ABSTRACT: Selection of the composite MARC III population for markers allowed better estimates of effects and inheritance of markers for targeted carcass quality traits (n = 254) and nontargeted traits and an evaluation of SNP specific residual variance models for tenderness. Genotypic effects of CAPN1 haplotypes (P = 0.12) on LM slice shear force (SSF) were similar in direction and size to previous reports. Effects of divergent CAPN1 haplotypes (1.15 kg) and additive effects of CAST (0.90 kg; P = 0.05) on SSF were large. Animals homozygous tender at both markers had 4.11 kg lower SSF than homozygous tough counterparts. The DGAT1 polymorphism affected fat thickness (P = 0.02) and VISNIR predicted SSF (P < 0.001), with both traits showing additive and dominance inheritance (P < 0.05). Genotype specific residual variance models for CAST fit SSF better (P < 0.001) than single residual variance models, with tougher genotypes having progressively larger residual (and hence phenotypic) variances.

Key words: beef tenderness; CAPN1; CAST; DGAT1

Introduction

Genetic markers in the µ-calpain (CAPN1), calpastatin (CAST), and diacylglycerol O-acyltransferase 1 (DGAT1) genes have been associated with important beef quality traits. Studies which survey industry populations often encounter small numbers of the rare homozygous genotype class. Recently, selection has been used to ensure representation of all genotypes in analysis (Bennett et al., 2013; Tait et al., 2014).

While validation of a purported effect across populations is important (Van Eenennaam et al., 2007; Johnston and Graser, 2010), characterization of effects on nontarget traits is also important. Knowledge of pleiotropic marker effects is critical for implementation of the genetic markers into the breeding objective.

This study selected a population of MARC III cattle (¼ Angus, ¼ Hereford, ¼ Red Poll, and ¼ Pinzgauer) to equalize marker frequency of CAPN1, CAST, and DGAT1 to 1) estimate marker frequency of CAPN1, CAST, and DGAT1 to 1) estimate effect size and mode of inheritance for previously reported markers on targeted beef carcass quality traits; 2) estimate effects of previously reported markers on nontarget performance or carcass quality traits; and 3) evaluate tenderness SNP specific residual variance models for tenderness.

Materials and Methods

Genetic markers. The CAPN1 haplotypes evaluated in this study were based on SNP CAPN1_316 and CAPN1_4751 (White et al., 2005). Haplotypes selected in this study were: CAPN1_316 allele C with CAPN1_4751 allele C (CAPN1hCC), CAPN1_316 allele G with CAPN1_4751 allele C (CAPN1hGC), and CAPN1_316 allele G with CAPN1_4751 allele T (CAPN1hGT). Additionally, a SNP in CAST, segregating C (CASTaC) and T (CASTaT) alleles (Casas et al., 2006), and a dinucleotide substitution in DGAT1, resulting in a lysine (DGAT1aK) or alanine (DGAT1aA) amino acid at the 232nd amino acid (Grisart et al., 2002) were selected. All three markers were selected for intermediate frequencies, primarily by using heterozygous sires.

Haplotype and allele frequencies achieved in the evaluation phase (birth years 2010, 2011, and 2012) (n = 627) were: CAPN1hCC = 0.267; CAPN1hGC = 0.326; CAPN1hGT = 0.385; CASTaC = 0.397; and DGAT1aK = 0.418.

Phenotype collection. Dams ranged in age from 2 to 13 yr and were defined as 2, 3, 4, or ≥ 5 yr for analysis. Records were removed for type of rearing, postnatal death, and lack of genotype information (n = 85). All calves had BW collected at birth and weaning (n = 542), with average age at weaning of 165 d. In 2010 and 2011, unselected bulls were castrated after weaning, whereas in 2012 all bull calves were castrated at birth. For steers, a yearling BW and a final BW were collected. All steers were harvested on a single day within each year (n = 254, average age = 452 d) to characterize carcass quality.

Carcass data, other than hot carcass weight, were collected at 36 h postmortem. Carcasses were ribbed between the 12th and 13th ribs and an image analysis based grading system (VBG2000; Shackelford et al., 2003) was used to assess adjusted fat thickness, LM area, USDA marbling score, and calculated vision yield grade. At the same time LM tenderness was predicted using visible and near-infrared reflectance spectroscopy (VISNIR; Shackelford et al., 2012a,b). A LM steak from the 13th rib region was returned to USMARC to evaluate slice shear force (SSF) at 14 d postmortem (Shackelford et al., 1999).

Analysis - genotype effects on means. All traits were analyzed with a mixed model using MTDFREML (Boldman et al., 1995). The model for preweaning traits accounted for birth year, age of dam, sex of calf from birth through weaning, age of calf (d), CAPN1 genotype, CAST genotype, DGAT1 genotype, and random additive polygenic effects. The pedigree went back 5 generations from animals evaluated and included 1,505 animals. Additive genetic variance estimates were constrained to 0.20 ≤ h² ≤ 0.70. Carcass traits were only collected on steers, so sex was removed from the model for carcass traits.

Additive and dominance effects were estimated for CAST and DGAT1. The difference between CAPN1hGT and CAPN1hCC haplotypes was estimated and the
CAPN1hGC difference from the midpoint of the CAPN1hGT and CAPN1hCC effect was estimated. An interaction between CAPN1 and CAST for SSF was also added to the model and tested for significance.

**Analysis - genotype effects on residual variance of SSF.** We further analyzed SSF for evidence of CAPN1 or CAST genotype specific residual variance. A single residual variance model (equivalent to the converged MTDFREML model) was analyzed with the MIXED procedure of SAS by providing the $A \sigma^2$ matrix as the animal random effect. Then genotype specific residual variance models were fit by adding heterogeneous residual variances based on CAPN1 or CAST genotypes to the MIXED analysis. A likelihood ratio test was performed to evaluate improvement of model fit.

**Results and Discussion**

**Marker effects on target traits.** Selection for 6 CAPN1 genotypes likely affected our power to detect the genotypic effect of CAPN1 on SSF ($P = 0.12$, Table 1). However, the CAPN1hGT effect relative to the CAPN1hCC haplotype of 1.153 kg for SSF (Table 2) was similar to the additive effect of 1.049 kg between the same haplotypes reported by Tait et al. (2014). Also, the CAPN1hGC effect was not significantly different ($P \geq 0.15$) from the intermediate point between CAPN1hGT and CAPN1hCC for any trait in this study; consistent with the claim of White et al. (2005) that CAPN1hGC is intermediate to the other 2 haplotypes for Warner-Bratzler shear force (WBSF). The effect of CAST on SSF was significant ($P < 0.01$, Table 1), with an additive inheritance pattern ($P = 0.05$, Table 2) and lack of evidence for dominance inheritance ($P = 0.22$). The additive effect of -0.902 kg (Table 2) CASTaT relative to CASTaC for SSF was smaller than the -1.257 kg effect reported by Tait et al. (2014).

In this study, the interaction between CAPN1 and CAST genotypes for SSF was not significant ($P = 0.40$). Interaction testing between CAPN1 haplotypes and CAST genotypes is challenging, even after selection to equalize frequencies (i.e. 3 of the 18 CAPN1 by CAST genotype combinations had < 1.2% of observations each).

There was a significant effect for DGAT1 on adjusted fat thickness ($P = 0.02$, Table 1), with evidence of additive ($P = 0.03$, Table 2) and dominance ($P = 0.03$, Table 2) modes of inheritance where homozygous DGATaK animals have more adjusted fat thickness than the other 2 DGAT1 genotypes. For the trait of % fat in milk of dairy cows, Kuehn et al. (2007) also identified dominance for the same marker in DGAT1, however they found the heterozygous animals to be closer to the DGATaK homozygotes, an opposite dominance effect.

However, this study did not find an effect of DGAT1 on marbling ($P = 0.87$, Table 1). While marbling and intramuscular fat are not the same trait, our results are consistent with the lack of an association for DGAT1 with intramuscular fat observed by Pannier et al. (2010).

**Marker effects on nontarget traits.** A significant association between DGAT1 and VISNIR predicted SSF ($P < 0.001$, Table 1) was identified, with evidence of a dominance mode of inheritance ($P < 0.001$) beyond the additive mode of inheritance ($P = 0.03$, Table 2). Direction of the effects of DGAT1 on VISNIR predicted SSF are the opposite direction of the adjusted fat thickness estimates (Table 2) and dominance was also present for the DGAT1 effect on adjusted fat thickness. Part of the DGAT1 effect on VISNIR predicted shear force might be a protective effect of subcutaneous fat to reduce sarcomere shortening.

**Table 1.** Significance ($P$-values) for genetic markers and heritability estimates for performance and carcass quality traits in MARC III cattle.

<table>
<thead>
<tr>
<th>Trait</th>
<th>CAPN1</th>
<th>CAST</th>
<th>DGAT1</th>
<th>h²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth BW</td>
<td>0.48</td>
<td>0.38</td>
<td>0.82</td>
<td>0.49</td>
</tr>
<tr>
<td>Weaning BW</td>
<td>0.86</td>
<td>0.89</td>
<td>0.32</td>
<td>0.70²</td>
</tr>
<tr>
<td>Yearling BW</td>
<td>0.79</td>
<td>0.72</td>
<td>0.47</td>
<td>0.20²</td>
</tr>
<tr>
<td>Final BW</td>
<td>0.44</td>
<td>0.63</td>
<td>0.47</td>
<td>0.20²</td>
</tr>
<tr>
<td>Dressing percent</td>
<td>0.52</td>
<td>0.79</td>
<td>0.12</td>
<td>0.51</td>
</tr>
<tr>
<td>Hot carcass weight</td>
<td>0.71</td>
<td>0.52</td>
<td>0.83</td>
<td>0.20²</td>
</tr>
<tr>
<td>Adjusted fat thickness</td>
<td>0.65</td>
<td>0.14</td>
<td>0.02</td>
<td>0.38</td>
</tr>
<tr>
<td>Marbling score</td>
<td>0.73</td>
<td>0.12</td>
<td>0.87</td>
<td>0.52</td>
</tr>
<tr>
<td>LM area</td>
<td>0.35</td>
<td>0.31</td>
<td>0.44</td>
<td>0.28</td>
</tr>
<tr>
<td>Vision yield grade</td>
<td>0.46</td>
<td>0.20</td>
<td>0.11</td>
<td>0.55</td>
</tr>
<tr>
<td>Slice shear force</td>
<td>0.12</td>
<td>&lt;0.01</td>
<td>0.55</td>
<td>0.22</td>
</tr>
<tr>
<td>VISNIRpredicted slice</td>
<td>0.85</td>
<td>0.01</td>
<td>&lt;0.001</td>
<td>0.20²</td>
</tr>
</tbody>
</table>

1 Genotypic values ≤ 0.05 in bold.  
2 Constrained to 0.20 ≤ h² ≤ 0.70.  
3 Visible and near-infrared reflectance spectroscopy (VISNIR; Shackelford et al., 2012a, b).

While a CAST genotypic effect on adjusted fat thickness was not supported in this study ($P = 0.14$, Table 1), the adjusted fat thickness estimate of an additive effect (-0.86 mm / CASTaT, $P = 0.05$, Table 2) was the same direction (-1.05 mm / CASTaT) as the suggestive CAST genetic effect ($P = 0.06$) identified by Tait et al. (2014).

**Marker effects on residual variance of SSF.** In comparison to the single residual variance model where $\sigma^2_e = 3.58$ kg for SSF (Fig. 1A), the CAST genotype specific residual variance model fit significantly ($P < 0.001$) better and effects were progressive, with $\sigma^2_e = 2.54$ kg, 3.98 kg, and 4.86 kg for CASTaT homozygotes, CASTaT:CASTaC heterozygotes, and CASTaC homozygotes, respectively (Fig. 1B). In comparison, there was less support ($P = 0.03$) for the CAPN1 specific residual variance model and the variances were not progressive. The significant improvement of CAST genotype specific residual variance models and the progressive increases in residual variance with mean CAST genotype effect is similar to our work in Angus cattle (Tait et al., 2014), reinforcing the 2 ways CASTaT may affect the risk of a tough eating experience for consumers.
Conclusion

This study is supportive of previous work on the importance of \textit{CAPN1} and \textit{CAST} genetic markers on mean levels of beef tenderness (VanEenennaam et al. 2007; Johnston and Graser, 2010; Tait et al., 2014). This work also replicates an effect of \textit{CAST} on variation in beef tenderness, suggesting more work should be done to validate the \textit{CAST} effects on residual variance in other resource populations. Similarities in estimated effects of \textit{CAST} on fat thickness between this study and previous work suggests this pleiotropic effect needs to be considered in selection schemes. While this study identifies a significant effect of \textit{DGAT1} on subcutaneous fat, the differences in direction of dominance effects of \textit{DGAT1} on fat thickness in beef cattle and % fat of milk in dairy cattle needs to be investigated further.

Acknowledgment

USDA is an equal opportunity provider and employer.

Literature Cited


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