Asian-Aust. J. Anim. Sci.
Vol. 20, No. 1 : 1 - 6
January 2007

www.ajas.info

# Association of SNP Marker in the Leptin Gene with Carcass and Meat Quality Traits in Korean Cattle

**S. C. Shin and E. R. Chung***

Division of Animal Science and Resources, College of Life Science and Natural Resources
Sangji University, Wonju 220-702, Korea

**ABSTRACT :** Leptin is the hormone product of the obese gene and is synthesized and secreted predominantly by white adipocytes and relates to the feedback system that regulates long-term body fat weight and composition. Therefore, the leptin gene could be an excellent candidate gene controlling fat deposition, carcass traits and meat quality in beef cattle. The objective of this study was to evaluate the association of 3 SNPs (A1127T and C1180T in exon 2 and C3100T in exon 3) in the bovine leptin gene with carcass and meat quality traits in Korean cattle. The C1180T SNP was associated with backfat thickness (BF) and marbling score (MS) ($p<0.05$). Animals with the genotype CC had higher BF than animals with TT genotype and higher MS compared with CT and TT genotypes. No significant associations were observed between the C3100T SNP and any carcass and meat quality traits analyzed. The effect of the A1127T SNP was not analyzed because the TT genotype was not detected and the AT genotype showed only 1.0% frequency. These results suggest that the C1180T SNP of the leptin gene may be useful as a genetic marker for carcass and meat quality traits in Korean cattle. (**Key Words** : Leptin Gene, SNP Marker, Carcass and Meat Quality Traits, Korean Cattle)

## INTRODUCTION

Meat quality and carcass composition are the most economically important traits in the beef cattle industry. However, genetic improvement of meat quality and carcass traits by selective breeding is difficult and expensive because of the difficulties in collecting the trait data. Marker or gene-assisted selection is a promising strategy for genetic improvement of such carcass traits. Molecular marker-assisted selection (MAS) will first require identification of candidate genes or anonymous genetic markers associated with the traits of interest. Candidate genes are those with a known relationship between a physiological or biochemical process and an economically important trait. The candidate gene approach allows the identification of single nucleotide polymorphisms (SNPs) in genes likely to cause variation in a trait based on physiological, immunological, or endocrine evidence. Meat quality traits are ideal candidate traits for the use of MAS and several quantitative trait loci (QTL) and genes affecting meat quality have been reported in beef cattle.

* Corresponding Author: E. R. Chung. Tel: +82-33-730-051, Fax: +82-33-730-0503, E-mail: erchung@sangji.ac.kr
Received March 21, 2006; Accepted July 10, 2006

Leptin is the hormone product of the obese gene synthesized and secreted predominantly by white adipose tissue (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 food intake, body composition, energy expenditure, reproduction and certain immune system functions (Nkrumah et al., 2005). Serum concentrations of leptin are correlated with carcass adipose depots and carcass composition in beef cattle (Geary et al., 2003). Leptin has been considered as a candidate gene for genetic variation in carcass and meat quality traits of beef cattle because of the essential role in physiological mechanisms related to adipocytes and fat deposition of mammals. Since the bovine leptin gene has been mapped on BTA4 (Pomp et al., 1997), several SNPs have been identified in both introns and exons of the leptin gene among different breeds of cattle (Lien et al., 1997; Konfortov et al., 1999; Buchanan et al., 2002). Associations of SNP alleles within exon 2 (Buchanan et al., 2002; Nkrumah et al., 2005) or the promoter region (Crews et al., 2004; Nkrumah et al., 2005) of the leptin gene with carcass and meat quality traits were reported in beef cattle, with some associations not being consistently verified across studies (Schenkel et al., 2005). Recently, fifty-seven SNPs

**Table 1.** Primer sequence, position and PCR product size used in RFLP and SSCP analysis of the leptin gene in Korean cattle

| Location[a] | Primer sequence (5'-3') | Nucleotide change | Restriction enzyme | Amplified region | Fragment size | Analysis method | GenBank accession No. |
|---|---|---|---|---|---|---|---|
| 877-1342 | GATTCCGCCGCACCTCTC<br>CCTGTGCAAGGCTGCACAGCC | A 1127 T | *Cla*I | Exon2 | 467 | RFLP | U50365 |
| 1008-1201 | ATGCGCTGTGGACCCCTGTATC<br>TGGTGTCATCCTGGACCTTCG | C 1180 T | *Kpn2*I | Exon2 | 94 | RFLP | U50365 |
| 2961-3456 | CCCTCTCTCCCACTGAGCTC<br>TAAAGGATGCCCACATAGGC | C 3100 T | | Exon3 | 496 | SSCP | U50365 |

[a] Location was based on the sequence of the bovine leptin gene from GenBank accession no. U50365.

including thirty-six novel and twenty-one known SNPs were identified in the leptin gene of Korean cattle (Yoon et al., 2005). Genetic information on SNP allele frequencies for different cattle breeds and evaluation of associations of leptin gene polymorphisms with economically important traits will be a prerequisite for implementation of MAS breeding strategies. The objective of this study was to evaluate the association of SNPs in the leptin gene with carcass and meat quality traits in Korean cattle.

## MATERIALS AND METHODS

### Animals and carcass data

Three hundred and nine Korean native steers, with pedigree information and performance records through the national progeny testing program, from the Hanwoo Experiment Station of the National Livestock Research Institute (NLRI) were used in this study. Meat samples were collected from the 13th thoracic rib to the first lumbar vertebrae of the steers within 24 h of slaughter and evaluated by mechanical and physical methods. The carcass data included were carcass weight (CW), carcass percentage (CP), M. *longissimus dorsi* area (LDA), backfat thickness (BF) and marbling score (MS). BF was measured at the 12th- and 13th- rib interface. LDA was measured at the 12th- and 13th- rib section using a tracing of the muscle. MS for quality grade is scored on a scale from 1 to 7 with 7 being associated with the most marbling.

### SNP identification and marker genotyping

For each animal, genomic DNA was extracted from blood peripheral lymphocytes using standard methods. SNPs were detected by direct sequencing using an ABI PRISM 3700 DNA analyzer (Applied Biosystems, Foster City CA). Genotyping of the gene-specific SNP marker for the bovine leptin gene was carried out using PCR-restriction fragment length polymorphism (RFLP) or PCR-single strand conformation polymorphism (SSCP) methods depending on the restriction site. Pairs of oligonucleotide primers used were designed and synthesized to amplify three leptin fragments based on the published nucleotide sequence information of exon 2 and exon 3 of the bovine leptin gene (GenBank accession No. U50365). The primer sequences, location, size of the amplified fragments and analytical method are shown in Table 1.

The PCR-RFLP was performed to detect the two SNPs in exon 2. The PCR amplification was performed in a 20 μl reaction volume containing 30 ng of genomic DNA, 10 pmol of each primer, 1× PCR buffer, 1.5 mM $MgCl_2$, 200 μM of each dNTP and 1.0 unit *Taq* polymerase. Amplification conditions were 94°C for 5 min followed by 35 cycles of 94°C for 30 sec, 60°C for 30 sec and 72°C for 30 sec, with a final extension at 72°C for 5 min. Amplified fragments were digested with restriction enzymes *Cla*I (A1127T) at 37°C or *Kpn2*I (C1180T) at 55°C for at least 4 h, respectively. The digested DNA fragments were separated by electrophoresis in 2% agarose gel or 6% polyacrylamide gel in 1×TBE buffer. Because no restriction site exists for SNP in exon 3, the PCR-SSCP analysis was used to genotype the animals. The PCR reaction used the same conditions as described above for the PCR-RFLP analysis. After PCR amplification, 4 μl of PCR product was mixed with 16 μl of gel loading solution containing 95% formamide, 20 mM EDTA, 0.05% bromophenol blue and 0.05% xylene cyanol. The mixture was then denaturated at 96°C for 7 min, cooled in ice for 5 min and loaded on a nondenaturing 12% polyacrylamide gels (49:1 acrylamide to bis-acrylamide). Electrophoresis was performed in 1× Tris borate (pH 8.3)-EDTA buffer at 250 V for 4 h at room temperature. After electrophoresis, the DNA fragments in the gel were detected by silver staining.

### Statistical analysis

The PROC GLM procedure of SAS (SAS, Inst. Inc., Cary NC) was used to test the association between SNP marker genotypes of the leptin gene and carcass and meat quality traits. The linear model used was as follows:

$$Y_{ijkl} = \mu + YS_i + P_j + G_k + e_{ijkl}$$

Where $Y_{ijkl}$ is the observation of the carcass trait, $\mu$ is the overall mean for each trait, $YS_i$ is the effect of $i_{th}$ year and season of calving, $P_j$ is the effect of $j_{th}$ parity, $G_k$ is the fixed effect of $K_{th}$ SNP genotype and $e_{ijkl}$ is the random residual effect.

**Table 2.** Genotype and allele frequencies of the leptin gene in Korean cattle

| SNP | Genotype frequency (%) | | | Allele frequency (%) | |
|---|---|---|---|---|---|
| A1127T | AA | AT | TT | A | T |
| | 98.0 | 2.0 | 0.0 | 99.0 | 1.0 |
| C1180T | CC | CT | TT | C | T |
| | 29.2 | 55.4 | 15.4 | 64.0 | 36.0 |
| C3100T | CC | CT | TT | C | T |
| | 23.1 | 52.4 | 24.5 | 49.3 | 50.7 |

## RESULTS

### SNP marker genotyping

A 467 bp fragment between nucleotides 877 and 1,342 and a 94 bp fragment between nucleotides 1,008 and 1,201 within exon 2, and a 496 bp fragment between nucleotides 2,961 and 3,456 within exon 3 of the leptin gene were amplified and sequenced, respectively. Three SNPs (A-T and C-T in exon 2 and C-T in exon 3) were found in exon sequences, containing the coding region, all of which resulted in amino acid changes. The A to T substitution detected at position 1,127 of the exon 2 creates a *Cla*I restriction site and was genotyped by PCR-RFLP (Figure 1). The A allele was cleaved into two fragments 215 and 252 bp, while the T allele remained uncut at 467 bp because of the absence of a *Cla*I recognition site. As a second SNP in exon 2, the C to T substitution at position 1180 creates a *Kpn2*I restriction site and was also genotyped by PCR-RFLP (Figure 2). The A allele yielded two bands of 19 and 75 bp, while the T allele showed a fragment of 94 bp. A transition of C to T was identified at position 3,100 of exon 3 and was genotyped by PCR- SSCP (Figure 3). Two alleles(C and T) were identified and all three possible genotypes, CC, CT and TT were observed.

The allele and genotype frequencies of three SNPs in exons 2 and 3 of the leptin gene in Korean cattle are shown in Table 2. At the A1127T SNP, only genotypes AA and AT were detected with frequencies of 98.0 and 2.0%, respectively; no individuals with TT genotype were observed. For the C1180T SNP, genotypes CC, CT and TT were detected with frequencies of 29.2, 55.4 and 15.4%.

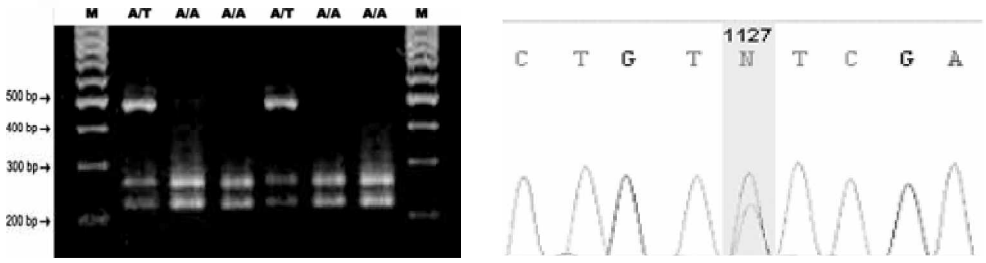


**Figure 1.** PCR-RFLP and sequence chromatogram for the detection of a A/T substitution at position 1127(A1127T SNP) within exon 2 region of the leptin gene in Korean cattle. M: molecular size marker.

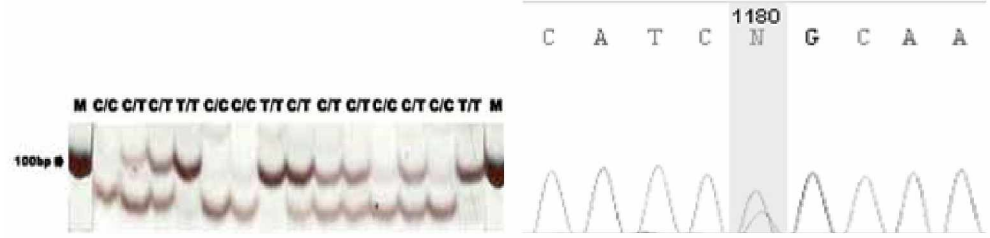


**Figure 2.** PCR-RFLP and sequence chromatogram for the detection of a C/T substitution at position 1180 (C1180T SNP) within exon 2 region of the leptin gene in Korean cattle. M: molecular size marker.

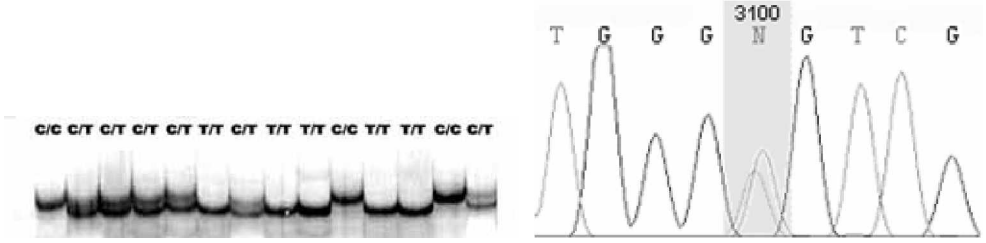


**Figure 3.** PCR-SSCP and sequence chromatogram for the detection of a C/T substitution at position 3100 (C3100T SNP) within exon 3 region of the leptin gene in Korean cattle

**Table 3.** Least squares means and standard errors for carcass traits and meat quality of different C1180T SNP genotypes in overall population

| Traits | SNP genotype | | | p-value | Additive effect | Dominance effect |
|---|---|---|---|---|---|---|
| | CC | CT | TT | | | |
| LW/kg | 549.987±7.832 | 543.116±5.082 | 530.000±36.173 | 0.7013 | 20.930±37.115 | 4.167±38.480 |
| CW/kg | 313.255±5.028 | 311.511±3.263 | 294.500±23.225 | 0.7245 | 19.360±23.830 | 13.933±24.707 |
| DP/% | 56.916±0.236 | 57.303±0.153 | 55.550±1.090 | 0.1322 | 1.380±1.113 | 2.109±1.154 |
| BF/cm | 0.722±0.040[a] | 0.617±0.026[ab] | 0.500±0.187[b] | 0.0472* | 0.230±0.194 | -0.004±0.202 |
| LDA/cm$^2$ | 73.555±1.256 | 75.847±0.815 | 73.000±5.801 | 0.2945 | 0.627±5.922 | 4.979±6.140 |
| MS/1-7 | 2.880±0.119[a] | 1.850±0.183[b] | 1.600±0.847[b] | 0.0388* | 0.406±0.900 | 0.887±0.933 |

LW: live weight; CW: carcass weight; DP: dressing percentage; BF: backfat thickness; LDA: M. *Longissimus dori* area; MS: marbling score.
* Effect was significant at $p<0.05$.
[a, b] Least square means within a row without a common superscript letter differ ($p<0.05$).

**Table 4.** Least squares means and standard errors for carcass traits and meat quality of different C3100T SNP genotypes in overall population

| Traits | SNP genotype | | | p-value | Additive effect | Dominance effect |
|---|---|---|---|---|---|---|
| | CC | CT | TT | | | |
| LW/kg | 544.678±8.420 | 551.763±5.755 | 529.799±8.675 | 0.1115 | 15.882±12.116 | 29.258±16.738 |
| CW/kg | 311.466±5.424 | 315.683±3.707 | 303.306±5.588 | 0.1852 | 8.784±7.801 | 16.723±10.777 |
| DP/% | 57.129±0.260 | 57.174±0.178 | 57.184±0.268 | 0.9869 | -0.043±0.373 | 0.038±0.515 |
| BF/cm | 0.677±0.044 | 0.642±0.030 | 0.624±0.046 | 0.6963 | 0.062±0.065 | -0.015±0.090 |
| LDA/cm$^2$ | 74.288±1.368 | 76.107±0.935 | 73.830±1.409 | 0.3159 | 0.511±1.957 | 4.108±2.704 |
| MS/1-7 | 1.842±0.200 | 2.147±0.137 | 2.156±0.206 | 0.4138 | -0.256±0.297 | 0.306±0.411 |

LW: Live weight; CW: Carcass weight; DP: Dressing percentage; BF: Backfat thickness; LDA: M. *Longissimus dori* area; MS: Marbling score.

respectively. At the C3100T SNP, the frequencies of CC, CT and TT genotypes were 23.1, 52.4 and 24.5%, respectively.

**SNP marker associations**

Results of the gene-specific SNP marker association analysis for the leptin gene are presented in Table 3 and 4. At the SNP marker of C1180T in exon 2 there was a significant effect on the BF and MS. The C allele was associated with a significant increase in BF and MS. Animals with the genotype CC had higher BF than animals with TT genotype ($p<0.05$) and higher MS compared with CT and TT genotypes ($p<0.05$). No significant associations were observed between C1180T SNP genotypes and other traits. The association analysis between the C3100T SNP and all traits examined in this study showed no significant genotype effects. On the other hand, the association test between A1127T SNP and carcass traits were excluded in this study because only limited SNP genotypes were available for the statistical analysis.

## DISCUSSION

Leptin, the product of the obese gene, is secreted predominantly from white adipose tissue and regulates feed intake, energy balance and body composition (Baile et al., 2000). Leptin is an important regulator of energy metabolism, adipocytes and reproduction, and is perhaps linked to meat quality determinants such as marbling (Hossner, 1998). The fact that leptin is found in adipose tissue makes it a logical choice to serve as an excellent genetic marker for carcass traits in beef cattle. In cattle, the leptin gene has been mapped to chromosome 4 and its full sequence is available in the GenBank database at accession number U50365. Several SNPs have been previously reported in the leptin gene among different breeds of cattle. In a previous study, thirty-six SNPs were newly identified, and twenty-one SNPs, which were reported in other breeds, were also confirmed in Korean cattle (Yoon et al., 2005). In this study, we investigated the effect on carcass traits and meat quality of three SNPs (two SNPs in exon 2 and one SNP in exon 3) reported previously in Korean cattle. The A1127T SNP in exon 2 results in a non-conservative amino acid substitution from tyrosine (TAT) to phenylalanine (TTT). The C1180T SNP in exon 2 causes a non-conservative amino acid substitution from arginine (CGC) to cysteine (TGC). The C3100T SNP in exon 3 results in a conservative valine (GTG) to alanine (GCG) amino acid change. Allele frequencies in the A1127T SNP were similar to those observed in five cattle breeds by Lagonigro et al. (2003), who reported that overall gene frequencies were 94% for the A allele and 6% for the T allele. In the C1180T SNP, gene frequencies of the C and T alleles were similar to those of Charolais and Simmental breeds, whereas a lower frequency of the T allele compared with that of Angus and Hereford breeds (Buchanan et al., 2002) was revealed. For the C3100T SNP, the frequencies of the C and T alleles were generally similar to those reported by Lagonigro et al. (2003).

The leptin gene itself is considered a potential QTL, influencing different production traits in cattle, for example, carcass and meat quality (Buchanan et al., 2002; Schenkel et al., 2005), milk production traits (Buchanan et al., 2002; Madeja et al., 2004), reproductive performance (Gonzalez et al., 2000; Almeida et al., 2003) and feed intake (Lagonigro et al., 2003). In the present study, we found significant associations between the C1180T SNP genotype in exon 2 and BF and MS. Animals with the CC genotype compared with the TT genotype had higher BF and MS. However, Buchanan et al. (2002) reported a significant genotype effect on grade fat and average fat, with the T allele being associated with higher fat, but with no significant association with carcass marbling score. They showed that the T allele was associated with fatter carcasses and the C allele with leaner carcasses. Schenkel et al. (2005) also reported that the T allele was associated with lower lean meat yield and higher fatness (fat yield and grade fat); however, the increased fatness did not translate into higher i.m. fat (marbling). Crews et al. (2004), however, did not find association of C1180T SNP (originally referred to as C305T SNP) with carcass traits of Charolais and Charolais-cross steers, which included backfat thickness and carcass marbling. Such discrepant results may be due to a number of factors, including breed differences or population composition and the small number of animals with the TT genotype. The frequencies of the SNP alleles within the breeds also supports an association with the exon 2 SNP and carcass fat content. The British breeds (Angus and Hereford) have a higher frequency of the T allele, whereas the Korean cattle have a higher preponderance of the C allele. British breeds are characterized by their early maturity, as compared to Korean cattle, with slow growth rate giving them the capacity to carry more fat at a young age (Gregory et al., 1994; Chung and Kim, 2005). Therefore, contrasting results such as these across studies illustrate the need to validate associations of SNP in the leptin gene with carcass and meat quality traits among different cattle breed populations before adoption is practical in industry breeding programs. In the A252T SNP (A1127T SNP in this study), Schenkel et al. (2005) also reported that the T allele was associated with less fat yield and grade fat and more lean yield compared with the A allele in crossbreds formed by several breeds. However, Lanonigro et al. (2003) did not find association of this SNP with the fat related traits from 169 Holstein-Charolais $F_2$ bull calves. The C3100T SNP in exon 3 was not significantly associated with any of the carcass and meat quality traits considered. However, further investigation in broader populations will be necessary to find the associations between this SNP and economically important traits. Although further efforts are required to validate our results, the association of leptin SNP markers with better carcass and meat quality traits was a very interesting finding and could be used as a predictor of carcass merits and composition in Korean cattle.

## ACKNOWLEDGEMENTS

This study was supported by a grant (Code# 20050301-034-442-016-01-00) from BioGreen 21 program, Rural Development Administration, Republic of Korea.

## REFERENCES

Almeida, S. E. M., E. A. Almedia, J. C. F. Moraes and T. A. Weimer. 2003. Molecular markers in the LEP gene and reproductive performance of beef cattle. J. Anim. Breed. Genet. 120:106-113.

Baile, C. A., M. A. Della-Fera and R. J. Martin. 2000. Regulation of metabolism and body fat mass by leptin. Annu. Rev. Nutr. 20:105-127.

Buchanan, F. C., C. J. Fitzsimmons, A. G. Van Kessel, T. D. Thue, D. C. W. Sim and S. M. Schmutz. 2002. Association of a missense mutation in the bovine leptin gene with carcass fat content and leptin mRNA levels. Genet. Sel. Evol. 34:105-116.

Chung, E. R. and W. T. Kim. 2005. Association of SNP marker in IGF-I and MYF5 candidate genes with growth traits in Korean cattle. Asian-Aust. J. Anim. Sci. 18:1061-1065.

Crews, D. H., J. D. Nkrumah, J. Yu and S. S. Moore. 2004. Association of single nucleotide polymorphisms in the bovine leptin gene with feedlot and carcass characteristics of crossbred steers. Can. J. Anim. Sci. 84:749-750 (Abstr.).

Geary, T. W., E. L. McFadin, D. M. MacNeil, E. E. Grings, R. E. Short, R. N. Funston and D. H. Keisler. 2003. Leptin as a predictor of carcass composition in beef cattle. J. Anim. Sci. 81:1-8.

Gonzalez, R. R., C. Simon, P. Caballero-Campo, R. Norman, D. Chardonnens, L. Devoto and P. Bischof. 2000. Leptin and reproduction. Hum. Reprod. 6:290-300.

Hossner, K. L. 1998. Cellular, molecular and physiological aspects of leptin: Potential applications in animal production. Can. J. Anim. Sci. 78:463-472.

Ji, S., G. M. Wills, R. R. Scott and M. E. Spurlock. 1998. Partial cloning and expression of the bovine leptin gene. Anim. Biotech. 9:1-4.

Konfortov, B. A., V. E. Licence and J. R. Miller. 1999. Re-sequencing of DNA from a diverse panel of cattle reveals a high level of polymorphism in both intron and exon. Mamm. Gen. 10:1142-1145.

Lagonigro, R., P. Wiener, F. Pilla, J. A. Woolliams and J. L. Williams. 2003. A new mutation in the coding region of the bovine leptin gene associated with feed intake. Anim. Genet. 34:371-374.

Lien, S. H. Sundvold, H. Klungland and D. I. Vage. 1997. Two novel polymorphisms in the bovine obesity gene (OBS). Anim. Genet. 28:238-246.

Madeja, Z., T. Adamowicz, A. Chmurzynska, T. Jankowski, J. Melonek, M. Switonski and T. Strabel. 2004. Effect of leptin gene polymorphisms on breeding value for milk production traits. J. Dairy Sci. 87:3925-3927.

Nkrumah, J. D., C. Li, J. Yu, C. Hansen, D. H. Keisler and S. S. Moore. 2005. Polymorphism in the bovine leptin gene promoter associated with serum leptin concentration, growth, feed intake, feeding behavior, and measures of carcass merit. J. Anim. Sci. 83:20-28.

Pomp, D., T. Zou, A. C. Clutter and W. Barendse. 1997. Rapid communication: mapping of leptin to bovine chromosome 4 by linkage analysis of a PCR- based polymorphism. J. Anim. Sci. 75:1427.

Schenkel, F. S., S. P. Miller, X. Ye, S. S. More, J. D. Nkrumah, C. Li, J. Yu, I. B. Mandell, J. W. Wilton and J. L. Williams. 2005. Association of single nucleotide polymorphisms in the leptin gene with carcass and meat quality traits of beef cattle. J. Anim. Sci. 83:2009-2020.

Yoon, D. H., B. H. Cho, B. L. Park, Y. H. Choi, H. S. Cheong, H. K. Lee, E. R. Chung, I. C. Cheong and H. D. Shin. 2005. Highly polymorphic bovine leptin gene. Asian-Aust. J. Anim. Sci. 18:1548-1551.