Identification and evaluation of polymorphisms in *FABP3* and *FABP4* in beef cattle

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ABSTRACT. Single nucleotide polymorphisms (SNPs) were screened in *FABP3* and *FABP4* by automatic sequencing of pools of DNA from crossbred animals whose phenotypes belonged to the upper and lower extremes for back fat and marbling, as well as of a pool of DNA from sires used for crossbreeding. Five SNPs were identified in *FABP3* and another nine SNPs were identified in *FAPB4*. Of these, only one SNP had no previous registry in the SNAP database (dbSNP). Three polymorphisms were selected for further evaluation of their association with production traits using restriction fragment length polymorphism-PCR (RFLP-PCR) or real-time PCR genotyping. All 3 markers were in Hardy-Weinberg equilibrium at the 5% significance level for all 7 genetic groups analyzed. Significant association was observed between *FABP3-G*/*A* with rib eye area (P = 0.035) and the rib eye area/hot carcass weight ratio (P = 0.025) and between *FABP4*/*Tas*I with marbling (P = 0.052) and meat texture (P = 0.053). No significant association was observed between the *FABP4-G/C* polymorphism and any of the observed traits. Previous association studies with allelic variants in these genes have shown mixed results,

probably because of the small effect of the genes for these traits, which suggests that results should be replicated in other populations.

Key words: *A-FABP*; Ribeye area; Back fat; *H-FABP*; Molecular marker; Marbling

INTRODUCTION

The beef cattle industry in Brazil has undergone intense transformation in response to increasing demands for a better quality product. Traits such as back and intramuscular fat deposition in beef cattle directly influence the choice of the consumer for succulence, taste, and tenderness (Luchiari Filho, 2000). Classic genetic animal improvement has given way to changes in the genetic composition of populations through selection and a breeding system intended to increase the frequency of favorable alleles and provide production increments along with improvement in traits of commercial interest (Euclides Filho, 1999; Ferraz and Eler, 2010).

Some traits, however, are tricky to improve upon by classic animal improvement, mainly when they present low heritability or are cumbersome or are measured at a late age (Davis and DeNise, 1998; Van Eenennaam, 2009). In these situations, marker assisted selection (MAS) complements traditional methods, allowing the selection of animals at an early age and with higher accuracy.

The identification of candidate genes responsible for phenotypic variations has been a challenge, mostly because of the quantitative nature of these variables; in other words, because they are controlled by many genes and are affected by the environment (Andersson, 2001). Several candidate genes have been identified and associated with carcass quality and fat deposition traits, such as *TG* (Barendse, 1999), *LEP* (Buchanan et al., 2002), *DGAT1* (Thaller et al., 2003), genes in the FABP (fatty acid-binding proteins) family, which encode small, highly conserved, cytoplasmic proteins that bind to long chain fatty acids and other hydrophobic ligands (Roy et al., 2003; Chmurzynska, 2006; Michal et al., 2006), and others.

The heart type FABP3 protein is present in several tissues with a high demand of fatty acids such as cardiac and skeletal muscle and lactating mammary gland. *FABP3* gene is involved in fatty acid transport from the cell membrane to intracellular sites (Veerkamp and Maatman, 1995; Roy et al., 2003). Fatty acid binding protein 4 (FABP4) is expressed in adipose tissue and plays an important role in lipid metabolism and homeostasis in adipocytes. *FABP4* gene has been shown to be associated with lipid metabolism (lipolysis and lipogenesis), marbling, and back fat deposition (Specht et al., 1996; Hertzel et al., 2006; Michal et al., 2006).

Therefore, our objective was therefore to identify single nucleotide polymorphisms (SNPs) in *FABP3* and *FABP4* by automatic sequencing, estimate allelic and genotypic frequencies, and evaluate association with meat and carcass quality traits in beef cattle.

MATERIAL AND METHODS

The experiment was carried out at Embrapa Gado de Corte, in Campo Grande, Mato Grosso do Sul (MS), Brazil, at the geographical coordinates 20° 27' S and 54° 37' W, and at an altitude of 530 m. The climate in this region is classified as tropical savanna and characterized by a well-defined dry period during the colder months and a rainy period during the summer months.

The production of experimental animals, maintenance, slaughter and harvesting of biological samples were performed as required by Brazilian Law No. 11.794 of October 8, 2008

and as by other rules applicable to the use of animals for scientific purposes, especially Normative Resolutions of National Council for Animal Experiments Control (CONCEA) and the Brazilian Guideline for Care and Use of Animals for Scientific and Teaching Purposes (DBCA).

Super-precocious steers evaluated in this work were obtained from the crossbreeding of 50 ½ Valdostana + ½ Nelore (VN) cows, 50 ½ Angus + ½ Nelore (AN) cows and 80 ½ Caracu + ½ Nelore (CN) cows. Breeding between maternal and terminal breeds was carried out between VN and AN cows inseminated by Caracu (CR) sires [yielding 75% taurine and 75% adapted (CRVN and CRAN) calves] and Canchim (CC) sires [yielding 56.25% taurine and 43.75% adapted (CCVN and CCAN) calves]. In parallel, CN cows were inseminated by Caracu sires [yielding 75% taurine and 100% adapted (CRCN) calves], by Canchim sires [yielding 56.25% taurine and 68.75% adapted (CCCN) calves], and by Angus sires [yielding 75% taurine and 50% adapted (RACN) calves]. In total, 201 calves divided into 7 genetic groups were obtained.

Male and female calves were weaned at 240 days, submitted to confinement in individual pens, and fed a diet based on sorghum, corn, and soybean meal fodder with and without the addition of cottonseeds and soybean hulls. During the experiment, animals were observed every 28 days for weighing and for taking ultrasound measurements of the longissimus dorsi muscle area (rib eye area, REA) and back fat (BF) thickness in the dorsal-lumbar region, between the 12th and 13th ribs. Animals were slaughtered when they reached a minimum of 5 mm of BF, thus determining the variable of days in confinement (DCONF).

Measurements of slaughter weight (SW), hot carcass weight (HCW), and dressing percentage (the ratio between SW and HCW = dress %) were taken at the time of slaughter. Twenty-four hours after slaughter, the longissimus dorsi muscle was analyzed between the 12th and 13th ribs of the right half-carcass. The measurements taken included carcass fat thickness (CFT), REA, marbling (MARB), physiological maturity (FISMAT), and texture (TEXT), as proposed by Muller (1980). Samples were also collected from the longissimus dorsi muscle for analysis of tenderness based on shear force (SF) measured in a Warner-Bratzler instrument as described by Menezes et al. (2009).

For genotyping, blood samples were collected from 201 animals into 4.5 mL vacutainer tubes containing K3 EDTA, and semen was collected from sires used for breeding. Genomic DNA was extracted by the method described by Regitano and Coutinho (2001). DNA was quantified on a NanoDrop spectrophotometer (Thermo Fisher Scientific Inc, Wilmington, DE, USA) and integrity was verified by electrophoresis on a 0.8% agarose gel.

Pools containing DNA from 14 animals each were created in order to screen for SNPs in *FABP3* and *FABP4*. The animals in each pool were balanced with respect to gender and genealogy, and they represented the upper and lower extremes for marbling and back fat. In order to determine the maternal or paternal origin of the SNPs, an extra pool containing DNA from the 18 sires that were used for crossbreeding was created. All pools were prepared in duplicate and contained 200 ng of DNA from each animal to minimize variation in composition and concentration.

Oligonucleotide pairs (Table 1) were designed to amplify regions of interest in sequences from *FABP3* and *FABP4* available in GenBank (www.ncbi.nlm.nih.gov) under Accession Nos. 281758 and 281759, respectively. The oligonucleotides used for sequence amplification flanked all exons and spanned both untranslated regions (UTRs) and parts of the intronic regions. These were designed using Primer 3 plus software available online (http://frodo.wi.mit.edu/primer3/) and specificity was verified using OligoAnalyzer v. 3.1, also available online (http://www.idtdna.com/analyzer/Applications/OligoAnalyzer/).

Table 1. Forward and reverse oligonucleotide sequences, and the size of each amplicon used to amplify regions of interest in sequences of *FABP3* and *FABP4* in beef cattle.

Gene	Fragment	Forward (5'-3')	Reverse (5'-3')	Amplicon (bp)
FABP3	1	GTGACACAGGGGCCTTTTA	GGGAACCAAGATCCCACAT	425
FABP3	2	CTCTTCTCCCTCCCCAACAT	CCCACACAGGCAACAGGTAGAT	481
FABP3	3	TCTGGCTCTCACACCATCAG	TGCAAGCACTCTCTCCTTCA	519
FABP3	4	CTGTCAAGAACCTGGCACAT	TGCTTCCTGAGTAGCAGTCTTT	528
FABP4	1	AAAAAGAGGCAGAAAGCCAAG	CAGAGGGAAAACTGCAGAGG	564
FABP4	2	ATGGAATGGCTTTCCTCCTT	TTGTGCCTTGGGTGTTCTTT	510
FABP4	3	CAAGGGCGATTGTCTCTATTTCTC	CCCCTATGATGCTATTCCACA	443
FABP4	4	TCCCTGAGAGGGTGTTGTTT	CCTGCTCAACATTGAAGGAGAC	548

PCR reactions were carried out in 25 µL reactions containing 80 ng genomic DNA, 0.164 µM each oligonucleotide, 10 mM Tris-HCl, pH 8.0, 50 mM KCl, 2.0 mM $MgCl_2$, 0.2 mM each dNTP, and 0.65 units (U) *Taq* DNA polymerase (Invitrogen™). PCR reactions were submitted to 2 min at 95°C for initial denaturing followed by 35 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 30 s. A final extension step at 72°C for 5 min was also included.

PCR products were purified using Exonuclease I (*Exo*I, USB Products) and shrimp alkaline phosphatase (SAP, USB Products) and submitted to a sequencing reaction using a BigDye® Terminator Cycle Sequencing Kit (v.3.1, Applied Biosystems, Foster City CA, USA). Automatic sequencing was carried out in an ABI 3130 instrument (Applied Biosystems). Each pool was sequenced using both the forward and reverse oligonucleotides separately and all reactions were carried out in duplicate.

SNP detection was carried out using CodonCode Aligner software (CodonCode Corporation, www.codoncode.com). Forward and reverse duplicate sequences were aligned for each pool generating a consensus sequence per pool. Consensus sequences were then compared among all pools generating a consensus sequence for all pools, which was then aligned to the *Bos taurus taurus* reference genome sequence obtained from NCBI.

Three of the 14 identified SNPs were selected and genotyped in 201 animals individually and their association with traits of interests was analyzed. Criteria for selection of SNPs included confirmation in duplicate pools, association to beef quality traits in previous studies, localization within the gene, and no previous identification in the SNP database (dbSNP; http://www.ncbi.nlm.nih.gov/projects/SNP/) or in the literature. Two of these SNPs were located on *FABP4* and identified herein as *FABP4/Tas*I (rs109346428) and *FABP4*-G/C (rs110383592). The third SNP, which was identified in *FABP3* (*FABP3*-G/A), had no previous registry in dbSNP.

The *FABP4*/*Tas*I SNP was genotyped using restriction length fragment polymorphism-PCR (RFLP-PCR) since it was located within the restriction site of endonuclease *Tas*I (Tsp509I). The forward and reverse oligonucleotides used for PCR were 5'-ATTATCCCCACAGAGCATCG-3' and 5'-TACTGCTGGGGGCACAGTAT-3', respectively, generating a 297-bp fragment. PCR was carried out in a final reaction volume of 25 µL containing 120 ng DNA, 0.164 µM each oligonucleotide, 10 mM Tris-HCl, pH 8.0, 50 mM KCl, 3.0 mM $MgCl_2$, 0.2 mM each dNTP, 1.0 U *Taq* DNA polymerase and submitted to the following program: 5 min for initial denaturing at 95°C, followed by 30 cycles at 94°C for 30 s, 63°C for 30 s, and 72°C for 30 s. Reactions were terminated after a final extension step at 72°C for 7 min.

Amplicons were digested in a reaction containing 7 µL PCR product and 1 U *Tas*I restriction enzyme. Reactions were incubated at 65°C for 3 h. Resulting fragments were separated on 3% agarose gels and stained with SybrGold (1:50,000). Gels were observed and photographed

by exposure to ultraviolet light. Two individuals belonging to each genotype were submitted to automatic sequencing to confirm the RFLP-PCR results. The GG genotype was characterized by the presence of 3 restriction fragments (136, 114, and 47 bp) and the AA genotype by 4 restriction fragments (136, 83, 47, and 31 bp). Heterozygous animals (GA) presented 5 fragments. The 136- and 47-bp fragments were common to all genotypes and were considered positive controls for efficient *Tas*I cleavage.

The other two selected SNPs (*FABP3*-G/A and *FABP4*-G/C) were genotyped by real-time PCR using TaqMan. Oligonucleotide and reporter sequences are detailed in Table 2, and they were used at concentrations of 36 and 8 µM, respectively. Genotyping was carried out in the Step One Plus instrument (Applied Biosystems). Reactions were carried out in a final volume of 5 µL containing 30 ng DNA, 2.5 µL TaqMan Master Mix, and 0.125 µL TaqMan assay. All PCR reaction plates contained positive controls for each possible genotype to facilitate grouping of tested samples and negative controls containing water in place of DNA.

Genotypic and allelic frequencies were calculated according to Weir (1996) and Hardy-Weinberg equilibrium was verified using the chi-square test. Discrepancies between expected and observed frequencies were analyzed at 5% significance.

Association studies were carried out using analysis of variance for traits related to beef and carcass quality using the general linear model (GLM) in the SAS statistical program (SAS Institute, 2003). The model considered the effect of each genotype on the polymorphism, year of birth (season), cattle lot (according to season of birth), sex, diet, genetic group of the individual, the sire, and the cow.

When significant effects ($P \leq 0.05$) were observed for the genotype, allelic substitution was estimated by substituting the effect of the genotype in the statistical model by co-variables representing the least frequent allele (0, 1, and 2).

Table 2. Oligonucleotide and reporter sequences used in real-time PCR to genotype two single nucleotide polymorphisms (SNPs) in *FABP3* and *FABP4* in beef cattle.

Gene	Forward	Reverse	Reporter 1	Reporter 2
FABP3	GGTCATTGAGCCCCTTGTACT	GAGGGAAGGGTATGAGCATGAG	TCGCCTCTCGCTCCAG (VIC)	TCGCCTCTCACTCCAG (FAM)
FABP4	TGGGATGACCTAGCACTAAAATCTAGAA	ACCATAAAGAGAAAACTCGTGGATGAT	AGAAGATACTCACGAGCACC (VIC)	AGAAGATACTCACCAGCACC (FAM)

VIC and FAM: fluorophores used to label SNP nucleotides.

RESULTS

The SNPs identified in *FABP3* and *FABP4* can be found in Table 3. Five SNPs were identified within *FABP3*, 4 of which had previously been deposited in dbSNP, and 1 of which is described in this study for this first time. This SNP was deposited in GenBank in November 2014 and it is available under acession No. KM 382065.1 (www.ncbi.nlm.nih.gov/nuccore/KM382065). In *FABP4*, 9 SNPs, all of which had previously been described and deposited in dbSNP, were identified. Three of the 9 SNPs in *FABP4* were located in the 3'UTR. A large number of observed SNPs were found in intronic regions.

After genotyping all 201 animals, both allelic variants from all 3 SNPs were found to be represented in the seven genetic groups studied. The allelic frequencies for the *FABP3*-G/A, *FABP4*-G/C and *FABP4/Tas*I polymorphisms within each of the seven genetic groups can be found in Table 4. For the *FABP3*-G/A SNP, the G allele was the most frequent in all genetic groups.

Groups RACN, CRAN, CRCN, and CRVN presented the highest frequencies of this allele at an average of 81.58%, while all other groups presented an average of 66.33%. For *FABP4*-G/C, the C allele was the most frequent and the groups with the highest frequencies for this allele were CCCN, RACN, and CCAN with an average of 86.80%, while in other groups this allele averaged 70.65%. The G allele was the most frequent for *FABP4/Tas*I and the groups with the highest frequency of this allele were CCVN, CRAN, CRCN and CRVN with an average of 57%, while the other groups averaged 44.3%.

Table 3. Description, localization, and single nucleotide polymorphism database (dbSNP) accession numbers for identified SNPs in *FABP3* and *FABP4* in beef cattle.

Gene	SNP	Localization	dbSNP accession No.
FABP3	C/G	INTRON I	rs210186725
FABP3	G/A	INTRON I	Not previously deposited
FABP3	A/G	INTRON III	rs109315289
FABP3	A/G	INTRON III	rs209338531
FABP3	C/T	INTRON III	rs210785101
FABP4	G/T	INTRON II	rs134173517
FABP4	A/T	INTRON II	rs109014985
FABP4	C/G	EXON III	rs110383592
FABP4	A/G	INTRON III	rs109388335
FABP4	A/G	INTRON III	rs109346428
FABP4	C/T	EXON IV	rs110370032
FABP4	A/G	3´UTR	rs109077068
FABP4	C/T	3´UTR	rs110266999
FABP4	C/G	3´UTR	rs109593774

Table 4. Allelic frequencies for the *FABP3*-G/A, *FABP4*-G/C, and *FABP4/Tas*I polymorphisms within each of the seven genetic groups and for the total population of beef cattle.

Marker	Allele	Genetic groups							Total (N = 201)
		CCAN (N = 26)	CCCN (N = 32)	CCVN (N = 25)	CRAN (N = 29)	CRCN (N = 28)	CRVN (N = 26)	RACN (N = 35)	
FABP3-G/A*	G	65.4	65.6	68.0	82.8	80.4	78.8	84.3	75.4
	A	34.6	34.4	32.0	17.2	19.6	21.2	15.7	24.6
FABP4- G/C*	C	80.8	95.3	68.0	72.4	76.8	65.4	84.3	80.0
	G	19.2	4.7	32.0	25.6	23.2	34.6	15.7	20.0
*FABP4/Tas*I	G	46.2	43.8	60.0	51.7	57.1	61.5	42.9	51.2
	A	53.9	56.3	40.0	48.3	42.9	38.5	57.1	48.8

*Significant differences ($P < 0.05$) were detected between allelic frequencies of genetic groups using the chi-square test. CCAN: Canchim-Red Angus/Nelore; CCCN: Canchim-Caracu/Nelore; CCVN: Canchim-Valdostana/Nelore; CRAN: Caracu-Red Angus/Nelore; CRCN: Caracu-Caracu/Nelore; CRVN; Caracu-Valdostana/Nelore; RACN: Red Angus-Caracu/Nelore.

All polymorphisms were shown to be in Hardy-Weinberg equilibrium ($P > 0.05$); however, significant differences were not observed for genotypic frequencies between genetic groups for any of the SNPs using the chi-square test, as seen in Table 5. Of all the animals genotyped for the *FABP3*-G/A polymorphism, 111 presented a GG genotype, 81 presented a GA genotype, and 9 exhibited an AA genotype. As for the *FABP4*-G/C SNP, 123 animals presented the CC genotype, 69 exhibited the GC genotype, and only 9 presented the GG genotype. All 3 genotypes of the *FABP4/Tas*I SNP were present in all genetic groups, where 45 animals presented the GG genotype, 116 presented the GA genotype, and 40 presented the AA genotype.

Table 5. Genotypic frequencies and level of significance for Hardy-Weinberg equilibrium for the polymorphisms *FABP3*-G/A, *FABP4*-G/C, and *FABP4/Tas*I within each of the seven genetic groups and for the total population of beef cattle.

Marker	Genotype	Genetic group							Total (N = 201)
		CCAN (N = 26)	CCCN (N = 32)	CCVN (N = 25)	CRAN (N = 29)	CRCN (N = 28)	CRVN (N = 26)	RACN (N = 35)	
FABP3-G/A	GG	46.1	43.8	40.0	65.5	60.7	57.7	68.6	55.2
	GA	38.5	43.7	56.0	34.5	39.3	42.3	31.4	40.3
	AA	15.4	12.5	4.0	0.0	0.0	0.0	0.0	4.5
	HW	0.745	0.985	0.358	0.533	0.433	0.392	0.544	
FABP4-G/C	GG	7.7	0.0	4.0	6.9	0.0	11.5	2.9	4.5
	GC	23.1	9.4	56.0	41.4	46.4	46.2	25.7	34.3
	CC	69.2	90.6	40.0	51.7	53.6	42.3	71.4	61.2
	HW	0.423	0.962	0.358	0.982	0.278	0.995	0.985	
*FABP4/Tas*I	GG	23.1	12.5	28.0	24.1	21.4	34.6	17.1	22.4
	GA	46.2	62.5	64.0	55.2	71.4	53.9	51.4	57.7
	AA	30.8	25.0	8.0	20.7	7.1	11.5	31.4	19.9
	HW	0.936	0.312	0.249	0.853	0.053	0.782	0.957	

CCAN: Canchim-Red Angus/Nelore; CCCN: Canchim-Caracu/Nelore; CCVN: Canchim-Valdostana/Nelore; CRAN: Caracu-Red Angus/Nelore; CRCN: Caracu-Caracu/Nelore; CRVN; Caracu-Valdostana/Nelore; RACN: Red Angus-Caracu/Nelore. HW: level of significance of Hardy-Weinberg equilibrium at the 5% level.

The results of the association study showed a significant effect between *FABP3*-G/A genotypes and REA (P = 0.035) (Table 6) and the REA/HCW ratio (P = 0.025; Table 7). In both cases, the AA genotype presented the highest phenotypic value; however, it only differed statistically from the heterozygous GA genotype. On the other hand, significant association was not observed between *FABP4*-G/C genotypes and any of the observed traits.

A significant effect was also observed between *FABP4/Tas*I genotypes and meat texture (P = 0.053) and marbling (P = 0.052) (Table 6). The AA genotype presented the highest scores for meat texture, even though average scores were statistically equivalent to those for the GG genotype. In contrast, heterozygous (GA) animals presented the highest scores for marbling.

Table 6. Significance (P) and mean ± standard error for phenotypic traits analyzed for the *FABP3*-G/A, *FABP4*-G/C, and *FABP4/Tas*I SNP genotypes in beef cattle.

Marker	Variable									
	DCONF (days)	SW (kg)	HCW (kg)	HCY (%)	PHYSMAT (score)	TEXT (score)	BF (mm)	REA (cm²)	MARB (escore)	SF (kg)
FABP3-G/A	P = 0.368	P = 0.384	P = 0.301	P = 0.450	P = 0.967	P = 0.370	P = 0.098	*P = 0.035	P = 0.545	P = 0.792
GG	136.50 ± 2.92	403.58 ± 4.92	221.62 ± 2.96	54.71 ± 0.23	14.04 ± 0.09	4.52 ± 0.06	5.56 ± 0.27	62.15 ± 1.13^{ab}	6.80 ± 0.37	5.61 ± 0.27
GA	1.16 ± 0.02	409.70 ± 5.23	224.53 ± 3.12	54.61 ± 0.25	14.06 ± 0.09	4.55 ± 0.06	5.10 ± 0.29	60.29 ± 1.19^{a}	6.35 ± 0.40	5.81 ± 0.28
AA	1.08 ± 0.06	417.31 ± 13.64	233.04 ± 8.15	55.41 ± 0.64	14.03 ± 0.23	4.33 ± 0.16	4.21 ± 0.74	67.62 ± 3.05^{b}	6.69 ± 1.01	5.60 ± 0.74
FABP4-G/C	P = 0.609	P = 0.866	P = 0.804	P = 0.295	P = 0.646	P = 0.573	P = 0.985	P = 0.881	P = 0.610	P = 0.589
GG	139.93 ± 7.64	401.15 ± 12.91	218.26 ± 7.72	53.88 ± 0.61	14.13 ± 0.23	4.41 ± 0.16	5.21 ± 0.75	59.99 ± 3.11	5.78 ± 1.01	6.41 ± 0.74
GC	134.28 ± 3.28	405.66 ± 5.55	223.43 ± 3.32	54.84 ± 0.26	14.09 ± 0.10	4.56 ± 0.06	5.33 ± 0.31	61.55 ± 1.29	6.53 ± 0.42	5.68 ± 0.30
CC	137.17 ± 2.94	407.59 ± 4.97	223.37 ± 2.99	54.65 ± 0.23	14.01 ± 0.09	4.52 ± 0.06	5.34 ± 0.28	61.58 ± 1.15	6.75 ± 0.37	5.63 ± 0.27
*FABP4/Tas*I	P = 0.919	P = 0.176	P = 0.507	P = 0.481	P = 0.169	*P = 0.053	P = 0.939	P = 0.876	*P = 0.052	P = 0.943
GG	136.93 ± 3.81	401.19 ± 6.38	220.46 ± 3.84	54.53 ± 0.30	14.20 ± 0.11	4.55 ± 0.07^{ab}	5.24 ± 0.35	61.64 ± 1.46	6.27 ± 0.47^{ab}	5.62 ± 0.35
GA	136.49 ± 3.01	404.67 ± 5.03	222.88 ± 3.04	54.83 ± 0.24	13.99 ± 0.09	4.45 ± 0.06^{b}	5.36 ± 0.28	61.67 ± 1.19	7.11 ± 0.38^{a}	5.70 ± 0.28
AA	135.02 ± 3.95	415.75 ± 6.61	226.32 ± 4.03	54.51 ± 0.32	13.99 ± 0.11	4.64 ± 0.07^{a}	5.37 ± 0.37	60.88 ± 1.51	6.02 ± 0.49^{b}	5.77 ± 0.36

DCONF = Days in confinement to reach 5 mm of back fat; SW = slaughter weight; HCW = hot carcass weight; HCY = hot carcass yield; PHYSMAT = physiological maturity (scale from 1 to 15 points split into classes A through E and in subclasses = lower, average, higher; 1 = older animal and 15 = youngest animal); TEXT = meat texture (scale from 1 to 5 points, 1 = rough and 5 = fine); BF = back fat thickness at the dorsal lumbar region on the carcass; REA = rib eye area measured on the carcass; MARB = marbling (values from 1 to 18, where 1 to 3 = traces, 4 to 6 = light, 7 to 9 = low, 10 to 12 = average, 13 to 15 = high, and 16 to 18 = abundant); SF = shear force. *P ≤ 0.05. ^{a,b,}-Values in the same column followed by different letters differ among themselves.

Table 7. Significance (P) and mean ± standard error of the ratio between rib eye area and hot carcass weight (REA/HCW), distribution of back fat on carcass (BFCARC), and gain in back fat per day of confinement (BF/D) for genotypes for markers *FABP3*-G/A, *FABP4*-G/C, and *FABP4/Tas*I.

Marker	Variable		
	REA/HCW (cm^2/kg)	BFCARC	BF/D (cm^2/day)
FABP3-G/A	*P = 0.025	P = 0.061	P = 0.143
GG	0.28 ± 0.004[a]	2.66 ± 0.14	4.44 ± 0.29
GA	0.27 ± 0.004[b]	2.38 ± 0.15	4.05 ± 0.31
AA	0.29 ± 0.01[a]	1.98 ± 0.38	3.04 ± 0.79
FABP4-G/C	P = 0.876	P = 0.971	P = 0.801
GG	0.28 ± 0.01	2.61 ± 0.38	3.80 ± 0.80
GC	0.28 ± 0.004	2.51 ± 0.16	4.33 ± 0.33
CC	0.28 ± 0.004	2.52 ± 0.14	4.23 ± 0.29
*FABP4/Tas*I	P = 0.093	P = 0.930	P = 0.940
GG	0.28 ± 0.005	2.56 ± 0.18	4.14 ± 0.37
GA	0.28 ± 0.004	2.53 ± 0.15	4.28 ± 0.31
AA	0.27 ± 0.005	2.47 ± 0.19	4.27 ± 0.39

*P ≤ 0.05. [a,b,-]Values in the same column followed by different letters differ among themselves.

When a markers genotype presented significant results (P ≤ 0.05), allelic substitution was estimated by substituting the effect of the genotype in the statistical model by covariates representing the quantity of the least frequent allele (0, 1, and 2), as shown in Table 8. This was made possible because alleles (but not genotypes) are transmitted to descendants; therefore, one can indicate an associated value for each allelic variant held by the individual (Falconer and Mackay, 1996). This analysis verified that the G allele of the *FABP3*-G/A SNP leads to a reduction in rib eye area while the A allele of the *FABP4/Tas*I SNP reduces marbling scores and increases texture. Nevertheless, these effects were small, and thus, no significant associations were observed in this analysis.

Table 8. Mean effect of allelic substitution ± standard error for polymorphisms with significant associations to carcass traits in beef cattle.

Marker	Allele	Variable	Effect of allelic substitution	P
FABP3-G/A	G	REA (cm^2)	-0.11 ± 1.06	0.9180
FABP3-G/A	G	REA/HCW	-0.004 ± 0.003	0.2766
*FABP4/Tas*I	A	TEXT	0.04 ± 0.05	0.3946
*FABP4/Tas*I	A	MARB (score)	-0.11 ± 0.30	0.7252

TEXT = meat texture (scale from 1 to 5 points, 1 = rough and 5 = fine); REA = rib eye area on carcass; MARB = marbling (values from 1 to 18, where 1 to 3 = traces, 4 to 6 = light, 7 to 9 = low, 10 to 12 = average, 13 to 15 = high, and 16 to 18 = abundant); REA/HCW = ratio between rib eye area and hot carcass weight.

DISCUSSION

SNP identification

Variants in *FABP3* have sparked interest because this gene is responsible for intracellular fatty acid availability, mobilization, and usage, mostly towards oxidation in the mitochondria (Glatz and van der Vusse, 1996; Glatz et al., 2003; Gardan et al., 2007). In swine, this gene has been considered a candidate for fat deposition traits; however, effects have been contradictory (Nechtelberger et al., 2001; Chmurzynska, 2006). Few studies have been published to date and results are equally inconsistent (Cho et al., 2008). Cho et al. (2008) identified 5 SNPs in *FABP3*,

only one of which was found in the present work (rs210186725). This SNP, however, did not present significant association with fat deposition traits in the previous study.

As for *FABP4*, of the 9 identified polymorphisms, SNP rs110383592 is noteworthy for having been studied by various authors in animals of different breeds (Cho et al., 2008; Barendse et al., 2009; Oh et al., 2012). Barendse et al. (2009) found this SNP to be associated with intramuscular fat deposition (marbling) in a population of Australian cattle. According to the authors, this SNP is found within a splice site between the third exon and the third intron, which makes it a functional polymorphism. On the other hand, this association has not been consistent among other studies. Oh et al. (2012) was unable to find significant association between this SNP and fat deposition traits, although it was shown to be associated to saturated and unsaturated fatty acid composition in the Hanwoo breed of cattle.

Most of the SNPs identified in both *FABP3* and *FABP4* were located in intronic regions. Nevertheless, several intronic markers have been described in the literature and shown to be associated with meat and carcass quality traits in beef cattle (White et al., 2005; Schenkel et al., 2006; Sherman et al., 2008).

Genotyping and association with phenotypic traits

For the *FABP3*-G/A polymorphism, the least frequent AA genotype was only observed in groups in which Canchim represented the terminal breed used for crossbreeding: CCAN (N = 4), CCCN (N = 4), and CCVN (N = 1). The A allele is apparently exclusive to zebuine breeds since Canchim sires (which hold a portion of zebuine genes: 5/8 Charolês and 3/8 zebu) were probably the only ones to transmit the allele to offspring. Considering that this was the first description of this SNP in the literature, new studies are needed to confirm this hypothesis.

Most previous association studies between SNPs in *FABP3* and traits of economic interest were carried out in swine (Gerbens et al., 1999; Chmurzynska et al., 2007); however, these results differ from those in the current study since all associations were made with either intramuscular or carcass distribution of back fat. Since the *FABP3*-G/A SNP is present in a gene involved in fat deposition and this is the first study to analyze this polymorphism, further studies are needed to validate these findings in different populations.

As for the *FABP4*-G/C SNP, the only genetic groups that did not present the GG genotype were CCCN and CRCN (Table 5). Oh et al. (2012) genotyped this same SNP in the Hanwoo breed of Korean cattle and found genotypic frequencies of 36.3, 47.7, and 16% for GG, GC, and CC, respectively, and they found the C allele to be the least frequent (44%). In Australian cattle from seven distinct breeds (Angus, Brahman, Belmont Red, Hereford, Murray Grey, Santa Gertrudis, and Shorthorn), Barendse et al. (2009) observed C allele frequencies ranging from 45 (Angus) to 85% (Brahman). In contrast to Oh et al. (2012) and Barendse et al. (2009), the lower frequency of the G allele observed in this study may indicate that this allele is either being introduced or removed from the studied population.

The results for the *FABP4*-G/C genotype and the traits studied herein are in accordance with Oh et al. (2012) who did not show association between this SNP and fat deposition traits in cattle of the Hanwoo breed, although he did observe an association with fatty acid composition. However, Barendse et al. (2009), found the SNP to be associated to intramuscular fat deposition (marbling) in different Australian bovine breeds.

For the *FABP4/Tas*I SNP we were unable to determine if this genotypic distribution was

present in other populations due to the lack of information about this SNP in the literature, nor was it possible to verify whether the association results corroborate those for other populations.

The small effects observed in this study are common due to the quantitative nature of these variables, which are generally controlled by many genes of lesser individual effect and influenced by environmental factors (Andersson, 2001). According to Barendse et al. (2009), the effect of *FABP4* is small, which hinders the ability to detect it. Cho et al. (2008) also argue that since statistical power to detect significant associations for both *FABP*4 and *FABP*3 are low, an alternative approach to confirm suggestive associations would be replication in larger populations with different breeds and larger phenotypic variation. Technologies are available that detect thousands of SNPs simultaneously with a reduction in cost and time. Therefore, it is possible to include information from thousands of SNPs associated with many traits of interest in the prediction of the genetic value of each animal, promoting significant advances in animal production.

The genes analyzed herein (*FABP3* and *FABP4*) appear to contribute to the development of general carcass traits, albeit through a small effect. Even though they may not be considered major genes in the development of these phenotypes, they may be used in haplotype studies or within a panel of markers whose cumulative effects may better explain the phenotypic variation observed between breeds and animals, which would lead to a more powerful analysis and enhance the accuracy of genetic prediction.

Conflicts of interest

The authors declare no conflict of interest.

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