1993). The molecular marker for the PIT1 gene was located within exon 6, and the alleles (Hinf+ and Hinf–) were defined by a silent mutation (Dierkes et al., 1998).

The LOKI software, version 2.4.5 (Heath, 2003), was used to exclude occurrences of possible genotyping errors, resulting in 1332; 748 and 517 animals with known genotype for IGF1, GH and PIT1, respectively. The allelic frequencies observed in the data files and the number of animals in each genotype for the genetic markers are presented in Tables 2 and 3.

#### 2.4. Allele substitution analyses

Analyses on the allele substitution effects of each genetic marker were performed using the GLM procedure of the SAS software (SAS 9.1, SAS Institute, Cary, NC, USA). The mean effects for the allele substitution on the EBVs were estimated by means of regression analysis, as deviations of the less frequent allele in relation to the more frequent allele (four alleles for the IGF1 marker and two for GH and PIT1), as described by Stear et al. (1989). If one of the genotypes was not observed, the regression analysis was performed using only two points. The dominance effect was assumed to be zero as the EBVs were considered as response variables. By definition, the EBVs are related to the additive effects and do not include the dominance deviation, which is defined as the difference between breeding values and genotypic values when

**Table 3**

Number of animals (N) in each genotype class for markers on IGF1, GH, and PIT1 genes in Canchim cattle.

IGF1		GH		PIT1	
Genotype	N	Genotype	N	Genotype	N
225/225	22	LL	568	Hinf+/Hinf+	331
225/227	82	LV	161	Hinf+/Hinf–	156
225/229	211	VV	19	Hinf–/Hinf–	30
225/231	28				
227/227	72				
227/229	353				
227/231	52				
229/229	405				
229/231	97				
231/231	10				
Total	1332	Total	748	Total	517

**Table 4**

Number of animals used for regression analyses on the IGF1, GH and PIT1 markers, with the estimated breeding values for birth weight (BW), weaning weight (WW), weight at 12 (W12) and 18 (W18) months of age, age (AFC) and weight (WFC) at first calving, scrotal circumference at 12 (SC12) and 18 (SC18) months of age, respectively.

Trait	Number of animals in the regression analyses		
	IGF1	GH	PIT1
BW	1327	746	515
WW	1325	747	517
W12	1326	747	516
W18	1329	747	516
AFC	1325	740	516
WFC	1327	742	517
SC12	1327	747	513
SC18	1325	744	515

only a single locus is under consideration (Falconer and Mackay, 1996).

To test the hypothesis that the regression coefficient for each equation was equal to zero, *t*-statistics was used. Observations for which the standardized residual was greater than three or less than minus three were excluded. The number of animals with EBVs that were used in each regression analysis is presented in Table 4. The regression model used was as follows:

$$y_{jk} = \mu + \sum_{j=1}^S \beta_j x_j + e_{jk}$$

where  $y_k$  is the EBV for each trait ( $k$ );  $S$  is the number of alleles minus one;  $\mu$  is the mean of the breeding values;  $x$  is the number of copies for a given allele ( $j$ );  $\beta$  is the linear regression coefficient (allele substitution effect), and  $e$  is the residual effect. For the marker IGF1, the analyses were performed taking into consideration the substitution of the most frequent allele ("229"), in relation to the others. For GH and PIT1, the most frequent alleles were "Leucine" and "Hinf+", respectively.

### 3. Results and discussion

The heritability estimates and the genetic and environmental correlations obtained in the two multi-trait analyses (BW, WW, W12, and W18; and AFC, WFC, SC12, and SC18) are presented in Tables 5 and 6, respectively. Similar results were found for BW and W12 (Mello et al., 2002), W12 (Mello et al., 2006), W18 (Toral et al., 2009), AFC and scrotal circumference adjusted at 420 days of age (Buzanskas et al., 2010), WFC (Gavioli et al., 2012), and SC12 (Gianlorenço et al., 2003) in the Canchim breed. All the traits evaluated, with the exception of AFC, presented moderate to high heritability (from 0.23 to 0.46), thus indicating that these traits could respond to selection.

The genetic correlations between BW, WW, W12, and W18 were all within the range found in the literature for various breeds (Castro-Pereira et al., 2007; Toral et al., 2006; Vargas et al., 2014). Boligon et al. (2007) studying Nellore breed, found negative and favorable genetic correlation between AFC and SC18 (–0.23) differing from the genetic correlation obtained in our study (0.08). For WFC and SC18, the same authors reported genetic correlation of 0.28. The genetic correlations for AFC and WFC with SC12 ( $0.00 \pm 0.20$  and  $-0.22 \pm 0.10$ , respectively) were lower than those reported by Castro-Pereira et al. (2007) and Silva et al. (2000). Due to the lack of heifers/cows that were direct descendants from bulls with records, the observed genetic correlations of AFC and WFC with SC12 and SC18 were unreliable because of the standard errors.

**Table 5**

Heritability estimates (diagonal), genetic (above the diagonal) and environmental (below the diagonal) correlations, and their respective standard errors for birth weight (BW), weaning weight (WW), weight at 12 (W12) and 18 (W18) months of age, respectively.

Trait	BW	WW	W12	W18	BW <sub>maternal</sub>	WW <sub>maternal</sub>
BW	0.35 ± 0.03	0.36 ± 0.05	0.36 ± 0.05	0.32 ± 0.06	0.06 ± 0.09	0.09 ± 0.07
WW	0.34 ± 0.02	0.42 ± 0.02	0.78 ± 0.02	0.71 ± 0.03	0.14 ± 0.08	–0.29 ± 0.05
W12	0.28 ± 0.02	0.82 ± 0.01	0.29 ± 0.02	0.98 ± 0.01	0.26 ± 0.07	0.33 ± 0.05
W18	0.25 ± 0.02	0.70 ± 0.01	0.77 ± 0.01	0.24 ± 0.02	0.27 ± 0.07	0.37 ± 0.05
BW <sub>maternal</sub>	–	–	–	–	0.10 ± 0.01	0.09 ± 0.08
WW <sub>maternal</sub>	–	–	–	–	–	0.09 ± 0.01

BW<sub>maternal</sub>=maternal genetic effect for BW; WW<sub>maternal</sub>=maternal genetic effect for WW. (–) Environmental correlations were set to zero.

**Table 6**

Heritability estimates (diagonal), genetic (above the diagonal) and environmental (below the diagonal) correlations, and their respective standard errors for age (AFC) and weight (WFC) at first calving and scrotal circumference at 12 (SC12) and 18 (SC18) months of age, respectively.

Trait	AFC	WFC	SC12	SC18
AFC	0.04 ± 0.02	0.13 ± 0.19	0.00 ± 0.20	0.08 ± 0.21
WFC	0.44 ± 0.04	0.31 ± 0.04	–0.22 ± 0.10	–0.13 ± 0.11
SC12	–	–	0.50 ± 0.06	0.85 ± 0.04
SC18	–	–	0.44 ± 0.07	0.54 ± 0.06

(–) Environmental correlations were set to zero.

Although the IGF1, GH, and PIT1 genes were found to be involved in the processes that regulate animal development (Oprzadek et al., 2003; Renaville et al., 2002), they may act differently on body tissues and at different stages of life. This could explain the different results found for each of the genetic markers used in our study. Association analyses between the IGF1, GH, and PIT1 markers and traits of economic interest in Canchim cattle were performed prior to the present study by Pereira et al. (2005), Andrade et al. (2008) and Carrijo et al. (2008). The present study differed from these authors particularly in the definitions of the animal's genetic groups and in the use of multi-trait analyses, which made it possible to use all the animals with genotype information for the allele substitution analyses.

The results from the allele substitution effect analyses for the IGF1 marker are presented in Table 7. The variance explained by the IGF1 marker was higher for WW and AFC, equal to 6% (coefficient of determination). However,  $R^2$  were low for all the traits studied. According to the results obtained for IGF1, the “225” allele was significantly associated ( $P \leq 0.05$ ) to higher EBVs for BW, BW<sub>maternal</sub>, WW, W12, W18, and SC18; and to lower EBVs for WW<sub>maternal</sub>. Thus, the “225” allele could be related to favorable responses for body weight and body development in Canchim cattle. The “231” allele was significantly associated ( $P \leq 0.05$ ) with

negative or close to zero EBVs for BW, WW, W12, W18, and WFC, respectively. The “227” allele did not present significant effect ( $P > 0.05$ ) with SC12 and SC18; while all others were significant ( $P \leq 0.05$ ). The EBVs for the “227” allele were intermediate when compared to “225” and “231”, respectively.

Similarly to the present study, the allele substitution analyses performed by Pereira et al. (2005) on Canchim cattle identified the alleles “225” and “231” as responsible for respectively higher and lower EBVs for BW, when studying IGF1. For W12, the “231” allele was also associated with lower EBVs. According to these authors, the most favorable allele for W12 was “229”, while our results showed that the most favorable allele for W12 was “225”, followed by “229”. Thus, the allele “229”, which is also the allele most frequently observed, is an allele assumed to be correlated with better reproduction and weight performance.

The results from the present study, with regard to the associations of the IGF1 marker, are in agreement with those reported in previous studies, in which the genotype class of this marker was seen to be correlated with BW and W12 (Pereira et al., 2005) and with BW (Andrade et al., 2008), respectively. In an experiment involving divergent selection for serum IGF1 concentration, Zhang et al. (2013) observed significant decrease ( $P < 0.001$ ) on age at first calving in Angus cattle heifers. Tizioto et al. (2012) found significant allele substitution effect for IGF1 and yearling weight ( $P \leq 0.017$ ) in the Nellore breed.

The allele substitution effect for the markers of GH and PIT1 genes are presented in Table 8. The variances explained by the GH and PIT1 markers were higher for AFC and WFC, respectively. However,  $R^2$  were low for all the traits studied. The effect observed on GH gene was significant ( $P \leq 0.05$ ) for the EBVs for AFC and WFC. The “valine” allele was responsible for lower EBVs for AFC and higher EBVs for WFC. The PIT1 gene presented a significant association ( $P \leq 0.05$ ) with the EBVs for WW, WW<sub>maternal</sub>, AFC, and WFC. The “Hinf–” allele was shown to be associated with lower EBVs for WW<sub>maternal</sub> and AFC. This allele was also significantly

**Table 7**

Mean allele substitution effect (represented by the regression coefficients and respective standard deviations) of the marker for the IGF1 gene (alleles 225, 227 and 231 in relation to 229), on the EBVs for birth weight (BW), weaning weight (WW), weight at 12 (W12) and 18 (W18) months of age, age (AFC) and weight (WFC) at first calving and scrotal circumference at 12 (SC12) and 18 (SC18) months of age in Canchim cattle.

Traits	225	P	227	P	231	P	$R^2$
BW (kg)	0.72 ± 0.12	< 0.01	0.28 ± 0.10	< 0.01	–0.60 ± 0.15	< 0.01	0.05
BW <sub>maternal</sub> (kg)	0.15 ± 0.05	< 0.01	0.11 ± 0.04	< 0.01	–0.10 ± 0.06	0.07	0.02
WW (kg)	3.81 ± 0.90	< 0.01	–4.20 ± 0.73	< 0.01	–4.57 ± 1.13	< 0.01	0.06
WW <sub>maternal</sub> (kg)	–0.69 ± 0.34	0.05	1.70 ± 0.28	< 0.01	–0.50 ± 0.43	0.25	0.04
W12 (kg)	2.66 ± 0.83	< 0.01	–2.00 ± 0.67	< 0.01	–5.05 ± 1.05	< 0.01	0.04
W18 (kg)	2.14 ± 0.91	0.02	–2.55 ± 0.73	< 0.01	–4.98 ± 1.14	< 0.01	0.03
AFC (days)	2.84 ± 0.49	< 0.01	3.21 ± 0.40	< 0.01	–1.07 ± 0.62	0.08	0.06
WFC (kg)	0.09 ± 0.85	0.92	–3.43 ± 0.68	< 0.01	–4.03 ± 1.06	< 0.01	0.03
SC12 (cm)	0.05 ± 0.07	0.50	–0.09 ± 0.06	0.09	0.03 ± 0.08	0.70	0.00
SC18 (cm)	0.27 ± 0.07	< 0.01	–0.01 ± 0.07	0.91	–0.09 ± 0.09	0.29	0.01

P=significance levels;  $R^2$ =coefficients of determination.



**Table 8**  
Mean allele substitution effect (represented by the regression coefficients and respective standard deviations) of the marker for the GH gene (“Valine” allele in relation to “Leucine”) and PIT1 gene (“Hinf–” allele in relation to “Hinf+”), on the EBVs for birth weight (BW), weaning weight (WW), weight at 12 (W12) and 18 (W18) months of age, age (AFC) and weight (WFC) at first calving, and scrotal circumference at 12 (SC12) and 18 (SC18) months of age in Canchim cattle.

Traits	GH			PIT1		
	“Valine”	P	R <sup>2</sup>	“Hinf–”	P	R <sup>2</sup>
BW (kg)	–0.04 ± 0.15	0.79	0.000	–0.26 ± 0.15	0.08	0.006
BW <sub>maternal</sub> (kg)	0.06 ± 0.06	0.31	0.001	–0.06 ± 0.05	0.25	0.002
WW (kg)	–1.92 ± 1.26	0.13	0.003	2.26 ± 1.16	0.05	0.007
WW <sub>maternal</sub> (kg)	–0.26 ± 0.42	0.53	0.000	–0.96 ± 0.40	0.02	0.011
W12 (kg)	–1.97 ± 1.11	0.08	0.004	0.86 ± 1.07	0.42	0.001
W18 (kg)	–1.89 ± 1.18	0.11	0.003	1.00 ± 1.16	0.39	0.001
AFC (days)	–1.91 ± 0.62	< 0.01	0.012	–1.85 ± 0.65	< 0.01	0.015
WFC (kg)	2.28 ± 1.12	0.04	0.006	3.50 ± 1.04	< 0.01	0.021
SC12 (cm)	–0.06 ± 0.08	0.46	0.000	0.05 ± 0.09	0.54	0.000
SC18 (cm)	–0.07 ± 0.09	0.45	0.000	–0.02 ± 0.09	0.80	0.000

P=significance levels; R<sup>2</sup>=coefficients of determination.

associated ( $P \leq 0.05$ ) with higher EBVs for WW and WFC.

Substitution of the “Leucine” allele of the GH gene by “Valine” led to a decrease of 1.91 days and an increase of 2.28 kg in the EBVs for AFC and WFC, respectively. Regarding to AFC and WFC, these traits are of great concern on beef cattle production, because it is desirable that heifers calves at an early age and not so heavy. According to Gavioli et al. (2012), dams with greater weight at maturity would show slow growth rate and later reproduction. Pereira et al. (2005) observed significant association ( $P \leq 0.05$ ) of GH markers with W12 and found that animals with the “Leucine/Valine” genotype had higher EBVs when compared to animals with the “Leucine/Leucine” genotype. However, significant allele substitution effect for GH was not detected for W12 in our study.

Arango et al. (2014) reported significant association ( $P < 0.01$ ) between GH markers and weight at first estrus and weight at first calving in Holstein heifers. Significant effect ( $P \leq 0.05$ ) between GH and longissimus muscle area was observed by Cardoso et al. (2014) in Nellore animals from three different lines (selection, traditional, and control). Furthermore, these authors found significant effect ( $P \leq 0.04$ ) of the interaction of GH and the growth hormone receptor (GHR) with weight at 550 days of age.

The substitution of the PIT1 allele “Hinf+” by “Hinf–” caused an increase of 2.26 kg and a decrease of 0.96 kg in the predicted EBVs for WW and WW<sub>maternal</sub>, respectively. The association between the “Hinf–” allele and higher EBVs for WW was similar to the results found by Carrijo et al. (2008), i.e. the animals that were homozygous for “Hinf–” had better performance for WW than the animals that were heterozygous and homozygous for “Hinf+”. We also observed that animals with the “Hinf–” allele had lower AFC and higher WFC. These results suggest that this allele substitution could lead to a better reproductive performance in Canchim heifers.

According to Cardoso et al., (2014), PIT1 markers (named as POU1F1) were monomorphic in Nellore animals, which was not assigned to the allele fixation due to selection. Zhang et al. (2009) found statistical difference ( $P < 0.05$ ) between AA and AB alleles for body weight at 4 months of age in crossed Qinchuan–Germany Yellow animals.

The effects of GH and PIT1 on AFC and WFC were similar, i.e. the most frequent alleles (“Leucine” and “Hinf+”, respectively). Evidence of associations were found for the same population data regarding IGF1 with birth weight and GH with weight at first calving and with weight at 12 months of age (Grossi et al., 2014). However, these authors highlighted that the markers of IGF1, GH, and PIT1 did not influence the phenotypic variance for growth and reproduction traits after Bonferroni correction for multiple testing.

#### 4. Conclusion

Important allele substitution effects were found for weight and reproduction traits in Canchim cattle. In general, markers on the IGF1 gene are related to higher EBVs for pre- and post-weaning body weight traits, while markers on the GH and PIT1 showed greater influence on the EBVs for age and weight at first calving. Adding molecular markers information on the selection process could result in increased genetic gains in Canchim cattle. Future studies using high-density genotyping platforms may contribute to our understanding of the effects of these genes on traits of economic importance in this breed.

#### Conflict of interest statement

The authors report that there are no conflicts of interest relevant to this publication.

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