.

Development of Molecular Markers to Improve Future Selection Methods for Carcass Traits in Brahman and Brahman Influenced Steers

A.M.Royer[1,2], C.Shivers[3], D. Riley[4], M. Elzo[5], and M.D. Garcia[1,2]

[1]Department of Animal Science, Louisiana State University, Baton Rouge, Louisiana 70803
[2]Central Research Station, LSU Agricultural Center, Baton Rouge, Louisiana 70820
[3]American Brahman Breeders Association
[4]Texas A&M Department of Animal Science
[5]University of Florida Department of Animal Science

Corresponding author information:

Dr. Matthew Garcia
105 J.B. Francioni Hall
Baton Rouge LA, 70803
Phone: 225-578-3436
Fax: 225-578-3279
Email: Mgarcia@agcenter.lsu.edu

ABSTRACT

Brahman cattle are important in tropical regions due to the breed's ability to tolerate excessive heat and parasite presence. However, Brahman cattle can exhibit lower carcass quality characteristics when compared to *Bos taurus* breeds. The objective of this study was to evaluate potential SNP associations on six candidate genes for carcass quality and composition traits in a population of Brahman and Brahman-influenced steers. Steers were evaluated through the American Brahman Breeders Association (ABBA) carcass evaluation project in Gonzales, Texas. Carcass traits measured included hot carcass weight (HCW), ribeye area (REA), marbling score (MARB), yield grade (YG), quality grade (QG), dressing percent (DRESS), and Warner-Bratzler shear force score (WBS). Six previously described candidate genes were chosen for SNP analysis based on previous association with growth and carcass traits. Candidate genes utilized in the current study included the calpastatin gene (CAST), the calpain gene (CAPN3), the thyroglobulin gene (TG), the growth hormone gene (GH-1), the insulin growth factor 1 gene (IGF-1) and the adiponectin gene (ADIPOQ). Six unique SNP from 3 candidate genes (TG, CAST and CAPN3) were identified as significantly associated ($P < 0.001$) with carcass quality traits (MARB, and QG). Genotypic effect was observed for all significant SNP, with differing levels of performance for animals inheriting different SNP genotypes. Although multiple SNP in the current study were significantly ($P < 0.001$) associated with growth and carcass traits, they must first be validated in much larger and diverse populations prior to implementation into selection strategies.

Keywords: Bos indicus, carcass traits, carcass quality

INTRODUCTION

Multiple tools have been developed to improve accuracy of animal selection and rate of improvement in economically important traits in beef cattle. Identification and utilization of molecular markers has been reported to increase the rate of genetic improvement as compared to other currently utilized selection tools (Davis et al., 1998). The candidate gene approach evaluates single nucleotide polymorphisms (SNPs) located on genes of known physiological function, analyzes potential associations with economically important traits. This may be especially useful for *Bos indicus* cattle, which can exhibit less desirable carcass characteristics when compared to *Bos taurus* cattle (Wheeler et al., 2001).

The current study evaluated SNP's located on six known candidate genes to evaluate potential associations with growth traits, feedlot performance, and carcass traits. The candidate genes included Adiponectin (ADIPOQ), Thyroglobulin (TG), Calpain-III (CAPN3), Calpastatin (CAST), Insulin like Growth Factor (IGF1), and Growth Hormone (GH1). The candidate genes were selected based on previous reports linking them to growth traits, and carcass quality and composition traits. Specifically, the candidate genes utilized herein have been previously reported to be associated with an animals growth curve (Mullen et al., 2010; Pereira et al., 2005; Machado et al., 2003, and Bauman et al., 1992), carcass traits such as marbling (Van Enennaam et al., 2007;Casas et al., 2005), rib eye area (Morsci et al., 2006), back fat thickness (Shin and Chung 2013) and tenderness (Shenkel et al., 2006; Koohmaraie et al., 2002).

Thus, the objective of the current study was to evaluate SNP located on six candidate genes and their potential associations with growth traits, feedlot performance, and carcass quality and composition traits in a population of Brahman and Brahman-influenced steers.

MATERIALS AND METHODS

Experimental Animals

All animals were treated and maintained in accordance with the principles and guidelines outlined in the Guide for the Care and Use of Agricultural Animals in Research and Teaching. The population of animals utilized included a total of forty-two Brahman (n=31) and Brahman influenced (n=11) steers born from 2009-2014 at the LSU AgCenter Central Research Station in Baton Rouge LA. After weaning steers were shipped to a commercial feedyard in Gonzales, TX, to complete the finishing process. Steers were processed individually upon arrival to the feedyard and were sorted into an appropriate pen based upon weight, frame size, and condition. When individual pens reached an average weight and body condition deemed acceptable (one centimeter of backfat and 544 kilograms body weight) animals were sent to a commercial packing plant for the collection of carcass quality and composition traits. Carcass traits collected included hot carcass weight (HCW), ribeye area (REA), marbling score (MARB), yield grade (YG), quality grade (QG), dressing percent (DRESS), and Warner-Bratzler shear force score (WBS).

DNA Extraction and Genotyping

Twenty milliliters of blood was collected from each steer via jugular venipuncture. After collection, blood was transferred to two 15ml tubes and centrifuged at 2800 x g for 20 minutes at 4 degrees Celsius. DNA was then extracted from white cell buffy coats using a Saturated Salt Procedure previously described by Miller et al. (1988). Purified DNA samples were diluted into 25ng/µl working solutions in preparation for genotyping.

Previously described SNP's were selected equidistantly across the TG, ADIPOQ, CAST, CAPN3, IGF-1, and GH1 genes for genotyping utilizing dbSNP

(http://www.ncbi.nlm.nih.gov/projects/SNP ; Supplemental tables 1-6). A total of 149 SNP's were selected from the six candidate genes and equidistant selection of SNP's was conducted to account for possible linkage between selected SNP's and potential causative mutations. Genotyping was performed by Neogen LLC (Lincoln, NE) utilizing a Sequenom genotyping platform (San Diego, CA).

Statistical Analysis

The mixed model procedure of SAS (version 9.4, SAS Institute, Cary, NC) was used to evaluate SNP associations from the candidate genes ADIPOQ, CAPN3, CAST, TG, IGF1, and GH1 with carcass quality and composition traits. Models were fitted individually for each trait. Traits were hot carcass weight (HCW), rib eye area (REA), marbling score (MARB), yield grade (YG), quality grade (QG), dressing percent (DRESS), and Warner-Bratzler shear force score (WBS). Fixed effects in the model were breed, breed*SNP genotype and SNP genotype. Random effects were sire and residual. The LSMEANS procedure and the pre-planned pairwise comparisons function were utilized to evaluate significant differences in performance for a trait when inheriting differing genotypes from a significant SNP. Any SNP with only one genotype or SNP that failed to generate genotypes for less than 50% of the experimental animals were excluded from the analysis due to lack of marker effects.

Results

SNP Associated with carcass traits

When evaluating SNP associations with HCW, REA, and YG, significance associations ($P < 0.05$) were identified for a total of 19 SNP located on 5 unique candidate genes (TG, CAST, CAPN3, IGF1, and GH1) (Table 1). Three SNP were associated with HCW ($P < 0.05$) and were all located on the CAST gene. The significant SNPs (rs109702795, rs134030456, and

rs137371179) exhibited similar effects on HCW(Table 2). Animals inheriting the heterozygous genotypes displayed significantly heavier hot carcass weights when compared to animals inheriting the major allele genotypes.

A total of 15 SNP from 5 candidate genes were identified as associated with REA ((P < 0.05; Table 1). Specifically, six SNP were located on the CAST gene, one was SNP located on the IGF1 gene, two were located on the CAPN3 gene, five were located on the TG gene, and one was located on the GH1 gene. Analyses of SNP located on the CAST gene (rs109020860, rs110386026, rs136873074, rs136882857, rs136982429, and rs137561617) revealed that animals inheriting the heterozygous genotype displayed significantly larger REA than animals inheriting the major allele genotype. An SNP located on the IGF1 gene (rs109022910) showed animals inheriting the minor allele genotype displayed significantly larger REA than animals inheriting the heterozygous or minor allele genotypes. Two markers located on the CAPN3 gene (rs109122904, rs137651874) were significantly associated with REA. Individuals inheriting the heterozygous genotype for rs109122904 showed larger REA than animals inheriting the major allele genotype, and an SNP located on CAPN3 (rs110452450) revealed animals inheriting the heterozygous or major allele genotypes displayed larger REA than animals inheriting the minor allele genotype. Five markers located on the TG gene (rs110553649, rs110946911, rs132813094, rs134743669, and rs386026054) displayed significant associations with REA. Analyses of TG markers (rs110946911 and rs386026054) revealed individuals inheriting the minor allele genotypes displayed larger REA than those individuals inheriting the respective heterozygous or major allele genotypes. Similarly, individuals that inherited the minor allele genotype of TG marker rs134743669 showed larger REA than individuals with the heterozygous genotype. Animals inheriting the minor allele genotype for TG gene marker rs132813094 displayed a

158 significantly larger REA than heterozygous animals. However, no significant differences were
159 detected when comparing the REA of animals that inherited the major allele genotype with the
160 REA of heterozygous or minor allele homozygous animals. Animals inheriting the heterozygous
161 genotype for TG gene marker rs110553649 exhibited significantly smaller REA than either
162 homozygous genotype, and there was no significant difference seen between the REA of the
163 major and minor allele genotypes. Finally for REA, one SNP from the GH1 gene (rs137651874)
164 revealed animals inheriting the heterozygous genotype displayed significantly larger REA than
165 animals inheriting the major allele genotype (Table 2).

166 One SNP located on the CAST gene was significantly associated ($P < 0.05$) with yield
167 grade (YG). Heterozygous animals for marker rs134030456 on the CAST gene displayed
168 significantly lower yield grade as compared to animals inheriting the major allele genotype
169 (Table 2).

170 Significant associations ($P < 0.05$) were found between carcass quality traits marbling
171 score, quality grade, and Warner-Bratzler shear force score and multiple SNP in several genes. A
172 total of 30 significant SNP were located on candidate genes TG, ADIPOQ, CAST, CAPN3,
173 IGF1, and GH1 (Table 3). A total of 13 SNP were found to be significantly associated ($P < 0.05$)
174 with marbling score (MARB). Single nucleotide polymorphisms from five candidate genes
175 utilized in the current study were significantly associated with marbling score. Specifically, one
176 located on the ADIPOQ gene, one located on the IGF1 gene, two located on the CAPN3 gene,
177 one located on the CAST gene, and eight located on the TG gene (Table 4). Analysis of SNP
178 rs383535987 located on the ADIPOQ gene showed that heterozygous animals exhibited larger
179 marbling scores than major allele homozygous animals. One SNP located on IGF1 gene
180 (rs109022910) indicated that animals with the minor allele genotype had greater marbling scores

than either heterozygous or major allele homozygous animals, with no significant difference between the heterozygous and major homozygous genotypes. The significant marker located on the CAST gene (rs110496242) showed that animals inheriting the major allele genotype had significantly larger marbling scores than the heterozygous animals. No significant difference was seen in comparing the minor allele genotype with the major or heterozygous genotypes. Two SNP were observed from the CAPN3 gene (rs109050259, rs134085397) Marker rs109050259 indicated animals with the minor allele genotype showed significantly smaller marbling scores than the heterozygous or major allele genotypes, with no significant difference between the heterozygous and major allele genotypes. Alternately for CAPN3 marker rs134085397, animals inheriting the minor allele genotype had significantly larger marbling scores than animals of the major allele genotype, and the animals inheriting the heterozygous genotype did not show significant difference in MARB than animals of either homozygous genotype. When evaluating the eight SNP associated with MARB located on the TG gene, a variety of genotypic effects were observed. Individuals inheriting the heterozygous genotype for rs109182502 and rs378900777 had higher MARB than those individuals inheriting the respective major allele genotypes. For marker rs386026054 on the TG gene, individuals inheriting the minor allele genotype displayed larger marbling scores than the heterozygous or major allele genotypes. Also, animals inheriting the heterozygous genotype showed significant increase in marbling score as compared to the major allele genotype. Marker rs378567477 from the TG gene indicated animals inheriting the minor allele showed significantly smaller marbling scores than animals inheriting the major allele genotype. The final four TG markers rs110501231, rs110553649, rs133980693, and rs135059985 indicated animals inheriting the respective minor allele genotypes showed

significantly larger marbling scores than animals inheriting the respective heterozygous or major allele genotypes. (Table 4)

A total of 14 SNP were identified as significantly associated ($P < 0.05$) with QG (Table 3). All six candidate genes were represented with one SNP from each IGF1, CAPN3, CAST, and GH1, three markers from ADIPOQ, and seven markers from the TG gene (Table 3). The SNP from IGF1 (rs109022910) showed animals inheriting the minor allele genotype exhibited better quality grade scores than animals of either the heterozygous or major allele genotypes, and no significant effect was seen between QG of heterozygous and major allele genotype animals. Alternately, the CAPN3 SNP (rs109050259) showed animals inheriting the minor allele genotype had lower QG scores than animals inheriting the heterozygous or major allele genotypes, with no significance seen between QG of the major allele genotypes and heterozygous genotypes. The SNP from the CAST gene rs110496242 revealed that animals inheriting the major allele genotype showed significantly higher QG than animals inheriting the heterozygous genotype. No significant differences were observed when comparing the QG of the minor allele genotype with the QG of the heterozygous or major allele genotypes. Marker rs137651874 from the GH1 candidate gene indicated a significant increase in QG for animals inheriting the major allele genotypes as compared to animals with the heterozygous genotypes. Two SNPs from the ADIPOQ candidate gene (rs380209068 and rs384076273) showed a significant increase in QG for animals inheriting the major allele genotype as compared to animals with the heterozygous genotype. A third SNP from the ADIPOQ gene (rs383535987) revealed that animals with the heterozygous genotype had a larger QG score than individuals that inherited the major allele genotype. When evaluating the seven SNP associated with QG located on the TG gene, a variety of genotypic effects were observed. Individuals inheriting the heterozygous genotype on

rs109182502 had higher QG scores than those individuals inheriting the major allele genotype. Individuals inheriting the minor allele genotype from four SNP (rs110501231, rs386026054, rs133980693, and rs135059985) had higher QG scores than those individuals inheriting the major allele genotype. No significant differences were identified when comparing individuals inheriting the heterozygous and the major allele genotypes from these four SNP. Marker rs378567477 on the TG gene revealed animals with the significantly higher QG inherited the major allele genotype, with no significance seen when comparing the heterozygotes with the minor allele homozygotes. Finally, SNP rs132813094 showed animals with the major allele genotype had greater QG scores than animals with the heterozygous genotype but had no significant difference compared to the minor allele genotype. The minor allele genotype also showed no significant difference when compared to the heterozygous genotype. (Table 4)

Three SNP from the IGF1, CAST, and ADIPOQ genes were identified as being significantly associated with WBS. Single nucleotide polymorphism rs110959643 from the IGF1 gene indicated a significantly higher shear force score for individuals inheriting the heterozygous genotype. Alternately SNPs rs137140434 from the CAST gene and rs383535987 from the ADIPOQ gene showed that heterozygous individuals had significantly lower WBS measurements when compared to those inheriting the major allele genotype. (Table 4)

Discussion

Several SNP located on the TG gene were significantly associated ($P < 0.05$) carcass quality and composition traits. Specifically, these traits included REA area, marbling score, and quality grade. A single SNP located on the TG gene has previously been reported to be significantly associated with REA (Casas et al., 2005). Previous studies have been variable in reporting favorable alleles located on the TG gene affecting marbling in *Bos indicus* and *Bos*

taurus cattle (Casas et al., 2005; Van Enennaam et al., 2007; Barendse et al., 1999). The primary factor contributing to the variable reports of the TG gene and its effects on carcass traits is that previous studies have reported that SNP effects in *Bos taurus* populations (Casas et al., 2005). Thus, for improved accuracy in *Bos indicus* populations, the development of different but appropriate markers may need to be developed for use in *Bos indicus* populations.

Multiple SNP on the ADIPOQ gene were significantly associated with carcass quality and composition traits including marbling, quality grade, and WBS. Previous reports of associations between SNP located on the ADIPOQ gene and REA, marbling, and backfat thickness were reported in multiple studies (Shin and Chung, 2013; Morsci et al., 2006), however, the current study only identified significant ADIPOQ gene SNP for REA and marbling.

Several SNP located on the CAST gene were identified as significantly associated with carcass quality and composition traits (HCW, REA, YG, QG, and WBS). These results were in agreement with previous studies that have reported SNP located on the CAST gene to be associated with tenderness in beef cattle (Smith et al., 2009; Schenkel et al., 2006; Café et al., 2010). Smith et al. (2009) reported an association with CAST SNP and tenderness in Brahman cattle. Schenkel et al. (2006) reported a higher frequency of favorable CAST allele ‘A’ resulted in lower percentage of tough steaks, higher fat content, and a difference in REA. A study conducted by Café et al. (2010) reported lower shear force scores in steaks from Brahman cattle inheriting the favorable allele.

Multiple CAPN3 SNP were significantly associated ($P < 0.05$) with carcass quality and composition traits (REA, marbling, and QG). Café et al. (2010) reported an increase in the frequency of the favorable ‘G’ allele increases meat tenderness. Furthermore, Barendse et al. (2008) reported that the CAPN3 gene was associated with meat tenderness in *Bos indicus* breeds.

However, no previous work reported significant associations between SNP's on the CAPN3 gene with REA, marbling and QG.

Multiple SNP located on the IGF-1 gene were significantly associated with carcass quality and composition traits including, REA, marbling, QG, and WBS. Previous studies indicated that SNP located on the IGF1 SNP were associated with growth and production traits (Pereira et al., 2005; Machado et al., 2003; Chang et al., 2009). Chang et al. (2009) reported a significant association between the IGF1 gene and carcass weight. However, Chang et al. (2009) found no association between SNPs located on the IGF1 gene with WBS, REA, marbling, and QG for SNPs identified as significant in the current study.

A single SNP (rs137651874) located on the GH1 gene in the current study was significantly associated with REA, and QG. This agreed with previous work that found GH1 to be a favorable candidate gene for cattle growth and carcass traits (Pereira et al., 2005; Mullen et al., 2010). However, no previous studies identified the SNP located on the GH1 gene that were significantly associated with REA and QG reported here.

Summary

Multiple SNP located on 5 candidate genes were identified as being significantly associated with carcass quality and composition traits in *Bos indicus* and *Bos indicus* influenced cattle. However, many of the significant SNP located on these five candidate genes identified as significant in the current study were located on genes that had not previously been described to have SNP associated with the specific carcass quality and composition traits evaluated here. Furthermore, many of the SNP identified as significant in the current study were novel in the sense that they were identified in a purebred *Bos indicus* and a *Bos indicus* influenced research population. Thus, because of the unique nature of the population and the small size of the

295 research population further validation of SNP identified here is warranted. Evaluations of these
296 SNP should be conducted in larger, more diverse populations, in multiple environments and
297 production schemes to further validate SNP associations identified with carcass quality and
298 composition traits.
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Table 1: Level of significance and frequency of animals from each genotype associated with carcass composition traits

Traits	Gene	SNP ID	Allele[4]	Minor Genotype Frequency[5]	Het Genotype Frequency[5]	Major Genotype Frequency[5]	SNP P-value	Breed x SNP P-value
HCW[1]	CAST	rs109702795	G/T	0	10	29	0.0303	0.2473
HCW	CAST	rs134030456	C/T	0	15	26	0.0544	0.1792
HCW	CAST	rs137371179	T/C	0	12	29	0.0324	0.2117
REA[2]	CAST	rs109020860	A/G	0	5	36	0.0425	0.1612
REA	CAST	rs110386026	C/T	0	6	35	0.0425	0.1612
REA	CAST	rs136873074	C/T	0	6	34	0.0425	0.1753
REA	CAST	rs136882857	C/T	0	6	35	0.0425	0.1612
REA	CAST	rs136982429	C/T	0	11	28	0.0032	0.2548
REA	CAST	rs137561617	T/A	0	6	31	0.0139	0.1775
REA	IGF-1	rs109022910	A/G	4	9	26	0.0459	0.1891
REA	CAPN3	rs109122904	A/G	0	5	36	0.0354	0.1713
REA	CAPN3	rs110452450	G/A	10	13	13	0.0367	0.0025
REA	TG	rs110553649	A/C	2	20	19	0.0333	0.1136
REA	TG	rs110946911	T/G	2	17	22	0.0113	0.2394
REA	TG	rs132813094	A/C	1	7	33	0.0400	0.1784
REA	TG	rs134743669	G/A	0	39	2	0.0076	0.1787
REA	TG	rs386026054	G/C	3	24	12	0.0221	0.4712
REA	GH-1	rs137651874	T/C	0	6	29	0.0401	0.3600
YG[3]	CAST	rs134030456	C/T	0	15	26	0.0443	0.1951

[1]Hot carcass weight

[2]Rib eye area

[3]Yield grade

[4]Minor allele represented on the left

[5]Number of animals inheriting each genotype

Table 2: Single nucleotide polymorphisms associated with carcass composition traits and least square means estimate comparisons between reported genotypes. Differing superscripts indicate a difference of means at P<0.05 within rows.

Traits	Gene	SNP ID	Allele[4]	Minor Genotype Mean	Het Genotype Mean	Major Genotype Mean
HCW[1]	CAST	rs109702795	G/T		396.30±20.60[a]	361.85±15.47[b]
HCW	CAST	rs134030456	C/T		401.51±17.97[a]	350.29±13.45[b]
HCW	CAST	rs137371179	T/C		393.32±20.42[a]	359.62±15.67[b]
REA[2]	CAST	rs109020860	A/G		36.68±1.72[a]	33.22±0.98[b]
REA	CAST	rs110386026	C/T		36.68±1.72[a]	33.22±0.98[b]
REA	CAST	rs136873074	C/T		36.81±1.71[a]	33.32±0.96[b]
REA	CAST	rs136882857	C/T		36.68±1.72[a]	33.22±0.98[b]
REA	CAST	rs136982429	C/T		36.03±1.29[a]	32.34±1.08[b]
REA	CAST	rs137561617	T/A		37.26±1.67[a]	33.05±0.90[b]
REA	IGF-1	rs109022910	A/G	37.87±1.60[a]	34.12±1.21[b]	33.19±0.82[b]
REA	CAPN3	rs109122904	A/G		36.88±1.56[a]	33.41±0.84[b]
REA	CAPN3	rs110452450	G/A	30.51±1.44[a]	34.68±0.88[b]	34.84±0.76[b]
REA	TG	rs110553649	A/C	37.45±2.47[a]	32.50±1.41[b]	34.93±1.56[a]
REA	TG	rs110946911	T/G	42.18±2.87[a]	34.49±1.15[b]	32.64±1.01[b]
REA	TG	rs132813094	A/C	37.14±2.76[a]	30.68±1.67[b]	33.42±1.01[ab]
REA	TG	rs134743669	G/A	40.01±2.74[a]	33.32±0.95[b]	
REA	TG	rs386026054	G/C	40.03±2.15[a]	33.18±1.12[b]	32.40±1.79[b]
REA	GH-1	rs137651874	T/C		37.42±1.49[a]	33.48±0.90[b]
YG[3]	CAST	rs134030456	C/T		4.11±0.36[a]	3.10±0.28[b]

[1]Hot carcass weight

[2]Rib eye area

[3]Yield grade

[4] Minor allele represented on the left

316 Table 3: Level of significance and frequency of animals from each genotype associated with carcass quality traits

Traits	Gene	SNP ID	Allele[4]	Minor Genotype Frequency[5]	Het Genotype Frequency[5]	Major Genotype Frequency[5]	SNP P-value	Breed x SNP P-value
MARB[1]	IGF-1	rs109022910	A/G	4	9	26	0.0022	0.0005
MARB	CAPN3	rs109050259	G/A	2	23	16	0.0136	0.1924
MARB	CAPN3	rs134085397	G/A	6	21	14	0.0227	0.0948
MARB	CAST	rs110496242	G/A	2	10	29	0.0105	0.1457
MARB	TG	rs109182502	T/C	0	3	38	0.0047	0.0166
MARB	TG	rs110501231	C/T	9	22	10	<0.0001	0.0127
MARB	TG	rs110553649	A/C	2	20	19	0.0492	0.2080
MARB	TG	rs386026054	G/C	3	24	12	0.0029	0.0017
MARB	TG	rs133980693	G/A	9	22	10	<0.0001	0.0127
MARB	TG	rs135059985	C/T	9	22	10	<0.0001	0.0127
MARB	TG	rs378567477	T/C	8	16	9	0.0007	0.0839
MARB	TG	rs378900777	T/C	0	2	39	0.0504	0.1321
MARB	ADIPOQ	rs383535987	G/A	0	3	38	0.0039	<0.0001
QG[2]	IGF-1	rs109022910	A/G	4	9	26	0.0286	0.0016
QG	CAPN3	rs109050259	G/A	2	23	16	0.0009	0.2217
QG	CAST	rs110496242	G/A	2	10	29	0.0003	0.0412
QG	TG	rs109182502	T/C	0	3	38	0.0235	0.0098
QG	TG	rs110501231	C/T	9	22	10	0.0020	0.0176
QG	TG	rs132813094	A/C	1	7	33	0.0416	<0.0001
QG	TG	rs386026054	G/C	3	24	12	0.0311	0.0047
QG	TG	rs133980693	G/A	9	22	10	0.0020	0.0176
QG	TG	rs135059985	C/T	9	22	10	0.0020	0.0176
QG	TG	rs378567477	T/C	8	16	9	0.0124	0.1284
QG	GH-1	rs137651874	T/C	0	6	29	0.0218	0.0007
QG	ADIPOQ	rs380209068	G/A	0	3	36	0.0363	<0.0001
QG	ADIPOQ	rs383535987	G/A	0	3	38	0.0199	<0.0001
QG	ADIPOQ	rs384076273	T/C	0	4	37	0.0556	<0.0001
WBS[3]	IGF-1	rs110959643	A/G	0	2	39	0.0537	0.3198
WBS	CAST	rs137140434	G/T	0	5	13	0.0431	0.7612
WBS	ADIPOQ	rs383535987	G/A	0	3	38	0.0293	0.3788

317 [1]Marbling
318 [2]Quality grade
319 [3]Warner-Bratzler shear force
320 [4]Minor allele represented on the left
321 [5]Number of animals inheriting each genotyope

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Table 4: Single nucleotide polymorphisms associated with carcass quality traits and least square means estimate comparisons between reported genotypes. Differing superscripts indicate a difference of means at P<0.05 within rows.

Traits	Gene	SNP ID	Allele[4]	Minor Genotype Mean	Het Genotype Mean	Major Genotype Mean
MARB[1]	ADIPOQ	rs383535987	G/A		560.00±37.70^{a}	437.05±12.06^{b}
MARB	IGF-1	rs109022910	A/G	565.00±31.66^{a}	457.08±24.18^{b}	426.56±16.01^{b}
MARB	CAPN3	rs109050259	G/A	285.91±50.09^{a}	422.02±23.75^{b}	440.23±23.94^{b}
MARB	CAPN3	rs134085397	G/A	491.66±29.14^{a}	435.56±20.71^{ab}	395.45±24.39^{b}
MARB	CAST	rs110496242	G/A	372.26±48.60^{ab}	335.12±32.79^{b}	441.65±21.33^{a}
MARB	TG	rs109182502	T/C		525.46±38.54^{a}	420.27±21.79^{b}
MARB	TG	rs110501231	C/T	547.50±21.59^{a}	433.15±16.07^{b}	371.11±32.19^{b}
MARB	TG	rs110553649	A/C	558.29±47.64^{a}	444.45±15.65^{b}	416.19±25.54^{b}
MARB	TG	rs133980693	G/A	547.50±21.60^{a}	433.15±16.07^{b}	371.11±32.19^{b}
MARB	TG	rs135059985	C/T	547.50±21.60^{a}	433.15±16.07^{b}	371.11±32.19^{b}
MARB	TG	rs378567477	T/C	372.86±34.61^{a}	446.11±21.58^{a}	547.50±22.89^{b}
MARB	TG	rs378900777	T/C		529.80±48.85^{a}	431.69±16.95^{b}
MARB	TG	rs386026054	G/C	553.33±36.01^{a}	443.33±14.23^{b}	369.50±32.71^{c}
QG[2]	IGF-1	rs109022910	A/G	755.00±19.20^{a}	703.29±14.76^{b}	694.06±9.71^{b}
QG	CAPN3	rs109050259	G/A	595.66±24.70^{a}	699.39±9.16^{b}	704.22±9.43^{b}
QG	CAST	rs110496242	G/A	668.14±24.91^{ab}	637.81±13.62^{a}	706.94±8.20^{b}
QG	TG	rs109182502	T/C		747.65±21.76^{a}	695.10±7.89^{b}
QG	TG	rs110501231	C/T	749.25±13.02^{a}	696.35±9.69^{b}	670.39±19.41^{b}
QG	TG	rs386026054	G/C	751.00±21.83^{a}	701.08±8.63^{b}	668.85±19.83^{b}
QG	TG	rs132813094	A/C	680.00±38.18^{ab}	656.33±15.59^{b}	701.36±7.10^{a}
QG	TG	rs133980693	G/A	749.25±13.02^{a}	696.35±9.69^{b}	670.39±19.41^{b}
QG	TG	rs135059985	C/T	749.25±13.02^{a}	696.35±9.69^{b}	670.39±19.41^{b}
QG	TG	rs378567477	T/C	671.93±21.56^{a}	701.94±13.45^{a}	749.25±14.26^{b}
QG	GH-1	rs137651874	T/C		742.83±16.17^{a}	798.64±8.42^{b}
QG	ADIPOQ	rs380209068	G/A		649.00±22.20^{a}	700.01±7.09^{b}
QG	ADIPOQ	rs383535987	G/A		753.33±21.60^{a}	697.82±6.91^{b}
QG	ADIPOQ	rs384076273	T/C		655.67±21.81^{a}	700.92±6.65^{b}
WBS[3]	IGF-1	rs110959643	A/G		9.92±1.13^{a}	7.79±0.50^{b}
WBS	CAST	rs137140434	G/T		5.99±0.61^{a}	7.62±0.38^{b}
WBS	ADIPOQ	rs383535987	G/A		5.66±1.10^{a}	8.00±0.54^{b}

[1]Marbling score

[2]Quality grade

[3]Warner-Bratzler shear force

[4] Minor allele represented on the left

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