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## Associations of a Leptin Gene Polymorphism with Beef Carcass Traits

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### Summary

The objective was to evaluate associations of leptin genotype with fat and muscle traits in cattle. A single nucleotide polymorphism located in exon 2 of the leptin gene in cattle codes for an amino acid change from arginine (R) to cysteine (C). Genotypes for the polymorphism were determined on 492 crossbred calves by *Acil* digestion of amplified PCR product (C allele: 130bp; R allele: 73bp and 57bp). Data were analyzed by least-squares, accounting for effects of genotype, sex, year, location, breed-type, and calf sire. Genotype was not significantly associated with carcass weight or ribeye area in any of the analyses. Associations of genotype with external fat thickness, KPH fat, and overall cutability were small and generally not statistically significant. Subjective marbling scores (assigned by USDA grader; 350 = slight 50, 400 = small 0, 450 = small 50) were higher ( $P = .02$ ) for CC (411 units) than for RR (388 units) genotype when adjusted to a constant slaughter age of 433 days. Similar differences between genotypes in marbling scores were observed when adjusting to a constant carcass weight or external fat thickness. Given the relatively modest association between genotype and marbling, potential application of the marker in the industry as a selection tool would be most relevant in herds with a large proportion of market animals possessing marbling scores that are near a price threshold level (e.g., select/choice quality grade).

Key Words: Cattle, Leptin, Gene marker

### Introduction

Improving product quality and efficiency of production are goals of the beef industry. The use of DNA-based markers could be particularly beneficial for genetic evaluation of economic traits for which phenotypic measurements are

difficult or expensive to obtain (e.g., meat quality and feed efficiency). A large number of DNA-based markers in livestock have been discovered to date, but relatively little is known about which markers could be useful in evaluation of specific traits. Leptin protein is synthesized in adipocytes, or fat cells, and has been implicated as a potential factor contributing to animal-to-animal variation in appetite, energy balance, and body composition. If variability (polymorphism) in the DNA sequence of the Leptin gene is associated with variability in production traits of interest, then a DNA-based diagnostic test could be a potentially useful tool for genetic evaluation. Therefore, the objective of the present study was to determine if a single nucleotide polymorphism in exon 2 of the Leptin gene is associated with variation in carcass traits in beef cattle.

### Materials and Methods

Calves used in this study were from dams produced in two-breed rotational mating systems involving crosses of Angus x Hereford, Simmental x Hereford, or Tarentaise x Hereford. Cows were mated either to rotational or terminal (Charolais) sires producing two- or three-breed crossbred calves. Calves were born and reared until weaning at the Antelope Range Livestock Station near Buffalo, SD or the Beef Breeding Research Unit at Brookings, SD. After weaning, calves were transported to either a feedlot located at the Brookings station or a commercial feedlot. Carcass traits were evaluated at a commercial packing facility at an average age of 433 days.

Blood was collected from each calf for subsequent DNA extraction and analysis. Animal genotype for the leptin polymorphism was determined by PCR-RFLP, a technique in which a 130-bp region of exon 2 of the leptin gene was amplified, exposed to digestion with the restriction enzyme *Acil*, and viewed following

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gel electrophoresis. The polymorphism (C allele: 130bp; R allele: 73bp and 57bp) consists of a single nucleotide switch (cytosine versus thymine), which is associated with an amino switch (arginine versus cysteine) in the Leptin protein molecule. Individual animals were genotyped as CC, CR, or RR.

Data were analyzed by least-squares procedures to evaluate associations of leptin genotype with carcass traits while adjusting for known sources of variation, including calf sex, birth year, breed-type, location of birth/feedlot, and calf sire. Additionally, data were adjusted to one of three endpoints: carcass weight (723 lb), fat thickness (.453 in), or slaughter age (433 days) in three separate analyses. The effects of allele substitution were evaluated in a second set of analyses in which the discrete effect of leptin genotype was replaced by the continuous effect of the number of 'C' alleles present in the genotype (i.e.; 0 in RR, 1 in CR, and 2 in CC). Linear and quadratic effects were evaluated in initial models as indicators of additive and non-additive effects, respectively.

### **Results and Discussion**

Presented in Tables 1-3 are least-squares means of carcass traits by genotype and regressions of each trait on the number of 'C' alleles in the genotype. Genotype was not significantly associated with carcass weight or ribeye area in any of the analyses. Associations of genotype with external fat thickness, KPH fat, and overall cutability were small and generally not statistically significant.

Associations of genotype with marbling score were statistically significant for the age- and fat-adjusted analyses and approached significance in the weight-adjusted analysis. Individuals with two copies of the 'C' allele produced carcasses with marbling scores of about 20 units higher, on average, than individuals with zero copies. This relatively small difference indicates that this marker accounts for a small fraction of the overall population variation in marbling and that other loci, perhaps many, are affecting the trait. Whether this magnitude of difference is sufficiently large to justify genotyping as a means of genetic evaluation in the beef industry depends, among other things, on the current level of marbling in the specific herd or population of interest. Herds in which a large proportion of market animals have marbling

scores near the price threshold levels for select/choice or standard/select quality grade would likely benefit more from genotype information compared to herds in which most animals are well below or above a quality grade/price threshold.

The reader should be cautioned that differences attributed to a particular gene marker could possibly be due to a different gene located in close proximity to the marker of interest. It is also possible, in studies utilizing multiple families and breeds, for population structure to cause "artificial" genotype effects. However, the inclusion of breed-type and sire in the statistical model should account for potential bias due to the effect of population structure in the present study. Additional analyses conducted on a within-sire basis tended to confirm the conclusion found in the original analyses: i.e., progeny of 'CC' sires tended to have larger marbling, in general, than those of 'RR' sires.

### **Implications**

The most important result observed in this study was a small association between genotype and marbling score. An increase of one 'C' allele in the genotype was associated with an increase of about 10 marbling units, on average, in this particular mixed-breed population. Results could be different in other populations. The magnitude of difference observed in this study is quite modest, but might be sufficient to justify genotyping in herds or populations in which most market animals have marbling scores near a quality grade/price threshold.

Table 1. Calf Leptin genotypic effects on carcass composition adjusted to a constant final age (433 days)

Trait	CC		CR		RR		F-test P-value	Regression coefficient <sup>a</sup>	
	N	LSMean±SE	N	LSMean±SE	N	LSMean±SE		Linear	Quadratic
Carcass weight, lb	118	719.57±6.92	276	721.36±5.21	98	721.24±7.40	0.968	NS	NS
Rib-eye area, in <sup>2</sup>	118	12.64±.13	276	12.75±.10	98	12.83±.14	0.520	NS	NS
Fat thickness, in	118	.502±.017	276	.468±.013	98	.470±.018	0.113	0.018±0.011 <sup>+</sup>	NS
KPH, %	118	2.74±.06	276	2.63±.04	98	2.69±.06	0.147	NS	0.091±0.046 <sup>+</sup>
Estimated cutability, %	118	49.84±.16	276	50.17±.13	98	50.18±.17	0.115	-0.184±0.104 <sup>+</sup>	NS
Marbling score <sup>b</sup>	118	410.9±6.85	276	404.76±5.31	98	387.21±7.31	0.017	9.73±4.50 <sup>+</sup>	NS
Choice grade, %	118	60.7±5	276	53.4±4	98	48.9±5	0.188	6.4±3.3 <sup>+</sup>	NS

<sup>a</sup> Trait value regressed on number of C alleles in genotype.

<sup>b</sup> Slight = 300 to 399, Small = 400 to 499.

<sup>+</sup>P < 0.10; \* P < 0.05.

Table 2. Calf Leptin genotypic effects on carcass composition, adjusted to a constant carcass weight (723 lb)

Trait	CC		CR		RR		F-test P-value	Regression coefficient <sup>a</sup>	
	N	LSMean±SE	N	LSMean±SE	N	LSMean±SE		Linear	Quadratic
Rib-Eye Area, in <sup>2</sup>	118	12.69±0.12	276	12.79±0.09	98	12.86±0.13	0.518	NS	NS
Fat thickness, in	118	.510±0.016	276	.474±0.012	98	.476±0.017	0.075	NS	NS
KPH, %	118	2.76±0.05	276	2.64±0.04	98	2.70±0.06	0.113	NS	0.090±0.045 <sup>+</sup>
Estimated cutability, %	118	49.78±0.15	276	50.13±0.12	98	50.14±0.16	0.064	-0.188±0.098 <sup>+</sup>	NS
Marbling score <sup>b</sup>	118	406.98±7.61	276	402.54±5.82	98	387.26±7.95	0.066	9.65±4.51 <sup>+</sup>	NS
Choice grade, %	118	57.8±5	276	50.7±4	98	46.2±5	0.206	5.8±3.3 <sup>+</sup>	NS

<sup>a</sup> Trait value regressed on number of C alleles in genotype.

<sup>b</sup> Slight = 300 to 399; Small = 400 to 499.

<sup>+</sup>P < 0.10; \* P < 0.05.

Table 3. Calf Leptin genotypic effects on carcass composition adjusted to a constant fat thickness (0.453 in)

Trait	CC		CR		RR		F-test P-value	Regression coefficient <sup>a</sup>	
	N	LSMean±SE	N	LSMean±SE	N	LSMean±SE		Linear	Quadratic
Carcass Weight, lb	118	710.69±6.70	276	716.93±5.02	98	716.61±7.14	0.653	NS	NS
Rib-Eye Area, in <sup>2</sup>	118	12.68±0.13	276	12.76±0.10	98	12.84±0.14	0.627	NS	NS
KPH, %	118	2.70±0.06	276	2.61±0.04	98	2.67±0.06	0.226	NS	0.076±0.044 <sup>+</sup>
Estimated cutability, %	118	50.25±0.09	276	50.30±0.07	98	50.34±0.10	0.750	NS	NS
Marbling score <sup>b</sup>	118	404.05±6.80	276	400.81±5.23	98	382.67±7.21	0.023	10.31±4.31 <sup>*</sup>	NS
Choice grade, %	118	54.5±5	276	49.4±4	98	44.7±5	0.339	NS	NS

<sup>a</sup> Trait value regressed on number of C alleles in genotype.

<sup>b</sup> Slight = 300 to 399; Small = 400 to 499.

<sup>+</sup>  $P < 0.10$ , <sup>\*</sup>  $P < 0.05$ .