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Association of leptin polymorphisms with production and reproduction traits in Iranian Holstein dairy cows

A R Heravi Moussavi[1], M Ahouei[2], M R Nassiry[1], A Javadmanesh[1]
[1]Excellence Center for Animal Science, Ferdowsi University, Mashhad, Khorasan, Iran, [2]Dept of Animal Science, University of Zabol, Zabol, Sistan Balouchestan, Iran
Email: bbheravi@yahoo.com

Introduction Leptin is a 16-kDa protein that is synthesized by adipose tissue and is involved in regulation of feed intake, energy balance, fertility and immune functions. It has been shown that leptin gene influences milk performance in cattle (Liefers *et al.*, 2002). Leptin is related to both energy metabolism and reproduction and it was shown that leptin polymorphisms had significant effect on calving interval and weight at first calving in beef cows (Almeida *et al.*, 2003). In cattle, the leptin gene is located on chromosome 4 and consists of three exons. The aim of this study was to indicate polymorphism at the bovine leptin gene locus in Iranian Holstein dairy cows and its contribution to the first cumulative 60-d milk yield, 305-d milk yield, days to first breeding (DFB), days open (DO), and days from first breeding to conception (DFBC) in the previous lactations.

Materials and methods In total, blood samples were collected from two hundred and thirty eight Iranian Holstein cows via venipuncture from coccygeal vessels. DNA extraction was done on the blood samples using guanidium thiocyanate-silica gel. PCR-RFLP method was used to detect the polymorphism of a 423 bp fragment from intron 2 of leptin gene. For the genotyped cow, the first cumulative 60-d milk yield, 305-d milk yield, DFB, DO, and DFBC using previous lactation records were also analyzed. For DFB and DO, the follow-up period started at 35 d after calving and ended at 200 and 365 d after calving, respectively. The DFB was defined as the interval from 35 d after calving to first breeding or end of follow-up period, whichever occurred first. The DO was defined as the interval from 35 d after calving to conception or end of follow-up period, whichever occurred first and the DFBC was the interval between DFB and DO. PopGen32 software was used to estimate the allele and genotype frequencies. Data from previous lactations were analyzed using Standard Least Square within mixed models using JMP software (version 4.0.4; SAS Institute Inc, NC. USA). Apart from the genotypic effect, the fixed effects were year, season, parity, and age at calving, and sire. For reproductive traits the cumulative first 60-d milk yield was also added to the model. Animal was fitted as a random effect.

Results Two genotypes, AA and AB, were distinguished which had the frequencies of 0.89 and 0.11, respectively. The genotypes were distributed according to the Hardy and Weinberg equilibrium ($P<0.05$). The results from previous lactations showed that the 305-d milk yield was affected by leptin polymorphism and the heterozygous produced more milk than the homozygous cows ($P<0.05$, Table 1). The 305-d milk yield was also affected by year, season, and parity at calving, and sire ($P<0.01$). The first 60-d cumulative milk yield was similar between two genotypes ($P=0.21$, Table 1) and tended to be higher in the heterozygous cows. The first 60-d cumulative milk yield was also impacted by year and parity at calving, and sire ($P<0.01$).

Table 1 Effect of the RFLP genotypes on milk production using previous lactation records.

	Genotypes		
Item	AA	AB	P
The first cumulative 60-d milk yield, kg	2288	2377	0.21
The 305-d milk yield, kg	9479	9550	0.03

There were no significant effects of genotype on reproductive performance (Table 2). The DFB was affected by sire, season and year of calving and age of cow at calving ($P<0.05$). The DO was influenced by sire, DFB and age at calving ($P<0.05$). Sire and DFB had significant effects on DFBC ($P<0.05$).

Table 2 Effect of the RFLP genotypes on reproduction traits using previous lactation records.

	Genotypes		
Item	AA	AB	P
Days from parturition to first breeding, d	71.04 ± 8.8	67.97 ± 12.2	0.77
Days open, d	126.75 ± 18.7	119.86 ± 28.1	0.79
Days from first breeding to conception, d	50.23 ± 20.7	46.18 ± 28.7	0.49

Conclusion The results of this study showed that leptin polymorphism was associated with 305-d milk production. It seems that breeding programs favouring the B-allele can yield a higher 305-d milk production without negatively affecting fertility.

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