Candidate genes for production traits in reference families of Nellore beef cattle

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Introduction

Brazil has the second largest herd of cattle in the world (Anualpec (2008)) with predominance of *Bos indicus* breeds which have a higher heat tolerance, resistance to parasites and fertility than cattle of European origin. Interest in improving carcass and beef quality is growing in the National beef industry, pressured by the requirements of meat markets.

The quantification of genetic variation and exploration of genomic segments that influence carcass and beef quality in the breeding population are essential for the establishment of quantitative and molecular criteria for selection.

The *PPARGC1A* (*peroxisome proliferative active receptor gamma coactivator 1 A*), *FABP4* (*fatty acid binding protein 4*) and *IGF-1* (*insulin-like growth factor*) genes are candidates to influence production traits in cattle. Association of polymorphisms in these genes with production traits were reported but studies with the populations where marker assisted selection is to be applied are still necessary before this information can effectively be used by producers.

The aim of this study was to investigate the presence of polymorphisms in the candidate genes *PPARGC1A*, *FABP4* and *IGF-1*in reference families of Nellore cattle and relate them to meat production traits in these animals.

Material and Methods

Animals. We used 270 steers descendents of 20 Nellore bulls, selected to represent the main genealogies marketed in Brazil. The animals were raised in three farms and allocated to two feedlots, in São Carlos, SP (21° 57' 33.32" S 47° 50' 33.28" W) and in Campo Grande, MS (21º 07' 16" S 56º 28' 55" O). The same experimental design will be repeated over three years as part of a National Research Network.

Phenotypic data. Backfat thickness (BFT) and ribeye area (REA) were measured by ultrasound when the animals were about 18 months, before entering in the feedlot, and after approximately 55 days of feedlot. Ultrasound images were taken at the cross section of the *Longissimus* muscle between the 12th and 13th rib. Fat gain (FG) was calculated as the difference from the first to the second ultrasound measure. Animals were weighed at

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weaning and at approximately 16 months. Weaning and yearling weight (WW and YW) were adjusted to 240 and 450 days respectively.

Sample collection and DNA extraction. Semen straws were used to obtain DNA from bulls by deproteinization with organic solvents. For the steers, DNA extractions were performed from 5 ml blood by a salting out method.

Genotypic data. The SNP (C/T) in intron 9 of the *PPARGC1A* gene (Weikard *et al.* (2005)) and the SNP (A/G) in exon 2 of the *FABP4* gene (Cho *et al.* (2008)) were genotyped by PCR-RFLP method, using restriction enzymes *BsuRI* and *NmuCI*, respectively. The microsatellite IGF-1 (Bishop *et al.* (1994)) was genotyped by capillary electrophoresis.

Statistical Analysis. A mixed model was used to evaluate the influence of markers on BFT and REA. Fixed effects were contemporary group composed by birth place, site of feedlot and genotypes, in addition to the random effect of bull. The age of the animal at the time of measurement was included as a covariate. For FG the model included fixed effects of site of feedlot and genotypes besides the random effect of bull. For WW and YW the model was fitted for fixed effects of birth place and genotypes, the age of the animal at measurement as covariate and the random effect of bull. The analysis was made by the maximum likelihood method using the procedure PROC MIXED of the Statistical Analysis System (SAS Institute Inc. (2003)). Genotypic means were calculated by the GLM procedure of SAS for the markers that had significant ($P < 0.05$) or suggestive effect ($P<0.10$) on any trait evaluated. A Tukey test was applied to confirm difference between means. Allele substitution effects were calculated for significant maker-trait associations by replacing the effect of marker genotype in the statistical model by covariates representing each allele in the genotype.

Results and Discussion

The reference families were segregating for the three marker loci studied. Allelic and genotypic frequencies are presented in Table 1.

Table 1. Allele and genotypic frequencies of *PPARGC1A*, *FABP4* and *IGF-1* polymorphisms.

GENE	FREQUENCY (%)				
	ALLELIC		GENOTYPIC		
PPARGC1A	C	T	CC	CT	TT
	85.40	14.60	72.20	26.36	1.44
FABP4	A	G	AA	AG	GG
	17.51	82.49	1.80	31.41	66.79
IGF-1	225	229	225/225	225/229	229/229
	73.55	26.45	54.34	38.41	7.25

PPARGC1A (*peroxisome proliferative active receptor gamma coactivator 1 A*); *FABP4* (*fatty acid binding protein 4*); *IGF-1* (*insulin-like growth factor*)

In the analysis of association (Table 2) *FABP4* genotypes were significantly associated ($P<0.05$) to BFT and suggestively associated ($P<0.10$) to FG.

Table 2. Results of the association between the marker and the characteristics evaluated

Markers	BFT1	BFT2	FG	REA1	REA2	WW	YW
PPARGC1A	0.499	0.737	0.548	0.570	0.412	0.285	0.774
FABP4	0.369	0.040	0.093	0.156	0.871	0.763	0.522
IGF-1	0.503	0.644	0.740	0.770	0.440	0.559	0.071

PPARGC1A (peroxisome proliferative active receptor gamma coactivator 1 A); *FABP4 (fatty acid binding protein 4)*; *IGF-1 (insulin-like growth factor)*; BFT1 and REA1= Backfat and Rib Eye Area at the first ultrasound measure, respectively; BFT2 and REA2= Backfat and Rib Eye Area at the second ultrasound measure, respectively

Polymorphisms in *FABP4* have been associated to BFT (Michall *et al.* (2006); Cho *et al.* (2008)), marbling (Michall *et al.* (2006)) and composition of palmitoleic and linoleic acid in the intramuscular fat (Hoashi *et al.* (2008)). In this work, despite no association was observed between *FABP4* and the first measure of BFT we found a significant effect of this marker on the second measure of BFT (BFT2) and suggestive effect on FG. The difference between the two analyses may reflect the low exposure of the genetic potential of the animals for fat deposition in the first measure, which was taken under pasture.

Significant difference between the means of genotypes AA and AG (P = 0.003) and AA and GG (P = 0.001) of *FABP4* gene were found for BFT2. There was no significant difference between AG and GG genotypes (P = 0.735), suggesting a dominant gene action. For FG, a suggestive difference between the means of AA and AG (P = 0.053) was found, significant difference between the means of AA and GG (P = 0.041) and no significant difference between the means of AG and GG genotypes (P = 0.793), consistent with the dominant effect observed for BFT2.

There was no significant association between *FABP4* and REA in this population of Nellore cattle, agreeing with other studies (Hoashi *et al.* (2008); Rezende *et al.* (2008)). No effect of *FABP4* polymorphism on growth traits WW and YW was observed. There was no significant effect of *FABP4* allele substitution on either BFT2 or FG. Since allele substitution is a measure of the additive effect of a *locus*, these results reinforce the dominant nature of the association between *FABP4* and BFT2.

Suggestive association was also found between YW and *IGF-1* gene (Table 2). Significant difference was not found between the means of genotypes of *IGF-1* gene for YW, but a significant effect of allele substitution for the *IGF-1* and YW (P=0.017). The mean allele substitution effect was 6.9 kilogram, with the 229 allele associated to reduced YW in this population of Nellore. The *IGF-1* gene has an essential role in the metabolism and growth of animals. Associations of this *IGF-1* polymorphism with growth traits (Ge *et al.* (2001); Pereira *et al.* (2005)) and residual feed intake (Wood *et al.* (2004)) have been described in cattle. Furthermore, Islam *et al.* (2009)) associated a SNP in the promoter region of *IGF-1* gene with BFT and other carcass traits. The microsatellite used in this study is also in the promoter region of *IGF-1* gene, than there is a high probability of being in linkage

disequilibrium with this SNP and behave as an indirect marker. We did not found significant effect of the microsatellite in the *IGF-1* gene on any carcass trait. However, for the growth traits we found a suggestive effect on YW and no effect was found for WW.

Although *PPARGC1A* is associated with energy metabolism and production traits and has been associated to fat deposition in the milk (Weikard *et al*. (2005); Schennink *et al*. (2009)) in the present work we did not find significant association with the traits studied, agreeing with other results obtained for carcass traits in cattle (White *et al.* (2006); Soria *et al*. (2009); Tizioto *et al*. (2009)).

Conclusion

FABP4 significantly affected BFT measured after 55 days on feedlot and had a suggestive effect on fat gain. In addition, a suggestive association between the *IGF-1* gene and YW was found in this population of Nellore breed. Extending this investigation to the next two years of progeny evaluation may allow for more accurate estimate of marker effect and application on breeding programs.

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