Indian Journal of Animal Sciences **90** (8): 1126–1129, August 2020/Article

Association of genetic polymorphism in leptin gene with growth, reproduction and production traits in Sahiwal cows

PRAJWALITA PATHAK[1]✉, ANUPAMA MUKHERJEE[2], VIVEK KUMAR NAYAK[3], SUCHIT KUMAR[4], SHABAHAT MUMTAZ[5] and ALOK KUMAR YADAV[6]

ICAR-National Dairy Research Institute, Karnal, Haryana 132 001 India

Received: 5 November 2019; Accepted: 2 December 2019

ABSTRACT

The present study explored the association between single nucleotide polymorphisms (SNPs) in leptin gene with various economic traits (body weight at different age, first lactation reproduction and production traits) in Sahiwal cows. PCR-RFLP of 522 bp fragment comprising partial intron 2 and exon 3 of leptin gene, carried out using *BsaAI* restriction endonuclease, revealed three genotypes (GG, AG and AA) at position G93263736A in chromosome 4 with genotypic frequencies of 0.39, 0.52 and 0.09 respectively. The allele frequencies were 0.65 for the G allele and 0.35 for the A allele. General Linear Model (GLM) analysis revealed significant association of SNP G93263736A with growth and first lactation production traits. The present study supports that SNP in leptin gene could be used as an aid to selection for improving different economic traits in Sahiwal cows.

Keywords: Growth, Heterozygote, Leptin, Polymorphism, Production traits, Reproduction

Primary goal of dairy farming is to improve quality and efficiency of increasing milk production. The conventional breeding programs require long period and significant cost to improve a particular trait. With fast developments in DNA based technologies, identifying polymorphism at DNA level regardless of sex, age and physiological status of animal in a population has been proved successful in selecting superior animals with favourable genotypes for enhancing the production. Till date, a large number of candidate genes have been reported among which leptin has been found to be critical for growth, reproduction as well as production traits (Dandapat *et al*. 2009, Clempson *et al*. 2011).

Leptin is a 16-kDa hormone produced by the obese gene (ob) (Zhang *et al*. 1994) primarily in white adipose tissue and is intimately related to regulation of feed intake, energy expenditure, growth, body composition, immunity and reproduction (Houseknecht *et al*. 1998). In growing dairy heifers, leptin was shown to play a role in the initiation of puberty (Chelikani *et al*. 2009). During early post-partum period, leptin concentrations were found to be positively correlated with pulsatile LH secretion and timing of the first ovulation after calving (Kadokawa *et al*. 2000). In cattle and sheep, leptin was shown to affect directly the ovarian steroidogenesis (Kendall *et al*. 2004, Nicklin *et al*. 2007). As there is a genetic correlation between start of luteal activity and energy balance, milk yield and live weight weight (Veerkamp *et al*. 2000), it could be hypothesized that polymorphisms at the leptin gene locus might influence phenotypic traits. Associations between leptin polymorphisms and milk yield, live weight, feed intake or fertility will provide insight into the underlying mechanisms of leptin and will be useful in breeding programs (Liefers *et al*. 2002). The association study carried out for LEP gene considering various body weights at different age is quite limited.

In the present study, we investigated single nucleotide polymorphisms in leptin gene and its possible associations between polymorphism and growth, reproduction and first lactation production traits using PCR-RFLP technique in Sahiwal cows.

MATERIALS AND METHODS

Animals and description of phenotypic data: DNA samples were isolated from blood collected from 103 Sahiwal cows of Indian origin belonging to the herd maintained at Livestock Research Complex, ICAR-National Dairy Research Institute, Karnal, Haryana, India. The geographical location of the farm is at an altitude of 250 metres above the mean sea level in the Indo-Gangetic alluvial plains (29°422 N latitude and 72°022 E longitude). The climate prevailing in this region is sub-tropical in nature. All the animals which have completed their first

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Present address: [1]ICAR-National Research Centre on Pig, Guwahati, Assam. [2]ICAR-National Dairy Research Institute, Karnal, Haryana. [3]National Bank for Agriculture and Rural Development, 6 Royd Street, Kolkata, West Bengal. [4]Bihar Veterinary College, Patna, Bihar.[5]ABAHC, Hirbandh, Bankura.[6]Central Cattle Breeding Farm, Andesh Nagar Lakhimpur Kheri, Uttar Pradesh. ✉Corresponding author e-mail: prajwalitapathak@gmail.com

lactation were maintained under the similar management regimen. The different traits considered for association analysis included body weight at different age i.e. Birth, 6 Months (6 M), 12 Months (12 M), 18 Months (18 M), 24 Months (24 M), 30 Months (30 M), Weight at First Service (WFS), Weight at First Calving (WFC); reproduction i.e. Age at First Service (AFS), Age at First Calving (AFC), First Service Period (FSP), First Calving Interval (FCI) and first lactation production traits, i.e. First Lactation 305Day or less Milk Yield (FL305DMY), First Lactation Total Milk Yield (FLTMY), First Lactation Length (FLL), First Lactation 305 day or less Fat Yield (FL305DFY), First Lactation 305 day or less SNF Yield (FL305DSNFY).

DNA extraction and genotyping of animal: The DNA was extracted from blood samples by commercially available kit (Genomic DNA Purification Kit Promega). The primers used for amplification were 5’-GTCTGGAGGCAAAGGGCAGAGT-3’ and 5’-CCACCACCTCTGTGGAGTAG-3’ as reported by Lien *et al.* (1997), which amplify a 522 bp fragment of leptin gene comprising partial intron 2 and exon 3. The reaction mixture contained 100 ng of genomic DNA, 30 pM of each primer, 12.5 µl Go Taq Green master mix 2× (Promega) and nuclease-free water to a final volume of 25 µl. Thermal cycling conditions included an initial denaturation for 5 min at 95°C followed by 32 cycles of 95°C (30 sec), annealing temperature 58.5°C (30 sec) and 72°C (30 sec) ending with a final extension for 10 min at 72°C.

The PCR products were confirmed by 1.5% agarose gel electrophoresis and were visualized under Gel Documentation System. The restriction fragment length polymorphism (RFLP) analyses of PCR product were carried out in a final volume of 20 µl reaction. For the RFLP technique, 10 µl of the PCR product was digested overnight at 30°C along with 2 U of the restriction enzyme *BsaAI*. The restriction fragments were separated in 2.5% agarose gel and visualized in a gel documentation system. Sizes of the digested fragments were estimated by comparing them against both 100 and 50 bp DNA ladder. The genotyping was done on the basis of restriction pattern of individual Sahiwal cows.

Statistical analysis: Genotypic frequencies for variant genotypes were calculated using the formula:

$$\text{Genotypic frequency} = \frac{\text{Number of animals with specfic (AA, AB and BB) genotype}}{\text{Total number of animals}}$$

Allelic frequencies were calculated as follows:

Allelic frequency of A = AA + ½ AB

Allelic frequency of B = BB + ½ AB

where AA and BB, genotypic frequency of homozygote; AB, genotypic frequency of heterozygote; A and B, allelic frequencies.

Least squares analysis of variance was carried out for unequal and non-orthogonal data using the technique described by Harvey (1990) to study effect of non-genetic factors like season and period. The model was used with assumptions that different components being fitted into the model were linear, independent and additive.

Association analysis: Association of the animal genotypes with growth, reproduction and milk production traits were determined by analyses of quantitative traits. Statistical analysis was carried out using SAS Enterprise Guide 4.2 software (SAS Institute Inc. 2009), and significance of differences based on genotypes effect of growth, reproduction and production traits were tested by following general linear models:

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

where $Y_{ij,}$ trait value of $_j^{th}$ animal of $_i^{th}$ genotype; m, overall mean; $G_{i,}$ effect of $_i^{th}$ genotypes and $e_{ij,}$ residual error NID $(0, \sigma^2{}_e)$.

RESULTS AND DISCUSSION

Genetic variability in LEP gene: PCR-RFLP method was performed to identify the polymorphism at a fragment of 522 bp comprising partial intron 2 and exon 3. Three *BsaAI* digestion patterns were observed indicating three genotypes, viz. an intact undigested fragment of 522 bp as AA genotype; an undigested fragment of 522 bp with two other fragments, one of 441 bp and the other of 81 bp as AG heterozygote; and a GG homozygote genotype with 441 and 81 bp fragments. The result is in agreement with Lien *et al.* (1997), who for the first time described a guanine (G) to adenine (A) substitution in intron 2 of the leptin gene of Norwegian cattle. Zetouni *et al.* (2013) also reported this variability in buffaloes. The same genotypes were also reported by Choudhary *et al.* (2005) in different breeds of *Bos taurus, Bos indicus* and *B. taurus* × *B. indicus* crossbreed, Rambachan *et al.* (2017) in Hariana cows and Pandey *et al.* (2017) in Sahiwal cattle.

The three distinct genotypic frequencies observed were 0.39 for genotype GG, 0.52 for AG and 0.09 for AA. PCR-RFLP analysis revealed a transition in a position G93263736A (intron 2) in the LEP gene. The frequency of heterozygote genotype in the present investigation was higher (0.52) than other two homozygote genotypes, which was in agreement with previous findings (Choudhary *et al.* 2005 and Rambachan *et al.* 2017) in Hariana cattle and by other researchers in various exotic cattle breeds (Souza *et al.* 2010, Azari *et al.* 2012, Rezaei *et al.* 2015).

The frequency of two alleles G (0.65) and A (0.35) observed in the present investigation is in concordance with the findings on different breeds of cattle (Almeida *et al.* 2003, Choudhary *et al.* 2005, Azari *et al.* 2012, Rezaei *et al.* 2015, Rambachan *et al.* 2017 and Pandey *et al.* 2017). The study suggests a selection force favouring the G allele in the studied population. On the contrary, lower frequency of LEP/*BsaAI* G 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).

Association of the leptin gene polymorphism with economic traits

Growth trait: The present study revealed that LEP SNP at position G93263736A was significantly associated with WFC ($P \leq 0.05$) (Table 1). The SNP G93263736A corresponded to three genotypes among which animals with GG genotype were associated significantly with higher WFC than the other two genotypes (AG and AA). Heterozygote genotype AG had intermediate body weight at first calving though there was no significance difference of WFC between AG and AA genotypes and this genetic variant was not found to be associated with weight at birth, 6 M, 12 M, 18 M, 24 M, 30 M and WFS. Souza *et al.* (2010) found an association (g.1620A>G) of the genotype with weaning weight in Nellore beef cattle.

Reproduction traits: Association study revealed no significant influence of LEP SNP at position G93263736A on different first lactation reproduction traits among Sahiwal cows (Table 2) and is in agreement with earlier findings (Dandapat *et al*. 2009, Komisarek and Antkowiak 2007, Zetouni *et al*. 2013 and Pandey *et al*. 2017). Findings of the current investigation disagree with the study conducted by Rambachan *et al*. (2017) that the SNP LEP/*BsaAI* significantly affected gestation period and dry period in Hariana cows but no significant difference was observed among the genotypes for the AFC and CI during the lactation. Liefers *et al*. (2002) reported significant effect of leptin polymorphism which was found to be associated with first postpartum luteal activity. SNPs of the leptin gene in Holstein cattle were analyzed by Clempson *et al*. (2011) and all of them showed associations with reproductive traits. In particular, LEP-1457 (A/G) SNP presented associations with calving interval duration.

Table 1. Means of the least squares for various growth traits in relation to leptin genotypes obtained in Sahiwal cows

Trait	Leptin genotype			P value
	GG (Mean±SD)	AG (Mean±SD)	AA (Mean±SD)	
Birth wt	19.92±0.78	19.06±0.72	19.15±0.82	0.331
6 M wt	77.23±4.58	72.91±4.22	79.62±4.78	0.346
12 M wt	123.90±6.84	119.14±6.31	124.99±7.14	0.612
18 M wt	169.68±9.21	179.31±8.50	192.11±9.62	0.118
24 M wt	232.27±10.11	234.24±9.32	243.17±10.56	0.701
30 M wt	279.03±11.25	285.76±10.38	293.08±11.75	0.543
WFS	304.12±11.34	290.66±10.46	300.77±11.84	0.297
WFC	376.51^{a}±12.31	363.96^{b}±11.36	357.11^{b}±12.86	0.021

^{a,b}Dissimilar superscript indicates significant difference.

Table 2. Means of the least squares for different reproduction traits in relation to leptin genotypes obtained in Sahiwal cows

Trait	Leptin genotype			P value
	GG (Mean±SD)	AG (Mean±SD)	AA (Mean±SD)	
AFS	1011.03±59.16	965.38±54.59	953.40±61.81	0.534
AFC	1295.78±59.31	1251.24±54.73	1240.47±61.96	0.555
FSP	294.81±54.98	295.31±50.73	268.08±57.44	0.533
FCI	584.30±55.43	587.92±51.14	546.11±57.91	0.839

Table 3. Means of the least squares for different first lactation production traits in relation to leptin genotypes obtained in Sahiwal cows

Trait	Leptin genotype			P value
	GG (Mean ±SD)	AG (Mean ±SD)	AA (Mean ±SD)	
FLTMY	2054.99± 133.47	2062.54± 107.70	1837.63± 148.40	0.735
FL305DMY	1904.89^{b}± 154.12	1943.62^{b}± 134.48	1739.75^{a}± 165.49	0.021
FLL	288.21± 33.84	281.30± 31.22	312.04± 35.35	0.78
FL305DFY	89.39^{b}± 11.52	87.49^{b}± 10.63	74.84^{a}± 12.04	0.025
FL305DSNFYY	180.05± 25.58	178.09± 23.61	141.52± 26.73	0.488

^{a,b}Dissimilar superscript indicates significant difference.

First lactation production traits: The association of LEP gene with various first lactation production traits is presented in Table 3. The present study revealed LEP SNP G93263736A among the Sahiwal cows corresponded to three genotypes and was found to be significantly associated ($P \leq 0.05$) with FL305DMY and FL305DFY. For this SNP, animals with heterozygote AG had significantly higher FL305DMY and FL305DFY than the other two homozygote genotype. There was no significant difference between genotype AG and GG genotype. The polymorphism had no association with FLTMY, FLL and FL305DSNFYY. Similar findings were described by Zetouni *et al*. (2013) that this polymorphism had no significant association with first lactation production traits. However, Tanpure *et al*. (2012), studied the same gene, found three variants in buffaloes that significantly associated with fat percentage. Results of the current study were also consistent with the study conducted by Rezaei *et al*. (2015) that the LEP-*BsaAI* polymorphism had a significant effect on milk yield, milk fat and protein percentage ($P < 0.01$) in native cattle of Iran. Similarly, Pandey *et al*. (2017) and Rambachan *et al*. (2019) revealed significant association of LEP/BsaAI genotypes with lactation period, total milk yield, milk yield in 300 days, peak yield and days to reach peak yield in Sahiwal and Hariana cattle respectively.

Based on present investigation, leptin gene polymorphisms showed significant association with different growth, reproduction and first lactation production traits. Heterozygote pertaining to SNPs G93263736A tended to have better performance than the other genotype. In breeding programme selection of animals with heterozygote genotype may be favourable for improving different economic traits in Sahiwal cattle with proper validation in larger population.

ACKNOWLEDGEMENTS

The authors are thankful to the Director of ICAR-NDRI and Head of the Division, AGB, NDRI, Karnal for providing the necessary facilities. We wish to acknowledge the hard work and sincerity of the staff of Livestock Research Centre.

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