



Association of *leptin* gene polymorphism with 305 days milk yield in Karan Fries cattle

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ABSTRACT

Leptin protein is synthesized by adipose tissue and is involved in regulation of milk yield. We investigated the association of genetic polymorphism in the bovine *leptin* gene with 305 days milk yield for the first 3 lactations. The study was conducted on 209 lactating Karan Fries cattle with known pedigree belonging to 3 genetic groups at an organized herd. All the cattle were genotyped by PCR-RFLP assay, and 3 genotypes, viz. TT, TC and CC were identified in the *exon-2* region of *leptin* gene. Representative PCR products of the genotyped animals were sequenced across the polymorphism. Karan Fries cattle with TT genotype showed significantly higher 305 days milk yield as compared to cattle with CC genotype.

Key words: Association, Bovine, Lactational yield, Leptin (Obese) gene, Variation

Leptin is a 16-kDa protein (Gong *et al.* 1996), involved in regulation of milk production besides feed intake, energy balance, fertility, and immune functions. Leptin protein, binds to a receptor mainly localized on Neuropeptide Y neurons, resulting in a reduction of feed intake and an increase of energy expenditure (Magni *et al.* 2000). The bovine leptin gene has variations (Pomp *et al.* 1997, Fitzsimmons *et al.* 1998, Hageman *et al.* 2000), which were found associated with fat deposition in beef cattle (Fitzsimmons *et al.* 1998). Since, evidence suggests that a genetic correlation exist between start of luteal activity and energy balance and milk yield, when only phenotypic records were analyzed (Veerkamp *et al.* 2000). Thus, it could be hypothesized that polymorphisms at the bovine *leptin* gene locus might play a role in milk production. If associations between *leptin* gene polymorphisms and milk yield exist, the association will provide insight into the underlying mechanisms of *leptin* gene and milk production, and results may be used in future breeding programs. The objective of this study was to evaluate the association of single nucleotide polymorphism in bovine *leptin* gene with 305 days milk yield from crossbred herd of Karan Fries cattle.

MATERIALS AND METHODS

Lactating Karan Fries cattle (209) were considered in the

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study maintained at cattle yard of National Dairy Research Institute, Karnal. All the cattle were screened for the presence of *leptin* gene polymorphism.

DNA isolation and polymorphism detection: The genomic DNA was isolated from aseptically collected venous blood using the standard phenol/chloroform method with minor modifications (Sambrook and Russel 2001). Forward and reverse primers (P_F 5'-GTGCCACGTGTGG TTTCTTC-3' and P_R 5'-CCTCCCTACCGTGTGTGAGA-3') with T_M of 61.57°C and 61.12°C respectively, were designed using Primer 3 software to amplify *exon-2* region of *leptin* gene from published NCBI (National Center of Bioinformatics, USA) sequences. The optimization of PCR was done to get the best possible amplification of the product, at an annealing temperature of 65°C for 1 min. The genotyping was carried out using PCR-RFLP (Polymerase Chain Reaction–Restriction Fragment Length Polymorphism) technique with *Aci1* restriction enzyme at 37°C for 6 to 8 h. The PCR products of different genotype were sequenced using the automated dye terminator cycle sequencing method with Ampli Taq DNA polymerase in ABI PRIZM 377 DNA sequencer.

Phenotypic information, statistical and genotype analysis: Performance records for 209 Karan Fries cattle, pertaining to 305 days milk yield, genetic group of the cattle, date of calving and date of dry for the first 3 lactations were collected from dairy cattle breeding division of National Dairy Research Institute, and were used for further analysis. Genotype and allele frequencies were calculated and compared by gene counting method (Falconer and Mackay

1996). To study the effect of non-genetic factors such as genetic group, year, season, age groups at calving and regression of dry period to overcome the non-orthogonality of effects due to unequal and disproportionate sub-class frequencies, least-squares analysis of fitting constants (Harvey 1987) was used (model not shown). The first 3 lactational 305 days milk yield records of all the cattle were adjusted for significant constants. All other analyses were performed using the statistical software SYSTAT (2007, version-12). To study the effect of *leptin* genotypes on adjusted 305 days milk yield in Karan Fries cattle, the model which included SNP genotypes as fixed effects was used.

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

where, Y_{ij} , j th cattle adjusted 305 days milk yield for i th genotype; μ , overall mean; G_i , effect of i th genotype on adjusted 305 days milk yield ($i = 1 - 3$); e_{ij} , random error associated with each observation assumed to be NID ($0, \sigma_e^2$).

The differences of least square means of lactational milk yield between subclasses of *leptin* genotype were tested for significance using Duncan's Multiple Range Test (DMRT) as modified by Kramer (1957).

RESULTS AND DISCUSSION

The PCR-RFLP of bovine *leptin* gene revealed 3 genotypes, viz. TT, TC and CC of *exon-2* region in bovine *leptin* gene. Frequency of TT, TC and CC genotypes were 0.13, 0.52 and 0.35, respectively. Sequencing results of amplified DNA revealed the two (T and C) alleles for the *exon-2* region of *leptin* gene in the Karan Fries cattle. T and C alleles of *leptin* gene in Karan Fries cattle were compared (Clustal W software) with *Bos taurus* (Fig.1) which showed variation at 1180th position. The sequences obtained for T and C alleles in Karan Fries cattle were submitted to NCBI-gene bank and their accession no (s) were obtained (GU907776 and GU907777). Translated products, showed difference in amino acid sequence of leptin protein. Amino acid *arginine* of the C allele was replaced with *cysteine* in T allele of Karan Fries cattle. This difference was due to presence of transition mutation (C to T) in the first base of 25th codon. Change in amino acid sequence of the leptin protein, can lead to difference in biological/physiological functions because *arginine* (positively charged, basic amino

Table 1. Least square means of adjusted 305 days milk yield corresponding to different *leptin* genotypes in Karan Fries cattle

Genotypes	Least square mean (kg) $\pm$ standard error		
	First lactation	Second lactation	Third lactation
TT	3860.01 $\pm$ 173.5 ^a (N=29)	4063.89 $\pm$ 265.1 ^a (N=27)	4894.14 $\pm$ 332.2 ^a (N=18)
TC	3743.78 $\pm$ 89.8 ^{ab} (N=108)	3959.89 $\pm$ 152.1 ^{ab} (N=82)	4383.73 $\pm$ 225.7 ^{ab} (N=39)
CC	3589.56 $\pm$ 110.1 ^b (N=72)	3714.05 $\pm$ 179.3 ^b (N=59)	3927.37 $\pm$ 271.3 ^b (N=27)

Figures in parenthesis indicate the number of animals; Means bearing the different superscript within column differ significantly at ($P < 0.05$).

acid) was replaced by polar and uncharged *cysteine* amino acid, which is a major amino acid change. Genotype frequencies obtained showed a close agreement with Buchanan *et al.* (2002) where they reported a single nucleotide polymorphism in the *exon-2* region of the *leptin* gene in Holstein Friesian. Cows with the T allele were found to have higher levels of leptin mRNA, thus suggesting that the T allele imparts a partial loss of biological function (Buchanan *et al.* 2002). Present results were also in close agreement with Chebel *et al.* (2008) where they reported the frequency of TT, TC and CC genotypes as 17.2%, 48.2% and 34.6% in Holstein cows. The allelic frequency of the *Kpn21* SNP in Holstein crossbreds as examined by Choudhary *et al.* (2005), and found the frequencies for the C allele in the crossbreds as 0.82 and T allele frequencies as 0.18. They inferred that the low presence of the T allele in the crossbreds of Holstein Friesian cattle was due to the lack of T alleles in the Haryana line of cattle. Our result did not agree with Choudhary *et al.* (2005) and both T and C allele are present in our crossbred Karan Fries cattle population. The adjusted 305 days milk yield were further analyzed to see the effect of different *leptin* genotypes as fixed effect. Estimated least square means of adjusted 305 days milk yield corresponding to TT, TC and CC genotypes in Karan Fries cattle for the first 3 lactations (Table 1). The mean 305 days milk yield for the TT genotype were 3860 kg, 4063 kg and 4894 kg and for TC genotype 3743 kg, 3959 kg and 4383 kg, in first, second and third lactation, respectively. Similarly, for CC genotype

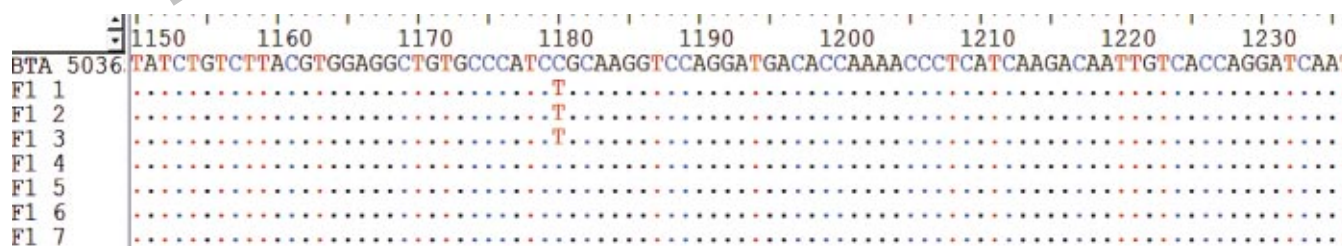


Fig 1. Nucleotide sequence alignment of *exon-2* region of *leptin* gene in Karan Fries cattle and their comparison with *Bos taurus*.

in first 3 lactations was found as 3589 kg, 3714 kg and 3927 kg. The lactational milk yield was maximum in third lactation and minimum in first lactation for all the genotypes of *leptin* gene. The TT genotype Karan Fries cattle were significantly different in 305 days lactational milk yield in comparison to CC genotype Karan Fries cattle and this estimate was about 271 kg (7.55%), 349 kg (9.40%) and 967 kg (24.62%) for first, second and third lactation, respectively. On comparing the TT genotype Karan Fries cattle for *leptin* gene with TC genotype Karan Fries cattle, it was revealed that cattle with TT genotype produced about 117 kg (3.13%), 104 kg (2.62%) and 511 kg (11.65%) more in first, second and third 305 day lactation milk yield, but this difference was nonsignificant. Also a nonsignificant increase in 305 days milk yield was observed in TC genotype when it was compared to CC genotype of *leptin* gene. Our results agreed with those of Buchanan *et al.* (2003), they reported that the animals homozygous for the T allele produced more milk (1.5 kg/day) vs. homozygous for C allele animals, without significantly affecting milk fat or protein percent over the entire lactation. Present results also seems to be in close agreement with Chebel *et al.* (2008), who reported that *leptin* genotype were associated with yields of milk and milk components, and cows homozygous for the C allele were less productive than those carrying the CT and TT genotypes. According to Chebel *et al.* (2008), 305-day yields of 3.5% fat-corrected milk was less in CC compared with CT cows by 258 kg. Whereas, Madeja *et al.* (2004) and Liefers *et al.* (2004) did not find any associations between the alleles of exon-2 region of *leptin* gene and lactational milk yields.

Results from the present study suggests that the TT genotype of bovine *leptin* gene may be associated with 305 days milk yield. Considering lack of systematic data recording and small sized dairy farms in India, these observations are of economic interest. Although in this study we measured the effect of *leptin* genotype on milk production in crossbred Karan Fries cattle only, but before this sort of genetic information can be used efficiently in breeding and management decisions, studies with different populations are required to properly characterize the robustness of the associations of *leptin* polymorphisms with 305 days milk yield and other economically important traits across cattle populations. This preliminary study suggests that the *leptin* genotype may play an important role in regulating 305 days milk yield in Karan Fries cattle.

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