Journal of Animal Research: v.7 n.3, p. 425-429. June 2017

DOI: 10.5958/2277-940X.2017.00063.8

Genetic Polymorphism of Leptin Gene in Relation with Reproduction Traits in Hariana Cows

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Received: 20 July, 2016 **Revised:** 21 July, 2016 **Accepted**: 04 August, 2016

ABSTRACT

Leptin gene is considered as marker of production and reproduction traits in dairy or beef cattle. The aim of this study was to investigate the genetic polymorphism in LEP gene in Hariana cows and their associations with reproduction traits. The blood samples were collected from 62 Hariana cows and analyzed in order to identify *LEP/BsaAI* (BTA 4, intron 2 and exon 3) genotypes using PCR-RFLP method. The allele frequencies observed were 0.37 and 0.63 for A and B *LEP* variants and genotypic frequencies were 9.67, 54.8 and 35.5 for AA, AB and BB genotype variants, respectively. Statistical analysis showed that SNP *LEP/BsaAI* significantly affected gestation period and dry period in analyzed population of cows. Observations of this investigation advocated that leptin is a candidate gene, which affects reproduction traits and might be implemented in breeding strategies to improve the reproductive performance of Hariana cattle breed.

Keywords: Hariana cows, leptin gene, reproduction, polymorphism

Leptin is a 16 kDa polypeptide hormone synthesized and secreted primarily from the adipose tissues (Forhead and Fowden, 2009) which has numerous physiological roles in the control of body growth, energy metabolism, feeding behaviour, reproduction and immune function (Moravcikova *et al.,* 2012). Leptin binds to a receptor on neuropeptide -Y- neurons in hypothalamus and plays a key role in the integration of feeding behaviour with internal signals of body energy status (Wayne *et al.,* 1995). An adequate amount of circulating leptin found to be essential for the attainment of puberty (Cunningham *et al.,* 1999). Besides, leptin may aid in regulation of steroidogenesis and ovarian development, and serve as either a primary signal initiating puberty, or as a permissive regulator of sexual maturation (Lindersoon *et al.,* 1998). In cattle, *LEP* gene is located on chromosome 4 which consists of three exons and two introns. The coding region of *LEP* gene (501 nucleotide length) is contained in exon 2 and 3 (Liefers *et al.,* 2002). Several mutations found in the bovine *LEP* gene reported to be associated with production and reproduction traits (Lagonigro *et al.,* 2003). The *LEP C (-963) T* polymorphism shown to be associated with milk yield (Glantz *et al.,* 2012), fat concentration (Giblin *et al.*, 2010), energy balance, feed intake and dry matter intake (Liefers *et al.,* 2005) and reproduction traits (Trakovicka *et al.*, 2013). Thus, the present study was designed with aim to elucidate the genetic polymorphism in LEP gene by PCR-RFLP assay and its association with reproduction traits in Hariana cattle.

MATERIALS AND METHODS

Sampling and DNA extraction

Blood samples were collected in 0.5% EDTA from 62 Hariaya cows and DNA was extracted according to phenol-chloroform extraction method (Sambrook and Russell, 2001). Quality and quantity of DNA were determined using spectrophotometer by measuring the optical density at wavelength of 260 and 280 nm.

PCR amplification

A fragment of 522 bp from intron 2 and exon 3 of the leptin gene was amplified using primers suggested by Lien *et al.* (1997).

Table 1: Primer sequence of LEP *BsaAI* loci

Locus	Primers
Leptin *BsaAI*	F (5´- GTC TGG AGG CAA AGG GCA GAG T-3´)
	R (5´-CCA CCA CCT CTG TGG AGT AG-3´)

One µl of DNA was amplified in a total volume of 25 µl PCR mix in thermocycler (Peqlab, USA). The PCR mix contained: 2.5 µl PCR dream buffer 10X, 200 µM dNTPs and 10 pM from each primer and 1 U *Taq* DNA polymerase. The PCR program was 94°C for 3 min, followed by 35 cycles of 94 °C for 30s, 58 °C for 30s and 72 °C for 30 sec. The final step prolonged for 5 min at 72°C.

Restriction reaction

Genotype analyses were carried out using the polymerase chain reaction – restriction fragment length polymorphism (PCR-RFLP) method. For this, the PCR products were digested using *BsaAI* (*Ppu21I*) restriction enzyme (Fermentas) at 30°C for 15 hours. Each digestion reaction contained 5 µl PCR products, 2 µl Buffer 10X, 5 U (0.5 µl) restriction enzyme and 9 µl deionized water. The fragments were separated by horizontal electrophoresis in 2% agarose gels in 1X TBE stained with Ethidium Bromide (10 mg/µl) (Fermentas) prior to visualization under UV light.

Statistical analysis

The allele and genotype frequencies of LEP/*BsaAI* polymorphism were examined for deviation from Hardy– Weinberg equilibrium using χ^2 test and statistical significance was determined by ANOVA followed by Tukey's post-hoc multiple comparison test using SPSS software for Windows (version 16.0). The data were presented as the mean ± SE and a p value <0.05 was considered to be statistically significant.

RESULTS

Table 2: Genotypic and allelic frequencies of LEP/*BsaA*I gene in Hariana Cattle

Breed (N=62)	Genotypic frequency (%)			Allelic Frequency		χ^2 value
	AA (n=6)	AB (n=34)	BB (n=22)	A	B	
Hariana	9.67	54.80	35.50	0.37	0.63	14.44

Where; N= Sample size, n= Number of animals in particular genotype.

The extracted *DNA* showed a good quality by spectrophotometer method and PCR resulted in clear bands (Fig. 1). *PCR-RFLP* of LEP/*BsaA*I result showed two alleles (A and B) and three genotypes (AA: 522 bp band; BB: 441 bp and 81 bp bands; AB: 522, 441, and 81 bp bands) (Fig. 2).

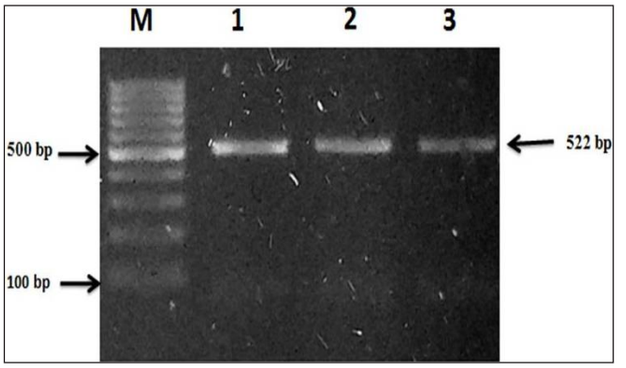

Fig. 1: Agarose (1%) gel electrophoresis showing PCR product of 522 bp; Lane M- DNA ladder (100 bp); Lane 1, 2, 3-LEP PCR product.

On the basis of the Hardy-Weinberg formulas, the expected frequencies of A and B alleles were 0.37 and 0.63, respectively. The expected frequencies of the three genotypes were 9.67% (AA), 54.8% (AB) and 35.5% (BB). The observed numbers of genotypes were 6 (AA), 34 (AB) and 22 (BB) (Table 2). The most frequent genotype for LEP/*BsaA*I loci in observed population was AB with 34 individuals. The calculated χ^2 test value was 14.44, indicating that selected population of Hariana cows was not in Hardy-Weinberg equilibrium.

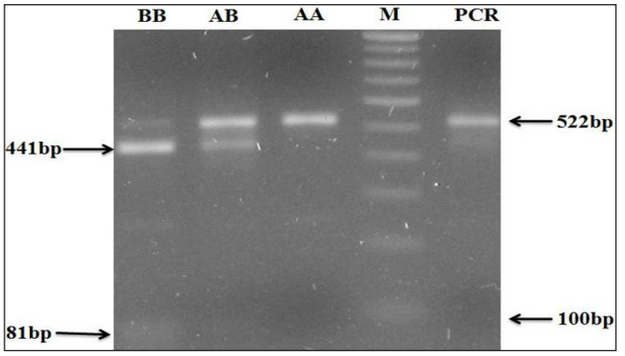

Fig. 2: LEP/BsaAI PCR-RFLP assay showing genotypic pattern in 2% agarose gel; Lane 1- BB genotype (441 & 81 bp), Lane 2- AB genotype (522, 441 & 81 bp), Lane 4- AA genotype (522 bp only); Lane M- DNA ladder (100 bp); Lane 5- PCR product

Association studies of LEP/*BsaAI* assay revealed significant influence of AA, AB and BB genotypes on gestation period (GP) and dry period (DP) while Age at first calving (AFC) and calving interval (CI) did not show significant influence of genetic polymorphism in LEP gene in analyzed population of cattle. The GP showed significant influence of SNP among AA, AB and BB genotypes in the first lactation while in second and third lactations GP did not show significant effect. DP of AA genotype was observed significantly lower than BB and AB genotypes in second and third lactation.

DISCUSSION

The frequency of AB genotype in the present investigation was higher (54.80) than other two genotypes AA and BB, which was in agreement with the observations of Choudhary *et al.* (2005) in Hariana cattle and by other authors in various exotic cattle breeds (Souza *et al*., 2010; Azari *et al.,* 2012; Rezaei *et al*., 2015). The value of BB genotype obtained in present study was 35.5%, which was comparable to the result of Choudhary *et al.* (2005) in Hariana cattle (40.0%). But the frequency of BB genotype was smaller than the findings of previous researchers in different indigenous and exotic cattle breeds. In their findings the value of BB genotype was <13.2% to 30.0% (Souza *et al*., 2010; Azari *et al.,* 2012; Rezaei *et al*., 2015). The present study suggests that this mutation might have occurred far back in evolution before the divergence of cattle into taurine and indicine cattle.

In present study, allelic frequency of LEP/*BsaA*I for A and B allele were 0.37 and 0.63 respectively, which was accordance with the findings of Choudhary *et al.* (2005) in Hariana and other breeds of cattle. Frequency pattern of B allele was higher than A allele which was similar to the findings of other workers in different breeds of cattle (Almeida *et al*., 2003; Azari *et al.,* 2012; Rezaei *et al*., 2015). It can be explained as there may be a selection force acting against the A allele or favouring the G allele

Table 3: Association studies of LEP/*BsaA*I genotypes with reproduction traits in three lactations

Lactation	Genotype	n	AFC (days)	GP (days)	DP (days)	CI (days)
I (N=49)	AA	5	1919.40±207.68	284.20±1.31^{b}	131.60±55.75	499.20±87.00
	AB	27	2128.07±68.40	276.67±1.51^{a}	254.17±33.77	546.92±30.35
	BB	17	1822.71±82.69	276.47±1.97^{a}	265.50±43.22	489.06±31.48
II (N=35)	AA	5	-----	276.60±3.04	90.20±13.84^{a}	527.40±46.93
	AB	18	-----	279.94±1.42	213.56±29.20^{b}	486.78±21.72
	BB	12	-----	278.50±1.61	292.00±63.22^{b}	463.17±25.95
III (N=22)	AA	3	-----	275.00±2.00	122.00±43.66^{a}	429.33±25.72
	AB	12	-----	280.42±1.76	244.50±32.20^{ab}	425.42±23.76
	BB	7	-----	282.29±1.99	336.14±47.87^{b}	422.00±30.05

AFC-Age at first calving GP- Gestation period DP- Dry period CI- Calving interval

Different superscript alphabet in a column differ significantly.

in these population. Contrary to the present findings, lower frequency of LEP/*BsaA*I B allele was reported in Nellore cattle (0.50), Mazandarani native cattle (0.44) and Iranian cattle by Souza *et al*. (2010), Azari *et al.* (2012) and Rezaei *et al*. (2015), respectively.

The reproductive parameters employed (AFC, DP, GP and CI) are indirect measurements of reproduction, also reflecting animal body conditions. Additionally LEP is related to both energy metabolism and reproduction (Houseknecht 1998). In the present study, no significant difference was observed among the genotypes for the AFC and CI in all the lactation. In contrast, Almeida *et al.* (2003) and Chebel and Santos (2011) observed significant association of CI and with LEP/*Sau3A*I genotypes and Dandapat *et al.* (2009) and Komisarek and Antkowiak (2007) did not reveal significant association of SNP in LEP gene with reproduction traits in Sahiwal and Jersey cows, respectively.

In conclusion, a leptin gene fragment of 522 bp was found polymorphic in Harian cow using restriction enzyme *BsaA*I. Leptin genotypes observed to have significant effect on gestation period and dry period during first lactation and during second and thrird lactation, respectively. Leptin genotypes were found non-significantly associated with reproduction traits (age at first calving and calving interval). The homozygous cows (AA) tended to have a shorter dry period than the heterozygous cows. This genetic information of bovine leptin gene could be useful in marker assisted selection to improve reproductive performance.

REFERENCES

Almeida, S.E.M., Almeida, E.A., Moraes, J.C.F. and Weimer, T.A. 2003. Molecular markers in the LEP gene and reproductive performance of beef cattle. *J. Anim. Breed. Genet.,* **12**: 106–113.

Azari, M.A., Hasani, S., Heidari, M. and Yousefi, S. 2012. Genetic polymorphism of leptin gene using PCR-RFLP method in three different populations. Slovak *J. Anim. Sci.,* **45**(2): 39-42.

Choudhary, V., Kumar, P., Bhattacharya, T.K., Bhushan, B. and Sharma, A. 2005. DNA polymorphism of leptin gene in *Bos indicus* and *Bos taurus* cattle. *Genet. Mol. Biol.,* **28**(4): 23-27.

Chebel, R.C. and Santos, J.E.P. 2011. Association between leptin single nucleotide polymorphism and reproductive performance of lactating Holstein cows. *Anim, Reprod. Sci.,* **127**: 126-134.

Cunningham, M.J., Clifton, D.K. and Steiner, R.A. 1999. Leptin'sactions on the reproductive axis: Perspectives and mechanisms. *Biol. Reprod.*, **60**: 216-222.

Dandapat, A., Kumar, D., Ghosh, A.K. and Banerjee, D. 2009. Association of leptin gene polymorphism with growth, milk production and reproduction traits in Sahiwal and crossbred cattle. *Indian J. Anim. Sci.*, **79**(9): 892-896.

Forhead, A.J. and Fowden, A.L. 2009. The hungry fetus? Role of leptin as a nutritional signal before birth. *J. Physiol.,* **587**: 1145-1152.

Giblin, L., Butler, S.T., Kearney, B.M., Waters, S.M., Callanan, M.J. and Berry, D.P. 2010. Association of bovine leptin polymorphism with energy storage traits in progeny tested Holstein- Fresian dairy cattle sires. *BMC Genet.,* **11**: 73-77.

Glantz, M., Mansson, H.L., Stalhammar, H. and Paulsson, M. 2012. Effect of polymorphism in the leptin, leptin receptor and acyl-CoA:diacylglycerol acyltransferase 1 (DGAT1) genes and genetic polymorphism of milk pro¬teins on bovine milk composition. *J. Dairy Res.*, **79**: 110-118.

Houseknecht, K.L., Baile, C.A., Matteri, R.L. and Spurlock M.E. 1998. The biology of leptin: a review. *J. Anim. Sci.,* **76**: 1405-1420.

Komisarek, J. and Antkowiak, I. 2007. The relationship between leptin gene polymorphisms and reproductive traits in Jersey cows. *Polish J. Vet. Sci.*, **10**: 193-197.

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

Liefers, S.C., Pas M.F.W., Veerkamp R.F. and Van der Lende T. 2002. Associations between leptin gene polymorphisms and production live weight energy balance, feed intake and fertilityin Holstein heifers. *J. Dairy Sci.*, **85**: 227-238.

Liefers, S.C., Veerkamp, R.F., Te Pas, M.F.W., Delavaud, C., Chilliard, Y., Platje, M. and van der Lende, T. 2005. Leptin promoter mutations affect leptin levels and performance traits in dairy cows. *Anim. Genet.*, **36** (2): 111-118.

Lien, S., Undvold, H., Lungland, H. and Vage, D.I. 1997. Two novel polymorphisms in the bovine obesity gene (OBS). *Anim. Genet.*, **28**: 45-48.

Lindersoon, M., Andersson-Eklund, L., Koning, D.J., Lunden, A., Maki-Tanila, A. and Andersson, L. 1998. Mapping of serum amylase-1 and quantitative trait loci for milk production traits to cattle chromosome 4. *J. Dairy Sci.,* **81**: 1454-1461.

Moravčíková, N., Trakovická, A. and Kasarda, R. 2012. Polymorphism within the intron region of the bovine leptin

gene in Slovak Pinzgau Cattle. *Anim. Sci. Biotechnol.*, **245**(1): 112-115.

Rezaei, M., Mirhosseini, S.Z. and Hossein-Zadeh, N.G. 2015. PCR-RFLP analysis of leptin gene and its association with milk traits in native cattle of Iran (*Bos indicus*). *Rev. Mex. Cienc. Pecu*., **6**(1): 15-24.

Sambrook, J. and Russel, D.W. 2001. Molecular Cloning: A Laboratory Manual. 3rd edn, Vol. 1, Cold Spring Harbor Laboratory Press, New York, USA.

Souza, F.R., Mercadante, M.E., Fonseca, L.F., Ferreira, L.M., Regatieri, I.C., Ayres, D.R., Tonhati, H., Silva, S.L., Razook, A.G. and Albuquerque, L.G. 2010. Assessment of DGAT1 and LEP gene polymorphisms in three Nelore (*Bos indicus*) lines selected for growth and their relationship with growth and carcass traits. *J. Anim. Sci.,* **88**: 435-441.

Trakovicka, A., Moravčikova, N. and Kasarda, R. 2013. Genetic polymorphisms of leptin and leptin receptor genes inrelation with production and reproduction traits in cattle. *Acta Biochim. Pol.*, **60** (4): 783-787.

Wayne, J., Kuenzel, W.J. and Fraley, G.S. 1995. Neuropeptid y: it´s in the neural regulation of reproductive function and feed intake in mammalian species. *Poultry Avian Biol. Rev.,* **6**: 185-209.