Polymorphisms in the bovine leptin promoter associated with serum leptin concentration, growth, feed intake, feeding behavior, and measures of carcass merit[1]

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ABSTRACT: Leptin is the hormone product of the obese gene synthesized and secreted predominantly by white adipocytes. It functions as a lipostatic signal regulating BW, food intake, energy expenditure, reproduction, and certain immune system functions. Although previous studies have identified polymorphisms in the coding regions of the leptin gene in cattle that show considerable associations with feed intake, milk quality and quantity, and carcass fatness, no such associations have been reported for the leptin promoter. The current study reports associations between SNP in the 5′ untranslated promoter region of the bovine leptin gene with serum leptin concentration, growth, BW, feed intake, feeding behavior, and carcass merit in hybrid cattle (n = 150). The study showed that animals with the TT genotype of a less frequent cytosine/thymine (C/T) substitution (UASMS2; frequency of thymine allele equals 0.21) detected at position 528 in the bovine leptin promoter (GenBank Accession No. AB070368) show 48 and 39% increases in serum leptin concentration ($P < 0.001$), 39 and 31% increases in backfat thickness ($P < 0.001$), and 13 and 9% increase in marbling score ($P = 0.01$), compared with CC or CT genotypes, respectively. Animals with the TT genotype also show significantly higher feed intake ($P < 0.001$), growth rate, metabolic BW ($P < 0.05$), and live weight at slaughter ($P < 0.10$). Animals with the GG genotype of a more frequent cytosine/guanine (C/G) substitution (UASMS3; frequency of G allele equals 0.59) at position 1759 in the bovine leptin promoter (GenBank Accession No. AB070368) also show higher feed intake ($P = 0.001$), growth rate ($P < 0.10$), and BW ($P < 0.01$). The thymine allele of UASMS2 and the guanine allele of UASMS3 were separately associated with higher feeding duration ($P < 0.05$). The two SNP show significant linkage disequilibrium and could also be relevant in predicting other characteristics, such as milk yield and quality in cattle. These results, however, represent the initial associations of the polymorphisms with these traits, and further efforts are required to validate these findings in other populations.

Key Words: Cattle Performance, Fatness, Leptin Promoter, Serum Leptin

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Introduction

Leptin is the hormone product of the obese gene synthesized and secreted predominantly by white adipocytes (Zhang et al., 1994; Ji et al., 1998). The role of leptin as a lipostatic signal regulating whole-body energy metabolism makes it one of the best physiological markers of BW, food intake, energy expenditure (Houseknecht et al., 1998; Woods et al., 1998), reproduction (Cunningham et al., 1999; Garcia et al., 2002), and certain immune system functions (Lord et al., 1998). Circulating leptin and adipose tissue leptin mRNA levels are correlated with BW, food intake, nutritional status, and adipose tissue mass in humans and animals (Larsson et al., 1998; Delavaud et al., 2002).

The leptin gene has been mapped to bovine chromosome 4 (Stone et al., 1996). Polymorphisms in the coding regions of the leptin gene in cattle have been associated with serum leptin concentration (Liefers et al., 2003a), feed intake (Liefers et al., 2002; Oprzadek et al., 2003), milk yield (Liefers et al., 2002; Buchanan et al., 2003), and body fatness (Buchanan et al., 2002; Nkrumah et al., 2004a). Although previous studies have focused on associations between polymorphisms in the coding regions of the leptin gene and economically important

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traits, studies in humans and other species have shown that polymorphisms in the leptin promoter may be of major importance. This is because such polymorphisms are generally associated with the sequence elements and factors regulating gene expression and may completely abolish the inducibility of the promoter (Miller et al., 1996) or decrease its activity significantly (Mason et al., 1998). The bovine leptin promoter has been sequenced (GenBank Accession No. AB070368) (Taniguchi et al., 2002). The objective of this study was to identify SNP in the leptin promoter and examine associations of such polymorphisms with performance, feed intake, feeding behavior, and ultrasound carcass merit.

Materials and Methods

Animals and Phenotypic Data

One hundred eighty hybrid cattle from an experimental population were used in the study. Detailed information about the animals used in the study and the data collection procedures have been described previously (Nkrumah et al., 2004b). Performance, feed intake, feed efficiency, feeding behavior, and ultrasound data were available on a total of 150 animals (131 steers and 19 bulls). Animals were progeny of the University of Alberta Hybrid dam line produced from more than 10 yr of crosses among three composite cattle lines, namely Beef Synthetic 1, Beef Synthetic 2, and Dairy × Beef Synthetic.

Briefly, Beef Synthetic 1 was composed of approximately 33% Angus and Charolais, approximately 20% Galloway, and the remainder of other beef breeds. The Beef Synthetic 2 composite was made up of approximately 60% Hereford and 40% other beef breeds. The Dairy × Beef Synthetic was composed of approximately 60% dairy breeds (Holstein, Brown Swiss, or Simmental) and approximately 40% beef breeds, mainly Angus and Charolais (Goonewardene et al., 2003). The animals were sired by Angus (n = 64), Charolais (n = 48), or University of Alberta Hybrid (n = 38) bulls. All animals used in the study were born in Spring 2002 and were managed and tested for growth and feed efficiency under feedlot conditions at the University of Alberta's Kinsella beef cattle research ranch. Animals weighed 325 (SD = 41.4) kg and were 248 (SD = 10) d of age at the beginning of the test.

All animals in the study were cared for according to the guidelines of the Canadian Council on Animal Care (CCAC, 1993). Animals were randomly assembled into two contemporary test groups (group 1 consisting of 67 steers and 19 bulls, and group 2 consisting of 64 steers) based on the observed capacity of the testing facility (Basarab et al., 2003). All 19 bulls were tested in the first batch to avoid potential aggressiveness problems towards other animals that were anticipated if bulls were included in the second test group. The animals were fed free choice a backgrounding diet of mainly alfalfa-brome hay with oats supplemented with corn grain and feedlot mineral supplement for approximately 30 d. This was followed by a 30-d pretest adjustment period, in which the proportion of corn in the backgrounding diet was gradually increased to introduce the animals to the test diet and the feeding system; this was done to allow them to adapt to the diet and learn to feed from the testing facility.

During the test period, a total mixed finishing diet composed of (as-fed basis) approximately 80.0% dry-rolled corn, 13.5% alfalfa hay, 5% feedlot supplement (32% CP beef mineral supplement containing Rumensin), and 1.5% canola oil was fed ad libitum. No growth-promoting implants were administered. Feed intake was measured for each animal using the GrowSafe automated feeding system (GrowSafe Systems Ltd., Airdrie, Alberta, Canada), which has been validated and used previously by Schwartzkopf-Genswein et al. (1999) and Basarab et al. (2003). Weight measurements of all animals during and after the test were taken weekly. Feeding, housing, and general management of the animals were as described by Nkrumah et al. (2004b).

Data analyzed include ADG, on-test metabolic midpoint weight (**MWT**), final BW (**FWT**), residual feed intake, feed conversion ratio, and daily DMI. Each animal's ADG during the test was computed as the coefficient of the linear regression of BW (kg) on time (d) using the regression procedure of SAS (SAS Inst., Inc., Cary, NC). The MWT of each animal over the test period was computed as the mid-point $BW^{0.75}$. The total feed intake of each animal over a 70-d test period was used to compute the daily DMI for each animal. Residual feed intake was computed for each animal as the difference between actual DMI and predicted expected daily DMI based on the ADG and MWT over the test period using procedures described by Arthur et al. (2001). Feed conversion ratio was computed as the ratio of DMI to ADG on test.

The behavior traits studied are daily feed bunk attendance and daily feeding duration. Procedures for determining feeding behavior from the GrowSafe System have previously been described (Basarab et al., 2003). Daily feed bunk attendance in this study is defined as the number of independent visits or attendance in a day by a particular animal to a feed bunk, regardless of the duration of the visit. Daily feeding duration was computed as the sum of the difference between feeding event end times and start times per day for each animal. It equals the total minutes in a day spent in feeding-related activities (prehension, chewing, backing away from the bunk and chewing, socializing, scratching or licking) at the feed bunk. Ultrasound measurements of 12th-/13th-rib fat thickness, LM area, and marbling score were taken approximately every 28 d with an Aloka 500V real-time ultrasound machine with a 17 cm, 3.5-MHz linear array transducer (Overseas Monitor Corp., Ltd., Richmond, British Columbia, Canada) using procedures detailed by Brethour (1992).

Blood Sampling, DNA Extraction, and Serum Leptin RIA

A 10-mL blood sample was collected by jugular venipuncture from each animal at the start of the feed efficiency tests from which genomic DNA was extracted using a standard saturated salt phenol/chloroform procedure. At the end of the feed efficiency tests, blood samples were collected from each animal by jugular venipuncture into evacuated tubes. Animals were bled in the morning, and all animals were allowed unrestricted access to feed and water before bleeding. Blood samples were allowed to clot for approximately 18 h at 4°C. Samples were centrifuged at 2,500 × *g* for 30 min, and serum was collected and stored at –20°C until assayed for leptin using the leptin radioimmunoassay described by Delavaud et al. (2000). Intra- and interassay CV for the leptin assays were less than 5%.

SNP Identification and Genotyping

Sixteen animals with extreme phenotypes for feed intake and ultrasound backfat thickness were selected from the population of 150 animals with performance data. Identification of polymorphisms in the bovine leptin promoter used the approximately 3.0-kb 5′ flanking region upstream from the putative transcription start site as reported by Taniguchi et al. (2002) (GenBank Accession No. AB070368). Primers were designed to cover the entire promoter region, and genomic DNA from the panel of 16 selected animals was amplified by PCR in two steps. A single PCR reaction (50 μL) of the first amplification step contained 1× high-fidelity PCR buffer, 250 μ*M* dNTP, 2 m*M* $MgSO_4$, 15 pmol of forward and reverse primers, 1 U of platinum high-fidelity *Taq* DNA polymerase (Invitrogen, Burlington, Ontario, Canada), and 20 to 60 ng of genomic DNA. Amplification was carried out on a GeneAmp 9700 (Applied Biosystems, Streetsville, Ontario, Canada) with the following conditions: 2 min at 94°C, five cycles of 94°C for 60 s, 55°C for 30 s, 68°C 30 s followed by 30 cycles of 94°C, 55°C, and 68°C at 30 s each, and a final step of 68°C for 5 min.

The second amplification step used the same conditions as the first except that nested forward and reverse primers were used, and the template was 2 μL of PCR product from the first amplification. In addition, the second thermal cycling step of five cycles at 94°C for 60 s, 55°C for 30 s, and 68°C for 30 s also was excluded. The PCR products were purified for sequencing by digesting with ExoI and shrimp alkaline phosphatase at 37°C for 15 min, and the enzymes deactivated at 85°C for 15 min. The PCR products from each animal were used for direct sequencing on a Beckman CEQ 8000 genetic analysis system (Beckman Coulter Canada, Inc., Mississauga, Ontario, Cananda). Forward and reverse sequences from each animal were comparatively aligned and analyzed for polymorphisms using the Discovery Studio Gene Version 1.1 DNA sequence analyses software (Accelrys, Inc., San Diego, CA).

The genotyping of each leptin gene-specific SNP was carried out using the 5′ nuclease allelic discrimination assay on an ABI PRISM 7700 sequence detector (Applied Biosystems). Forward and reverse primers (Table 1) were designed to amplify each polymorphism using genomic DNA from each animal. Additionally, two ABI TaqMan fluorogenic probes (with a different reporter dye on each probe) were designed to target the two alleles of each SNP (Table 1). A perfect match of a probe sequence to the target sequence will result in amplification, during which cleavage and release of the reporter dye occurs. A substantial increase in fluorescence signal for one or the other of the two dyes indicates homozygosity for a particular allele of the SNP, whereas an increase in both dyes represents heterozygosity.

A subset of the genotyped animals was sequenced across each polymorphism and the sequence results were used to confirm the genotypes obtained by discrimination assays. In addition to the experimental herd used in the association study, 160 animals from five commercial lines of relatively unrelated cattle (selection lines M1, M2, M3, M4, and TX of Beefbooster, Inc., Calgary, Canada) were also genotyped to confirm the segregation of the SNP and their Mendelian inheritance in the breeds used to create the selection lines. Foundation breeds were Angus for **M1**, Hereford for **M2**, various small breeds for **M3**, Limousin and Gelbvieh for **M4**, and Charolais for **TX** (MacNeil and Newman, 1994.)

Statistical Analyses

The genotype frequencies of each polymorphism were examined for deviations from Hardy-Weinberg equilibrium within both the experimental and commercial populations by χ^2 tests. Tests of linkage disequilibrium between pairwise genotype combinations of the SNP were also performed using χ^2 procedures. Single marker association analyses were carried out to evaluate the relationship between different genotypes of each SNP and serum leptin concentration, growth rate, BW, feed intake, feed efficiency, feeding behavior, and ultrasound traits of the animals in the experimental population. Data were analyzed using the Mixed procedure of SAS. The statistical model used included fixed effects of SNP genotype (three genotype classes per SNP), breed of sire (Angus, Charolais, or Hybrid), contemporary group (groups one and two), sex of animal (bull and steer), all possible two-way interactions among fixed effects, and age of dam and age of animal as covariates. The residual effect was the random term in the model, and tests of fixed effects of genotype were carried out with the residual as error term and account for multiple testing within SNP.

All interactions that were not significant ($P > 0.20$) for a trait were dropped from the final model. Additive and nonadditive genetic effects were estimated for traits that were different ($P < 0.05$) between animals with different SNP genotypes. Additive genetic effects

Table 1. Primer and probe sequences (5′ to 3′) for genotyping each polymorphism

SNP[a]	Forward primer	Reverse primer	Probe 1[b]	Probe 2[b]
UASMS1	ggcacaatcctgtgtattggtaaga	gtccatgtaccattgcccaatttt	ctttcacctagtat**a**tctag	tctttcacctagtat**g**tctag
UASMS2	aggtgcccagggactca	caacaaaggccgtgtgaca	caagctctagagcct**g**tgt	aagctctagagcct**a**tgt
UASMS3	atgtatattttggtgtgagagtgtgtgt	agctggaaagaacggattataaaatggt	cacacatt**c**caatcaa	cacatt**g**caatcaa

[a]UASMS1 (cytosine [C]/thymine [T] substitution), UASMS2 (C/T substitution) and UASMS3 (C/guanine [G] substitution) are located at positions 207, 528 and 1759, respectively, of the bovine leptin promoter according to GenBank Accession No. AB070368.
[b]Probe nucleotides in bold target the specific alternative alleles of a particular SNP.

were computed as the difference between the solutions of the estimate for the trait effect of the two homozygous genotypes. Dominance deviation was computed by subtracting the average of solutions of the estimate for the trait effect for homozygous genotypes from that of the heterozygote genotype.

Results

The analysis identified three new single nucleotide polymorphisms, namely UASMS1, UASMS2, and UASMS3, located at positions 207 (cytosine [**C**]/thymine [**T**] substitution), 528 (C/T substitution) and 1759 (C/guanine [**G**] substitution), respectively, in the bovine leptin promoter (GenBank Accession No. AB070368). Observations of the genotypes for animals in both the experimental and commercial populations revealed that all animals that had genotypes CC, CT, or TT for UASMS1 also had genotypes CC, CG, or GG for UASMS3, respectively. The UASMS1 SNP was not analyzed further as its Mendelian inheritance and trait associations were identical to that of UASMS3. Table 2 shows the genotype and allele frequencies of UASMS2 and UASMS3 in the experimental and commercial populations of cattle.

The frequency of the T allele of UASMS2 was 21 and 20%, and that of the G allele of UASMS3 was 59 and 48% in the experimental and commercial populations, respectively. The genotype frequencies of the two SNP were distributed according to Hardy-Weinberg equilibrium proportions in both populations ($P > 0.10$). Pairwise comparison of genotypes of UASMS2 and UASMS3 revealed the existence of significant linkage disequilibrium between the SNP (i.e., certain pairwise genotype combinations were observed more than expected; $P = 0.002$). Allele frequencies of UASMS2 ($\chi^2 = 5.71$; $P < 0.05$) and UASMS3 ($\chi^2 = 9.17$; $P = 0.01$) differed among the different selection lines of the commercial population. Frequency of the T allele of UASMS2 was lower for the M1 line compared with the M2 line ($P < 0.05$), M3 line ($P < 0.10$), and TX line ($P < 0.05$). The G allele of UASMS3 was lower in the M1 line compared with the TX (Charolais; $P < 0.004$), M2 line ($P < 0.10$), M3 line ($P < 0.02$), and M4 line ($P < 0.04$). Differences in allele frequencies among the other lines were not significant ($P > 0.10$).

The overall averages and standard deviations for the traits tested are presented in Table 3. Most of the traits tested showed considerable variation among the animals, which gave desirable ranges for comparing them among animals with different genotypes. The effects of UASMS2 and UASMS3 genotype on the traits analyzed are presented in Tables 4 and 5, respectively, whereas Table 6 shows estimates of the additive or dominance

Table 2. Genotype and allele frequencies of the various single nucleotide polymorphisms in an experimental population and in different lines of a commercial population of cattle

	Genotype frequencies						Allele frequencies	
	UASMS2[a]			UASMS3[b]			UASMS2[a]	UASMS3[b]
Population	CC	CT	TT	CC	CG	GG	T allele	G allele
Experimental[c]	0.63	0.32	0.05	0.18	0.46	0.36	0.21	0.59
Commercial[d]	0.63	0.34	0.03	0.26	0.52	0.22	0.20	0.48
M1 line	0.77	0.23	0.00	0.42	0.52	0.06	0.11^{x}	0.32^{x}
M2 line	0.58	0.33	0.09	0.24	0.58	0.18	0.26^{y}	0.47^{y}
M3 line	0.58	0.39	0.03	0.23	0.48	0.29	0.23^{y}	0.53^{yz}
M4 line	0.70	0.27	0.03	0.21	0.58	0.21	0.17^{xy}	0.50^{yz}
TX line	0.50	0.50	0.00	0.19	0.47	0.34	0.25^{y}	0.58^{z}

[a]UASMS2 located at position 528 (cytosine [C]/thymine [T] substitution) in the bovine leptin promoter according to GenBank Accession No. AB070368.
[b]UASMS3 is located at position 1759 (C/guanine [G] substitution) in the bovine leptin promoter according to GenBank Accession No. AB070368.
[c]University of Alberta hybrid cattle.
[d]Beefbooster, Inc., Calgary, Alberta, Canada.
[x,y,z]Within the commercial populations in a column, frequencies without a common superscript letter differ, $P < 0.05$.

Table 3. Overall means (n = 150) and standard deviations of traits analyzed in the experimental population of hybrid cattle

Trait	Mean	SD
Serum leptin concentration, ng/mL	14.99	6.60
ADG, kg/d	1.42	0.25
Metabolic BW, $kg^{0.75}$	86.67	10.21
Final BW, kg	506.21	55.33
DMI, kg/d	10.29	1.62
Feed:gain	7.27	1.00
Residual feed intake, kg/d	0.00	0.83
Daily feeding duration, min/d	57.05	10.38
Daily feed bunk attendance, events/d	31.00	6.15
Ultrasound backfat thickness, mm	7.11	2.63
Ultrasound marbling score	5.01	1.64
LM area, cm^2	72.44	6.88

deviation of each of the two SNP for traits showing significant differences among genotypes. The T allele of UASMS2 was significantly associated with serum leptin concentration ($P < 0.001$) and showed significant additive and dominance effects (Table 6). Serum leptin concentration was higher for animals with genotype TT than for those with genotype CC ($P < 0.001$). Serum leptin also was higher ($P = 0.04$) in CT animals than in CC animals. During the performance and efficiency testing period, MWT differed among genotypes of UASMS2 ($P = 0.05$) and was higher for animals with genotype TT than for CC. Average daily gain differed ($P = 0.002$) among genotypes and was higher for animals with genotype TT and CT than for animals with genotype CC. Animals with the TT genotype of UASMS2 also had higher FWT compared with genotype CT or CC ($P = 0.10$).

During the testing period, animals with TT and CT genotypes for UASMS2 consumed more DM daily than CC animals ($P = 0.001$). Ultrasound backfat thickness was higher ($P < 0.001$) for animals with the T allele of UASMS2 than for animals with the C allele. Similarly, the T allele of UASMS2 was associated with higher ultrasound marbling score ($P = 0.01$) compared with the C allele. The significant increase in body fatness in animals with the T allele of UASMS2 was associated with slight decreases in ultrasound LM area, although these differences were not statistically significant. Daily feeding duration was higher ($P = 0.02$) in animals heterozygous for UASMS2, but it did not differ between TT and CC animals. The number of daily feed bunk attendances, however, did not differ among genotypes of UASMS2. Residual feed intake tended to differ ($P = 0.06$) among UASMS2 genotypes and was less in CT animals than in the homozygotes. Feed conversion ratio, however, did not differ among genotypes of UASMS2.

As shown in Tables 5 and 6, genotypes of UASMS3 also showed associations with several of the traits considered in the study. Animals with genotype GG consumed more feed on test ($P < 0.001$), tended to have higher residual feed intake ($P = 0.09$), were heavier during the test (i.e., higher MWT; $P = 0.02$), tended to grow faster ($P = 0.08$), and had a greater FWT ($P = 0.04$) than CG or CC animals. Feeding duration was higher ($P = 0.005$), whereas the number of feed bunk attendance events was lower ($P = 0.09$) for GG animals than for animals with other genotypes of UASMS3. Backfat thickness was also higher ($P = 0.04$) in GG animals than in CC or CG animals; however, animals with different genotypes of UASMS3 did not differ in

Table 4. Association of UASMS2 genotypes (least squares means ± SE) with measures of serum leptin concentration, performance, feeding behavior, and carcass merit of hybrid cattle

	UASMS2 genotype[a]			
Trait	CC	CT	TT	*P*-value[b]
No. of animals	99	45	6	
Serum leptin, performance, feed efficiency, and behavior				
Serum leptin, ng/mL	11.97 ± 0.82^z	14.10 ± 0.96^y	23.22 ± 2.44^x	<0.001
Metabolic BW, $kg^{0.75}$	85.13 ± 1.60^y	87.51 ± 1.77^y	91.40 ± 2.26^x	0.05
ADG, kg/d	1.33 ± 0.03^y	1.47 ± 0.04^x	1.42 ± 0.10^x	0.002
Final BW, kg	483.12 ± 11.6^z	499.69 ± 12.7^y	527.71 ± 23.51^x	0.10
DMI, kg/d	9.62 ± 0.20^y	10.53 ± 0.21^x	10.19 ± 0.53^x	0.001
Residual feed intake, kg/d	–0.43 ± 0.19^x	–0.08 ± 0.21^y	–0.63 ± 0.38^x	0.06
Feed:gain	7.36 ± 0.15	7.25 ± 0.16	7.28 ± 0.41	0.81
Feeding duration, min/d	51.54 ± 2.34^y	56.95 ± 2.58^x	52.38 ± 4.77^y	0.02
Feed bunk attendance, events/d	36.48 ± 1.56	34.78 ± 1.71	32.37 ± 3.16	0.22
Ultrasound measurements				
Backfat thickness, mm	5.59 ± 0.48^z	6.30 ± 0.51^y	9.19 ± 0.91^x	0.001
Marbling score	4.63 ± 0.12^z	4.82 ± 0.13^y	5.30 ± 0.25^x	0.01
LM area, cm^2	73.05 ± 1.45	72.17 ± 1.60	70.03 ± 2.94	0.40

[a]UASMS2 polymorphism is a cytosine (C)/thymine (T) substitution located at position 528 of the bovine leptin promoter according to GenBank Accession No. AB070368.
[b]Testing differences among different single nucleotide polymorphism genotypes in trait means.
[x,y,z]Least squares means within a row without a common superscript letter differ, $P < 0.05$.

Table 5. Association of UASMS3 genotypes (least squares means ± SE) with measures of serum leptin concentration, performance, feeding behavior, and carcass merit of hybrid cattle

	UASMS3 genotype[a]			
Trait	CC	CG	GG	*P*-value[b]
No. of animals	27	68	55	
Serum leptin, performance, feed efficiency, and behavior				
Serum leptin, ng/mL	12.16 ± 1.41	12.13 ± 0.96	13.65 ± 0.99	0.30
Metabolic mid-weight, $kg^{0.75}$	83.59 ± 2.08^{y}	85.05 ± 1.58^{y}	87.75 ± 1.71^{x}	0.02
ADG, kg/d	1.26 ± 0.07^{z}	1.38 ± 0.04^{y}	1.42 ± 0.04^{x}	0.08
Final BW, kg	475.82 ± 15.01^{y}	480.74 ± 11.41^{y}	500.70 ± 12.38^{x}	0.04
DMI, kg/d	9.10 ± 0.31^{z}	9.6 ± 0.30	10.1 ± 0.33	0.007
Residual feed intake, kg/d	–0.59 ± 0.25^{x}	–0.37 ± 0.19^{xy}	–0.16 ± 0.21^{y}	0.09
Feed:gain	7.26 ± 0.29	7.19 ± 0.22	7.48 ± 0.24	0.29
Feeding duration, min/d	48.99 ± 3.06^{z}	51.96 ± 2.33^{y}	56.72 ± 2.53^{x}	0.005
Feed bunk attendance, events/d	39.16 ± 1.56^{x}	36.24 ± 1.07^{y}	35.96 ± 1.10^{y}	0.09
Ultrasound measurements				
Backfat thickness, mm	5.73 ± 0.56^{y}	5.29 ± 0.38^{y}	6.39 ± 0.39^{x}	0.04
Marbling score	4.56 ± 0.16	4.60 ± 0.12	4.72 ± 0.13	0.36
LM area, cm^2	73.34 ± 1.90	72.36 ± 1.45	73.35 ± 1.58	0.64

[a]UASMS3 polymorphism is a cytosine (C)/guanine (G) substitution located at position 1759 of the bovine leptin promoter according to GenBank Accession No. AB070368.
[b]Testing differences among different single nucleotide polymorphism genotypes in trait means.
[x,y,z]Least squares means within a row without a common superscript letter differ, $P < 0.05$.

serum leptin concentration, feed conversion ratio, marbling score, or LM area.

Discussion

The present study detected three bi-allelic single nucleotide substitutions, namely UASMS1, UASMS2, and UASMS3, in the bovine leptin promoter located at nucleotide positions 207, 528, and 1,759 (according to GenBank database Accession No. AB070368), respectively. Our analysis revealed significant linkage disequilibrium between UASMS2 and UASMS3, and both SNP separately show associations with performance, feed intake, and feeding behavior in the experimental cattle population. In addition, the UASMS2 SNP shows significant associations with serum leptin concentration and measures of body fatness.

With respect to UASMS2, the results presented showed that serum leptin concentration was 48 and 39% higher for TT animals than for CC or CT animals, respectively. Daily DMI was 6 and 5.7% lower by CC animals than by CT or TT animals, respectively, and ADG was 9 and 6% greater for CT and TT animals than for CC animals, respectively. Final BW was 17 kg and 38 kg higher in TT animals than CT or CC animals, respectively, and ultrasound backfat thickness was 39 and 31% higher for TT animals than for CC or CT animals, respectively. Additionally, marbling score was

Table 6. Additive effect (*a*) and dominance deviation (*d*) of UASMS2 and UASMS3 on traits that showed differences ($P < 0.10$) among genotypes

	UASMS2		UASMS3	
Traits[a]	*a* ± SE	*d* ± SE	*a* ± SE	*d* ± SE
SLPT, ng/mL	–11.24 ± 2.43***	–4.49 ± 1.48**	—	—
ADG, kg/d	–0.10 ± 0.04*	0.11 ± 0.06	–0.12 ± 0.05*	0.03 ± 0.04
MWT, $kg^{0.75}$	–5.94 ± 2.98*	–0.79 ± 1.85	–4.16 ± 1.67**	–0.78 ± 1.15
FWT, kg	–34.07 ± 21.56*	–9.28 ± 13.07	–24.96 ± 12.07**	–8.69 ± 8.28
DMI, kg/d	–0.39 ± 0.57	0.59 ± 0.24*	–1.01 ± 0.32***	–0.01 ± 0.22
RFI, kg/d	0.24 ± 0.34	0.47 ± 0.20*	–0.43 ± 0.20*	–0.002 ± 0.14
DUR, min/d	–0.054 ± 4.34	5.31 ± 2.63*	–7.73 ± 2.45**	–0.92 ± 1.68
DFA, events/d	—	—	3.19 ± 1.60*	–1.32 ± 1.13
UBF, mm	–3.58 ± 0.92***	–1.10 ± 0.56	–0.66 ± 0.57	–0.76 ± 0.4*
MAR	–0.68 ± 0.22**	–0.34 ± 0.14**	—	—

[a]SLPT = serum leptin concentration; FWT = final BW; UBF = backfat thickness; MAR = marbling score; MWT = metabolic midpoint weight; RFI = residual feed intake; DUR = daily feeding duration; and DFA = daily feed bunk attendance.
*$P < 0.05$.
**$P < 0.01$.
***$P < 0.001$.

also 13 and 9% higher for TT animals than for CC or CT animals, respectively. Significant differences were also detected in feeding behavior among the different genotypes of UASMS2. Correspondingly, animals with the GG genotype of UASMS3 (compared with CG or CC, respectively) had higher MWT (3 and 5%), higher FWT (4 and 5%), higher growth rate (3 and 11%), increased daily DMI (5 and 10%), and higher feeding duration (8 and 14%).

The significant linkage disequilibrium between the two polymorphisms is not surprising as the two SNP are only 1,231 bp apart, and it suggests that the effect of one of the SNP may reflect an indirect effect of the other. Indeed, the effect of the polymorphisms on performance seems to be similar (that is both affect feed intake, BW, and growth). However, because UASMS2 shows stronger associations with serum leptin concentration and body fatness, it may be speculated that UASMS2 may be more functionally significant. Significantly higher growth rates, BW, and body fatness of animals with higher serum leptin concentration and genotype TT for UASMS2 is consistent with previous evidence in humans (Larsson et al., 1998) and cattle (Geary et al., 2003; Liefers et al., 2003a) showing that serum leptin concentration is positively related to BW and body fatness.

Nonetheless, the observed higher daily feed consumption of animals with higher serum leptin concentration and genotype TT for UASMS2 (as well as previous evidence in dairy cattle indicating a positive relationship between serum leptin concentration and feed intake) (Liefers et al., 2003a) is surprising and in contrast to evidence in humans (Larsson et al., 1998). The latter authors reported that higher serum leptin concentration was associated with lower habitual food consumption in humans. However, underestimation of self-reported food intake, which may be as high as 20% or more in obese human subjects (Lichtman et al., 1992), could partially explain the negative correlation between leptin and food intake in humans, as leptin levels are generally higher in obese subjects. The phenotypic correlation between serum leptin concentration and daily DMI in the present study was positive ($r = 0.26$; $P < 0.01$; data not shown).

There is considerable interest in the application of molecular technologies in the form of specific DNA markers that are associated with various QTL to promote more efficient and relatively easy selection and breeding of farm animals with an advantage for an inheritable trait of growth rate, BW, carcass merit, feed intake, and milk yield and composition (Spelman and Bovenhuis, 1998). Several QTL for performance and meat production in cattle have been identified, and a number of potential candidate genes have been identified and selected for analyses based on a known relationship with physiological or biochemical processes and production traits. It is seldom reported that one particular polymorphism in a candidate gene would influence several traits of economic importance in livestock at the same time. The leptin gene, however, seems to be one of the exceptions, as its involvement in the regulation of several biologically important processes in the body makes it, perhaps, one of the best physiological gauges for energy balance, BW, and body fat content in mammals. Several studies have been conducted to characterize the relationship of circulating leptin with traits of economic importance in beef cattle. For example, Ehrhardt et al. (2000) and Delavaud et al. (2002) showed that circulating leptin levels are correlated with BW, food intake, nutritional status, and adipose tissue mass. Circulating leptin concentrations are also correlated with the regional distribution of body fat (Yamada et al., 2003) and could be used as a predictor of carcass merit in cattle (Geary et al., 2003).

Several polymorphisms have been described in the bovine leptin gene (Pomp et al., 1997; Konfortov et al., 1999; Haegeman et al., 2000). Fitzsimmons et al. (1998) reported a positive association between a microsatellite marker (*BM1500*; located approximately 3.6 kb away from the leptin gene) and body fatness in cattle. Polymorphisms have also been reported in the coding regions of the leptin gene that show considerable associations with feed intake (Liefers et al., 2002; Lagonigro et al., 2003; Oprzadek et al., 2003), carcass merit (Buchanan et al., 2002; Nkrumah et al., 2004a), milk quantity and quality (Buchanan et al., 2003; Liefers et al., 2003b), and serum leptin (Liefers et al., 2003b) in cattle.

The exact molecular and physiological mechanisms underlying the association of the polymorphisms with the variety of traits reported in the present study are unknown. The possible functionality of the promoter variants can only be appreciated from in vivo and in vitro experiments. The association of UASMS2 with serum leptin concentration as well as body fatness provides indirect in vivo evidence on the potential biological role of the UASMS2 mutation. It is speculated that the location of the present SNP, especially UASMS2, in the regulatory region of the leptin gene makes them potential regulators of leptin expression in cattle or serve as surrogates for causative SNP that are yet to be detected. Several putative Sp1, CCAAT/enhancer binding protein (**C/EBP**), and TATA box binding sequences were detected in the vicinity of the SNP reported in the present study (Taniguchi et al., 2002). Studies in humans have shown that mutations in the C/EBP-α region of the leptin promoter abolished the inducibility of the promoter by C/EBP-α (Miller et al., 1996). Mason et al. (1998) showed that mutations in the C/EBP-α and TATA motifs, as well as in a consensus Sp1 site of leptin, decreased promoter activity by 10-, 10-, and 2.5-fold, respectively, and abolished binding of these factors.

In addition, a common promoter variant of the human leptin gene has been shown to be significantly associated with serum leptin concentration in obese girls (Le Stunff et al., 2000). The SNP reported in the present study may be associated with other characteristics such as milk yield and composition due to the associations

with BW, feed intake, body fatness, and circulating leptin concentrations. The present study was conducted to independently test the association of different gene-specific polymorphisms with traits that are obviously correlated. The appropriateness of correction for multiple testing in this case is unclear and currently controversial (Perneger, 1998). A conservative criterion to control for multiple testing has therefore not been applied to the results presented here. However, if a highly conservative method of correction, such as the Bonferroni adjustment, is applied to the number of independent tests carried out, certain of the significant associations detected may no longer be significant, whereas others would remain unchanged.

Implications

Gene-specific single nucleotide polymorphisms in the regulatory region (promoter) of the bovine leptin gene were detected that show associations with serum leptin concentration, growth rate, body weight, feed intake, feeding behavior, and measures of carcass merit. The polymorphisms may also be predictive of other important traits in cattle such as milk yield and composition. These results represent the initial associations of the polymorphisms with these traits and further efforts are required to validate these findings in other populations before their application in marker-assisted selection.

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