

## LEPTIN GENE POLYMORPHISMS HAD NO EFFECTS ON OPEN DAYS AND CALVING INTERVAL

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### SUMMARY

Leptin plays an important role in the regulation of feed intake, energy metabolism, growth and reproduction of cattle. We used the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique to screen for DNA polymorphisms of the leptin gene in 255 cows of Iranian Holstein. Amplified region is located in exon three of leptin gene. The genomic bovine leptin sequences, which consist of three exons, were obtained from Gene Bank (Accession number U50365). Genotype frequencies were 0.588, 0.388 and 0.024 for AA, AB and BB respectively and allelic frequencies were 0.782 and 0.218 for A and B, respectively. We investigated effect of A59V polymorphism in the leptin gene on two reproduction traits. Significances of the genotype effects were tested using approximated F-statistic (SAS GLM procedure). This study showed that genotype had no effect on open days and calving interval (NS). Animals with the AB genotype had higher open days and calving interval.

### INTRODUCTION

Leptin is a 16-kDa polypeptide hormone synthesized and secreted predominantly by Adipose tissue. It functions regulating body weight, food Intake, energy expenditure, reproduction and immune system functions. Leptin was first identified, as gene product found deficient in obese (ob/ob) mice. A single base mutation of the leptin gene at the codon 105, as observed in the ob/ob mouse involved C/T mutation and replacement of arginine by a premature stop codon and a subsequent production of an inactive form of leptin (Zhang *et al.* 1994). The genetically obese ob/ob mouse exhibits obesity, infertility, hyperglycemia, impaired thyroid function and hyperinsulinemia with insulin resistance (Dubuc 1976). Treatments of the ob/ob mice with recombinant leptin reduce feeding and body weights (Halaas *et al.* 1995). Leptin treatment of animals has been shown to cause a decrease in food intake, body weight loss, fat deposit weight loss and increase in energy metabolism, therefore leptin not only causes reduced food intake, but the potential body weight losses are enhanced due to an increased metabolic rate (Houseknecht *et al.* 1998). The gene encoding Leptin was mapped to Bovine chromosome 4 and it consists 3 exons and 2 introns of which only two exons are translated into protein. The coding region of the leptin gene (501 nucleotides in length) is contained in exon 2 and 3, which are separated by intron of approximately 2 kb. Leptin is considered as a candidate gene for milk performance related traits in cattle. Several polymorphism in this gene have been found (Liefers *et al.* 2002 ). In exon three, A59V polymorphism, causes as amino acid change from alanine to valine. These amino acids both belong to the group of aliphatic amino acids, but valine is more hydrophobic. Aim of this study is to analyze A59V Polymorphism in exon 3 of leptin gene in Iranian Holstein cattle.

### MATERIALS AND METHODS

Blood was collected from 255 Holstein cattles of four different herd managements in Isfahan province. Simple statistics is presented in Table 1. Genomic DNA extracted from whole blood. Genotypes of A59V polymorphism were identified with PCR-RFLP technique. Amplified region

is located in exon three of Leptin gene. The genomic bovine leptin sequences, which consist of three exons, were obtained from GeneBank (Accession number U50365). The polymerase chain reaction was used to amplify the 331 bp DNA fragments from genomic DNA. The PCR reaction contained 100 ng of genomic DNA, 0.3  $\mu$ M of each primer, 1.5 mM MgCl<sub>2</sub>, 200  $\mu$ M dNTP, 10mM Tris HCl, 50 mM KCl and 1 U *Taq*-polymerase in total volume of 20  $\mu$ L. Sequences of primers that were used in PCR were reported previously by Haegeman et al. (2000). The sequence of the forward and reverse primers, respectively were: 5'-GGG AAG GGC AGA AAG ATA G-3' and 5'-TGG CGA ACT GTT GAG GAT C-3'. Conditions for PCR were 94°C for 2 min, followed by 35 cycles of 94°C for 30 s, 55°C for 1 min, and 72°C for 30 s. Followed by final extension for at for 15 min 72°C. Digestion of PCR product of 331 bp with with 5 U of *HphI* (Fermentas) in 20  $\mu$ L of reaction volume at 37°C for 8 h and analysed on 8% nondenature polyacrylamide gel. Allele A in the A59V polymorphism was the allele not digested by restriction enzyme, allele B was the restriction enzyme-digested PCR product. Digestion revealed 3 genotypes, AA (331 bp), AB (331, 311, and 20 bp), and BB (311 and 20 bp). The PCR reaction result in an artefact band of 600 bp which did not interfere it was not digested by the enzyme. The allele and genotype frequencies of A59V polymorphism were examined for deviation from Hardy-Weinberg equilibrium using  $\chi^2$  test. Data were analyzed using PROC GLM of SAS (2000), using the following linear model:

$$Y_{ijk} = \mu + G_i + H_j + b_1(X_{ijk} - \bar{X}) + b_2(Z_{ijk} - \bar{Z}) + e_{ijk}$$

Where  $Y_{ijklmn}$  = traits measured on each animal

$\mu$  = Overall mean

$G_i$  = Effect of Genotype

$H_j$  = Effect of herd

$X$  = Effect of dry period

$b_1$  = Linear regression for dry period trait

$Z$  = Effect of Lactation period

$b_2$  = Linear regression for lactation period trait

$e_{ijk}$  = Residual error

## RESULTS AND DISCUSSION

Genotype and allele frequencies of the A59V polymorphism are listed in Table 2. Genotype frequencies in all herds were 0.588, 0.388 and 0.024 for AA, AB and BB, respectively and allelic frequencies were 0.782 and 0.218 for A and B, respectively. Allelic frequency analysis have shown that frequencies ranged from 0.759 to 0.824 for allele A and 0.176 to 0.241 for allele B in all herds. In 2th herd we did not found BB genotype. The genotype frequencies were distributed according to Hardy-Weinberg equilibrium proportions in every four herd but were not in all herds ( $p < 0.05$ ). Open days was higher in heterozygous genotype. Calving interval did not differ among genotypes and was less in BB animals than in heterozygotes (Table 3). Woodside et al. (1998) showed that leptin influences the length of the anestrus period in rats suffering from severe negative energy balance due to food deprivation. As high producing cows also suffer from a negative energy balance due to lactation, leptin may influence the postpartum anestrus period in early-lactating cows.

Our findings for A59V polymorphism in bovine leptin gene are similar to those of Hanna Kulig (2005) who reported A and B allele frequencies of 0.760 and 0.240, respectively. Liefers *et al.* (2002) found a frequency of 0.747 for the A allele and of 0.254 for the B allele Result show that

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allele B has lower frequency in all study. Nassiry et al (2008) reported that allele C in Sarabi, Taleshi, Sistani, Golpayegani, Brown Swiss and Holstein cattle with 68, 55, 69, 71, 55 and 57% value were the most frequent alleles. Observed heterozygosities were highest in Golpayegani (57.89%).

Two days of total feed restriction in 11–12-month-old heifers markedly reduced leptin mRNA in adipose tissue, as well as circulating concentrations of leptin IGF-I, and insulin, and reduced the frequency of LH pulses compared to controls (Amstalden et al., 2000). In contrast to the prepubertal heifer, short-term fasting (60 h) did not attenuate pulsatile LH release in the mature cow. Central administration of leptin increased plasma LH in fasted but not in control-fed cows (Amstalden et al., 2002). Short-term (72-h) fasting and fasting-mediated reductions in LH pulse frequency were attenuated by peripherally administered recombinant leptin (Nagatani et al., 2000).

**Table 1. Mean  $\pm$  SD and CV for open days and calving interval**

Trait	N	Mean $\pm$ SD	Min	Max	CV
Open days	248	142.69 $\pm$ 4.05	40	393.5	44.62
Calving interval	248	418.35 $\pm$ 4.14	303.67	666.5	15.57

**Table 2. Genotype and allele frequencies of the A59V polymorphism**

Herd	N	Genotype			Allele	
		AA	AB	BB	A	B
1	58	0.552	0.414	0.034	0.759	0.241
2	54	0.648	0.352	0.000	0.824	0.176
3	40	0.650	0.325	0.025	0.813	0.188
4	103	0.553	0.417	0.029	0.762	0.238
Total	255	0.588	0.388	0.024	0.782	0.218

**Table 3. Effect of the A59V polymorphism on reproductive traits**

Trait	Genotype		
	AA	AB	BB
Calving interval	407.51 $\pm$ 5.6 <sup>a</sup>	416.05 $\pm$ 6.8 <sup>a</sup>	394.3 $\pm$ 26.3 <sup>a</sup>
Open days	128.15 $\pm$ 6.1 <sup>a</sup>	135.72 $\pm$ 7.3 <sup>a</sup>	119.71 $\pm$ 28.6 <sup>a</sup>

a,b Least squares means within a row without a common superscript letter differ,  $P < 0.05$ .

## **CONCLUSIONS**

This polymorphism could be further evaluated for marker assisted selection. Polymorphisms had no effect on open days and calving interval (NS). For finding the evolutionary relationships among closely populations, Leptin is a suitable and informative marker system.

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