Association of a single nucleotide polymorphism in the bovine leptin gene with feed intake, feed efficiency, growth, feeding behaviour, carcass quality and body composition

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Nkrumah, J. D., Li, C., Basarab, J. B., Guercio, S., Meng, Y., Murdoch, B., Hansen, C. and Moore, S. S. 2004. **Association of a single nucleotide polymorphism in the bovine leptin gene with feed intake, feed efficiency, growth, feeding behaviour, carcass quality and body composition**. Can. J. Anim. Sci. **84**: 211–219. Leptin is a 16-kDa-hormone product of the obese gene synthesized and expressed predominantly by adipose tissues, which has been shown to play major roles in the regulation of body weight, feed intake, energy balance, fertility, and immune system functions. We report an investigation into the association of a previously identified cytosine to thymine missense mutation in exon 2 of the bovine leptin gene with feed intake, feed efficiency, growth, feeding behaviour, carcass quality and body composition in five genetic selection lines of a commercial population of beef cattle. Differences among genotypes in growth, feed intake and feed efficiency were not significant ($P > 0.10$) though steers homozygous for the thymine allele had positive residual feed intake (i.e., consumed more feed than expected) (+ 0.19 kg d^{-1}) whilst steers homozygous for the cytosine allele had negative residual feed intake (–0.18 kg d^{-1}). Steers carrying the thymine allele had a higher rate of gain in ultrasound backfat ($P = 0.02$), ultrasound backfat thickness ($P = 0.06$), higher carcass grade fat (backfat) ($P = 0.005$), lower yield grade ($P = 0.01$) and lower lean meat yield ($P = 0.007$). The thymine allele also tended to be associated with higher loin subcutaneous fat ($P = 0.07$) and was associated with higher brisket subcutaneous fat ($P = 0.01$), and brisket body cavity fat ($P = 0.0001$). No associations were found among the different genotypes and real-time ultrasound marbling, ultrasound longissimus thoracis area, carcass marbling and carcass longissimus thoracis area ($P > 0.10$). Our results show that animals carrying the thymine allele versus the cytosine allele may produce carcasses with poorer grades and lower lean meat yields but do not differ in carcass marbling or other fat depots.

Key words: Beef cattle, leptin, feed intake, carcass merit

Nkrumah, J. D., Li, C., Basarab, J. B., Guercio, S., Meng, Y., Murdoch, B., Hansen, C. et Moore, S. S. 2004. **Association du polymorphisme d'un nucléotide du gène de la leptine bovine à la prise alimentaire, à la valorisation des aliments, à la croissance, aux habitudes alimentaires, à la qualité de la carcasse et à la composition corporelle**. Can. J. Anim. Sci. **84**: 211–219. La leptine est un dérivé hormonal 16-kDa du gène de l'obésité synthétisé et principalement exprimé par les tissus adipeux. On sait qu'elle joue un rôle important dans la régulation du poids, la prise d'aliments, le bilan énergétique, la fécondité et les fonctions du système immun. Les auteurs ont étudié l'association d'une mutation faux-sens entre la cytosine et la thymine rapportée antérieurement dans la partie exon 2 du gène de la leptine bovine avec la prise alimentaire, la valorisation des aliments, la croissance, les habitudes alimentaires, la qualité de la carcasse et la composition corporelle de cinq lignées d'une population commerciale de bovins de boucherie. Le génotype ne varie pas de manière significative ($P > 0,10$) pour la croissance, la prise alimentaire et la valorisation des aliments bien que les sujets homozygotes pour l'allèle de la thymine se caractérisent par une prise d'aliments résiduelle positive (à savoir, ils mangent plus que prévu) (+ 0,19 kg par jour) alors que les homozygotes pour l'allèle de la cytosine se trouvent dans la situation inverse (–0,18 kg par jour). Les bouvillons portant l'allèle de la thymine présentent un taux de gain supérieur pour le gras dorsal mesuré aux ultrasons ($P < 0,02$), une couche de gras dorsal mesurée aux ultrasons plus épaisse ($P < 0,06$), une meilleure couche de gras au classement (gras dorsal) ($P < 0,005$), un rendement plus faible au classement ($P < 0,01$) et un rendement plus faible en viande maigre ($P < 0,007$). L'allèle de la thymine a aussi tendance à être associé à une plus grande quantité de graisse sous-cutanée dans la longe ($P < 0,07$) et dans la pointe de poitrine ($P < 0,01$) ainsi qu'avec une quantité supérieure de graisse viscérale au niveau de la pointe de poitrine ($P < 0,0001$). Les auteurs n'ont relevé aucune association entre les divers génotypes et le persillé mesuré en temps réel aux ultrasons, la superficie du *Longissimus thoraci* mesurée aux ultrasons, le persillé réel de la carcasse et la superficie réelle du *Longissimus thoraci* ($P > 0,50$). Les résultats indiquent que les animaux portant l'allèle de la thymine pourraient donner une carcasse de catégorie inférieure à celle des animaux portant l'allèle de la cytosine, et se caractériser par un rendement plus faible en viande maigre, même si le persillé et les autres dépôts adipeux ne varient pas.

Mots clés: Bovins de boucherie, leptine, prise alimentaire, valeur de la carcasse

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Abbreviations: **DM**, dry matter; **MAS**, marker assisted selection; **ME**, metabolizable energy; **SNP**, single nucleotide polymorphism

Leptin is a 16-kDa cytokine-like hormone product of the obese gene (Zhang et al. 1994) thought to be an essential physiological factor in the lipostatic regulation of body weight and in nutritive status signalling in mammals (Woods et al. 1998). Leptin is synthesized and expressed predominantly by adipocytes (Houseknecht et al. 1998). It functions as a potent efferent regulator through both central and peripheral pathways to affect body weight, feed intake, feeding behaviour, energy expenditure, fertility, and immune system functions (Baile et al. 2000; Rahmouni and Haynes, 2001).

Research data from swine (Robert et al. 1998; Ramsay et al. 1998), sheep (Boquier et al. 1998; Kumar et al. 1998), and cattle (Delavaud et al. 2002; Ren et al. 2002; Geary et al. 2003) indicate that plasma and serum leptin concentrations and tissue mRNA levels are highly correlated with intake, carcass quality as well as adipose tissue mass. For instance, in pigs, Robert et al. (1998) reported a positive association between leptin mRNA levels and backfat thickness. Similarly, Daniel et al. (2002) showed that plasma leptin concentrations in ewes are influenced by nutritive state and rib fat thickness. Geary et al. (2003) reported positive correlations between serum leptin concentrations and marbling score, backfat thickness, quality grade, and yield grade and a negative correlation between serum leptin concentration and longissimus muscle area.

Polymorphisms in the leptin gene have been shown to be associated with body fatness in mice (Hamann and Mathaei 1996) and humans (Oshiro et al. 2000). Similarly, Jiang and Gibson (1999) have described polymorphisms in the swine leptin gene that are associated with body composition, especially with body fat. Several polymorphisms have been described in the bovine leptin gene (Pomp et al. 1997; Fitzsimmons et al. 1998; Haegeman et al. 2000). In addition, Fitzsimmons et al. (1998) reported a positive association between a microsatellite marker (*BM1500*), [located ~3.6 kb away from the leptin gene on bovine chromosome 4 (Stone et al. 1996a, b)], and body fatness in cattle.

In dairy cattle, Liefers et al. (2002) showed positive tendencies and associations between different polymorphisms in the leptin gene and feed intake, milk yield and composition. Recently, a cytosine (C) to thymine (T) mutation in exon 2 of the bovine leptin gene that encoded an amino acid change of arginine to cysteine has been proposed as a causative mutation imparting a potential partial loss of biological function that is associated with fatter carcasses and higher leptin mRNA levels (Buchanan et al., 2002).

Results presented so far do not indicate the specific effect of this leptin exon 2 single nucleotide polymorphism (SNP) on carcass and meat quality. As well, data on the potential implications of this finding on overall animal performance and production system efficiency are lacking. We hypothesized that polymorphisms in the leptin gene may be associated with differences in animal performance, efficiency and body composition. Our aim therefore is to study the segregation of the leptin exon 2 SNP reported by Buchanan et al. (2002) in five genetic selection lines of a commercial population of beef cattle and to examine its associations with feed efficiency, feed intake, growth, feeding behaviour and different measures of carcass quality and body composition.

MATERIALS AND METHODS

Animals and Phenotypic Data

Detailed information about the animals used in the study and the data collection procedures have been described previously (Basarab et al. 2003). Briefly, phenotypic data were collected in a 2-yr serial slaughter experiment on 7- to 8-mo-old crossbred steers at the Agriculture and Agri-Food Canada, Lacombe Research Centre. The genetic structure of the animals in each year group consisted of animals from each of the five BeefBooster genetic selection lines (M1, M2, M3, M4, and TX). Foundation breed(s) were Angus for M1, Hereford for M2, various small breeds for M3, Limousin and Gelbvieh for M4, and Charolais for TX (Kress et al. 1996). MacNeil and Newman (1994) have described indices used as the selection criteria along with independent culling levels for birth weight, weaning weight, post-weaning rate of gain, scrotal circumference and feet, leg and disposition scores for the selection lines.

Animals were cared for according to the guidelines set by the Canadian Council on Animal Care (CCAC 1993). In all, a total of 144 animals and their phenotypic records from the 2-yr serial slaughter experiment were used in this study. Specifically, 73 steers from the study in year 1 (13 from M1 line and 15 each from the M2, M3, M4 and TX lines) and 71 steers from the study in year 2 (13 each from the M1 and M4 lines and 15 each from M2, M3 and TX lines) were used. A 10-mL blood sample was collected from each animal at start of the feed intake test from which genomic DNA was extracted and used in genotyping each animal.

Traits Analyzed

Detailed information as well as the relationships between the phenotypic data have been published (Basarab et al. 2003). Briefly, residual feed intake is a feed efficiency trait measured as the difference between an animal's actual feed intake and the expected intake of the same animal based on its growth rate and body size. Dry matter (DM) intake of each animal was calculated from the total feed intake of each animal (75.1% DM). Metabolizable energy (ME) intake was determined from the DM intake of each animal by multiplying DM intake by ME content of the diet (11.77 and 11.16 MJ ME kg^{-1} DM in years 1 and 2, respectively). Average daily gain and metabolic midweight were computed from the growth curve of each animal modelled over time. Feed-to-gain ratio was calculated by dividing intake by average daily gain. Ultrasound longissimus thoracis area, backfat thickness and marbling score were taken with an Aloka 500V real-time ultrasound with a 17-cm, 3.5-MHz linear array transducer (Overseas Monitor Corporation Ltd., Richmond, BC). Carcass traits were obtained as standard Canadian meat industry carcass measurements (Agriculture Canada 1992). Final real-time ultrasound measurements were taken just before slaughter. Measurement and/or determination of body composition have been thoroughly described (Basarab et al. 2003).

Marker Information and Genotyping

The genotyping of the leptin exon 2 gene-specific SNP was carried out using an ABI PRISM™ 7700 sequence detector based on allelic discrimination using the 5' nuclease assay (Applied Biosystems). A forward primer [5' ggctttggccctatctgtcttac 3'] and a reverse primer [5' cttgatgagggttttggtgtca 3'] were designed to flank the thymine to cytosine single nucleotide polymorphism in exon 2 of the genomic *Bos taurus* leptin gene sequence as described by Buchanan et al. (2002). The original sequence of the bovine leptin gene was obtained from GenBank (Accession No. U50365). Additionally, two ABI TaqMan® fluorogenic probes were designed to target the two alleles of the SNP, with VIC™ reporter dye for allele T and FAM™ reporter dye for allele C. The sequences of the TaqMan® fluorogenic probes designed to target alleles T and C were [5' ccttgc**a**gatggg 3'] and [5' ccttgc**g**gatggg 3'], respectively.

Amplification reactions were monitored in real-time and a perfect probe-target match and subsequent annealing will result in the cleavage and release of the reporter dye during amplification. Data acquisition during the assay is based on fluorescence resonance energy transfer (FRET). Thus, a substantial increase in fluorescence signal for either the VIC or FAM dye indicates homozygosity for the VIC-specific allele (allele T) or the FAM-specific allele (allele C), respectively. An increase in both VIC and FAM signals above a specified threshold indicates heterozygosity. The real-time amplification plot of DNA from each animal was used to confirm its marker genotype.

Statistical Analysis

All statistical analyses of the data were carried out with SAS (version 8.1) (SAS Institute, Inc. 1999). The Categorical Model (CATMOD) Procedure of SAS was used to test the differences among selection lines in allele frequency of the mutation. The same model was used to test the deviations of genotype frequencies within each selection line from the expectations. The General Linear Model (GLM) Procedure of SAS was used to test the associations among different SNP genotypes and feed intake, feed efficiency, growth, real-time ultrasound measurements, feeding behaviour, carcass traits, and body composition. The data were subjected to an analysis of covariance with fixed effects of year of study (two levels), fixed effect of selection line (M1, M2, M3, M4 and TX), herd of origin nested within selection line and year, fixed effect of SNP genotype (CC, CT and TT), interactions between fixed effects, linear covariates of initial weight of animal, days on test and age of dam and residual error.

Each trait was tested for the effect of the mutation using the above model independently of the other traits in the study. The Mixed Model Procedure of SAS was also used to test the effects of the mutation on each individual trait using the above factors. In each case, the results from the Mixed Model Procedure were not different from the results from the GLM Procedure so only results from the GLM Procedure are reported. The residual error was used as the error term to test the effect of all fixed factors in the model except the effect of selection line, which was tested with herd of origin nested within selection line and year as error term. The initial statistical model included the effect of age of dam, but dam age was excluded from the final model, as it had no significant effect on any of the traits analyzed ($P > 0.20$). All *F*-tests were carried out using Type III sums of squares. Multiple comparisons of trait means for SNP genotypes were analyzed by least squares. Additive genotypic values and dominance deviations were estimated for traits that were or tended to be significantly different ($P < 0.10$) among different SNP genotypes. We estimated additive genotypic value as half the difference between the genotypic values of the TT and CC genotypes. We also estimated dominance deviation as the deviation of the CT genotypic value from the midpoint between the TT and CC genotypic values (Falconer and Mackay 1996).

RESULTS

Frequencies of the three genotypes (TT, CT, and CC) were approximately distributed according to Hardy-Weinberg proportions in the BeefBooster selection lines used in the study. Observed genotype frequencies for all selection lines did not differ from the expectations ($P > 0.10$) (data not shown). Allele frequencies of the leptin SNP for various selection lines are listed in Table 1. There were differences in allele frequencies among selection lines ($\chi^2 = 10.96$, $P < 0.02$). The Angus-based selection line (M1) had a higher frequency of the thymine allele compared to lines based on various small breeds (M3) ($P < 0.02$), Gelbvieh and Limousin (M4) ($P < 0.01$) and Charolais (TX) ($P < 0.002$), but not to the Hereford-based line (M2) ($P > 0.05$). The Charolais-based selection line (TX) had a lower frequency of the thymine allele compared to all the other selection lines ($P < 0.01$). The differences in allele frequencies among the other selection lines were not significant ($P > 0.05$). Generally, lines based on Angus and Hereford had higher carcass and body fat and lower carcass lean ($P < 0.001$) compared to the lines based on the Gelbvieh, Limousin and Charolais (data not shown).

The effects of the different leptin SNP genotypes on feed intake, feed efficiency, and growth, feeding behaviour, real-time ultrasound measurements and carcass traits are presented in Table 2. Feed intake, feed efficiency and feeding behaviour were not different among the different leptin SNP genotypes ($P > 0.10$). However, animals homozygous for the thymine allele (TT) consumed 0.19 kg d^{-1} more feed than expected (had a positive residual feed intake) whilst animals homozygous for the cytosine allele (CC) consumed 0.18 kg d^{-1} less feed than expected (had a negative residual feed intake) ($P > 0.20$). A similar relationship was observed for feeding behaviour (feeding duration and feeding frequency) with animals carrying the thymine allele having a higher feeding duration and feeding frequency compared to animals with the cytosine allele, though not significantly so.

Of the growth traits considered, metabolic mid-weight, average daily gain and daily gains in longissimus thoracis area were not different among different genotypes ($P > 0.20$). In addition, partial correlations between leptin genotype and residual feed intake ($r = 0.01$), dry matter ($r = –0.06$), feeding duration ($r = –0.17$), feeding frequency ($r = 0.003$), average daily gain ($r = –0.08$) and feed conver-

Table 1. Frequencies of bovine leptin SNP alleles in different selection lines

Selection line	Number of animals	T allele	C allele
M1	26	0.71*a*	0.29
M2	30	0.55*ab*	0.45
M3	28	0.48*b*	0.52
M4	30	0.47*b*	0.53
TX	30	0.42*c*	0.58
Total	144	0.52	0.48

a–c Frequencies in columns followed by different letters are different (overall $\chi^2 = 10.96$, $P < 0.02$).

sion ($r = 0.02$) were not significant ($P > 0.10$). There were differences among genotypes in daily gain in real-time ultrasound backfat thickness ($P < 0.02$). Animals homozygous for the thymine allele had a faster rate of gain in backfat compared to animals that were heterozygous ($P < 0.05$) and to animals homozygous for the cytosine allele ($P < 0.001$). Final real-time ultrasound longissimus thoracis area and ultrasound marbling scores were not different among genotypes ($P > 0.10$). However, consistent with the rate of gain in backfat, final real-time ultrasound backfat thickness scores tended to be higher in thymine homozygotes compared to other genotypes ($P < 0.10$). Thymine homozygotes had higher ultrasound backfat than heterozygotes and cytosine homozygotes ($P < 0.05$).

Slaughter weight, carcass weight, carcass longissimus thoracis area and carcass marbling score did not differ among different genotypes ($P > 0.50$) (Table 2). On the other hand, leptin genotype had significant associations with carcass grade fat ($P < 0.005$), carcass yield grade ($P < 0.01$) and lean meat yield ($P < 0.007$) (Table 2). Thymine homozygotes (TT) had more carcass grade fat compared to heterozygotes ($P < 0.05$) and to animals homozygous for the cytosine allele ($P < 0.001$). Heterozygotes in turn had more carcass grade fat compared to cytosine homozygotes ($P < 0.05$). Additionally, thymine homozygotes had lower lean meat yield and yield grades compared to heterozygotes ($P < 0.05$) and cytosine homozygotes ($P < 0.001$).

In our study, no differences were observed among different genotypes in empty body composition (water, fat, protein and ash), body composition (lean, bone, subcutaneous fat, intermuscular fat, and body cavity fat), distribution of carcass fat (subcutaneous fat, intermuscular fat, and body cavity fat), and total carcass lean, carcass fat and carcass bone (as gram per kilogram of carcass) (Table 3). However, most of the fat-related traits were consistently higher in animals homozygous for the thymine allele compared to animals homozygous for the cytosine allele. This relationship tended to be opposite for lean-related traits.

The nine wholesale cuts of the beef carcass round, butt, loin, flank, plate and brisket (as percent of the whole carcass) did not differ among genotypes (Table 3). Whilst chuck tended to be higher in animals homozygous for the thymine allele ($P < 0.10$), shank tended to be higher in animals homozygous for the cytosine allele ($P < 0.10$) compared with other genotypes. Animals homozygous for cytosine had lower rib cuts compared with heterozygotes ($P < 0.01$) and with animals homozygous for thymine ($P < 0.01$). The effect of the different leptin genotypes on the composition of various wholesale cuts is presented in Table 4. No genotype differences were observed for percent composition of wholesale cuts with the exception of subcutaneous fat of loin ($P < 0.10$), lean of plate ($P < 0.10$), subcutaneous fat of brisket ($P < 0.01$) and body cavity fat of brisket ($P < 0.0001$),

Different genotypes did not differ in lean, bone, subcutaneous fat, body cavity fat and intermuscular fat composition of various wholesale cuts ($P > 0.10$), though percent subcutaneous fat compositions of most wholesale cuts tended to be higher in animals homozygous for the thymine allele compared with animals that are homozygous for the cytosine allele (Table 4). Percentage of subcutaneous fat was lower in the loin of animals homozygous for the cytosine allele compared to heterozygotes ($P < 0.05$) and thymine homozygotes ($P < 0.05$). Subcutaneous fat percent was also higher in the brisket of heterozygous animals compared with cytosine homozygotes ($P < 0.05$) and thymine homozygotes ($P < 0.001$). Body cavity fat of brisket was higher in thymine homozygotes compared with the other two genotypes ($P < 0.0001$).

The results presented here were consistent for all the genetic selection lines in this study. However, some of the traits in this study showed (or tended to show) SNP genotype-by-selection line interactions. These included ME intake ($P < 0.04$), lean meat yield ($P < 0.10$), ultrasound backfat ($P < 0.02$), ultrasound l. thoracis area ($P < 0.05$), percentage rib cut ($P < 0.02$), percentage brisket ($P < 0.05$), and percentage lean of rib ($P < 0.01$), percentage body cavity fat of brisket ($P < 0.0001$), percentage subcutaneous fat of brisket ($P < 0.001$), percentage intermuscular fat of rib ($P = 0.01$) and percentage intermuscular fat of butt ($P < 0.02$). These differences may however, just reflect the differences in allele frequencies of the mutation in different selection lines.

DISCUSSION

The identification of genetic markers that are positively associated with economically important traits in livestock species has the potential to significantly alter the rate of genetic improvement through the use of marker-assisted selection (MAS). In this study, we investigated the segregation of a previously reported single nucleotide polymorphism in exon 2 of the bovine leptin gene and its associations with various traits. Our results confirm the segregation of the SNP reported by Buchanan et al. (2002) in all five genetic selection lines of BeefBooster Inc. The distribution of the SNP allele frequencies among the various selection lines is also consistent with those reported by Buchanan

Table 2. Effect of bovine leptin SNP on least square means of feed intake, feed efficiency, growth, feeding behaviour, ultrasound measurements and carcass traits of steers

			SNP genotype[z]				
Traits	Mean[u]	SD[t]	CC	CT	TT	SED[y]	*P* value
Number of steers			32	74	38		
Feed intake/efficiency							
Residual feed intake (kg d^{-1})	0.00	0.66	–0.18	0.05	0.19	0.05	0.23
Feed:gain ratio (kg DM kg^{-1} gain)	5.67	0.63	5.66	5.66	5.77	0.05	0.70
DM intake (kg d^{-1})	8.17	1.05	8.16	8.23	8.25	0.09	0.88
ME intake (kg d^{-1})	93.33	11.08	92.79	92.93	94.37	0.92	0.88
DM intake [g $(kg^{0.75}\ d)^{-1}$]	90.21	6.81	89.48	90.69	91.37	0.56	0.49
ME intake [MJ $(kg^{0.75}\ d)^{-1}$]	1031.97	64.43	1023.11	1037.17	1043.93	5.30	0.50
Growth traits							
Metabolic mid-weight ($kg^{0.75}$)	87.76	7.74	87.54	88.74	88.39	0.64	0.43
Average daily gain (kg d^{-1})	1.57	0.25	1.57	1.57	1.54	0.02	0.88
Gain in backfat thickness (mm d^{-1})	0.06	0.04	0.06[c]	0.07*b*	0.09*a*	0.003	0.02
L. thoracis area ($cm^2\ d^{-1}$)	0.04	0.03	0.06	0.05	0.04	0.003	0.16
Feeding behaviour							
Feeding duration (min d^{-1})	89.52	18.49	88.30	91.83	90.44	1.54	0.69
Feeding frequency (events d^{-1})	9.30	2.38	8.87	9.31	9.34	0.20	0.88
Real-time ultrasound measurements							
Ultrasound backfat thickness, mm	8.95	3.01	8.66	8.79	10.11	0.25	0.07
Ultrasound l. thoracis area (cm^2)	85.52	8.84	85.72	84.85	84.84	0.71	0.90
Ultrasound marbling score	5.19	0.70	5.27	5.13	5.31	0.06	0.54
Carcass traits							
Slaughter weight (kg)	459.44	65.74	456.27	457.96	461.18	5.48	0.73
Cold carcass weight (kg)	293.52	44.47	290.66	292.33	295.14	3.71	0.61
L. thoracis area (cm^2)	76.64	9.88	76.09	76.27	74.52	0.82	0.74
Grade fat (backfat) (mm)	9.81	3.87	8.65*c*	10.10*b*	11.47*a*	0.32	0.005
Marbling score[x]	445.99	59.29	442.20	4452	442.18	4.94	0.74
Yield grade[w]	1.64	0.65	1.52*b*	1.64*b*	2.00*a*	0.055	0.01
Lean meat yield[v] (%)	57.81	3.06	58.58*a*	57.64*a*	56.25*b*	0.26	0.007

[z]Leptin marker genotypes are defined as CC = homozygous normal, CT = heterozygous, TT = homozygous mutant.
[y]Standard error of the difference between least squares means.
[x]Marbling score is a measure of intramuscular fat: trace marbling or less = 100 to 399 (Canada A quality grade); slight marbling = 400 to 499 (Canada AA quality grade); small to moderate marbling = 500 to 799 (Canada AAA quality grade); slightly abundant or more marbling = 800 to 1100 (Canada Prime).
[w]Yield grade refers to lean meat yield: 1 = 59% or better, 2 = 54% to less than 59%, and 3 = less than 54% lean meat yield.
[v]Lean meat yield, % = 57.96 + (0.202 × *L. thoracis* area, cm^2) – (0.027 × warm carcass weight, kg) – (0.703 × average backfat thickness, mm) (Jones et al. 1984).
[u]Overall trait mean.
[t]Overall trait standard deviation.
a–c Least squares means in rows followed by different letters are different.

et al. (2002). Lines based on British breeds (Angus and Hereford) have a higher frequency of the thymine allele compared to lines based on continental breeds (Limousin, Gelbvieh and Charolais). Consistent with this is the observation that selection lines with higher frequencies of the thymine or cytosine allele also had fatter or leaner carcasses, respectively. It could be suggested from the observed distribution of alleles across selection lines that, with the exception of the Angus-based selection line, previous selection in the overall lines considered had left the frequencies of this SNP unaffected.

Also, consistent with the findings of Buchanan et al. (2002), our results show that the thymine allele of the leptin SNP is positively associated with the daily rate of gain in ultrasound backfat (additive genotypic value = 0.03 ± 0.01), ultrasound backfat thickness (additive genotypic value = 0.73 ± 0.04), and carcass grade fat (backfat) (additive genotypic value = 1.41 ± 0.04) as well as the subcutaneous and body cavity fat of certain wholesale cuts (Table 5). The increase in backfat resulted in significant reductions in yield grade (additive genotypic value = 0.24 ± 0.08) and lean meat yield (additive genotypic value = –1.17 ± 0.36 in animals carrying the thymine allele (Table 5). The genotypic value of the heterozygous genotype was mostly systematically in-between alternate homozygotes. With the exception of a few of the body composition traits, none of the dominance deviations (dominance genotypic values) proved to be significantly different from zero, indicating the possible absence of dominance.

However, our results show that the significant increase in backfat in animals carrying the thymine allele does not result in significant increases in marbling scores, intermus-

Table 3. Effect of bovine leptin SNP on least square means of empty body composition, carcass composition, distribution of carcass fat, body components and distribution of wholesale cuts in steers

			SNP genotype[z]				
Traits	Mean	SD	CC	CT	TT	SED	*P* value
Number of steers	144		32	74	38		
Empty body composition (g kg^{-1} EBW)							
Water	517.92	38.15	517.57	518.67	522.23	3.18	0.79
Fat	276.32	46.57	274.96	275.55	271.92	3.88	0.89
Protein	165.62	14.08	166.30	165.50	165.98	1.17	0.97
Ash	40.83	7.05	42.48	40.79	40.32	0.59	0.46
Carcass composition (g kg^{-1})							
Lean	567.28	36.17	569.85	566.11	562.50	3.01	0.67
Bone	151.43	13.16	152.62	149.95	152.91	1.20	0.42
Subcutaneous fat	84.69	17.38	81.39	85.90	85.68	1.45	0.17
Intermuscular fat	172.23	26.60	170.98	171.77	175.62	2.22	0.78
Body cavity fat	24.64	6.68	24.88	25.13	24.89	0.56	0.97
Distribution of carcass fat (g kg^{-1} carcass fat)							
Subcutaneous fat	298.85	28.56	292.74	301.82	297.80	2.38	0.46
Intermuscular fat	610.99	25.94	607.16	612.53	613.85	2.16	0.58
Body cavity fat	90.15	12.75	89.41	91.02	93.41	1.06	0.60
Body component (% of final liveweight)							
Carcass lean	33.07	2.24	33.09	32.98	33.01	0.19	0.98
Carcass fat (dissectible fat)	16.42	2.70	15.88	16.48	16.66	0.23	0.34
Carcass bone	8.81	0.70	8.83	8.74	8.96	0.06	0.29
Distribution of wholesale cuts (g kg^{-1} carcass)							
Round	241.18	17.30	240.62	239.50	239.01	1.44	0.92
Butt	82.50	4.18	83.01	82.44	83.70	0.35	0.57
Loin	67.88	5.85	67.36	68.48	67.27	0.49	0.62
Flank	59.38	8.30	61.42	59.08	58.41	0.69	0.26
Chuck	287.46	11.35	288.33	286.06	293.53	0.95	0.08
Rib	98.06	6.43	96.15*c*	98.77*b*	101.12*a*	0.54	0.03
Plate	68.15	8.64	67.93	69.59	66.08	0.72	0.17
Brisket	58.06	6.55	57.32	58.56	55.77	0.55	0.29
Shank	37.33	4.38	37.85	37.51	35.11	0.37	0.07

[z]Leptin marker genotypes are defined as CC = homozygous normal, CT = heterozygous, TT = homozygous mutant.
a–c Least square means in rows followed by different letters are different.

cular fat or total carcass fat. Our results are consistent with the results of Buchanan et al. (2002), as well as generally accepted literature evidence indicating the negative genetic and phenotypic relationship between carcass backfat thickness on the one hand and rib eye area and lean meat yield on the other (Arnold et al. 1991; Morris et al. 1999; Bertrand et al. 2001; Boyles 2002). As a result of this known relationship, external fat depth is a negative factor in the formula used for calculating lean meat yield in most carcass evaluation systems, including the Canadian carcass grading system (Jones et al. 1984).

On the other hand, the correlation between backfat thickness and marbling score has been reported to be either very low in magnitude or not different from zero (Bertrand et al. 2001; Boyles 2002). Though positive phenotypic correlations between carcass backfat thickness and marbling score have been reported (Hamilton 1995; Crews and Kemp 2001), it is known that such correlations usually result when cattle are slaughtered at higher levels of finish and that backfat thickness, by itself, may account for only a small proportion of the variation in marbling (Bertrand et al. 2001).

According to Edwards and Page (1994) the estimated total genetic gain expected with MAS may be very high depending on the type of model and the strength of the marker-trait association. In addition, the heritability of carcass backfat thickness, rib eye area and lean meat yield has been shown to range from moderate to high (Morris et al. 1999; Bertrand et al. 2001; Crews and Kemp 2001).

The implication of the present results is that selection for animals carrying the thymine allele of the SNP described here may result in a population of beef cattle with higher subcutaneous fat depths and lower lean meat yields and poorer yield grades without a corresponding increase in intermuscular fat or marbling. The tendency for this polymorphism to preferentially influence subcutaneous fat instead of other body fat depots needs to be further investigated. However, the results presented here emphasize that care needs to be taken in the interpretation of results and its application to marker-assisted selection,

Table 4. Effect of bovine leptin SNP on least square means of the composition of different wholesale cuts

			SNP genotype[z]				
Traits	Mean	SD	CC	CT	TT	SED	*P* value
Number of steers	144		32	74	38		
Round (g kg^{-1} carcass)							
Lean	228.83	38.32	227.44	225.19	222.43	3.19	0.87
Subcutaneous fat	32.48	8.9	29.90	33.10	31.90	0.75	0.18
Intermuscular fat	26.49	5.83	25.15	26.69	26.64	0.49	0.35
Body cavity fat	2.17	0.82	1.99	2.24	2.16	0.07	0.55
Butt (g kg^{-1} carcass)							
Lean	72.54	11.70	71.77	72.55	73.26	0.98	0.85
Subcutaneous fat	12.66	4.23	12.11	12.56	13.30	0.35	0.51
Intermuscular fat	13.26	3.40	13.71	13.39	13.22	0.28	0.83
Body cavity fat	4.94	1.60	5.07	4.96	4.83	0.13	0.87
Loin (g kg^{-1} carcass)							
Lean	55.53	8.51	53.88	56.27	53.86	0.71	0.22
Subcutaneous fat	16.07	5.03	14.55	16.56	17.02	0.42	0.07
Intermuscular fat	66.85	2.48	67.77	66.35	67.12	0.21	0.97
Body cavity fat	6.81	2.12	6.55	6.63	6.93	0.18	0.78
Chuck (g kg^{-1} carcass)							
Lean	251.36	39.23	249.03	251.31	251.33	3.27	0.94
Subcutaneous fat	25.34	8.69	24.12	25.74	26.13	0.72	0.57
Intermuscular fat	79.62	19.12	77.31	78.52	84.18	1.59	0.12
Body cavity fat	4.87	2.90	4.95	4.81	4.14	2.45	0.69
Rib (g kg^{-1} carcass)							
Lean	70.69	11.31	73.81	70.53	68.62	0.94	0.14
Subcutaneous fat	17.34	5.12	16.76	17.50	18.82	0.43	0.19
Intermuscular fat	23.75	7.56	21.93	24.18	23.32	0.63	0.28
Body cavity fat	5.10	1.59	5.19	5.22	4.58	0.13	0.19
Brisket (g kg^{-1} carcass)							
Lean	36.56	8.19	36.68	36.99	33.38	0.68	0.12
Subcutaneous fat	13.50	1.22	12.33*b*	15.28*a*	12.29*b*	0.10	0.01
Intermuscular fat	20.09	6.29	19.33	19.78	20.16	0.52	0.88
Body cavity fat	2.25	1.22	2.07*b*	2.16*b*	3.30*a*	0.10	0.0001
Plate (g kg^{-1} carcass)							
Lean	45.38	9.60	47.04	44.40	46.07	0.80	0.07[†]
Subcutaneous fat	5.63	2.75	5.41	5.89	5.30	0.23	0.51
Intermuscular fat	27.32	9.31	26.54	27.60	26.99	0.78	0.76
Body cavity fat	10.53	3.49	10.10	10.66	10.17	0.29	0.61

[z]Leptin mutation genotypes are defined as CC = homozygous normal, CT = heterozygous, TT = homozygous mutant.
a–c Least square means in rows followed by different letters are different

especially when only one genetic marker is being considered. Though taking animals with the thymine allele to a higher level of finish on feedlots might generally result in increases in overall carcass fat and therefore carcass marbling, the economic implications in terms of additional feeding and management costs and subsequent potential losses in yield and carcass grade may render such an option prohibitive for the meat industry.

One of our primary objectives in this study was to test the possibility of a significant association between this exon 2 SNP and feed intake and efficiency in feedlot cattle. Our inability to demonstrate a significant effect of this missence mutation on feed intake and feed efficiency, despite effects on carcass quality may be attributed to many reasons including the fact that animals carrying the thymine allele may be compensating for the possible reduction in biological function of leptin by synthesizing more of the hormone and thus lowering the overall effect of the polymorphism. This may be true since the study by Buchanan et al. (2002) indicated that animals carrying the thymine allele also expressed higher levels of leptin mRNA in tissues.

Furthermore, it has been shown by Delavaud et al. (2002) that the relationship between leptin and feeding level may be partly independent of the relationship with adiposity and that long-term effects on adiposity may prevail over the effects on daily food intake. Our inability to show significant differences in feed intake may also be due to the small data set used in this study. However, our results for both feed intake and efficiency indicated that animals homozy-

Table 5. Effect of leptin SNP on ultrasound quality, carcass quality and various wholesale cuts

Traits	a^z (± SD)	Prob^x	d^y (± SD)	Prob^x
Gain in backfat thickness (mm d^{-1})	0.02 ± 0.005	0.006	0.003 ± 0.01	0.58
Ultrasound backfat thickness (mm)	0.73 ± 0.36	0.05	–0.59 ± 0.49	0.23
Grade Fat (Backfat) (mm)	1.41 ± 0.0.42	0.001	0.03 ± 0.57	0.96
Yield grade	0.24 ± 0.08	0.005	–0.12 ± 0.11	0.29
Lean meat yield (%)	–1.17 ± 0.36	0.002	0.22 ± 0.49	0.65
Brisket subcutaneous fat (g kg^{-1} carcass)	–0.3 ± 0.64	0.62	2.64 ± 0.86	0.003
Brisket body cavity fat (g kg^{-1} carcass(	0.61 ± 0.16	0.0002	–0.53 ± 0.22	0.02
Plate lean (g kg^{-1} carcass)	–0.67 ± 1.08	0.54	3.31 ± 1.47	0.03
Loin subcutaneous fat (g kg^{-1} carcass)	1.24 ± 0.58	0.04	0.77 ± 0.79	0.33
Chuck (g kg^{-1} carcass)	2.51 ± 0.94	0.02	4.87 ± 2.56	0.06
Rib (g kg^{-1} carcass)	2.49 ± 0.99	0.01	–0.14 ± 1.28	0.91
Shank (g kg^{-1} carcass)	–1.37 ± 0.66	0.04	–1.04 ± 0.90	0.25

[z] a, additive genotypic value = $(\Phi_{TT} - \Phi_{CC})/2$ = half the difference between the genotypic values of the TT and CC genotypes (Falconer and Mackay 1996).
[y] d, dominance deviation = $\Phi_{CT} - (\Phi_{TT} - \Phi_{CC})/2$ = deviation of the CT genotypic value from the midpoint between the TT and CC genotypic values (Falconer and Mackay 1996).
[x] Probability of an additive or dominance genotypic value.

gous for the thymine allele may consume 0.37 kg d^{-1} more feed (less efficient) compared to animals homozygous for the cytosine allele, though this result was only a trend.

SUMMARY

Alleles of the single nucleotide polymorphism identified by Buchanan et al. (2002) were segregating in all five genetic selection lines of the BeefBooster cattle population. Lines based on Angus and Hereford had higher frequencies of the thymine (mutant) allele whilst lines based on continental breeds have higher frequencies of the cytosine (normal) allele. The thymine allele is significantly associated with higher rates of ultrasound backfat gain, higher carcass grade fat (backfat), poorer yield grades and lower lean meat yields compared to the cytosine allele. Higher carcass backfat in animals with the thymine allele did not translate into higher carcass marbling or intermuscular fat. Higher feed intake and feed efficiency were observed in animals carrying the thymine allele compared to the cytosine allele, though this relationship was only a trend. The prospects for selecting for animals carrying the thymine allele, compared to the cytosine allele, reduced saleable meat yields with no improvement in overall carcass quality. More information is required to confirm the findings of the studies on this SNP, assess the potential of linked genes to affect the traits under investigation, as well as to determine the economic value of the different alleles of the SNP under different contexts.

ACKNOWLEDGEMENTS

This work was supported through grant number 2000M624 awarded to Dr. S. S. Moore through the Alberta Agricultural Research Institute and grant number 2000AB364 through the Canada Alberta Beef Industry Development Fund. The authors thank Dr. Erasmus K. Okine for critically reviewing and providing invaluable comments on the manuscript.

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