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μ-Calpain (*CAPN1*), *calpastatin* (*CAST*), and *growth hormone receptor* (*GHR*) genetic effects on Angus beef heifer performance traits and reproduction

Richard G. Tait Jr. [1], Robert A. Cushman, Anthony K. McNeel [2], Eduardo Casas [3], Timothy P.L. Smith, Harvey C. Freetly, Gary L. Bennett*

USDA, Agricultural Research Service, U.S. Meat Animal Research Center, Clay Center, NE 68933, United States

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ABSTRACT

Genetic marker effects and type of inheritance are estimated with poor precision when minor marker allele frequencies are low. An Angus population was subjected to marker assisted selection for multiple years to equalize *CAPN1* haplotypes, *CAST*, and *GHR* genetic marker frequencies. The objective was to estimate the pleiotropic effects of these carcass quality oriented markers for body weight, reproduction, and first calf performance traits in 174 replacement beef females which were managed under 2 post-weaning development protocols. Heifers were weighed at 11-, 12-, and 13-mo, at first breeding season pregnancy evaluation, and prior to first calving season. Pubertal status was determined at 11-, 12-, and 13-mo of age. Antral follicles were counted, reproductive tracts were scored, and tract dimensions were measured at 13-mo. Body condition and hip height were scored and measured at pregnancy evaluation and prior to calving season. Heifer pregnancy and weaning rates and ordinal birth date were recorded. Calf body weights at birth and weaning were analyzed. Single df linear contrasts for recessive effects of the *GHR* heterozygous genotype showed significant decreases of 2.5–3.6% in 11-, 12-, and 13-mo heifer body weights and heifer weight prior to calving. The additive differences between *GHR* homozygotes were small and not significant for all body weights measured but a 1 wk difference in calf birth date was significant. For all 13-mo uterine measurements, scores, and antral follicle counts, only the *CAST* dominance contrast for medium antral follicle count was significant. The *CAPN1* haplotype with a strong additive effect for increased beef tenderness also had a significant additive effect on calving date. Heifers homozygous for the tender haplotype calved 7.9 days later than heifers homozygous for the tough haplotype. Most heifer reproductive traits were not significantly affected by *CAST* and *CAPN1* markers that are widely used to improve beef tenderness by selection and breeders should not be concerned with how these markers affect reproduction and other heifer traits with the possible exception of *CAPN1* effects on calving date.

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1. Introduction

Genetic markers associated with beef carcass and meat quality traits create a selection opportunity if marker effect sizes for these traits can be validated across populations [1–3]. Characterizing these same genetic marker effects for nontarget traits like reproduction is also important to their use for selection, but these effects are often not investigated or reported. For example, an *MSTN* marker that increases muscling was also reported to increase age at puberty [4]. Marker effect information on several traits is needed to optimize selection for multi-trait objectives. A difficulty in obtaining this information from industry populations is that one

* Corresponding author.
E-mail addresses: rtait@neogen.com (R.G. Tait), bob.cushman@ars.usda.gov (R.A. Cushman), anthony.mcneel@zoetis.com (A.K. McNeel), eduardo.casas@ars.usda.gov (E. Casas), tim.smith@ars.usda.gov (T.P.L. Smith), harvey.freetly@ars.usda.gov (H.C. Freetly), gary.bennett@ars.usda.gov (G.L. Bennett).
[1] Present address: Neogen, GeneSeek Operations, 4131 N. 48th St, Lincoln, NE 68504, United States.
[2] Present address: Zoetis, Inc., 333 Portage St., Kalamazoo, MI 49007, United States.
[3] Present address: United States Department of Agriculture, Agricultural Research Service, National Animal Disease Center, Ames, IA 50010, United States.

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homozygous genotype is often rare resulting in poor estimates or omitting that genotype from analyses. Recently, selection to increase minor allele frequency in experimental populations has been used to ensure representation of all genotypes in data for analysis [5–7]. Haplotypes in *μ-calpain* (***CAPN1***) [8] and SNP in *calpastatin* (***CAST***) [9] have been associated with beef tenderness in cattle [1,2,6]. *Growth hormone receptor* (***GHR***) SNP have been associated with milk yield and milk composition traits in dairy cattle [10,11] but also with body composition and yield traits in beef cattle [12] suggesting potential for pleotropic effects. Frequencies of the minor SNP alleles in an experimental Angus population were 0.08 for CAST and 0.18 for GHR. Selection increased these frequencies to 0.35 for CAST, 0.42 for GHR, and the combined frequencies of two haplotypes for CAPN1 from 0.70 to 0.89 [6]. The objectives of this study were to estimate the effects of these carcass and meat quality oriented markers on performance, reproductive, and first calf performance traits in Angus beef heifers to determine if these traits need to be considered when using these markers for carcass and beef quality selection.

2. Material and methods

2.1. Cattle population

The U.S. Meat Animal Research Center (**USMARC**) Animal Care and Use Committee approved the procedures used in this experiment.

Heifers in this study were contemporaries of the males utilized by our group [6] to evaluate the genetic effects of *CAPN1*, *CAST*, and *GHR* on carcass traits in steers. Briefly, this is an Angus population with about 210 calves born each year. Approximately 15 sires born within the herd were used each year for AI and natural service breeding. These heifers were from 25 sires with 1–13 heifer progeny.

2.2. Genetic markers

Markers chosen for this experiment were SNP haplotypes in *CAPN1* and single SNP within *CAST* and *GHR*. The *CAPN1* haplotype evaluated in this study was based on 2 previously identified SNP: CAPN1-316 (BTA 29; rs17872000; [13]) and CAPN1-4751 (BTA 29; rs17872050; [8]). CAPN1_316 and CAPN1_4751 SNPs were used to define haplotypes within the *CAPN1* gene. For reference, the Illumina A and Illumina B alleles were determined using the definitions from Illumina [14]. Haplotypes of interest in this study were: CAPN1_316 allele C (alanine amino acid; Illumina B allele) with CAPN1_4751 allele C (Illumina B allele) (**CAPN1-CC**) and CAPN1_316 allele G (glycine amino acid; Illumina A allele) with CAPN1_4751 allele T (Illumina A allele) (**CAPN1-GT**). Among *CAPN1* haplotypes with >1% frequency, White and coworkers [8] reported the largest difference for 14-d Warner-Bratzler shear force between CAPN1-CC haplotype and CAPN1-GT haplotype, neither of which were the most common haplotype in that study. Therefore, divergent haplotypes were selected to increase their frequencies in this population. Haplotypes not of interest in this study were: CAPN1_316 allele C with CAPN1_4751 allele T (**CAPN1-CT**) and CAPN1_316 allele G with CAPN1_4751 allele C (**CAPN1-GC**) and their frequencies were reduced by selection. Additionally, a SNP in *CAST* (BTA7; rs109221039; [9]) segregating C (Illumina B allele; [14]) (**CAST-C**) and T (Illumina A allele; [14]) (**CAST-T**) alleles was selected to increase the frequency of CAST-C in this population. Also, a SNP in *GHR* (BTA20; rs385640152 [commonly referred to as F279Y]) segregating T (Illumina B allele; [14]; phenylalanine amino acid) (**GHR-T**) and A (Illumina A allele; [14]; tyrosine amino acid) (**GHR-A**) alleles [12] causing a phenylalanine to tyrosine substitution at the 279th amino acid [10] was selected to increase the frequency of GHR-T in this population.

Samples of DNA were extracted from blood or semen. Extraction of DNA was performed using a Qiagen QIAmp DNA mini blood kit (Qiagen, Valencia, CA). Blood samples were collected in 10 mL syringes with 4% EDTA. Blood was frozen until DNA was extracted. Genotyping was performed using a primer extension method with mass spectrometry-based analysis of the extension products on a MassArray system as suggested by the manufacturer (Sequenom, Inc., San Diego, CA) and described by Stone and coworkers [15]. When necessary, genotype assays were repeated to reduce missing genotypes.

2.3. Base, selection, and evaluation phases

As described previously [6] this experiment consisted of 3 phases: base, selection, and evaluation. Cattle were selected to increase CAPN1-GT haplotype, CAST-C allele, and GHR-T allele frequencies. Allele frequencies of the population during base, selection, and evaluation phases of the experiment were previously reported [6].

In the evaluation phase, sires mostly heterozygous for *CAPN1*, *CAST*, and *TG* markers were bred to heifers and cows whose allele frequencies were near the target frequencies of 0.5 for each allele in each gene. Heifers for this experiment were sampled within year of birth from heifers that 1) were born as singles and raised by their own dam; 2) survived past weaning to be genotyped; 3) were successfully genotyped for all four markers; 4) did not have an uninformative *CAPN1* haplotype (CAPN1-CT or CAPN1-GC) and 5) were from sires with heifer progeny representing multiple genotypes. To balance power across genotypes, heifers with high frequency genotypes (heterozygous and homozygous major allele) were randomly selected within sire for evaluation and all heifers from low frequency genotypes were evaluated. This design was used to increase the statistical power of genotype comparisons from the limited number of heifers that could be retained for breeding.

This study utilized 174 heifers from the evaluation phase born in 2009 (n = 56), 2010 (n = 58), and 2011 (n = 60). Genotype frequencies for *CAPN1*, *CAST*, and *GHR* genetic markers of evaluation heifers are reported in Table 1. These heifers were progeny of 25 sires and 142 dams (16 of which were evaluation heifers themselves). Dams ranged in age from 2 to 11 y, however, for analysis they were defined as 2, 3, 4, or ≥5 y. All heifers were managed to

Table 1
Evaluation phase Angus heifer genotype frequencies (n = 174) for *CAPN1*, *CAST*, and *GHR* in a population selected to increase minor genotype and haplotype frequencies.

Genotype	n	%
CAPN1[a]		
CAPN1-CC:CAPN1-CC	65	37.4
CAPN1-CC:CAPN1-GT	75	43.1
CAPN1-GT:CAPN1-GT	34	19.5
CAST[b]		
CAST-C:CAST-C	21	12.1
CAST-C:CAST-T	94	54.0
CAST-T:CAST-T	59	33.9
GHR[c]		
GHR-A:GHR-A	55	31.6
GHR-A:GHR-T	86	49.4
GHR-T:GHR-T	33	19.0

[a] Haplotype of two SNP within the *CAPN1* gene. CAPN1-CC = CAPN1_316 C allele with CAPN1_4751 C allele and CAPN1-GT = CAPN1_316 G allele with CAPN1_4751 T allele.
[b] CAST-C = C allele of *CAST* SNP and CAST-T = T allele of *CAST* SNP.
[c] GHR-A = A allele of *GHR* SNP and GHR-T = T allele of *GHR* SNP.