Original Research

Study of Genetic Polymorphism in Leptin Exon 3 Region and its Association with Milk Production and Reproduction Traits in Indian Sahiwal Cattle

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Abstract

This study investigated the status of leptin (LEP) exon 3 polymorphism in Indian Sahiwal cattle breed (n = 124) using hphI/PCR-RFLP assay and its association with milk production and reproduction traits. The 454 bp amplified fragment upon restriction digestion revealed two types of genotypes; namely; CT genotype (454, 309 and 145 bp fragments) and TT genotype (309 and 145 bp fragments) with frequencies 29.03% and 70.97%, respectively. We could not find any CC genotype (454 bp; uncut fragments) in screened population. Allelic frequency of C and T alleles were 0.15 and 0.85, respectively. Chi square analysis revealed that screened population was not found in Hardy-Weinberg equilibrium. Association studies reveled that TT genotype was associated with shorter age at first calving (AFC) and days to reach peak yield (DRPY) in the first lactation, while TT has shorter dry period (DP) than CT genotype in all the five lactation. TT genotype was also associated with shorter calving interval (CI) in all the studied lactation except lactation I. Therefore, the present study indicating that T allele of this SNP in LEP exon 3 regions could be used as a potential genetic marker for improvement in reproduction and production performance in cattle.

Key words: Cattle, *hph*I, Leptin, Milk Production Traits, PCR- RFLP, Sahiwal

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Introduction

Leptin is a 16-kDa polypeptide hormone product of the leptin (*LEP*) gene. It is synthesized and secreted predominantly by adipose tissue and regulating body weight, food Intake, energy expenditure, reproduction and immune system. The milk production traits are quantitative traits and these are directly related to feed intake and energy balance (Block *et al*., 2001). It was observed that the feed intake-induced leptin increase was eliminated during lactation and they speculated that the hypo-leptinemia may

be an important factor promoting the hyperphagia of lactation. This data demonstrated that the onset of the negative energy balance is largely responsible for the declining leptin concentrations towards parturition and the low leptin level during lactation probably induces the hyperphagia of lactation. Therefore, *LEP* may be important in regulating metabolic adaptation of nutrient partitioning during the energy-consuming processes of pregnancy and lactation (Moschos *et al*., 2002). The gene encoding leptin was mapped to bovine chromosome 4 and it consists of 3 exons and 2 introns, of which only two exons are translated into protein. The coding region of the *LEP* gene (501 nucleotides length) is contained in exon 2 and 3, which are separated by intron of approximately 2 kb (Stone *et al*., 1996) and it is considered as a strong candidate gene for milk performance related traits in cattle.

Several single nucleotide polymorphisms (SNPs) in the *LEP* gene have been identified by previous authors (Liefers *et al*., 2002; Madeja *et al*., 2004, Yoon *et al*., 2005; Oztabak *et al*., 2010). However, research on associations between *LEP* gene polymorphism and milk production traits in dairy cattle is rather scanty (Buchanan *et al*., 2003; Sadeghi *et al*., 2008; Dandapat *et al*., 2009; Singh *et al*., 2014). A SNP resulting from a *C>T* substitution, causes amino acid change from alanine to valine (A59V) has been observed in *LEP* exon 3 in various cattle breeds (Haegeman *et al*., 2000; Madeja *et al*., 2004; Kulig and Kmiec, 2009; Oztabak *et al*., 2010; Silva *et al*., 2014), but a few reports about this *LEP* exon 3 polymorphism and its association with milk production and reproduction traits have been reported in Sahiwal cattle breed (Dandapat *et al*., 2009; Dandapat *et al*., 2010; Singh *et al*., 2014). Hence, the present study was undertaken with the objective of investigation of this SNP (A59V) within *LEP* exon 3 region and its association with milk production and reproduction traits in Indian Sahiwal cattle.

Materials and Methods

Animals and DNA Isolation

A total of 124 animals (only female) of Sahiwal breed of cattle were utilized in the present investigation, out of them 92 maintained at Instructional Dairy Farm, National Dairy Research Institute (NDRI), Karnal (Haryana) and remaining 32 maintained at Instructional Livestock Farm Complex (ILFC), DUVASU, Mathura (Uttar Pradesh). Genomic DNA was isolated using the standard phenol–chloroform extraction method (Sambrook and Russel, 1991).

Amplification of *LEP* Exon 3 Gene Fragment and *Hph*I/PCR-RFLP Assay

C>T SNP containing region of *LEP* gene fragment has been amplified from isolated DNA as per Oztabak *et al*., 2010 (Table 1). The restriction digestion was carried out at 37ºC for 14-16 hr in a total volume of 15µl containing 10µl PCR products, 0.5 µl *Hph*I (Fermentas, USA; 10U/ µl) and 1.5µl 10X RE buffer. For restriction fragment analysis, digested products were checked on 1.5% agarose gel in 1X TAE buffer for 4-5 hrs at 5 V/cm.

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Table 1: SNPs, Primers, annealing temperature and restriction enzyme details of amplified regions of *LEP* gene

SNP	Region	Amino Acid Change	Primer Sequence	Product Size	Ann Temp	RE
C>T	Exon 3	A59V	5'- GGGAAGGGCAGAAAGATAG-3' 5'- CCAAGCTCTCCAAGCTCTC-3'	454 bp	57°c 30s	*Hph*I

Statistical Analysis

The data will be generated by estimating the frequency of different amplified products. The allelic frequency and genotypic frequencies of *LEP* gene was estimated by standard procedure (Falconer and Mackay, 1996) using following formulae:

$$\text{Gene Frequency} = (2D + H)/ 2N$$

Where, D = No. of homozygote of particular gene, H = No. of heterozygote having that gene, N = Total No. of individuals

$$Genotypic\ Frequency = \frac{Total\ no.of\ individual\ of\ particular\ genotype}{Total\ no.of\ individuals\ of\ all\ genotype}$$

The chi square (χ^2) test ($P \leq 0.05$) was also performed to test whether the distribution of the genotype frequencies was in the Hardy-Weinberg equilibrium (Snedecor and Cochran, 1989).

Association Study

The association study of LEP/*hph*I genotypes with the following milk production and reproduction traits: age at first calving (AFC), dry period (DP), lactation period (LP), total milk yield (TMY), milk yield in 300 days (MY300), calving interval (CI), peak yield (PY), days to reach peak yield (DRPY). Analysis of associations between the *LEP* genotypes, and production traits, were carried out with the General Linear Model (GLM) using SPSS software (ver. 16). The following linear model was applied:

$$Y_{ij} = \mu + G_i + e_{ij}$$

where: Y_{ij}– observed trait value of i^{th} genotype in animal; μ – mean trait value; G_i – effect of i^{th} genotype; e_{ij}– random error. Significant differences among least square means of different genotypes were calculated using Duncan's multiple-range test, and *P* values of 0.05 were considered statistically significant.

Results and Discussion

The amplified fragments of the *LEP* gene revealed 454 bp product (Fig. 1). The LEP/*Hph*I PCR-RFLP assay revealed two types of banding pattern (genotypes); one of them was of 309 and 145 bp (TT genotype); second of 454, 309 and 145 bp (CT genotype) and wild type homozygous pattern (CC

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genotype) was completely absent in the sample population (Fig. 1). This revealed that the Sahiwal cattle used in the present study were polymorphic in nature with two types of alleles C and T.

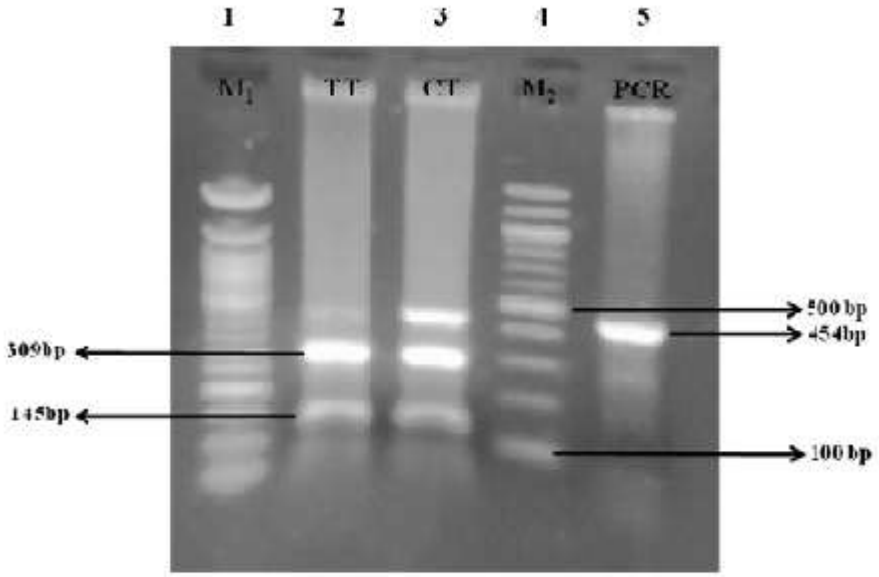

Fig. 1: LEP/*Hph*I PCR-RFLP assay showing genotype pattern in 1.5% agarose gel; Lane 1: M_1: 50bp ladder, 2: TT genotype (309 & 145 bp), 3: CT genotype (454, 309 & 145 bp) 4: M_2 - 100 bp ladder, 5: Undigested PCR product (454 bp).

LEP/*Hph*I PCR-RFLP assay revealed that the TT genotype was the most frequent (70.97%) in all the screened samples, followed by the heterozygote CT (29.03%), whereas the CC genotype was not found in these samples. The frequency of LEP/*Hph*I C and T alleles was 0.15 and 0.85, respectively. Chi square test revealed that $\chi^2_{(cal)} > \chi^2_{(tab)}$ at 1% level of significance for degree of freedom 1 indicating that screened population of Sahiwal cattle was not found in Hardy- Weinberg equilibrium. The means with standard errors of mean (Mean ± S.E.M.) for each trait related to each genotype in all the five lactations are presented in Table 2. In the present study, the CC genotype was not found (0.0%), which was similar to study in Nellore bulls (Silva *et al*., 2014). This report was dissimilar with the findings in Sahiwal cattle; monomorphic; 100% (Dandapat *et al*., 2009; 2010), 96.0% (Singh *et al*., 2014) and in other breeds of cattle; Polish Black and White bull (42.7%; Madeza *et al*., 2004), Jersey X HF X Sahiwal crossbred (57.0%; Dandpat *et al*., 2010), Iranian Holstein (58.8%; Yazdani *et al*., 2010), Frieswal (58.0%; Singh *et al*., 2014). However, its frequency was 100% in Mehsana buffalo (Deshpande *et al*., 2014). It might be possible due to small population size and selective breeding towards T allele.

The genotypic frequency of CT genotype in the present investigation was 29.03%, which was in the range (30 - 47%) with the observations in different breeds of cattle; Polish Black and White bull (47.0%, Madeza *et al*., 2004), Jersey X HF X Sahiwal crossbred (36.0%; Dandpat *et al*., 2010), Iranian Holstein

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(38.8%; Yazdani *et al*., 2010), Frieswal (38.0%; Singh *et al*., 2014) and Nellore bulls (30.0%; Silva *et al*., 2014).

Table 2: Association studies of LEP/*Hph*1 genotypes with milk production and reproduction traits in Sahiwal cattle

Lactation	Genotype	n	AFC (days)	DP (days)	LP (days)	TMY (liters)	MY300 (liters)	CI (days)	PY (liters)	DRPY (days)
I	TT	88	1103.6±10.69**	133.78±4.41*	347.85±5.98	1617.3±42.9	1424.52±37.36	481.64±6.87	6.34±0.16	54.38±0.81*
(N=124)	CT	36	1550.8±42.3**	176.11±16.7*	348.53±12.5	1550.1±90.58	1409.44±80.72	524.64±21.9	5.8±0.34	58.06±1.59*
II	TT	70	-----	133.81±5.13**	319.99±5.24	1613±51.60	1513.09±44.95	452.51±6.53**	6.91±0.2	53.05±1.06*
(N=88)	CT	18	-----	228.17±22.83**	350.56±20.9	1480.2±161.97	1355.44±127.79	584.28±25.56**	5.64±0.66	59.22±2.0*
III	TT	51	-----	129.04±7.87*	328.33±6.24	1648.6±54.12	1518.24±48.45	456.39±9.09*	6.79±0.24	52.53±1.27
(N=62)	CT	11	-----	221.27±34.04*	323.27±19.15	1495.8±181.59	1409.18±171.16	544.55±31.79*	5.59±0.90	59.73±4.08
IV	TT	32	-----	130.29±7.93*	328.26±6.45	1664.6±71.23	1550.60±64.13	455.92±10.63*	7.18±0.28	47.29±1.31
(N=50)	CT	18	-----	227.67±34.29*	317.75±17.21	1419.6±172.74	1328.42±137.21	545.42±31.04*	7.33±0.56	50.83±2.69
V	TT	32	-----	125.22±6.82*	324.25±7.66	1688.2±73.59	1587.97±72.45	449.78±10.47*	7.31±0.33*	50.19±1.81
(N=41)	CT	9	-----	219.01±31.46*	318.22±17.19	1344.0±169.92	1266.33±140.91	537.22±26.17*	5.22±0.78*	60.0±4.45

Superscript (*) and (**) of a given row indicates significant difference between genotypes at P<0.05 and at P<0.01, respectively, n — number of individuals in particular genotype, N — total number of individual in particular lactation, n= number of animals; GP= Gestation Period; LP= Lactation Period; TMY= Total Milk Yield; MY300= Milk Yield in 300 days; DP= Dry Period; CI= Calving Interval; PY= Peak Yield; DRPY= Days to Reach Peak Yield

But it was much higher than the previous findings in Sahiwal; 4.0% (Singh *et al*., 2014). In contrast, Dandapat *et al*. (2009) and (2010) could not observe any CT and TT genotypes in studied Sahiwal cattle. The value of TT genotype obtained in present study was 70.97%, similar to result in Nellore bulls (70.0%; Silva *et al*., 2014). This study was not consistent with the results of previous reports. It was much higher than the other findings in Sahiwal; 0.0% (Dandpat *et al*., 2009; 2010; Singh *et al*., 2014) and other cattle breeds; Polish Black and White bull (10.25%, Madeza *et al*., 2004), Jersey X HF X Sahiwal crossbred (7.0%; Dandpat *et al*., 2010), Iranian Holstein (2.4%; Yazdani *et al*., 2010), Frieswal (4.0%; Singh *et al*., 2014). However, its frequency was 100% in Murrah buffalo (Datta *et al*., 2013). Since in India, Sahiwal cattle are reared for milch purpose, this mutant allele might have undergone selection.

In the present study, allelic frequency of LEP/*Hph*I for C and T allele were 0.15 and 0.85, respectively. In contrast, higher frequency of LEP/*Hph*I C allele was reported in Sahiwal; 1.0 (Dandpat *et al*., 2009; 2010), 0.98 (Singh *et al*., 2014) and all other breeds of cattle; Polish Black and White bull (0.66, Madeza *et al*., 2004), Jersey X HF X Sahiwal crossbred (0.75; Dandpat *et al*., 2010), Iranian Holstein (0.782; Yazdani *et al*., 2010), Nellore (0.99; da Silva *et al*., 2012), Frieswal (0.77; Singh *et al*., 2014). It might be possible due to small sample population and selective breeding towards T allele.

Association analysis revealed that TT genotype had significantly (*P = 0.000*) smaller AFC in comparison to heterozygote CT. These results of AFC were dissimilar to the findings in Jersey X HF X Sahiwal (Dandpat *et al*., 2009) and frieswal (Singh *et al*., 2014) crossbred cattle. It might be due to difference in

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crossbred and indigenous cattle population. GP were not affected by genotypes in all the five lactations, which was also reported in crossbred cattle (Dandpat *et al*., 2009), while CC genotype had significant higher GP than other genotypes in Iranian Holstein cattle (Yazdani *et al*., 2010). LP was not affected by genotypes in all the lactations but CT genotype had higher LP in crossbred cattle (Dandpat *et al*., 2009). However, DP of TT genotype was significantly *(P<0.05)* lower than CT genotype in all the lactations. It can be explained by the selective breeding towards T allele, while in Iranian Holstein cattle there was no significant difference for DP among genotypes (Yazdani *et al*., 2010). CI of TT genotype (except Lactation I) was also significantly (*P<0.05*) lower than CT genotype in all the lactations, while in Iranian Holstein (Yazdani *et al*., 2010) and crossbred (Dandpat *et al*., 2009) cattle, CI was not affected by the genotypes. In the present study, no significant difference was found for TMY and MY300 between CT and TT genotypes in all the lactations, which was similar to the findings in Frieswal cattle (Singh *et al*., 2014) but CT genotype had higher milk yield in first and second lactation in crossbred cattle (Dandpat *et al*., 2009).

In the current study, T allele was found to be responsible for higher milk production without negatively affecting energy balance and fertility, which was similar to previous findings (Liefers *et al*., 2002, Madeja *et al*., 2004). Finally, allele T of *LEP* gene can be considered as a good indicator for milk production in the Sahiwal cattle breed.

Conclusion

Only two genotypes (CT and TT) in *LEP* exon 3 was identified in studied Sahiwal cattle population using *hph*I/PCR-RFLP assay. Association studies confirmed that C>T/*hph*I genotypes had significant effect on milk related traits. TT genotype had shorter AFC and DRPY in first lactation, while TT genotype has shorter DP than CT genotype in all the five lactation. TT genotype was also associated with shorter CI in all the lactation except lactation I. LEP/*hph*I genotypes had no effect on LP, TMY, MY300, GP and PY. Sahiwal cows with TT genotype tended to have a better reproduction performance than the heterozygote cows. Therefore, this SNP could be useful in marker assisted selection to improve the reproduction and milk production performance in dairy cattle.

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References

1. Block SS, Butler WR, Ehrhardt RA, Bell AW, Van Amburgh ME and Boisclair YR. 2001. Decreased concentration of plasma leptin in periparturient dairy cows is caused by negative energy balance. Journal of Endocrinology. 171: 339–348.
2. Buchanan FC, Van Kessel AG, Waldner C, Christensen DA, Laarveld B and Schmutz SM. 2003. An association between a leptin single nucleotide polymorphism and milk and protein yield. Journal of Dairy Science. 86: 3164-3166.
3. da Silva RCG, Ferraz JBS, Meirelles FV, Eler JP, Balieiro JCC, Cucco DC, Mattos EC, Rezende FM and Silva SL. 2012. Association of single nucleotide polymorphisms in the bovine leptin and leptin receptor genes with growth and ultrasound carcass traits in Nellore cattle. Genetics and Molecular Research. 11: 3721-3728
4. Dandapat A, Kumar D, Ghosh AK and Banerjee D. 2009. Association of leptin gene polymorphism with growth, milk production and reproduction traits in Sahiwal and crossbred cattle. Indian Journal of Animal Sciences. 79(9): 892- 896.
5. Dandapat A, Kumar D, Ghosh AK and Umapathi V. 2010. A note on a DNA polymorphism study of leptin gene in Sahiwal and crossbred cattle using PCR-RFLP technique. Livestock Research for Rural Development. 22(1): 2010.
6. Datta S, Verma A, Chatterjee P and Pal A. 2013. Molecular characterization of the leptin gene in riverine buffalo. Buffalo Bulletin. 32(3): 196-211.
7. Despande M, Rank DN, Vataliya PH and Joshi CG. 2014. Study of leptin gene polymorphism in Mehsana buffalo (*Bubalus bubalis*). Buffalo Bulletin. 33(1): 115-119.
8. Falconer DS and Mackay TFC. 1996. Introduction to Quantitative Genetics. 4th edn., pp. 56. Addison Wesley Longman Limited, English.
9. Haegeman A, Van Zeveren A and Peelman LJ. 2000. New mutation in exon 2 of bovine leptin gene. Animal Genetics. 31:79.
10. Kulig H and Kmieć M. 2009. Association between leptin gene polymorphisms and growth traits in Limousin cattle. Genetika. 45: 838-841.
11. Liefers SC, Te Pas MFW, Veerkamp RF and Van Der Lende T. 2002. Associations between leptin gene polymorphisms and production, live weight, energy balance, feed intake and fertility in Holstein heifers. Journal of Dairy Science. 85: 1633.
12. Madeja Z, Adamowicz T, Chmurzynska A, Jankowski T, Melonek J, Switonski M and Strabel T. 2004. Effect of leptin gene polymorphisms on breeding value for milk production traits. Journal of Dairy Science. 87(11): 3925- 3927.
13. Moschos S, Chan JL and Mantzoros CS. 2002. Leptin and reproduction: A review. Fertility and Sterility. 77: 433-444.
14. Oztabak K, Toker NY, Cemal UN, Akis I, Mengi A, Karadag O and Soysal D. 2010. Leptin gene polymorphisms in native Turkish cattle breeds. *Kafkas* Üniversitesi Veteriner Fakültesi Dergisi . 16(6): 921- 924.
15. Sadeghi M, Babak MMS, Rahimi G, Javaremi AN. 2008. Effect of leptin gene polymorphism on the breeding value of milk production traits in Iranian Holstein. Animal. 2(7): 999-1002
16. Sambrook J and Russel DW. 2001. Molecular Cloning: A laboratory Manual. Cold Spring Harbour. Tab. Press, New York, pp. 6.4-6.11.
17. Silva DBS, Crispim BA, Silva LE, Oliveira JA, Siqueira F, Seno LO and Grisolia AB. 2014. Genetic variations in the leptin gene associated with growth and carcass traits in Nellore cattle. Genetics and Molecular Research. 13(2): 3002-3012.
18. Singh U, Kumar S, Deb R, Mann S and Sharma A. 2014. Genotypic profiling of coding region of leptin gene and their association studies on reproductive and milk production traits in Sahiwal and Frieswal cattle of India. African Journal of Biotechnology. 12(42): 6140- 6146.
19. Snedecor GW and Cochran WG. 1989. Statistical Methods. 8th Ed. Ames: Iowa State Press.

DOI 10.5455/ijlr.20170504011835

20. Stone RT, Kappes SM and Beattle CW. 1996. The bovine homolog of the obese gene maps to chromosome 4. Mammalian Genome. 7: 399-400.
21. Yazdani H, Rahmani HR, Edris MA and Dirandeh E. 2010. Association between A59V polymorphism in exon 3 of leptin gene and reproduction traits in cows of Iranian Holstein. African Journal of Biotechnology. 9(36): 5997- 6000.
22. Yoon DH, Cho BH, Park BL, Choi YH, Cheong HS, Lee HK, Chung ER, Cheong IC and Shin HD. (2005). Highly polymorphic bovine leptin gene. Asian Australian Journal of Animal Science. 18(11): 1548:1551.

DOI 10.5455/ijlr.20170504011835