ISSN 1682-296X (Print)
ISSN 1682-2978 (Online)

Bio Technology

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Biotechnology 7 (2): 347-350, 2008
ISSN 1682-296X

Association of SNP in the ExonII of Leptin Gene with Milk and Reproduction Traits in Holstein Iranian Cows

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Abstract: The objective of this study was to consider the genetic variations in the exonII region of the leptin gene for the Iranian Holstein cows. Association between leptin genotype with milk production and reproductive traits were analyzed by a univariate linear model. Result is shown that the T allele had significant effect on milk yield versus C allele. This effect was severe particularly for cows which had 60 and 100 milking days ($p<0.028$). Homozygous animals for the TT genotype produced more milk (1.6 kg day^{-1}) than CC animals. However the association analysis showed no significant effect between two genotypes for 305 day-milk yield and open days.

Key words: Leptin, polymorphism, PCR-RFLP, Iranian Holstein

INTRODUCTION

Since its discovery in 1994, a protein hormone synthesized and segregated by adipose tissue, has been shown to regulate feed intake in several species such as cattle, sheep and pig (Spicer, 2001). Other important roles of leptin are control of body weight, feed intake, immune function and reproduction (Buchanan *et al*., 2002, 2003; Liefers *et al*., 2003). The leptin gene has been mapped in the bovine chromosome 4 (BTA), linked to markers IDVGA51 and DIK26 (Nkrumah *et al*., 2004; Pomp *et al*., 1997). QTL for milk production traits on chromosome 4 is a region where the leptin gene and serum amylase 1 gene are located (Madeja *et al*., 2004). This hormone product of the obese gene located on BTA4 and is play a role in the regulation of appetite, energy partition and body composition (Schenkel *et al*., 2005). Leptin binds to a receptor mainly localized on neuropeptide Y-neurons, which results in a reduction of feed intake and an increase of energy expenditure neuropeptid Y is also involved in the control of reproductive function (Liefers *et al*., 2002). Ob/ob male mice lack functional leptin and are hyperphagic, morbidly obese and exhibit impaired reproductive function. Leptin deficiency in male mice is associated with impaired spermatogenesis, increased germ cell apoptosis and up regulated expression of proapoptotic genes within the testes (Ganapathy *et al*., 2006).

In dairy cattle, the increase in milk yield has been accompanied by more negative energy balance during early lactation and a decrease in fertility. Leptin hormone concentration were high during late pregnancy and declined to a nadir at parturition (Liefers *et al*., 2005a). Leptin gene has three exons and two introns. Several polymorphisms (~20) for this gene have been found so far. The frequency of this polymorphism was 1 per 84 bp in exonic sequences (Liefers *et al*., 2005b). ExonI was not sequenced because it is a non-coding exon (Buchanan *et al*., 2002). In exon2 RFLP: *Cla*1 that by Madeja *et al*. (2004) was described (an A/T substitution resulting in change from tyrosine to phenylalanine). *Knp*21 that by Buchanan *et al*. (2002) was described (an C/T substitution resulting in change from Arginine to Cysteine). The Cysteine/Arginine change in exonII is non-conserved substitution and is more likely to alter the functioning of the leptin hormone (Buchanan *et al*., 2002). In exonIII two SNPS were found *Nru*1 (a C/T substitution resulting in a change from Valine to Alanine) by Lagonigro *et al*. (2003) and *Hph*1 (a C/T substitution resulting in change from Alanine to Valine) by Madeja *et al*. (2004). In the leptin prompter 20 SNPs with 1.6 kbp were discovered (Liefers *et al*., 2005a). In introns the more polymorphism were found which include intron 1103 and 126 SNPs intron 2 RFLP lep *Sau*A1 and lep*BsaA*1. The aim of this study was to investigate the effect of the exonII polymorphism on milk production and reproduction in Iranian Holstein cows.

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MATERIALS AND METHODS

Blood sample: Blood samples were obtained from 161 Iranian Holstein cows that had been high number of parturient and are kept in the ASTAN-GHODS farm which is located in North East of Iran in September 2006. Blood was obtained from sacral vein by venoject using K_2EDTA (BD Vacutainer UK) as anticoagulant. Samples were stored at -20°C until needed for DNA extraction.

DNA extraction: Genomic DNA was extracted from peripheral blood leukocytes by the method (Boom *et al*., 1990) used diatom kit (Iso-gene Co., Moscow). Purity assessed by 1% Agarose gel (2 g Agarose + 2 mL $TBE10_X$ + 18 mL dH_2O).

PCR reaction: A 94 bp region of exon2 of the leptin gene was amplified using of primer that described by Buchanan *et al*. (2002). A final volume of PCR 20 μL (Gene Pak PCR Universal). The amplification of this region was performed by thermal cycling. The amplification program included an initial denaturation at 94°C for 3 min, followed by 35 cycles of 94°C for 1 min, 56°C for 45 sec, 72°C for 45 sec and final extension for 5 min at 72°C. The PCR products were separated by 2% agarose gel electrophoresis at 80 V for 15 min.

PCR product digestion: 5-8 μ PCR product was digested with 0.5 unit of *Bsp13* I restriction enzyme at 50°C for 6 h in incubator. The digested products were loaded in 8% poly acryl amide gel (6 mL poly acryl amide 39:1 + 3 mL $TBE10_x$ + 21 mL dH_2O) and run at 120 V for 1 h and 30 min and visualized with silver staining M50 bp were used as molecular weight marker. Then genotype and allele frequency of leptin gene were analyzed using the Pop-Gene program.

Statistical analysis: The effect of genotype on milk production and reproductive traits was analyzed by mixed model used from JMP5.1 program.

RESULTS AND DISCUSSION

This SNP could be digestion with the restriction enzyme *BSP13I*. The C allele was cleaved in two fragments of 75 and 19 bp, while the T allele uncut at 94 bp (Fig. 1).

The least squares means and standard errors of milk production which include milk yield of 60, 100 and 305 days were shown in Table 1. Cows with genotype TT of leptin had significantly higher milk 60 and 100 days than the genotype CC ($p<0.05$) however no significant

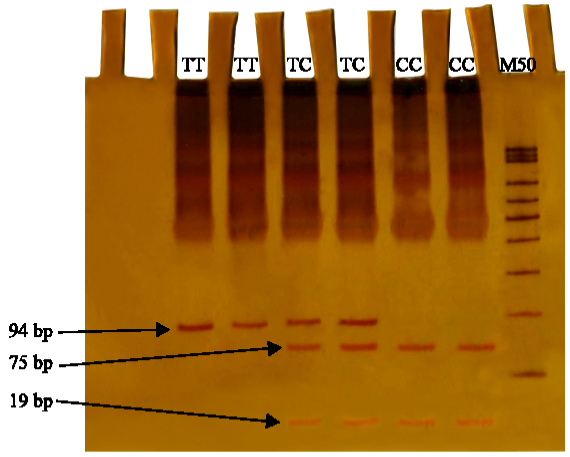

Fig. 1: An 8% acryl amide gel displaying a *BSP13I* restriction digests on An amplified portion of bovine leptin exonII

difference observed between genotypes in milk 305 day. This effect was prominent in 60 day (1.7 kg day^{-1}), declining to 1.5 kg day^{-1} in 100 day. The effect of leptin genotype on reproduction traits is shown in Table 2. For trait of open days no significant differences between genotypes were observed.

This study showed a genetic association between TT genotype and 60 and 100 day of milk which is confirm the results of Buchanan *et al*. (2003), Madeja *et al*. (2004), Liefers (2004) and Liefers *et al*. (2005a, b) did not find any associations between the Kpn2I polymorphism and milk production and reproduction traits. Such different results may be due to many factors such as breed differences, small number of animals studied (161 cows) and small number of animals with the TT genotype (27). In addition many other factors influence milk yield and reproduction traits such as environmental factors, age of cow, lactation parity and stage, animal's health with special reference to the mammary gland and interaction between the non genetic factors and genetic components (Madeja *et al*., 2004). Buchanan *et al*. (2002) verified a correlation between Kpn2I SNP alleles and the bovine carcass fat composition and suggested that the C/T change may be the causative mutation. Almeida *et al*. (2007) did not find any association between Kpn2I SNP polymorphism and the weight gain. Silva *et al*. (2002) did not find any effect of the HphI polymorphism, although they pointed that the Sau3AI can be a possible marker for milk and protein yield. Madeja *et al*. (2004) reported that the HphI polymorphism (the TT genotype) had an effect on the breeding values of production traits. Animals with the TT genotype had approximately two times higher EBV for milk and protein yields. Nkrumah *et al*. (2004) reported

Table 1: Least Square Means (LSM) and Standard Error (SE) for 60, 100 and 305 day milk yield

	Milk 60 day		Milk 100 day		Milk 305 day	
Genotype	LSM	SE	LSM	SE	LSM	SE
CC	2408.5527[a]	225.70538	223.26685[a]	144.65539	9195.9684[a]	678.59999
TC	2688.5087[b]	191.92515	3406.0053[ab]	144.62834	8827.9510[a]	663.34061
TT	2730.5089[b]	223.26685	3809.8806[b]	194.79186	9200.8870[a]	894.27898

[a,b]: Means with a different superscript are significantly different ($p<0.05$)

Table 2: Least square means (LSM) and standard error (SE) for trait of open days

Genotype	LSM	SE
CC	70.354331[a]	9.680972
TC	71.871495[a]	9.105133
TT	66.898553[a]	10.023633

association between SNP in the 5′ untranslatable promoter region of the bovine leptin gene with serum leptin concentration, growth, birth weight, feed intake and carcass merit in hybrid cattle. The study revealed that animals with the TT genotype in comparison to CC and CT genotypes showed increases in serum leptin concentration, backfat thickness and marbling score. Animals with the TT genotype also show significantly higher feed intake, growth rate, metabolic BW and live weight at slaughter (Nagatani *et al*., 2000). Liefers *et al*. (2003) reported that Sau3AI-AB genotype product 1.32 kg day^{-1} more milk and consume 0.37 kg day^{-1} more food compared with the sau3AI-AA genotype. Liefers *et al*. (2005a) was found association between three SNP in leptin promoter with fertility, energy balance and protein yield. Adamowicz *et al*. (2006) reported that C/G substation in promoter region of the leptin gene result in decreased the leptin gene promoter binding capacity for nuclear proteins and showed that the leptin expression level was higher in cattle with the CC than with the GG genotype. Lagonigro *et al*. (2003) was found association between A/T substation in exon2 of the leptin gene with feed intake and reported that genotype of AT in this position had 19% greater mean feed intake than genotype AA.

ACKNOWLEDGMENT

This investigation was supported by grants from Center of Excellence at the Animal Science Department, Agriculture College, Ferdowsi University of Mashhad in Iran.

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