Association of *Leptin gene* polymorphisms with carcass traits in Nellore cattle[1]

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Introduction

Nellore breed (*Bos indicus*) is the most numerous breed in Brazilian beef production systems mainly due to its adaptability to tropical environment (Ferraz and Felicio (2010)). In the modern beef production system it is necessary to be more efficient, shortening of productive cycle and offering better quality products. To reach that goal, it is essential to use of genetically superior animals. There are several tools to support the genetic selection of animals, including the marker assisted selection. Several studies have indicated the association of *leptin gene* polymorphisms with production of leptin, fat deposition and growth in cattle, especially in European breeds (*Bos taurus*), fed in feedlots (Fitzsimmons *et al.* (1998); Buchanan *et al.* (2002); Nkrumah *et al.* (2004); Liefers *et al.* (2005); Nkrumah *et al.* (2005)). This study was designed to evaluate the association of leptin gene polymorphisms with carcass traits in Nellore cattle and the feasibility of using these markers in breeding programs in Brazil, aiming to improve selection of Nellore cattle for economically relevant traits, like ultrasound carcass measurements, indicators of carcass merit and carcass yield.

Material and Methods

Samples and traits. Data on 1856 Nellore young bulls, raised under pasture conditions until 18 months of age and, after, fed in feedlots with medium energy diet based on corn silage, with ages varying from 21 to 28 months and live weights around 560 kg were used in this research. *Longissimus dorsi* muscle area (LMA) and backfat thickness (BF) were obtained from a cross-sectional image on the muscle, measured between the 12th and 13th ribs. The rumpfat thickness (RF) was measured at the intersection between the *Gluteus medius* and *Biceps femoris* muscles located between the hooks and pin bones. Backfat thickness was estimated at the 3/4 position from the chine bone end of the *Longissimus dorsi* muscle using the cross-sectional ribeye image. Real-time ultrasound images were collected using Piemedical Scanner 200 VET equipped with a linear probe of 17.8 cm and a 3.5-MHz transducer. Vegetable oil was applied and the area was curried free of dirt and debris before transducer placement. For collection of LMA and BF images, a standoff pad was used to guarantee acoustic contact between the linear probe and the natural body shape of the animal. Transducer placement was first determined by palpating the left side of the animal between the 12th and 13th ribs. The ultrasound probe was moved toward the midline between and parallel to the 12th and 13th rib bones and then laterally until the LM came into full view on

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the screen. The RF images were obtained by placing the transducer at the insertion of the *Gluteus medius* and *Biceps femoris* muscles without the use of the acoustic coupler.

Genotyping. DNA samples were obtained from blood or hair follicles. DNA was extracted from blood samples collected using EDTA vacuum tubes and impregnated on FTA cards by NaCl extraction and precipitation method described for Olerup and Zetterquist (1992). Four markers were studied in this research, the marker *E2FB* in the exon 2 (Buchanan *et al.* (2002)) and the *C963T*, *A1457G* and *UASMS1* in the promoter region (Liefers *et al.* (2005), Nkrumah *et al.* (2005)) of *leptin gene*. Genotypes came from DNA mass spectrometry (Sequenom iPlexTM Mass Spec), carried out in laboratories, located in USA and licensed by IGENITY® (Duluth, Georgia), a Merial Ltda subdivision, the company that owns the exploration licenses rights on markers analyzed.

Statistical analyses. Allelic and genotypic frequencies for each marker were estimates by simply counting of different alleles and genotypes using PROC ALLELE from SAS/Genetics, version 9.1.3. Markers' effects on carcass traits were evaluated using mixed model methodology, in a sire model, using PROC MIXED of SAS, using the model:

$$Y_{ijk} = \mu + C_i + \beta_1\left(I_{ijk} - \bar{I}\right) + \beta_2\left(M_{ijk} - \overline{M}\right) + S_j + e_{ijk}$$

where Y_{ij} the phenotypic value of an animal, μ is the general mean of the trait, C_i is the fixed effect contemporary group, β_1 is the coefficient for the covariate age at exam, β_2 is the regression coefficient for a given genetic marker, S_j is the coefficient associate to random effect of sire, and e_{ij} is the random effect of the residual. Allele substitution effect was estimated as suggested by Falconer (1981), using β_2. F-statistic was considered significant for allelic substitution effect if the nominal P-value was lower between 0.05 and 0.01 (*) or lower than 0.01 (**).

Results and discussion

The descriptive statistics of the traits analyzed are presented in Table 1.

Table 1: Descriptive statistics for ultrasound traits of Nellore young bulls

TRAITS	N	AVG	STD	MIN	MAX
LMA (cm²)	1856	65.09	7.42	40.20	93.80
BF (mm)	1856	2.17	1.27	0.00	7.60
RF (mm)	1856	3.82	1.86	0.00	11.90

N = nº of observations; AVG = average; STD = standard deviation; MIN, MAX = minimum or maximum values

Number of genotyped animals and allelic and genotypic frequencies of genetic markers on analyzed population are presented on Table 2. These markers, initially discovered in *Bos taurus*, were analyzed in Nellore cattle, a *Bos indicus* breed. The frequencies for *C963T* and *UASMS1* were low, with the minor allele frequencies of these markers near 0.02. However, for *E2FB* and *A1457G* markers there were observed high variability on allele frequencies. These genetic markers were used for allele substitution effect analysis.

The estimated allele substitution effects, that represent the substitution of one allele by other on each marker, were described in Table 3, showing the association of the three single nucleotide polymorphisms on analyzed traits. Any marker presented allele substitution effect for backfat thickness. The *A1457G* polymorphism was not associated with evaluated traits.

Table 2: Allelic and genotypic frequencies of genetic markers on a Nellore young bull population

Marker	N	Genotypic frequencies	Allelic frequencies
E2FB	1856	F(CC) = 0.876 F(CT) = 0.119 F(TT) = 0.005	f (C) = 0.936 f (T) = 0.064
C963T	1853	F(CC) = 0.960 F(CT) = 0.038 F(TT) = 0.002	f (C) = 0.979 f (T) = 0.021
A1457G	1856	F(AA) = 0.602 F(AG) = 0.340 F(GG) = 0.058	f (A) = 0.772 f (G) = 0.228
UASMS1	1856	F(CC) = 0.001 F(CT) = 0.041 F(TT) = 0.958	f (C) = 0.022 f (T) = 0.978

E2FB presented significant effect for LMA and RF. Buchanan *et al.* (2002), Nkrumah *et al.* (2004) and Schenkel *et al.* (2005) reported significant association of the *E2FB* marker with red meat yield, fat yield and scores.

C963T and *UASMS1* markers presented significant effect for LMA. In other study with *Bos taurus* cattle, *C963T* marker was associated significantly with plasmatic leptin concentration and T allele was favorable to increase the plasmatic concentration (Liefers *et al.* (2005)). Nkrumah *et al.* (2005) using data of crossbreed animals (only *Bos taurus* breeds) reported that UASMS1 marker was associated with final body weight and backfat thickness. In Nellore cattle, studies have shown other polymorphisms related to leptin metabolism or in the *leptin gene* associated with carcass traits (Ferraz *et al.* (2009); Souza *et al.* (2010)).

Table 3: Estimated allele substitution effects on Longissimus muscle area (LMA) and Rump Fat (RF) in Nellore animals

Marker	LMA	RF
	$\beta_1 \pm$ SE	$\beta_1 \pm$ SE
E2FB	1.05 ± 0.47*	0.28 ± 0.13*
C963T	2.08 ± 1.04*	NS
UASMS1	2.35 ± 0.99**	NS

* $0.01<P\leq0.05$, ** $P\leq0.01$, NS = Not signifficant

Conclusion

Leptin gene markers *E2FB*, *UASMS1* and *A1457* had effects on important economic ultrasound measured carcass traits. These findings support the potential use of these genetic markers in marker-assisted selection for improvement of such traits but more studies are necessary to evaluate the combined effect of these markers, searching for non additive effects. New samples, with larger number of animals and phenotypes of animals with larger capacity for fat deposition, as well as largest ribeye area, which is favorably correlated with the percentage of the commercial cuts can positively impact the Brazilian beef production system in all its segments.

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