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South Dakota Beef Report, 2006

Animal Science Reports

2006

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Recommended Citation

Sanborn, Angela M.; Rosa, Artur J.M.; Linblom, Stacy J.; and Taylor, Anna R., "Association of Leptin Gene Markers with Carcass Traits in Beef Cattle" (2006). *South Dakota Beef Report, 2006*. Paper 6.

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Association of Leptin Gene Markers with Carcass Traits in Beef Cattle¹

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BEEF 2006 – 05

Summary

The objective of this study was to evaluate four genetic markers on the leptin gene for association with carcass traits in three crossbred families. Three half-sib families were developed from crossbred sires. Families 1, 2, and 3 comprised 26, 21, and 66 offspring, respectively ($n = 113$). The genetic background of the sires, dams, and offspring was 1/3 Angus, 1/3 Hereford, 1/3 Simmental. Carcass traits collected were finished weight, hot carcass weight (HCW), marbling score, Quality Grade, Longissimus muscle area (LMA), rib fat, Yield Grade, and percent kidney, pelvic, and heart fat (KPH). The four markers analyzed were located on the exon 2, exon 3, and promoter region of the leptin gene. There was an association of marbling score with leptin exon 3 ($P < 0.05$), and ability to grade choice with leptin exon 2 ($P < 0.05$), exon 3 ($P < 0.001$), and promoter ($P < 0.01$) in family 2. Family 2 also displayed allelic effects for ability to grade choice ($P < 0.01$) with leptin exon 3 and promoter. Family 3 showed an association between leptin exon 2 ($P < 0.05$) and marbling score. No association was detected ($P > 0.05$) on family 1.

Introduction

The leptin gene is a candidate gene for association with carcass traits as the leptin protein has been shown to affect various metabolic activities. Leptin is a protein that is secreted by white adipocytes and has receptors located in many types of cells throughout the body. Leptin has been shown to contribute to intake regulation as well as energy balance, including energy expenditure, in humans and rodents. Therefore, this gene may also be of use in advancing efficiency of production, carcass

quality, and overall health in livestock selection applications. (Houseknecht et al., 1998)

Associations of a marker in exon 2 of the leptin gene with carcass traits were previously observed in calves produced at the South Dakota State University Beef Breeding Unit, Brookings, SD from 1995 to 1999. Cows arising from a two-breed rotational system involving crosses of Angus x Hereford, Simmental x Hereford, and Tarentaise x Hereford breed types were mated to rotational type or terminal Charolais sires. The calves produced were either two- or three-breed crosses. Significant association was found between genotype and marbling score ($P = 0.02$) when adjusted for slaughter age, HCW, and back fat. Suggestive relationships were noted for back fat, KPH, and percent cutability. (Bierman, 2001; Bierman et al., 2003)

The objective of this study was to determine association between carcass traits and four leptin gene markers, including the marker previously found to be associated with marbling score in the same herd.

Materials and Methods

A reference population of 162 offspring born from 2001 to 2004 from three sires was developed at the South Dakota State University Beef Breeding Unit, Brookings, SD. The three sires and all offspring were comprised of 1/3 Angus, 1/3 Hereford, and 1/3 Simmental genetic material. The offspring and mated dams of each sire were identified as families 1, 2, and 3. Family 1 corresponds to sire 988083, family 2 corresponds to sire 999114, and family 3 corresponds to sire 988042.

Cattle were weaned at approximately 185 days of age. Following weaning, cattle were fed a corn based diet consisting of 12.5% crude protein and 94.2 Mcal/cwt NE_m for about 110 days. Harvest criteria included an average ultrasound determined rib fat measurement of

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0.30 inches and average finished weight of at least 1,000 pounds for the group.

Cattle were marketed to Tyson Fresh Meats, Dakota City, NE or PM Beef, Windom, MN. Animal identification numbers were matched to plant carcass identification numbers at the time of harvest for cross-referencing pre- and post-mortem phenotypes. Phenotypic data was collected for finished weights and post-mortem measures of HCW, marbling score, Quality Grade, LMA, rib fat, percent KPH, and Yield Grade. Finished weight was determined just prior to harvest, while HCW was determined at the time of harvest. Carcasses were chilled for at least 24 hours prior to collection of the additional carcass data. LMA and rib fat measures were taken by South Dakota State University personnel, while marbling score,

Quality Grade, percent KPH, and Yield Grade were determined by a USDA grader.

Whole blood was collected from each calf by jugular or tail vein venapuncture at weaning and again at 5 to 6 months into the feeding period. Samples of about 10 ml were collected in evacuated tubes with 15% EDTA. Blood was stored at 4°C for no more than 24 hours prior to buffy coat (white blood cell) extraction by centrifugation. DNA was extracted from the isolated buffy coats using a saturated salt DNA extraction procedure (Miller et al., 1988).

The four markers analyzed were located on the exon 2, exon 3, and promoter regions of the leptin gene. The primers used are noted in Table 1.

Table 1. Primers for leptin gene markers

Marker name	Primers	Annealing temperature	Reference
Leptin exon 2	Forward 5'-CTGTATCGATTCTGTGGCTTTGG-3' Reverse 3'-GCGTGTGTGAGATGTCATTGATCC-3'	60°C	Fitzsimmons and Schmutz, 1999
Leptin exon 3	Forward 5'-CCCTCTCTCCCACTGAGCTC-3' Reverse 5'-TAAAGGATGCCACATAGGC-3'	63°C	Konfortov et al., 1999
Leptin promoter	Forward 5'-AGGCAGGATGTTTAGTCGCAGCAT-3' Reverse 5'-TGTGAGCTGGAAAGAACGGA-3'	60°C	(designed for this study)

The marker on exon 2 was previously described by Bierman and in 2001 and 2003. The markers on exon 3 and promoter were detected by DNA sequencing of the three herd sires on an ABI 3730 DNA Analyzer (Hitachi, Ltd.) at the Nevada Genomics Center. Sequences were aligned and compared using Sequencher 4.6 software (Gene Codes Corporation, 2006). Single nucleotide polymorphisms (SNPs) were detected on the exon 3 and promoter regions of the leptin gene and restriction enzymes were selected based on the sequences of DNA nucleotides surrounding the SNPs. A guanine-adenine (GA) deletion marker was detected on the leptin promoter region, and was heterozygous in sires 1 and 3. Genotyping of the SNP markers was conducted by restriction fragment length polymorphism (RFLP) polymerase chain reaction (PCR) with visualization by electrophoresis on 3% agarose gels with ethidium bromide staining. Genotyping of the GA deletion marker was completed by PCR amplification with incorporated fluorescent dye. PCR products were sent to the Iowa State University Genomics Facility for testing.

Returned gel files were aligned and genotyped using GeneMarker 1.5 (SoftGenetics LLC, 2006). Assigned genotypes were verified against sire and dam genotypes, with those not in agreement excluded from the analysis (excluded $n = 49$).

Statistical analysis of associations between the gene markers and phenotypic traits was conducted using General Linear Model (GLM) procedure of SAS (SAS Institute Inc., 2001). Differences in sex, year, age at finishing, and the specific marker were accounted for in the model, with age at finishing used as the adjustment factor. Representative *P*-values and Least-squares means were determined.

Results and Discussion

A total of 113 offspring, from families 1 ($n = 26$), 2 ($n = 21$), and 3 ($n = 66$), were included in the analysis of genotypic and allelic effects on carcass traits. The SNP marker on leptin exon 2 was genotyped and analyzed in all three

families, as all three sires were heterozygous for the marker. The SNP markers on leptin exon 3 and leptin promoter were genotyped and analyzed only in family 2, as they were informative only for the sire of that family. Tables

2 through 4 show the genotypic and allelic effects of the leptin gene markers with carcass traits where significance ($P < 0.05$) was detected.

Table 2. Leptin exon 2 genotypic effects on carcass phenotypes in families 2 and 3

Trait	Family 2						P-value
	CC ^a		CR ^a		RR ^a		
	n	LS Mean	n	LS Mean	n	LS Mean	
Finished weight, lb	6	1091	10	1072	5	1060	0.7906
HCW, lb	6	642	10	611	5	654	0.1219
Marbling score ^b	6	369	10	339	5	345	0.4426
Choice ^c	6	0.83	10	0.10	5	0.20	0.0165
LMA, in ²	6	12.7	10	12.4	5	12.9	0.8330
Fat, in
YG ^d	6	1.95	10	1.64	5	1.80	0.4180
KPH, %	6	2.25	10	2.06	5	2.25	0.7772

Trait	Family 3						P-value
	CC ^a		CR ^a		RR ^a		
	n	LS Mean	n	LS Mean	n	LS Mean	
Finished weight, lb	14	1072	29	1061	24	1088	0.4497
HCW, lb	14	650	28	653	23	647	0.9300
Marbling score ^b	12	379	28	425	24	385	0.0151
Choice ^c	14	0.47	29	0.56	24	0.53	0.8925
LMA, in ²	12	12.3	26	12.2	24	12.8	0.4270
Fat, in	10	0.40	17	0.48	14	0.47	0.2534
YG ^d	12	2.20	26	2.23	22	2.24	0.9855
KPH, %	8	2.31	23	2.68	20	2.45	0.0674

^a genotype; ^b slight = 300-399, small = 400-499; ^c choice = 1, not choice = 0; ^d USDA 1, 2, 3, 4, 5

Table 3. Leptin exon 3 and promoter genotypic effects on carcass phenotypes in family 2

Trait	Leptin exon 3						P-value
	CC ^a		CT ^a		TT ^a		
	<i>n</i>	LS Mean	<i>n</i>	LS Mean	<i>n</i>	LS Mean	
Finished weight, lb	2	1015	11	1082	8	1070	0.5064
HCW, lb	2	660	11	619	8	627	0.3809
Marbling score ^b	2	398	11	323	8	364	0.0233
Choice ^c	2	0.50	11	0.00	8	0.75	0.0005
LMA, in ²	2	12.8	11	12.4	8	12.8	0.8583
Fat, in
YG ^d	2	2.10	11	1.57	8	1.87	0.1425
KPH, %	2	2.54	11	2.03	8	2.16	0.4603

Trait	Leptin promoter						P-value
	AA ^a		AG ^a		GG ^a		
	<i>n</i>	LS Mean	<i>n</i>	LS Mean	<i>n</i>	LS Mean	
Finished weight, lb	.	.	12	1059	9	1085	0.4067
HCW, lb	.	.	12	624	9	631	0.6521
Marbling score ^b	.	.	12	334	9	357	0.2274
Choice ^c	.	.	12	0.08	9	0.67	0.0024
LMA, in ²	.	.	12	12.4	9	12.8	0.3976
Fat, in
YG ^d	.	.	12	1.70	9	1.74	0.7991
KPH, %	.	.	12	2.17	9	2.08	0.6893

^a genotype; ^b slight = 300-399, small = 400-499; ^c choice = 1, not choice = 0; ^d USDA 1, 2, 3, 4, 5

Table 4. Leptin exon 3 and promoter allelic effects on carcass phenotypes in family 2

Trait	Leptin exon 3				P-value
	C ^a		T ^a		
	n	LS Mean	n	LS Mean	
Finished weight, lb	9	1015	8	1022	0.8303
HCW, lb	9	610	8	608	0.9377
Marbling score ^b	9	356	8	377	0.3330
Choice ^c	9	0.11	8	0.75	0.0068
LMA, in ²	9	11.9	8	12.3	0.5276
Fat, in
YG ^d	9	1.82	8	1.99	0.4112
KPH, %	9	2.32	8	2.26	0.8069

Trait	Leptin promoter				P-value
	A ^a		G ^a		
	n	LS Mean	n	LS Mean	
Finished weight, lb	12	1059	9	1085	0.4067
HCW, lb	12	624	9	631	0.6521
Marbling score ^b	12	334	9	357	0.2274
Choice ^c	12	0.11	9	0.75	0.0024
LMA, in ²	12	12.4	9	12.8	0.3976
Fat, in
YG ^d	12	1.70	9	1.74	0.7991
KPH, %	12	2.17	9	2.08	0.6893

^a base substitution; ^b slight = 300-399, small = 400-499; ^c choice = 1, not choice = 0; ^d USDA 1, 2, 3, 4, 5

Only 2 offspring from family 2 had rib fat data, so analysis of that trait was not possible. The leptin promoter AA genotype was not present in the offspring from family 2, though a small number of dams were genotyped AA.

Of the traits analyzed, marbling score and ability to grade choice showed the greatest association with the four leptin markers. Family 2 exhibited significant genotypic effects for marbling score for leptin exon 3 ($P < 0.05$), and ability to grade choice for leptin exon 2 ($P < 0.05$), exon 3 ($P < 0.001$), and promoter ($P < 0.01$). Family 2 also displayed significant allelic effects for ability to grade choice ($P < 0.01$) for leptin exon 3 and promoter. Family 3 showed a significant association between genotype and marbling score for leptin exon 2 ($P < 0.05$). Family 1 had a significant association of the leptin promoter GA deletion marker with Yield Grade ($P < 0.05$).

No associations between the markers and finished weight, HCW, LMA, rib fat, and percent KPH were detected.

The results of this study are supportive of the previous studies (Bierman, 2001; Bierman et al., 2003) conducted with the same population at the South Dakota State University Beef Breeding Unit, Brookings, SD, for an association of significance between genotype for the marker on leptin exon 2 and marbling score. However, comparison of least square means for each genotype was not consistent between families. Significant association with marbling score and ability to grade choice, were observed for all four markers analyzed by genotype. Significant association with ability to grade choice was noted in leptin exon 3 and leptin promoter when analyzed by sire allele.

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