

POLYMORPHISMS IN THE LEPTIN GENE AND THEIR ASSOCIATIONS WITH PERFORMANCE, FEED EFFICIENCY, AND CARCASS MERIT OF BEEF CATTLE

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INTRODUCTION

Leptin is an adipocyte-derived hormone product of the obese gene (Zhang *et al.*, 1994). Leptin circulates in the serum in the free and bound forms and functions as a lipostatic signal regulating whole body energy metabolism through interactions with the leptin receptor in the hypothalamus. In cattle, circulating leptin levels are correlated with body weight, feed intake, and body fatness (Geary *et al.*, 2003). Single nucleotide polymorphisms (SNP) in the leptin gene have been shown to be associated with several traits in cattle (Buchanan *et al.*, 2002; Liefers *et al.*, 2003; Nkrumah *et al.*, 2005). We report associations of two leptin SNP and their genotype combinations with serum leptin, performance, feed efficiency, and carcass merit of beef cattle.

MATERIALS AND METHODS

Animals and Phenotypic Data. Growth, feed intake, ultrasound, and carcass data were collected on 464 beef steers produced from a cross between a hybrid dam line and Angus, Charolais or University of Alberta Hybrid bulls. Animals were managed and tested under feedlot conditions over three yrs at the University of Alberta using the Growsafe automated feeding system. Two tests (approximately 80 animals per test) were conducted each year. Detailed procedures, including those for obtaining measures of performance, feed efficiency, and carcass merit have been reported by Nkrumah *et al.* (2004).

Blood Collection, DNA Isolation, Serum Leptin Assay, and SNP Genotyping. A 10-mL blood sample was collected from each animal for genomic DNA extraction using a standard saturated salt phenol/chloroform procedure. One week prior to slaughter, blood samples were collected for serum leptin assays. Serum samples were assayed for leptin using the RIA kit described by Delavaud *et al.* (2000). Intra- and interassay coefficients of variation were less than 5%. The first SNP tested (UASMS2) is a C-T substitution at nucleotide 528 (GenBank accession #AB070368) in the bovine leptin promoter (Nkrumah *et al.*, 2005). The second SNP (A59V) is a C-T mutation at position 321 (GenBank accession #BTA512639; EMBL Accession #AJ512639) in exon 3 of the bovine leptin gene, which results in an alanine to valine substitution at amino acid 59 in the β -helix region in the leptin molecule (Liefers *et al.*, 2003). Genotyping of all was carried out using the Illumina GoldenGate assay on the BeadStation 500G Genotyping System (Illumina Inc., San Diego, CA).

Statistical Analyses. Tests of linkage disequilibrium (LD) and Hardy-Weinberg equilibrium were performed using PROC ALLELE of SAS/Genetics 9.1.3 (SAS Institute, Inc., Cary, NC).

Associations of genotypes or haplotypes of the SNP with each trait was tested by least squares procedures using PROC MIXED of SAS. The model used included fixed effects due to SNP genotype or haplotype, breed of sire (Angus, Charolais, and Hybrid), year of test (three levels), test group nested within year (two levels per year), all possible interactions when ($P < 0.05$), and linear and quadratic effects of age. Additive genetic effects (a) were computed as the difference between the solutions of the estimate for the trait value of the two homozygous genotypes.

RESULTS AND DISCUSSION

Frequencies of the T alleles were 18% and 75% for the UASMS2 and A59V SNPs, respectively. Both SNP were in Hardy-Weinberg equilibrium ($P > 0.10$). However, there was a highly significant LD between the two SNPs ($\chi^2 = 29.83$, $P < 0.001$). Animals with the TT genotype of UASMS2 (Table 1) had significantly higher serum leptin concentration ($a = 6.40$ ng/mL; $P < 0.001$), daily DMI ($a = 0.76$ kg/d; $P = 0.036$), ultrasound backfat ($a = 2.43$ mm; $P = 0.017$), and ultrasound marbling score ($a = 0.51$; $P = 0.023$).

Table 1. Association of UASMS2 SNP in the leptin promoter with different test traits (LS means \pm SE).

Trait	UASMS2 SNP genotype			P value ^A
	CC	CT	TT	
Number of animals	306	146	12	–
Serum leptin level, ng/mL	13.04 \pm 0.38 ^c	13.94 \pm 0.54 ^c	19.20 \pm 1.53 ^b	<0.001
FCR, kg DM/kg gain ^B	7.21 \pm 0.11	7.37 \pm 0.14	7.22 \pm 0.33	0.47
Dry matter intake, kg/d	10.33 \pm 0.13 ^c	10.71 \pm 0.17 ^{bc}	11.09 \pm 0.42 ^b	0.036
Ultrasound backfat, mm	8.93 \pm 0.20 ^d	9.09 \pm 0.28 ^c	11.38 \pm 0.83 ^b	0.017
Ultrasound marbling score	5.07 \pm 0.06 ^c	5.22 \pm 0.08 ^b	5.58 \pm 0.20 ^b	0.023
Number of animals	255	118	8	–
Carcass grade fat, mm	10.32 \pm 0.30 ^c	10.58 \pm 0.44 ^c	13.37 \pm 1.36 ^b	0.09
Carcass marbling score	2.47 \pm 0.04	2.54 \pm 0.06	2.65 \pm 0.17	0.35
Lean meat yield	58.17 \pm 0.17	57.98 \pm 0.44	55.88 \pm 1.26	0.17

^A P value from overall F test; ^{b, c, d} Means in rows followed by different superscripts are different ($P < 0.05$).

^B FCR = Feed conversion ratio.

Similarly, the TT genotype of A59V (Table 2) was associated with higher serum leptin concentration ($a = 3.89$ ng/mL; $P = 0.003$), ADG ($a = 0.13$ kg/d; $P = 0.04$), and FCR ($a = -0.68$ kg DM/kg gain; $P = 0.005$). The A59V SNP was also associated with ultrasound backfat thickness ($a = 1.53$ mm; $P = 0.014$) and ultrasound LM area ($P = 0.011$) but not with ultrasound marbling score ($P > 0.10$). The association of A59V with serum leptin concentration is consistent with the findings of Liefers et al. (2003), who reported higher serum leptin levels in TT animals during late pregnancy. There were no significant associations with BW, daily DMI or residual feed intake ($P > 0.10$). Differences were also observed among genotypes of A59V in carcass grade fat ($P = 0.10$), average carcass backfat ($P = 0.039$; $a = 1.92$ mm), carcass LM area ($a = -1.57$ cm²; $P = 0.015$), carcass lean meat yield ($a = 1.66\%$; $P = 0.024$), and yield grade ($P = 0.10$).

Table 2. Association of A59V in leptin exon 3 with different test traits (LS means \pm SE)

Trait	A59V SNP genotypes			P value ^A
	CC	CT	TT	
Number of animals	31	174	259	–
Serum leptin level, ng/mL	10.80 \pm 0.98 ^d	13.40 \pm 0.40 ^c	14.43 \pm 0.37 ^b	0.0029
Average daily gain, kg/d	1.36 \pm 0.05 ^c	1.48 \pm 0.03 ^b	1.50 \pm 0.03 ^b	0.039
FCR, kg DM/kg gain	7.96 \pm 0.23 ^b	7.26 \pm 0.12 ^c	7.20 \pm 0.12 ^c	0.005
Ultrasound backfat, mm	7.93 \pm 0.55 ^d	8.74 \pm 0.23 ^c	9.46 \pm 0.21 ^b	0.014
Ultrasound LM area, cm ²	82.20 \pm 1.42 ^c	84.55 \pm 0.61 ^b	83.24 \pm 0.57 ^{bc}	0.011
Number of Animals	26	143	212	–
Carcass grade fat, mm	9.52 \pm 0.79	10.15 \pm 0.36	10.94 \pm 0.33	0.10
Average carcass backfat, mm	10.63 \pm 0.78 ^d	11.64 \pm 0.34 ^c	12.55 \pm 0.30 ^b	0.039
Carcass LM area, cm ²	84.40 \pm 1.64 ^{bc}	85.56 \pm 0.81 ^b	83.83 \pm 0.75 ^c	0.015
Lean meat yield	59.13 \pm 0.74 ^b	58.56 \pm 0.34 ^{bc}	57.47 \pm 0.31 ^c	0.024
Carcass yield grade	1.67 \pm 0.14	1.59 \pm 0.06	1.76 \pm 0.06	0.10

^A P value from overall F test;

^{b, c, d} Means in rows followed by different superscripts are different ($P < 0.05$).

In addition, carcass grade fat ($a = 3.04$ mm) and average carcass backfat ($a = 2.99$ mm) tended to be higher ($P < 0.10$) in TT animals compared to CC animals of UASMS2. Associations of UASMS2 with the remaining test traits were not significant ($P > 0.10$). The results of the haplotype (genotype combinations) associations (Table 3) indicated that the T alleles of both SNP show associations with higher serum leptin concentration, with the highest effect observed for genotype TT-TT ($P < 0.01$). There were significant differences among the different genotype combinations in ADG ($P = 0.04$) and feed conversion ratio ($P = 0.019$). On the other hand, the T alleles of both SNP were associated with increased ultrasound backfat ($P = 0.005$) and marbling score ($P < 0.001$). The T alleles of both SNP were also associated with increased carcass backfat and marbling and with lower lean yield.

Table 3. Association of UASMS2 and A59V genotype combinations with Various test traits in cattle

Trait ^A	UASMS2 and A59V haplotype						Effect ^B , %	P value ^B
	CCCC	CCCT	CCTT	CTCT	CTTT	TTTT		
<i>Animals</i>	31	127	148	47	99	12	—	—
SLPT	10.37 ^e	12.91 ^{de}	14.10 ^d	14.01 ^d	14.11 ^d	19.39 ^c	8.97	<0.001
ADG	1.36 ^d	1.47 ^c	1.51 ^c	1.46 ^c	1.47 ^c	1.51 ^c	0.87	0.042
FCR	7.89 ^c	7.24 ^d	7.08 ^d	7.49 ^{dc}	7.42 ^{dc}	7.22 ^d	0.50	0.019
UBF	7.82 ^c	8.55 ^{de}	9.42 ^d	9.16 ^d	9.07 ^d	11.38 ^c	6.68	0.005
UMAR	5.15 ^c	5.02 ^e	5.11 ^c	5.26 ^{de}	5.20 ^c	5.55 ^c	6.26	0.001
ULMA	82.32 ^d	84.07 ^{cd}	82.19 ^d	85.71 ^c	82.96 ^d	81.64 ^d	0.91	0.01
<i>Animals</i>	26	109	120	34	84	8	—	—
CGF	9.66 ^e	9.89 ^{de}	10.89 ^d	10.84 ^d	10.55 ^d	13.51 ^c	5.53	<0.001
CMAR	2.42 ^e	2.41 ^e	2.48 ^{de}	2.56 ^{cd}	2.51 ^{cd}	2.72 ^c	4.16	0.01
CREA	84.33 ^{cd}	85.16 ^{cd}	82.60 ^d	86.09 ^c	82.86 ^d	83.70 ^d	4.85	0.01
CYG	1.67	1.57	1.74	1.72	1.65	2.04	4.20	<0.001
LMY	59.07 ^c	58.70 ^{cd}	57.53 ^d	58.08 ^{cd}	57.80 ^d	55.59 ^e	8.92	<0.001

^A SLPT = serum leptin concentration (ng/mL); DMI = dry matter intake ((kg/d); ADG = average daily gain (kg/d); FCR = feed conversion ratio (kg DM/kg gain); UBF = ultrasound backfat (mm); UMAR = ultrasound marbling score; ULMA = ultrasound LM area (cm²); CGF = carcass grade fat (mm); CMAR = carcass marbling; CLMA = carcass LM area (cm²); CYG = carcass yield grade; LMY = lean meat yield (%).

^B P values and haplotype effects are from haplotype regression and expressed as % of total phenotypic variation in the trait.

^{c, d, e} Means in rows followed by different superscripts are different ($P < 0.05$).

CONCLUSION

Two mutations in the leptin gene show associations with serum leptin, performance and carcass merit. These mutations may be useful for marker assisted selection in beef cattle.

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