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Associations of DNA polymorphisms in growth hormone and its transcriptional regulators with growth and carcass traits in two populations of Brangus bulls

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ABSTRACT. Sequence polymorphisms in the growth hormone (GH) gene and its transcriptional regulators, Pit-1 and Prop-1, were evaluated for associations with growth and carcass traits in two populations of Brangus bulls Chihuahuan Desert Rangeland Research Center (CDRRC, N = 248 from 14 sires) and a cooperating breeding program (COOP, N = 186 from 34 sires). Polymorphisms were SNP mutations in intron 4 (C/T) and exon V (C/G) in GH, A/G in exon VI in Pit-1, and A/G in exon III in Prop-1. In the COOP population, bulls of Pit-1 GG genotype had a significantly greater percentage of intramuscular fat than bulls of the AA or AG genotype, and bulls of the Prop-1 AA genotype had significantly greater scrotal circumference than bulls of AG or GG genotypes at ~365 days of age. Also, heterozygous genotypes for the two GH polymorphisms appeared advantageous for traits of muscularity and adiposity in the COOP population. The heterozygous genotype of GH intron 4 SNP was associated with advantages in weight gain, scrotal circumfer-

ence, and fat thickness in the CDRRC population. The two GH polymorphisms accounted for ≥27.7% of the variation in these traits in the CDRRC population; however, R^2 was <5% in the COOP population. Based on haplotype analyses the two GH SNPs appeared to be in phase; the haplotype analyses also paralleled with the genotype analyses. Polymorphisms in GH and its transcriptional regulators appear to be predictors of growth and carcass traits in Brangus bulls, particularly those with heterozygous GH genotypes.

Key words: Brangus, Growth hormone, DNA, SNP, Growth, Carcass

INTRODUCTION

Growth hormone (GH) has been used as a functional and positional candidate gene in genotype to phenotype association studies in several species, including bovines (i.e., *Bos taurus* and *Bos indicus*) and *Homo sapiens* (Ge et al., 2003; Beauchemin et al., 2006; Rudd et al., 2006). Rationale for choosing this hormone as a candidate gene includes its role in growth, lactation, carbohydrate metabolism, and many other aspects of homeorhesis (Ohlsson et al., 1998; Akers, 2006; Ayuk and Sheppard, 2006). Synthesis and secretion of GH are regulated by hypothalamic releasing factors, somatotrophic transcription factors, as well as a plethora of endocrine feedback signals (Giustina and Veldhuis, 1998; Pfaffle et al., 1999; Fodor et al., 2006).

The GH locus is on bovine chromosome 19; Lagziel et al. (2000) suggested that a sequence polymorphism in intron 4 of the GH gene could be used to differentiate humped (*B. indicus*) from humpless (*B. taurus*) cattle. Other comparisons among these types of cattle, particularly Angus and Brahman breeds, revealed phenotypic differences in carcass traits (Morrison, 2005). Admixed populations of British-*B. taurus* cattle (i.e., Angus or Hereford) and *B. indicus* cattle (i.e., Brahman) have been useful for various investigations, particularly quantitative trait locus (QTL) detection (Casas et al., 2003; Kim et al., 2003) and physiology delineations. In brief, Brahman cattle tend to have lower levels of adiposity and serum concentrations of leptin, but greater serum concentration of GH than do Angus cattle (Carroll, 1996; Thomas et al., 2002; Lopez et al., 2006).

Association studies and validation procedures using small numbers of DNA polymorphisms (i.e., markers) typically assume additivity, if the markers are within a gene or in linkage (Van Eenennaam et al., 2007). However, in admixed populations of *B. taurus* and *B. indicus* cattle, heterozygous genotypes appear to be advantageous for the prediction of physiological measurements and performance traits (Thomas et al., 2004; Pereira et al., 2005). This effect is probably due to allele interactions. Creating admixed populations with Zebu (i.e., *B. indicus*) cattle most likely augments this effect as these cattle were found to have high frequencies of homozygosity in single nucleotide polymorphisms (SNPs) discovered in sequencing projects of *B. taurus* cattle. This is particularly true for polymorphisms in genes of the GH axis (Beauchemin et al., 2006). Herein, we evaluated frequencies and associations of genotypes and haplotypes to phenotypes of bull development in a composite *B. indicus* x *B. taurus* breed of cattle (Brangus = 3/8 Brahman x 5/8 Angus).

MATERIAL AND METHODS

Animal model

Phenotype information on 434 Brangus bulls was collected based on guidelines described by the Beef Improvement Federation (BIF, 2006) for gain-tested bulls. From 1997 to 2003, the tested bulls were from the New Mexico State University Chihuahuan Desert Rangeland Research Center (CDRRC; progeny of 14 sires). From 2004 to 2005, the test included bulls from a cooperative breeding program (COOP; Mound Creek Ranch, Leona, TX; progeny of 34 sires). The two time periods were considered as separate populations/data sets. Complete pedigree information was known for all bulls, and bulls were registered with the International Brangus Breeders Association (San Antonio, TX). Subsets of bulls from reference sires in each breeding program were included in both performance tests from 2004 to 2005 (the COOP population). Reference sires included at least two bulls from the CDRRC breeding program and two bulls from the Mound Creek breeding program. A reference sire had to have at least two progeny at the two bull test locations. In the COOP population, contemporary groups were assigned based on location of weaning and location of post-weaning gain test. A brief description of the two breeding programs follows:

Chihuahuan Desert Rangeland Research Center

This Brangus herd was initiated in 1966; cows were managed as spring-calving range cattle with limited supplementation and assistance with dystocia. The annual breeding season was from May 1 to August 1 with artificial insemination and natural service matings. Within two days after birth of each calf, date of birth, birth weight, and calf gender were recorded and calves were assigned a unique identification number. Three to four months following birth, calves were vaccinated for clostridial, complex viral, and pasturella diseases. Vaccinations were repeated two to four weeks after the first vaccination. Calves were weighed at weaning at ~205 days of age. Post-weaned calves were given a two- to four-week acclimation period prior to the start of a gain test. The gain test ration was formulated to achieve a 1.5 kg head^{-1} day^{-1} gain with a corn-alfalfa-based diet (14.9% protein and 75% total dissolved nitrogen) for 112 days. Bulls were weighed every 28 days to obtain a linear plot of days versus gain to estimate average daily gain (ADG; slope of the line from $y = mx + b$). At ~365 days of age, bulls were given a breeding soundness exam to measure scrotal circumference and insure reproductive functionality. Simultaneously, carcass traits were measured with ultrasound.

Mound Creek Ranch

This organization has been breeding Brangus cattle for 17 years. For spring calving, breeding season was from May 21 to August 21 with the use of AI and natural service matings. Cows were maintained on Bermuda (*Cynodon dactylon*) and Bahia (*Paspalum notatum*) grass pastures and supplemented with hay of these grasses in winter as needed. Date, weight, and hair color code were recorded at birth and calves assigned their individual identification. Three to four months after birth, calves were provided creep feed and administered vaccinations for clostridial, complex viral, and pasturella diseases. At ~205 days of age, vaccines were

re-administered, calves were de-wormed, and weaned. Post-weaned bull calves were weighed and allowed to graze Bermuda grass *ad libitum*. Prior to the start of this test, calves were acclimated with a medicated-receiving ration for seven days, followed by a two-week acclimation period with a ration formulated for a 1.5 kg head^{-1} day^{-1} gain. This was a corn-based supplement (12.0% CP and 70.7% TDN) to grazing on Bermuda grass. When the calves reached ~365 days of age, they were weighed, scrotal circumference recorded, and carcass traits measured via ultrasound. At this time, bulls were re-vaccinated for clostridial and respiratory diseases and de-wormed. Bulls were weighed at the beginning and end of the gain test and these data were used to estimate average daily gain (ADG = (final weight - initial weight)/number of days on test).

Carcass measurements

Carcass trait data included real-time ultrasound measurements of fat thickness at the 12th and 13th rib, longissimus muscle area/kg of body weight, and intramuscular fat percentage of the longissimus muscle by a Centralized Ultrasound Processing certified technician (Perkins et al., 1992).

DNA extraction and genotyping

Genotyping and DNA extraction were done using procedures described by Beauchemin et al. (2006). In brief, blood was collected with vacuum tubes coated with ethylenediaminetetraacetic acid, the post-centrifugation white blood cell supernatant (i.e., buffy coat) was recovered, and then DNA extracted with QIAamp DNA Blood Mini Kit (#51104, Qiagen, CA, USA). The genotypes were determined (Table 1). Allelic discrimination was accomplished using fluoroprobes and real-time PCR or PCR, followed by digestion of amplicon with target restriction enzyme and subsequent fragment separation using ethidium bromide-stained 3% NuSieve agarose gels and electronic imaging. Two of the sequence polymorphisms were assayed as SNP, GH leucine (C) to valine (G) SNP and Prop-1 histadine (A) to arginine (G). The GH sequence polymorphism in intron 4 was assayed as an RFLP and presented as a C/T SNP. The *Hin*f-I RFLP in Pit-1 was represented as an A/G SNP. Data were represented as SNP for ease of presentation as haplotypes. The two sequence polymorphisms in the GH gene were in intron 4 and exon V, which were 1547 and 1758 bp from the 5' start of the gene.

Table 1. Gene, DNA sequence polymorphism, locus, functional region, and common literature description of sequence polymorphisms of growth hormone (GH) and its transcription factors.

Gene	Sequence polymorphism	Chromosome locus (~Mb)	Gene region	Common description in literature[a]
GH	C/T	19, 40.5 Mb	intron 4	Msp-1 RFLP
GH	C/G	19, 40.5 Mb	exon V	Leucine/Valine SNP
Pit-1	A/G	1, 19.4 Mb	exon VI	*Hin*f-I RFLP
Prop-1	A/G	7, 18.4 Mb	exon III	Histadine/Arginine SNP

[a]Beauchemin et al., 2006.

Statistical analysis

Prior to statistical analyses, birth weight, 205-day weight, and 365-day weight traits were adjusted by age and age of dam according to BIF guidelines (2006). Statistical analyses were conducted for each population of bulls using SAS (SAS Inst. Inc., Cary, NC, Ver 9.1.3), which includes functions for genetic analyses (Saxton, 2004). Assumptions of normality of data distribution and equality of variances within contemporary group, sire, and genetic categorizations were tested (Littell et al., 2002). A sire was required to have at least two progeny to be included in these analyses. Allelic, genotypic, and haplotype frequencies were estimated using the program routines, proc allele and proc haplotype. Linkage disequilibrium was estimated for the two loci in the GH gene. This procedure yielded tests of chi-square distribution, correlation coefficient (r^2), and Lewontin's D' estimate.

The genetic effect of the sequence polymorphisms was evaluated in prediction analyses using mixed model methodology (Littell et al., 1996). The model was:

$$y_{ijklmn} = \mu + A_i + B_j + C_k + D_l + E_m + F_n + e_{ijklmn}$$

where y_{ijklmn} = phenotypic value of trait,
μ = population mean,
A_i = fixed effect of genotype, haplotype, or probability of haplotype,
B_j = fixed effect of contemporary group,
C_k = fixed effect of year,
D_l = covariate of Julian birthday (i.e., age of bull),
E_m = fixed effect of the age of dam categories (BIF, 2006),
F_n = random effect of sire nested within contemporary group using the *Z* statistic to test if
Ho: $\sigma_w^2 = 0$ (Littell et al., 1996), and
e_{ijklmn} = random residual error.

If the trait was adjusted for known environmental effects prior to analyses (e.g., age of dam adjustments for birth weight and age of calf adjustments for weaning weight), Julian birthday and age of dam terms were omitted from the model. Year was only included in predictions involving the CDRRC population, since the data collected from the COOP population was for a single year. The genetic terms (i.e., A_i) were evaluated in four formats: 1) fixed effect of genotype; this was the genotype of a single locus for Pit-1 and Prop-1; however, for GH, this term involved the combination of two loci (i.e., prior to combining these two genotypes, the interaction of the two genotypes was determined to be significant; $P < 0.05$), 2) fixed effect of a single haplotype, 3) fixed effect of the four haplotypes fitted simultaneously, and 4) effect of the probability of an individual possessing each of the four haplotypes fitted as covariates (i.e., these four haplotypes were fitted in the model simultaneously). If the variances of the genetic terms were heterogenous then a repeated measures analysis was used that accounted for that heterogeneity. The interactions of the four SNPs were also tested with this model. If the genetic term (i.e., A_i) or an interaction of the terms were detected as significant ($P < 0.05$) source(s) of variation, means were separated with pre-planned pair-wise comparisons of least squares means. To evaluate the proportion of variation attributed to a specific genotype, simple predictions were conducted using a general linear model and ascertaining the coefficient of determination (R^2).

RESULTS

Growth and carcass traits (Table 2) and allelic and genotypic frequencies (Table 3) were recorded. Combined genotypes for the two loci in the GH gene and haplotypes were also recorded (Table 4). Haplotypes from the two GH gene sequence polymorphisms appeared to be in linkage disequilibrium in both populations of bulls ($\chi^2 \approx 36$, $P < 0.001$; $r^2 \approx \pm 0.4$; $D' \approx \pm 0.8$).

Sire and year were significant ($P < 0.05$) sources of variation in the prediction of traits in the CDRRC population. Sire and contemporary group were significant predictors ($P < 0.05$) of traits in the COOP population. Age and age of dam were not significant sources of variation for traits that were not pre-adjusted for age of the animal and age of dam; however, these terms were included in these models because of their biological relevance. Pit-1 and Prop-1 genotypes were not significant sources of variation in prediction analyses in the CDRRC population (Table 5). However, in the COOP population, Pit-1 genotype was a significant ($P < 0.05$) source of variation in prediction of intramuscular fat (%), as was the Prop-1 genotype for prediction of scrotal circumference. Specifically, bulls of the Pit-1 GG genotype appeared to have greater ($P < 0.05$) intramuscular fat (%) than bulls of the AA or AG genotype, and bulls of the Prop-1 AA genotype appeared to have greater ($P < 0.05$) scrotal circumference than bulls of the AG or GG genotype (Table 5). Interactions were detected ($P < 0.07$) among genotypes of Pit-1 and Prop-1 and among the two genotypes with the combined genotypes in the GH gene in prediction of ADG. This interaction was detected in both populations; however, a mean separation test did not reveal a consistent observable trend inferring an advantageous genotype combination. Coefficient of determination from a simple prediction of traits with Pit-1 and Prop-1 genotypes accounted for less than 5% of the variation in these two populations.

Table 2. Arithmetic mean ± standard error for growth and carcass traits in two populations of Brangus bulls.

Trait	Population	
	CDRRC (N = 248)	COOP (N = 186)
Birth weight (kg)	39.01 ± 0.30	37.15 ± 0.04
205-day weight (kg)	255.02 ± 3.25	308.08 ± 2.54
365-day weight (kg)	452.07 ± 2.63	526.78 ± 3.86
ADG (kg/day)	1.52 ± 0.01	1.91 ± 0.03
Scrotal circumference (cm)	34.59 ± 0.21	35.10 ± 0.23
Intramuscular fat (%)	3.39 ± 0.14	3.75 ± 0.05
Backfat (cm)	1.58 ± 0.37	0.63 ± 0.01
LMA/BW (cm^2/kg)	0.16 ± 0.001	0.17 ± 0.001

ADG = average daily gain; LMA/BW = longissimus muscle area per unit of body weight. CDRRC = Chihuahuan Desert Rangeland Research Center; COOP = Cooperating breeding program.

The combined genotypes of the sequence polymorphisms in the GH gene were significant ($P < 0.01$) predictors of birth weight, scrotal circumference, and fat thickness in the CDRRC population. These genotypes also tended ($P < 0.09$) to predict ADG, while the significance level

Table 3. Allelic and genotypic frequency percents of DNA sequence polymorphisms in the growth hormone (GH) gene and its transcriptional regulators in two populations of Brangus bulls.

Polymorphism Population	Allele frequency (%)		Genotypic frequency (%)		
GH intron 4	C	T	CC	CT	TT
CDRRC	68.9	31.1	51.5	34.8	13.7
COOP	79.1	20.9	63.8	30.7	5.5
GH exon V	C	G	CC	CG	GG
CDRRC	60.0	40.0	37.5	45.1	17.4
COOP	73.9	29.1	57.1	33.7	9.2
Pit-1 exon VI	A	G	AA	AG	GG
CDRRC	81.9	18.1	65.6	32.6	1.8
COOP	77.5	22.5	59.9	35.2	5.0
Prop-1 exon III	A	G	AA	AG	GG
CDRRC	92.3	7.7	84.6	15.4	-
COOP	75.2	24.8	55.3	39.6	5.0

CDRRC = Chihuahuan Desert Rangeland Research Center; COOP = Cooperating breeding program.

Table 4. Genotype and haplotype frequency percents for sequence polymorphisms in the growth hormone gene in two populations of Brangus bulls.

Item	Population	
	CDRRC	COOP
Genotype[1]		
CCCC	11.7	27.6
CCCG	25.4	27.0
CCGG	15.7	9.2
CTCG	9.8	7.4
CTCC	13.7	23.3
TTCC	12.2	4.9
TTCG	1.5	-
TTGG	-	0.6
Haplotype[2]		
CC	31.2	54.0
CG	37.8	25.1
TC	27.9	19.9
TG	3.0	1.0

[1]Order of genotypes include intron 4 locus followed by exon V locus.
[2]Order of haplotypes include intron 4 locus followed by exon V locus.
CDRRC = Chihuahuan Desert Rangeland Research Center; COOP = Cooperating breeding program.

Table 5. Least squares means ± pooled standard error (SE) of performance traits by genotypes of transcriptional regulators of growth hormone in two populations of Brangus bulls.

Trait	Pit-1 genotype				Prop-1			
	AA	AG	GG	Pooled SE	AA	AG	GG	Pooled SE
CDRRC population								
Birth weight (kg)	39.4	38.6	39.5	1.1	39.2	38.4	-	0.7
205-day weight (kg)	256.5	252.8	268.5	4.07	255.9	249.7	-	3.8
365-day weight (kg)	455.0	448.4	473.8	6.3	453.2	448.1	-	5.0
ADG (kg/day)	1.5	1.5	1.6	0.04	1.5	1.5	-	0.03
Scrotal circumference (cm)	35.0	34.2	35.7	0.9	34.8	34.4	-	0.5
Intramuscular fat (%)	3.7	3.8	-	0.2	3.7	4.1	-	0.2
Fat thickness (cm)	0.5	0.5	0.5	0.04	0.5	0.5	-	0.03
LMA/BW (cm^2/kg)	0.2	0.2	0.2	0.005	0.2	0.2	-	0.003
COOP population								
Birth weight (kg)	37.5	38.7	38.8	1.3	37.3	37.9	36.8	1.3
205-day weight (kg)	295.3	296.3	302.4	7.8	294.6	294.4	281.6	7.7
365-day weight (kg)	506.7	511.0	523.0	10.0	511.5	503.5	496.8	12.4
ADG (kg/day)	1.7	1.7	1.7	0.1	1.7	1.7	1.8	0.1
Scrotal circumference (cm)	35.0	34.8	35.1	0.6	35.8^a	34.3^b	34.4^b	0.1
Intramuscular fat (%)	3.7^a	3.7^a	4.0^b	0.1	3.7	3.6	3.6	0.2
Fat thickness (cm)	0.7	0.7	0.7	0.06	0.7	0.6	0.6	0.06
LMA/BW (cm^2/kg)	0.2	0.2	0.2	0.00	0.2	0.2	0.2	0.004

Within a row under genotype of transcriptional regulator of growth hormone, means without a common superscript differ ($P < 0.05$).
ADG = average daily gain; LMA/BW = longissimus muscle area per unit of body weight. CDRRC = Chihuahuan Desert Rangeland Research Center; COOP = Cooperating breeding program.

of genotype was 0.15 for 365-day weight and 0.18 in the prediction of intramuscular fat (%). In the COOP population, GH genotype was a significant predictor ($P < 0.05$) of intramuscular fat (%), fat thickness, and longissimus muscle area per unit of body weight. The mean separation test includes eight columns of genotypes for comparison (Table 6), which makes it difficult to select advantageous genotype(s) for each trait; however, in general, it appears that heterozygous (CT) genotypes at the intron 4 locus have the most advantageous ($P < 0.05$) production trait levels when paired with the CC genotype of the exon V locus in the CDRRC population and the heterozygous CG genotype of the exon V locus in the COOP population. Simple prediction analyses of these data suggested these combined genotypes accounted for ≥27.7% of the variation in traits of the CDRRC population. The intron 4 locus accounted for ≥20.0% of this variation; however, coefficients of determination were <5% in the COOP population.

When haplotype was tested as a single-fixed effect in prediction analyses, the CG haplotype was detected as a significant ($P < 0.05$) predictor of fat thickness and the TC haplotype was detected as a significant ($P < 0.05$) predictor of longissimus muscle area per unit of body weight in the CDRRC population (Table 7). The CC haplotype was a significant ($P < 0.05$) predictor of fat thickness in the COOP population. The CC haplotype was a significant ($P < 0.05$) predictor of longissimus muscle area per unit of body weight in the CDRRC population, whereas it predicted fat thickness in the COOP population. Models attempting to evaluate the four haplotypes simultaneously as fixed effects were unsolvable, which was probably due to the presence of rare haplotypes. Nonetheless, when haplotypes were fitted based on the probability of the individual possessing the four haplotypes as covariates, the four haplotypes predicted ($P < 0.05$) intramuscular fat (%) and longissimus muscle area per unit of body weight in the COOP population. The CC, CG, and TG haplotypes predicted ($P < 0.05$) intramuscular fat (%) and the TG haplotype predicted ($P < 0.05$) longissimus muscle area per unit of body weight in the CDRRC population.

DISCUSSION

Growth hormone is transcribed and translated in the somatotrophs of the anterior pituitary gland. Transcription is regulated by two powerful DNA-binding factors, known as Pit-1 and Prop-1. Pituitary secretion of GH is stimulated by hypothalamic secretion of GH-releasing hormone, but inhibited by hypothalamic secretion of somatostatin (Giustina and Veldhuis, 1998; Pfaffle et al., 1999; Fodor et al., 2006). An illustration of the many genes involved in the growth hormone-IGF-I endocrine axis/pathway was presented by Farber et al. (2006). Genetic selection for enhanced growth and body leanness has been associated with increased pituitary secretion of GH (Bunger and Hill, 1999; te Pas et al., 2001, 2004). Administration of recombinant GH enhances lactation and carcass lean meat yield (i.e., bovine somatotropin; Etherton and Bauman, 1998; Akers, 2006). Knowledge of these relationships provided rationale for targeting GH as both a functional and positional candidate gene relevant for genetic selection programs in Brangus cattle.

Detection of QTL and association testing of sequence polymorphisms in candidate genes are two approaches used to develop tools to improve complex traits of animal production (Andersson and Georges, 2004). In some initial candidate gene studies, which involved only a few sequence polymorphisms within or linked to a known gene, associations were typically tested in statistical models that assumed that the effects of alleles were additive. Data were

Table 6. Least squares means ± pooled standard error (SE) by genotypes in the growth hormone (GH) gene for growth and carcass traits in two populations of Brangus bulls.

Trait	GH genotypes[1]								
	CCCC	CCCG	CCGG	CTCG	CTCC	TTCC	TTCG	TTGG	Pooled SE
CDRRC population									
Birth weight (kg)	40.56^{a}	38.37^{b}	38.82^{a,b}	39.0^{a,b}	40.47^{a}	38.78^{b}	42.95^{a,b}	-	0.96
205-day weight (kg)	257.07	252.54	257.16	256.74	256.01	260.75	253.45	-	6.36
365-day weight (kg)	446.85	446.29	451.66	458.03	465.29	457.00	435.73	-	7.54
ADG (kg/day)	1.51^{a}	1.50^{a}	1.49^{a}	1.53^{a}	1.61^{b}	1.50^{a}	1.41^{a}	-	0.04
Scrotal circumference (cm)	33.98^{a}	34.67^{a}	33.81^{a}	35.26^{a,b}	36.12^{b}	36.56^{b}	35.45^{b}	-	0.74
Intramuscular fat (%)	3.70	3.72	3.62	4.06	3.79	4.42	3.88	-	0.23
Fat thickness (cm)	0.42^{a}	0.54^{b}	0.51^{b}	0.55^{b}	0.60^{b}	0.51^{b}	0.42^{a}	-	0.04
LMA/BW (cm^2/kg)	0.16	0.16	0.16	0.16	0.16	0.16	0.16	-	0.003
COOP population									
Birth weight (kg)	36.65	36.66	39.07	36.04	36.69	36.92	-	38.56	0.62
205-day weight (kg)	300.19	311.10	313.85	297.44	305.49	288.80	-	333.91	10.19
365-day weight (kg)	519.04	532.90	515.79	521.61	521.03	510.43	-	557.70	16.37
ADG (kg/day)	1.92	1.86	1.69	1.93	1.85	1.99	-	2.06	0.15
Scrotal circumference (cm)	34.83	34.47	34.52	34.72	34.57	35.59	-	35.29	1.06
Intramuscular fat (%)	3.61^{a}	3.59^{a}	3.69^{a,b}	4.05^{b}	3.77^{a,b}	3.82^{a,b}	-	3.62^{a,b}	0.21
Fat thickness (cm)	0.59^{a,b}	0.63^{b}	0.49^{a}	0.66^{b}	0.63^{b}	0.68^{b}	-	0.35^{a,b}	0.07
LMA/BW (cm^2/kg)	0.17^{a}	0.17^{a}	0.16^{a}	0.18^{b}	0.18^{b}	0.17^{a}	-	0.14^{a}	0.004

Within a row, means without a common superscript differ ($P < 0.05$).
[1]Order of genotypes include intron 4 locus followed by exon V locus.
ADG = average daily gain; LMA/BW = longissimus muscle area per unit of body weight.
CDRRC = Chihuahuan Desert Rangeland Research Center; COOP = Cooperating breeding program.

Table 7. Least squares means ± pooled standard error (SE) by haplotypes in the growth hormone (GH) gene for growth and carcass traits in two populations of Brangus bulls.

Trait	GH haplotypes[1]				
	CC	CG	TC	TG	Pooled SE
CDRRC population					
Birth weight (kg)	39.30	38.37	39.13	39.19	0.79
205-day weight (kg)	254.08	255.11	256.85	251.38	4.19
365-day weight (kg)	451.91	452.47	456.38	450.30	5.06
ADG (kg/day)	1.52	1.51	1.57	1.56	0.03
Scrotal circumference (cm)	34.65	34.63	35.60	33.83	0.43
Intramuscular fat (%)	3.81**	3.84**	3.89	3.74**	0.23
Fat thickness (cm)	0.52	0.56*	0.57	0.53**	0.03
LMA/BW (cm^2/kg)	0.16**	0.16	0.16*	0.16**	0.003
COOP population					
Birth weight (kg)	36.38	37.30	37.09	36.97	1.12
205-day weight (kg)	301.48	305.69	300.89	296.20	7.04
365-day weight (kg)	523.70	523.12	517.75	517.33	10.61
ADG (kg/day)	1.94	1.87	1.89	1.99	0.10
Scrotal circumference (cm)	34.76	34.61	34.65	35.02	0.72
Intramuscular fat (%)	3.81**	3.84**	3.93**	3.96**	0.05
Fat thickness (cm)	0.62*	0.59	0.61	0.56	0.05
LMA/BW (cm^2/kg)	0.17**	0.17**	0.17**	0.17**	0.004

*Significance ($P < 0.05$) of single haplotype in prediction.
**Significance ($P < 0.05$) of haplotype probability in prediction.
[1]Order of haplotypes include intron 4 locus followed by exon V locus.
ADG = average daily gain; LMA/BW = longissimus muscle area per unit of body weight.
CDRRC = Chihuahuan Desert Rangeland Research Center; COOP = Cooperating breeding program.

presented based on regression on the number of favorable alleles (Van Eenennaam et al., 2007). This procedure was effective in association analyses involving additive SNPs within the μ-calpain gene that were in linkage disequilibrium (Page et al., 2002, 2004; White et al., 2005).

Crossbred cattle of the Angus and Brahman parent breeds are known to exhibit heterosis (Morrison, 2005). Thus, it is possible that in admixed, *B. taurus* x *B. indicus* cattle populations such as Brangus cattle, alleles are not additive. Thomas et al. (2004) and Pereira et al. (2005) suggested that heterozygous genotypes could be favorable in associations with performance traits or physiological measurements. It is possible that the alleles from these GH loci in these Brangus populations have some type of allele interaction that influences the phenotypes. Knowledge of these potential allelic interactions led to the design of the statistical models that we used in this study.

Extensive linkage disequilibrium should be expected in admixed/crossbreed populations (Zhao et al., 2003). These types of populations have proven to be useful for QTL detection, even though chromosomal coverage was somewhat limited at the time these initial studies were conducted (Casas et al., 2003; Kim et al., 2003). In general, preliminary evidence suggests cattle have a high level of linkage disequilibrium (~13 Mb; Khatkar et al., 2006). Linkage disequilibrium was detected among the two GH loci that we evaluated in the two populations in our study. This result was expected as the loci of these sequence polymorphisms were only separated by 211 bp. Additional research is needed to delineate the genetic effects of heterosis and allele interactions to define the role of linkage disequilibrium when testing for associations of genotypes with phenotypes in admixed populations. An analysis that involves identity by descent of haplotypes or haplotype blocks could help with these statistical challenges. Li et al. (2002) and Stone et al. (2005) successfully used these approaches for the detection of associations of haplotype with phenotype; however, the cattle evaluated in those studies were primarily composites of *B. taurus* breeds.

Associations involving transcriptional regulators of the GH gene, Pit-1 and Prop-1, were single locus associations in our study. Only in the COOP population involving 34 sires, was there enough of a distribution of these bi-allelic genotypes to detect an association. Typically, the minor allele frequency must be greater than 10% for association testing (Abecasis et al., 2001). Nonetheless, there must have been a large enough effect of the GG genotype on the Pit-1 and AA genotype for Prop-1 to detect significant association with intramuscular fat (%) and scrotal circumference in this population. Since the frequency of the GG genotype in Pit-1 was quite low, these results suggest that selective breeding for this genotype would effectively improve the intramuscular fat (%) trait. However, this may not be feasible biologically. The Pit-1 protein localized in regions of the somatotrope nucleus conducting transcription and expression of this gene in its wild-type form was not found to be correlated with pituitary secretion levels of GH in a study of growing wethers (Mancini et al., 1999; Thomas et al., 2000).

Most of our knowledge on the endocrine importance of Pit-1 was derived from mutated/non-functional protein studies. This is also true for the prophet of Pit-1, Prop-1, which binds upstream to Pit-1 in the GH gene promoter/enhancer region (Pfaffle et al., 1999; Guy et al., 2004). Pit-1 has not been found to be a strong predictor of growth and carcass traits or has lacked presence of at least one of the three genotypes in association studies involving beef cattle (Moody et al., 1996; Zhao et al., 2004; Curi et al., 2006).

Our study is one of the first reports of an association study involving Prop-1 in cattle. Showalter et al. (2002) described the DNA sequence mutations in Prop-1 and provided evi-

dence from transcriptional activation assays suggesting that the histadine to arginine non-synonymous SNP at position 173 of the coding sequence greatly influences the ability of this protein to initiate transcription. Non-synonymous SNPs generally infer a gene structure to function relationship and have become a focal point of genomic projects attempting to use SNP data to predict phenotypes of complex traits (Crawford et al., 2005). Cumulatively, the data of this study, together with reports of QTL detection for ovulation rate in cattle (Kirkpatrick et al., 2000; Arias and Kirkpatrick, 2004), suggest that additional fine mapping and(or) candidate gene investigations are justifiable for this region of chromosome 7. The interactions of Pit-1 and Prop-1 with GH genotypes also suggest that the chromosomal regions containing these transcription factors may influence the GH gene through epistasis.

Growth hormone was one of the initial targets in candidate gene association studies in cattle (Taylor et al., 1998; Parmentier et al., 1999; Vukasinovic et al., 1999). Additional reports were published by Barendse et al. (2006), Gao et al. (2006) and Li et al. (2006). There is also evidence suggesting that the exon V non-synonymous SNP coding for leucine versus valine influences pituitary secretion of GH (Grochowska et al., 1999; Sorensen et al., 2002). In our study, the polymorphisms in intron 4 and exon V both appeared to be predictors of growth and carcass traits. Specifically, the heterozygous genotype appears advantageous in the COOP population, whereas the CTCC genotype is notable in predictions involving the CDRRC population. In these two populations, the effects of these two bi-allelic GH loci are more detectable in measures of body fat than for growth traits. This could be explained by the fact that these two breeding programs have been selecting for growth for many generations, but for carcass traits for only a few generations. Thus, there is probably less variation available for partitioning to genotype terms for the growth traits (i.e., 205- and 365-day adjusted weights) than for carcass traits. Note that scrotal circumference is included in these discussions of growth traits, as it has been found to be strongly correlated with growth traits in the CDRRC population (Thomas et al., 2002).

Results from our mixed model analyses suggested that haplotypes are significant predictors of carcass traits. However, an advantageous haplotype was not easily observed, nor were there any similar observable patterns among the two populations when the means were plotted. Since the model assumed allele interaction rather than additivity, haplotypes were fitted as covariates; so mean separation test(s) were not applicable. Haplotype analyses are becoming the norm in association studies, which will continue to develop to account for linkage disequilibrium and identification of tagging SNP (Wall and Pritchard, 2003; Zhao et al., 2003). The reports of Bosse et al. (2005) and Wagner et al. (2005) are examples of the use of haplotypes in association studies involving GH and(or) measures of body fat in humans. Our research demonstrates that haplotypes may contain important information for the prediction of body fat and muscling traits; however, the project needs additional chromosomal coverage to determine if these SNPs are important in a haplotype block on chromosome 19 and to unravel the challenges of determining the significance of a causal SNP mutation within a haplotype block.

A noteworthy observation in our study was the coefficient of determination ($R^2 \geq 27.7\%$) in the prediction of growth and carcass traits based on genotypes or haplotypes in the CDRRC population. Most of this value was also attributed to the polymorphism in intron 4. This is probably a unique finding in a relatively small population, and initially, we would expect the non-synonymous SNP in exon V to be more biologically relevant. However, current literature suggests that polymorphisms in introns have substantial relevance, through mechanisms such as

alternative splicing and exon shuffling (Roy and Gilbert, 2006). When these markers were tested in the COOP population with a larger number of sires, the coefficient of determination was minimal in the association tests of genotypes/haplotypes with phenotypes. However, though these GH bi-allelic loci were significant sources of variation in these sire-based predictions, the results did not reveal an advantageous haplotype. In summary, polymorphisms in GH and its transcriptional regulators appear to predict growth and carcass traits in Brangus bulls, particularly heterozygous GH genotypes.

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