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Prediction of lactation persistency in crossbred cattle using genotype profile of lactation curve traits

VIKAS VOHRA[1], ALKA CHOPRA[2] and A K CHAKRAVARTY[3]

ICAR-National Bureau of Animal Genetic Resources, Karnal, Haryana 132 001 India
and
ICAR-National Dairy Research Institute, Karnal, Haryana 132 001 India

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ABSTRACT

The objective of the present study was to identify the best fit lactation model in relation to bovine leptin gene and to assess lactation persistency based on lactation curve traits in crossbred Karan Fires cattle. Incomplete gamma (Wood) function and exponential (Wilmink) function tests were used to describe the characteristics of lactation curve in first lactation. Woods model showed a comparatively better fit. Different types of lactation curves depicted by these cattle using data spread over a period of 15 years (1994 to 2009) were grouped into desired and non-desired type of lactation curve. Subsequently, genotype profiling was done using PCR-RFLP. A single nucleotide polymorphism identified in exon-2 region of bovine leptin gene, was associated with desired type of lactation curve and animals having TT genotype showed better persistency of milk yield. The results validated in test population had shown positive relationship between leptin genotypes and lactation curve traits. The inference from work has a potential application in breeding program of the country, where it may give support to existing expected producing ability (EPA) based selection methodology followed for selection of dairy animals, by adding leptin genotype as one additional selection criterion for early selection in crossbred dairy bulls and cattle.

Key words: Lactation curve, Leptin gene, Mathematical modelling, Persistency, Woods model

India is home to world's largest cattle population and majority of the country's cattle milk is sustained by crossbred cattle, which is about 21% of total cattle strength (DAHD&F 2012). Economics of milk production is a key for successful dairy industry and it greatly depends on lactation yield and its persistency in dairy animals. Lactation persistency is the ability of cattle to maintain milk at higher level after the peak of lactation, which is depicted in lactation curve through relatively slow rate of decline after attaining the peak in the curve. Lactation curves can be depicted using empirical mathematical models, where test day milk yield is plotted as function of time. According to Kellogg (1977), lactation curve models are a concise summary of the pattern of milk yield determined by the biological efficiency of the cattle. Knowledge about the shape of lactation curves in a dairy herd reveals useful information and can be used in genetic evaluations along with identification of lactation persistency (Strucken *et al*. 2011). Various models have been used to fit a lactation curve (Wood 1967, Wilmink 1987, Olori *et al*. 1999) by utilizing lactation yield as function of time in different dairy breeds of cattle, and stability of lactation curve is considered proportional to milk persistency (Olori *et al*. 1999). Literature on lactation curves suggests that dairy cattle with stable or flat lactation curve are more persistent compared to those with the same lactation yield (Tekerli *et al.* 2000). Identifying cattle with particular pattern and higher persistency of lactation milk yield can improve the economics of a dairy herd.

Our previous results (Vohra *et al*. 2010) indicate that crossbred cattle herd maintained at ICAR-National Dairy Research Institute (NDRI), Karnal (Haryana) revealed the presence of four types of lactation curves (continuously increasing type, standard type, continuously decreasing type and reverse standard type) using different mathematical models. Besides phenotypic variability, genetic variability does exist in dairy cattle with respect to lactation curve traits (Sotia *et al*. 2004) and this variability need to be explored among crossbred dairy animals in India. Single nucleotide variation (SNP) in growth related genes – IGF2, lactoferin gene, feed balance and fertility gene – leptin gene, had been associated with milk production and production diseases in HF crossbreds (Berkowicz *et al*. 2011, Vohra *et al*. 2011, Chopra *et al.* 2015). However, Madeja *et al*. (2004) and Liefers *et al.* (2004) working independently did not find any such associations of exon-2 of bovine leptin with lactation milk yield.

Leptin gene is on the 4th chromosome (Barendse *et al*.

Present address: [1]Senior Scientist (vohravikas@gmail.com), Animal Genetic Resource Division, [2]DST Post Doctorate Fellow (virgoalka@gmail.com), ABTC, [3]Principal Scientist and Head (ak_chakravarty@yahoo.co.in), DCB Division.

1994) in the bovines and its gene product is 16 kDa protein (peptide hormone) that is primarily produced by white adipocytes (Houseknecht *et al*. 1998). It has been hypothesized that peptide hormone acts on the central nervous system as satiety signal through specific receptors, that are mainly expressed in the hypothalamus and is responsible for feed intake and negative feed balance in cattle. Therefore, information about the types of lactation curve, their goodness of fit and their corresponding genotypes, when combined, will aid in developing the selection criteria for dairy animals. Keeping the above facts in mind, present work was attempted to evaluate whether *leptin* gene plays any significant role in variability of lactation curve types and traits, and to develop the selection aid for higher milk yield and persistency in crossbred Karan Fries (KF) cattle from North India.

MATERIALS AND METHODS

Population studied: In the present study, crossbred KF animals (cross of exotic Holstein Friesian bull with indigenous Tharparkar dam), maintained at the organized herd of ICAR-NDRI were taken for lactation curve study and genotype analysis. The breeding policy at ICAR-NDRI herd, for improvement of the crossbred KF population is based on *inter-se* mating to maintain 50–75% level of exotic germplasm.

Phenotypic and pedigree records: Fortnightly test day (TD) milk yield records of about 330 lactating KF cattle were initially considered for the study. After standardization, about 260 KF cattle's records were retained. About 5460 TD milk records of 209 lactation spread over a period of fifteen years (1994 to 2009) were further used for association studies. The reason attributed to this lower herd size is limited availability of dairy farms with structured breeding records in the country. These animals were selected as a representative of the herd/reference population. First record was taken on the 5th day after calving and subsequently, TD milk yields at 15 day, interval up to 305 days were recorded for each cattle. Therefore, total of 21 TD milk yields records/cattle were considered. For controlling the effect of various genetic and non-genetic factors, subclass effect of genetic group, season, age were classified. Three sub-classes of genetic group based on their pedigree were 50%, 62.5% and 75% of exotic germplasm. Winter (Dec. to March), summer (April to June), rainy (July to Sept.) and autumn (Oct. to Nov.) were taken as subclass of season. Age at calving was taken as fixed effect. To quantify these effects, TD milk records were subjected to the least square analysis (Harvey 1987) using the following model.

$$Y_{ikln} = \mu + G_i + S_k + A_l + e_{ikln} \qquad \text{….(1)}$$

where, Y_{ikln}, nth test day milk yield for lth age group, kth season, ith genetic group; μ, overall mean; G_i, effect of ith genetic group on test day milk yield (i=1-3); S_k, effect of kth season on test day milk yield (k=1–4); A_l, effect of lth age groups in each calving on test day milk yield (l=1–3); e_{ikln}, random error associated with each observation assumed to be NID (0, σ_e^2).

The significance of subclass least-squares mean test day yield for different genetic and non-genetic factors was tested using Duncan's Multiple Range Test (Kramer 1957). The TD milk yield records were adjusted for significant effects thus, obtained, before subjecting them to lactation curve analysis.

Fitting of lactation curve: Incomplete gamma functions as proposed by Wood's (Wood 1967) and exponential function as proposed by Wilmink (Wilmink 1987) were chosen to model our test day milk yield. Both are three parameter based functions, and had the advantage of having a limited number of parameters with an assessed direct reference to main features of lactation curve (Rekaya *et al*. 2000). Functions equations (2a and 3, as below) were fitted to standardized and adjusted TD milk records.

Woods model	$Y_t = at^b\, e^{-ct}$	...(2)
Log linear Woods model	$\log Y_t = \log a + b \log t + ct$	...(2a)
Wilmink model	$Y_t = a + be^{-kt} + ct$	...(3)

where, Y_t, test day yield at day t from parturition; a, initial milk yield; b, ascending phase of lactation; c, descending phase of lactation, e, base of natural logarithm; k, 0.05; t, days in milk.

Three lactation curve traits i.e. initial milk yield (a; where $a>0$), rate of incline to reach the peak yield (b) and rate of decline after attaining the peak yield (c), were generated using Wood's and Wilmink function. Based on the relationship between the lactation curve traits, as suggested by Macciotta *et al.* (2005), types of lactation curve were detected. Different lactation curves thus obtained were grouped into desired and non-desired types.

DNA extraction and SNP genotyping: Genomic DNA was isolated using Phenol-chloroform method as suggested by Sambrook and Russel (2001) with minor modifications. Forward and reverse primers (PF 5' GTGCCACGTGTGGT TTCTTC-3' and PR 5' CCTCCCTACCGTGTGTGAGA-3') were designed using Primer-3 software to amplify exon-2 region of leptin gene from published NCBI sequences. The optimization of PCR was done at an annealing temperature of 65°C for 1 min. The genotyping screening was carried out using PCR-RFLP technique with *Aci1* restriction enzyme at 37°C for 6 to 8 h. The representative samples of different genotypes obtained were sequenced to confirm the presence of SNP revealed through PCR-RFLP. Automated dye terminator cycle sequencing method was used. The desired and non-desired type of lactation curves were grouped according to their genotype of leptin gene.

Validation of results: Test population of 30 KF heifers were randomly selected from the same herd. Genotyping with respect to exon-2 polymorphism of bovine leptin gene was completed, before the onset of first lactation. Later, fortnightly test day milk yield data was recorded. Average lactation traits were obtained from reference population using the Woods function were taken to estimate different types of curves.

RESULTS AND DISCUSSION

Least square analysis (LSA) was done to quantify effect of genetic and non-genetic factors on TD milk yield records in KF cattle. Using model-1, the fixed effects, genetic group, season of calving and age at calving were classified. LSA was also attempted with period as fixed effect on fortnightly TD milk yields, but due to skewed frequency and inability to classify the KF cattle into the relevant periods, the effect of period/year of calving was dropped. Season and genetic group showed significant effect on majority of TD milk yields, whereas, the age at calving showed a non-significant effect except for the TD1 and TD 15–21, where it showed significant effect at 5% level of significance. Overall, least square mean for TD milk yield in KF cattle ranged from 9.88 kg to 14.17 kg. The TD1 milk yield had lowest mean and peak milk yield is attained around fifth test day (TD5), which had highest mean. It was estimated that in KF cattle average days to attain peak yield was around 65 day, and average peak milk yield was 14.17 kg for first lactation. Maximum variability of 60.61% was present in 7th TD milk yield, and minimum of 27.87% was observed in 3rd TD milk yield. The data was adjusted for significant non-genetic effects and was further analyzed for fitting of lactation curves using different functions.

Modeling lactation curves: Based on lactation curve traits, four types of lactation curve were detected (Fig. 1). Lactation curve depicted were then grouped into desired and non-desired type of lactation curves (Fig. 2). Both the functions could depict the four types of curve but Woods function showed higher R^2 value than Wilmink function. Our results suggests that Wood function can be effectively used for identifying the standard type of lactation curve both in reference and test population of KF cattle, whereas, the Wilmink function showed better goodness of fit for the non-desired types of lactation curves when compared to Woods function. Although Wood function can give an acceptable fit to milk yield data from a given lactation, it tends to over predict during early and late lactation and under predict data during mid-lactation (Grossman and Koops 1988). In our results, comparatively higher R^2 value is obtained, thus it can be inferred that Wood function fitted well to the studied reference population. Hence, was used to associate and compare the SNP of leptin gene. The average lactation curve traits namely a, b and c estimated by Wood function after classifying the population as desired and non-desired based on model properties of the function. Our results were similar to Koko and Chakravarty (2008) who reported that the lactation curve parameters in the first lactation had higher 'Log a' (initial milk yield), a lower 'b' (rate of incline to reach the peak yield) and 'c' (rate of decline after peak yield) values using Wood function. However, the initial test day monthly milk yield (parameter 'a') was found lower in first lactation using Wilmink function. Macciotta *et al*. (2005) fitted Wood function to

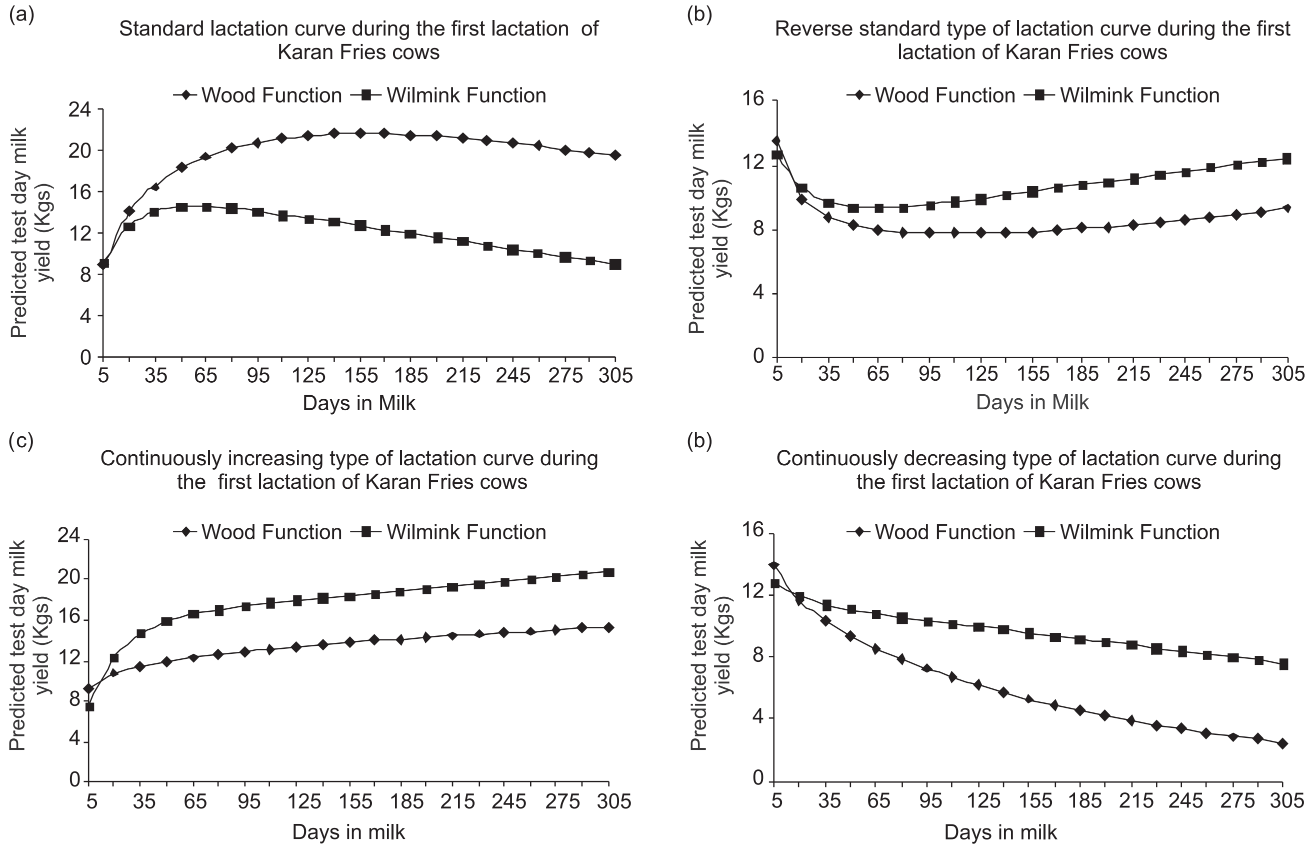
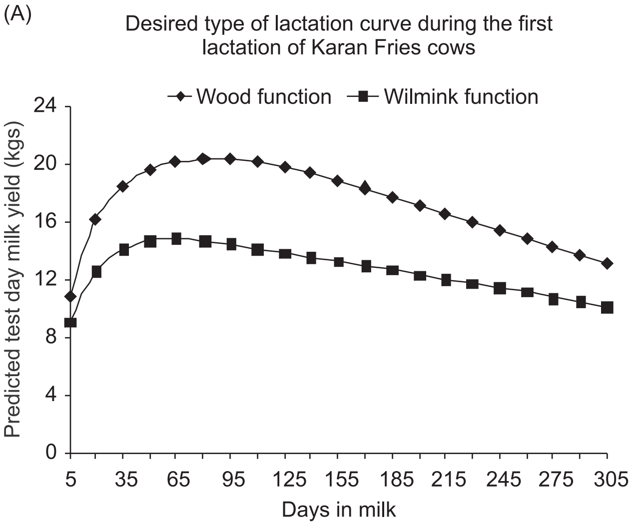


Fig. 1. (a-d). Different types of lactation curve in reference population of Karan Fries cattle.

test day records of Italian Simmental cattle and found that the Wood and Wilmink function could detect two main groups of curve shape i.e. standard and atypical/ continuously decreasing curve besides reverse standard and continuously increasing types of lactation curve. The types of lactation curve obtained in our study were comparable to the ones obtained by Macciotta *et al*. 2005. The standard and continuously increasing type of lactation curve were comparable to desired type of lactation curve and atypical curve was similar to our non-desired type of lactation curves.

Polymorphism in exon 2 of leptin gene: All the KF cattle were screened for the presence or absence of SNP in exon-2 region of *leptin* gene. It was found that leptin is polymorphic in crossbred KF cattle herd. Three genotypes for exon-2 were noted with different frequencies, namely, TT, TC and CC genotype. Genotype TC showed the highest frequency (51.6%), followed by CC genotype (35.2%) and TT genotype was identified to be rare, with 13.2% frequency. Genotype frequencies obtained for exon-2 region of leptin gene 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. Cattle 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) in Holstein cattle. Choudhary *et al.* (2005) 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 Hariana line of cattle. Our result does not agree with Choudhary *et al.* (2005) and both T and C allele were present, probably because the studied crossbred KF cattle population has Tharparkar blood along with Holstein Friesian. Here it is important to mention



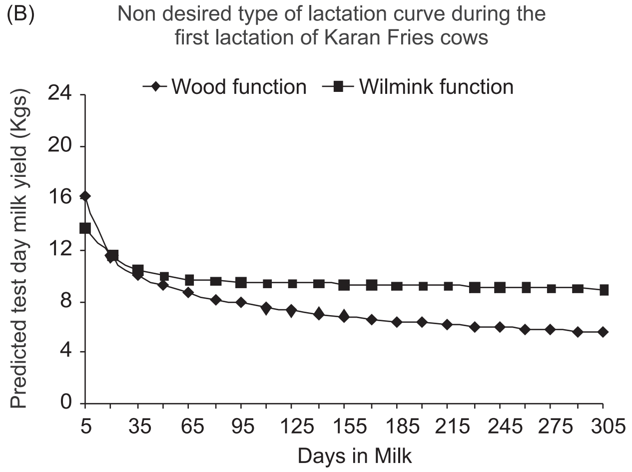


Fig. 2. (A) Desired/typical type of lactation curve in reference population of Karan Fries cattle, (B) Non-desired/atypical type of lactation curves in reference population of Karan Fries cattle.

Table 1. Lactation curve traits estimated by Woods function under different leptin genotypes in Karan Fries cattle

Population	Leptin genotype	Desired type of curves				Leptin genotype	Non-desired type of curves			
		a	b	c	R^2 (%)		a	b	c	R^2 (%)
Reference (N = 209)	TT (100)	5.67	0.35	-0.00152	61.60	TT (0.0)	-	-	-	-
Test (N = 30)	TT (100)	9.72	0.13	-0.00100	40.00	TT (0.0)	-	-	-	-
Reference (N = 209)	TC (84.70)	6.77	0.32	-0.00174	58.77	TC (15.30)	26.99	-0.2300	-0.00033	36.00
Test (N = 30)	TC (86.66)	9.20	0.16	-0.00110	45.83	TC (13.34)	18.89	-0.0797	-0.00034	22.60
Reference (N = 209)	CC (90.16)	6.46	0.34	-0.00169	54.94	CC (9.84)	18.01	-0.2500	0.00085	34.00
Test (N = 30)	CC (77.78)	6.96	0.18	-0.00114	55.83	CC (22.22)	18.35	-0.1276	0.00100	26.55

Figures in parenthesis indicates percentages of Leptin genotypes under desired and non-desired curves. N, number of Karan Fries cattle.

that Tharparkar breed has higher milch potential compared to Hariana cattle. Sequencing of DNA revealed T and C alleles of leptin gene. Both alleles of crossbred KF cattle when compared with *Bos Taurus* (using Clustal W), showed variation at 1180th position. The sequences obtained for T and C alleles in KF cattle was submitted to NCBI-gene bank and their accession numbers (GU907776 and GU907777) were obtained. On comparing the T and C alleles, it was revealed that C to T transition had led to non-synonymous change. The change from cytosine to thymine in T allele has resulted the change in amino acid arginine (positively charged; basic amino acid) to cysteine amino acid (polar and uncharged). This major change in amino acid can affect the production potential and thus, shape of lactation curve in bovines (Vohra *et al.* 2011).

Genotype vis-à-vis lactation curve in reference population: The average lactation curve traits viz. a, b and c estimated in reference population using Wood function were classified under different leptin genotype. The TT genotype of leptin gene was found to be present only in desired type of lactation curves and was absent in non-desired type of lactation curve. The percentage of curves classified under TC and CC genotypes were 84.70% and 90.16%, respectively. The initial TD milk yield (a) for desired type of lactation curves were lowest for the KF cattle having TT genotype, whereas TC genotype showed maximum initial TD milk yield (Table 1). The TT genotype had shown highest rate of incline to reach the peak yield (b) for desired type of lactation curve, and had lowest rate of decline after attaining the peak milk yield (c) [Fig. 3]. Animals having TT, TC and CC genotype showed R^2 value of 61.60%, 58.77% and 54.94%, respectively. The goodness of fit for non-desired type of lactation curves was found to be less than 40% (Table 1).

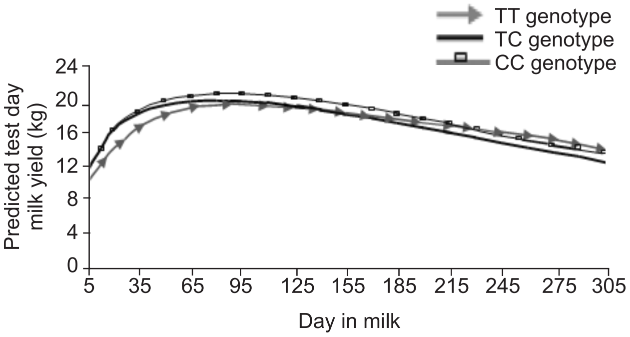


Fig. 3. Genotype profiling of desired type of lactation curves based on polymorphism in exon-2 of bovine leptin gene using Wood's function in reference population of Karan Fries cattle.

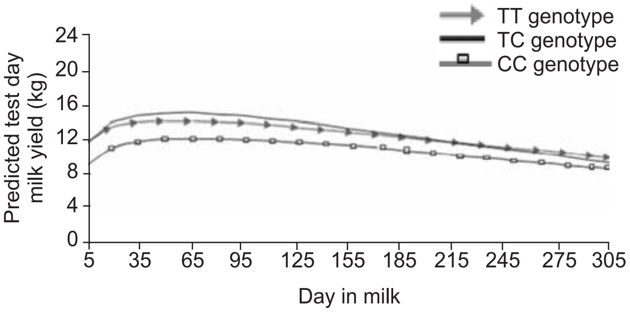


Fig. 4. Genotype profiling of desired type of lactation curves based on polymorphism in exon-2 of bovine leptin gene using Wood's function in test population of Karan Fries cattle.

Genotype vis-à-vis lactation curve in test population: Average lactation curve traits in test population and classified as desired and non-desired, based on model properties of the Wood's function. The results in test population were found to be on the lines of reference population (Fig. 4). Almost similar percentage of curves were classified under TT (100%) and TC (86.66%) genotypes, however, the CC genotypes had markedly lower percentage (77.78%) of lactation curves. Moreover, TT genotype in test population showed higher persistency in test day milk yield followed by TC and CC genotypes (Fig. 4). The goodness of fit for predicting the test day milk yield in test population was little lower than reference population (Table 1). The initial TD milk yields and rate of incline to reach the peak yield for desired type of lactation curves were not similar in reference and test population for TT and CC genotypes of leptin (Figs. 3,4). However, for both reference and test populations with TT genotype had shown lowest rate of decline (c) (Table 1) Figs. 3,4. Since, the rate of decline in a lactation curve determines the persistency of the dairy animal, therefore, it can be concluded cattle bearing TT genotype of leptin gene were more persistent milk producers. Our results are in agreement with Soita *et al.* (2004) who studied influence of leptin single nucleotide polymorphism (C/T transition that results in an Arg25Cys) on lactation curve traits and they reported that TT/TC genotypes attained earlier peak yield and were more persistent than CC genotypes of leptin gene.

Woods model can be successfully used to depict different pattern of lactation curves and lactation persistency. Our results report for the first time that TT genotype of leptin gene (exon 2) is related to desired type of lactation curves and contributes in maintaining the persistency of crossbred KF cattle. TT genotype of leptin gene showed lowest rate of decline, after peak yield is attained, in both the reference as well as test populations as compared to the animals with TC and CC genotypes of the leptin gene. Therefore, the study indicates that the genetic variability of bovine leptin genes determining lactation curve traits can be used for developing an aid to existing EPA based selection criteria of crossbred KF animals for higher milk yield and persistency.

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