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Genetic variation in intronic region of GH1 gene and its potential as a marker for test day milk production traits in Holstein Friesian crossbred cattle

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ABSTRACT

The study was conducted to find out the genetic variability in intron 3 region of GH1 gene and to estimate its effect on test day milk production traits in Karan Fries (Holstein Friesian crossbred) cattle. PCR-RFLP using *MspI* revealed two genotypes, namely BB and AB with frequencies as 0.18 and 0.82, and the frequency of B and A alleles as 0.59 and 0.41, respectively. Season of calving was found significant only for test day (TD) fat yield of TD3, TD6 and TD10. High association was found between test day milk traits and average lactation milk yield from TD3 onwards. TD1 and TD5 were found to be good for selection of animals as TD milk yield increased by 1.9352 kg and 0.9271 kg, TD fat yield by 101.2 g and 10.6 g and TD SNF yield by 188.8 g and 93.7 g respectively. The genetic variability in the targeted region could be further explored as a potential marker.

Key words: Genetic variation, GH1 gene, HF crossbred cattle, Intron 3, Test day milk production traits

India's milk production rose from 55.6 million tonnes in 1991–92 to 165.4 million tonnes in 2016–17 (BAHS 2017). This means that, on an average, milk production increased by more than 4% per year. This spurt in milk production was facilitated by crossbreeding programme in the country. According to the 19th Livestock Census (Anonymous 2012), the crossbred/exotic animals comprise only 21% of the total cattle population, yet their contribution in milk production from cattle is as high as 53.7% (BAHS 2017).

Though India boasts of being the world's highest milk producing country, yet the genetic progress in India has been slow due to small population size even in organised herds, large generation interval and low replacement rate. Most of the countries have embarked on the use of molecular data in their selection programmes. The integration of molecular marker information along with the phenotypic information has improved the accuracy of selection and reduced the generation interval. Marker Assisted Selection (MAS) is one such selection procedure involving genetic markers associated with the traits. In India, research is going on to search the markers for milk production traits.

Growth hormone (GH1) gene can be considered as a genetic marker of milk productivity in cattle (Khatami *et al.* 2005). It has an important role in milk production and composition traits. Variations in intron 3 region of GH1 gene have potential usefulness as genetic markers and could help in genetic improvement of populations (Mohammadabadi *et al.* 2010). The present study was carried out to see the genetic variability in intron 3 region of GH1 gene and to estimate its effect on milk production traits in Karan Fries (KF) cattle, which is a cross of Holstein Friesian males and Tharparkar females, maintained by inter-se mating in ICAR-National Dairy Research Institute (NDRI), Karnal, Haryana, India.

MATERIALS AND METHODS

Sample collection: The blood samples of 78 pedigreed Karan Fries (HF crossbred) cattle including 22 male calves, 17 female calves and 39 dams were collected from Livestock Research Centre (LRC) of ICAR-NDRI, Karnal.

DNA isolation and quality checking: The DNA was isolated from blood using phenol-chloroform method with minor modifications (Sambrook and Russel 2001). Agarose gel electrophoresis (0.8%) was carried out for checking the quality of DNA. Sharp and intense bands were characteristic of good quality DNA. Quality and concentration of DNA was also estimated by Nanodrop spectrophotometer method (ND 1000). The concentration of DNA was calculated as: Concentration of DNA (μg/ml) = (OD_{260}× 50 × Dilution Factor) / 1000.

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The Forward (P1) and Reverse (P2) set of primers covering intron 3 region of GH1 gene were designed using primer3 software. Primers were procured from Sigma Aldrich Chemicals Pvt. Ltd (Bengaluru, India). The sequence of primers and their nucleotide numbers were as follows: F- CCCACGGGCAAGAATGAGGC (20) and R-TGAGGAACTGCAGGGGCCCA (20).

PCR-RFLP of the targeted region: The targeted region (intron 3) of GH1 gene was amplified by standardizing PCR program using genomic DNA at an annealing temperature of 58°C for 30 sec and corresponding set of forward and reverse primers in a fixed reaction mixture with the help of thermal cycler. The PCR amplified product was checked on 1.7% agarose gel to verify the amplification of target region. PCR-RFLP was carried out using restriction endonuclease to generate a unique restriction polymorphic profile. NEB cutter and cleaver were used to search the suitable restriction enzyme for typing SNPs.

Estimation of gene and genotype frequency: The frequency of gene and genotype were calculated by Gene Counting method (Falconer and Mackay 1996)

Gene frequency = (2D + H) / 2N

$$\text{Genotype frequency} = \frac{\text{Total number of animals of a particular genotype}}{\text{Total number of animals of all genotypes}}$$

where D, Number of homozygote; H, Number of heterozygote; N, Total number of animals.

Phenotypic traits of Karan Fries cattle: The first and second lactation 305 day milk yield (kg) data of Karan Fries cattle were collected from Livestock Record Cell of Animal Genetics and Breeding Division and the data regarding ten monthly test day milk yield, test day fat percentage and test day SNF percentage of first and second lactation were collected from the record room of Livestock Production and Management Section at ICAR-NDRI, Karnal. The traits like test day fat yield (kg) and SNF yield (kg) were generated for both first and second lactation.

Data adjustment: The normalisation of traits was done (Snedecor and Cochran 1994), using mean and standard deviation of the traits. The effect of non-genetic factors on test day milk production and milk composition traits was analysed by least-squares analysis for non-orthogonal data (Harvey 1990). The model used was

$$Y_{ijk} = \mu + S_i + P_j + e_{ijk}$$

where Y_{ijk}, observation of k^{th} KF cattle under i^{th} season of calving and j^{th} parity; μ, overall mean; S_i, fixed effect of i^{th} season of calving; P_j, fixed effect of j^{th} parity and e_{ijk}, random error~NID (0, σ^2_e).

Correlation analysis: The correlation analysis was carried out in order to find the association between test day traits and average lactation milk yield of pooled first and second lactations in Karan Fries cattle. It was helpful to select early test days for selection of young Karan Fries animals.

Effect of SNP on milk production traits: The effect of SNP of GH1 gene (intron 3) on test day milk production traits was assessed using the regression model

$Y_{ij} = a + b_1 SNP_1 + e_{ij}$,

where Y_{ij}, test day milk production traits; a, intercept; b_1, regression coefficients for SNPs; SNP_1, effect of SNP_1; e_{ij}, random residual error NID (0, s^2_e) (Wang *et al.* 2011).

RESULTS AND DISCUSSION

Polymerase Chain Reaction amplification revealed product of size 329 bp fragment of intron 3 region of GH1 gene at an annealing temperature of 58°C for 30 sec. PCR-RFLP of the same region using *MspI* digestion revealed three products of sizes 329, 224 and 105 bp. Two genotypes namely, BB and AB were identified in the 329 bp fragment. The genotype BB had 329 bp fragments whereas the genotype AB had 329, 224 and 105 bp fragments. Several workers have found all the three genotypes in intron 3 region of GH1 gene using *MspI* enzyme. Zhou *et al.* (2006) reported three genotypes namely, AA, AB and BB by digestion of the same targeted region with *MspI* enzyme. Nam *et al.* (2014) also reported three genotypes, viz. +/+, +/- and -/- for the same region.

The frequency of B allele and A allele was estimated to be 0.59 and 0.41, respectively whereas the frequencies of BB and AB genotypes were estimated as 0.18 and 0.82. Zhou *et al.* (2006) reported allele frequencies of B and A allele as 0.125 and 0.875, respectively and genotype frequencies of A/A, A/B and B/B genotypes as 0.77, 0.21 and 0.02, respectively, in China Holstein cows.

The average test day milk yield was estimated to be 12.69±0.49 kg. The test day milk yield (TDMY) ranged from 10.06±0.58 kg in TD10 to 15.23±0.69 kg in TD2. The average test day fat yield was 531.32±19.14 g. The test day fat yield (TDFY) ranged from 432.29±2.84 g in TD10 to 619.63±32.93 g in TD2. The average test day SNF yield was calculated as 1.11±0.04 kg. The test day SNF yield (TDSNFY) ranged from 0.87±0.05 kg in TD10 to 1.31±0.06 kg in TD2. A general trend was observed in a way that the monthly TDMY attained peak at TD2 (15.23±0.69 kg) and thereafter, a gradual decline was noticed. The trend of average test day milk yield for Karan Fries cattle, as reported by several past workers has been close to the average TDMY found in this study. Sarkar *et al*. (2006) reported the average test day milk yield in Karan Fries cattle as 11.19±0.70 kg. Tripathy (2015) estimated average test day milk yield and average test day fat yield in Karan Fries cattle as 12.21±0.22 kg and 520.10±9.21g, respectively.

The least squares means of different test day milk yield varied from 10.30±0.81 kg in TD10 to 14.64±1.89 kg in TD5, different test day fat yield varied from 0.37±0.09 kg in TD9 to 0.68±0.06 kg in TD4 and test day SNF yield varied from 0.98±0.16 kg in TD10 to 1.28±0.17 kg in TD5. The least squares means for test day milk yield, fat yield and SNF yield are given in Table 1.

Test day milk yield was not significantly affected by season of calving. The highest and lowest test day milk

Table 1. Least-squares means of test day milk and milk composition traits in Karan Fries (HF crossbred) cattle

Test day	Number of observations (N)	TDMY Mean±SE (kg)	TDFY Mean±SE (kg)	TDSNFY Mean±SE (kg)
TD1	32	13.02±2.00	0.52±0.10	1.12±0.18
TD2	35	14.16±1.23	0.57±0.06	1.21±0.11
TD3	33	14.42±1.19	0.59±0.05	1.25±0.11
TD4	35	14.40±1.29	0.68±0.06	1.25±0.11
TD5	30	14.64±1.89	0.50±0.09	1.28±0.17
TD6	36	13.13±1.03	0.54±0.05	1.13±0.09
TD7	30	11.58±1.08	0.45±0.04	1.01±0.09
TD8	26	11.04±2.04	0.43±0.08	0.98±0.18
TD9	25	11.38±1.96	0.37±0.09	0.98±0.16
TD10	19	10.30±0.81	0.42±0.04	0.88±0.07

TDMY, Test day milk yield; TDFY, Test day fat yield; TDSNFY, Test day solid non fat yield.

yield values were observed on TD3 in winter (16.19±1.70 kg) and TD10 in autumn (8.31±1.14 kg) respectively. Season of calving was found significant (P≤0.05) for fat yield of TD3, TD6 and TD10. The highest test day fat yield was observed on TD3 in winter (0.70±0.07 g) and TD4 in summer (0.70±0.07 g) whereas the lowest test day fat yield was observed on TD9 in autumn (0.29±0.12 g). Season of calving was not found significant for different test day SNF yield. The highest and lowest test day SNF yield values were observed on TD3 in winter (1.42±0.16 kg) and TD10 in autumn (0.83±0.22 kg) respectively. Parity was not found significant for any of the traits. Season of calving was reported to have a significant effect on first lactation average test day milk yield and first lactation average test day fat yield in Karan Fries cattle by Tripathy (2015).

The correlation between test day traits and average milk yield of first and second lactation was high, from TD3 onwards. The correlation between average test day milk yield and average lactation milk yield of pooled first and second lactations was very high (0.7372). The maximum correlation was found between TD8MY (r=0.8258) and average lactation milk yield. The correlation between average test day fat yield and average lactation milk yield of pooled first and second lactations was very high (0.7419). The maximum correlation was found between TD8FY (r=0.8360) and average lactation milk yield. The correlation between average test day SNF yield and average lactation milk yield of first and second lactations was also found to be very high (r=0.7148). The maximum correlation was found between TD8SNFY (r= 0.8326) and average lactation milk yield.

The effect of identified SNP of GH1 gene (intron 3) on milk production traits was analysed for different test days through regression analysis (Table 2). For test day milk yield, good influence of SNP in the targeted region was observed on TD1, TD5 and TD8. The test day milk yield increased by 1.9352 kg in TD1 (b=1.9352), 0.9271 kg in TD5 (b=0.9271) and 1.3542 kg in TD8 (b=1.3542). Similarly, high effect of SNP on test day fat yield and test day SNF yield was observed on TD1, TD5 and TD8. The test day fat yield increased by 101.2 g in TD1 (b=0.1012), 10.6 g in TD5 (b=0.0106) and 79.9 g in TD8 (b=0.0799). The test day SNF yield increased by 188.8 g in TD1 (b=0.1888), 93.7 g in TD5 (b=0.0937) and 118.5 g in TD8 (b= 0.1185). For early selection of animals based on the phenotypic information and the effect of the identified genetic marker, TD1 and TD5 were found to be suitable. Kour *et al.* (2017) estimated the effect of genetic variability in the exon 5 region of GH1 gene and reported that the effect of SNP on overall test day milk yield, test day fat yield and test day SNP yield was 1.05 kg, 43.2 g and 103 g respectively. No reference was found regarding effect of SNP in intron 3 region of GH1 gene on milk production traits.

Table 2. Regression Coefficients (b) of SNP of GH1 gene (intron 3) on test day milk yield and milk composition traits in Karan Fries (Holstein Friesian crossbred) cattle

Test day	Number of observations (N)	TDMY (kg) Regression Coefficients (b)	TDFY (kg) Regression Coefficients (b)	TDSNFY (kg) Regression Coefficients (b)
TD1	32	1.9352	0.1012	0.1888
TD2	35	–0.3839	–0.0531	–0.0074
TD3	33	0.0092	0.0807	0.0112
TD4	35	–0.4138	–0.0176	–0.0267
TD5	30	0.9271	0.0106	0.0937
TD6	36	–0.1500	0.0144	–0.0089
TD7	30	–0.7500	–0.0378	–0.0649
TD8	26	1.3542	0.0799	0.1185
TD9	25	0.5875	0.0221	0.0511
TD10	19	–0.2760	–0.0209	–0.0331

TDMY, Test day milk yield; TDFY, Test day fat yield; TDSNFY, Test day solid non fat yield.

The study identified the genetic marker in intron 3 region of GH1 gene in relation to monthly test day production performance in pedigreed Karan Fries cattle. PCR-RFLP carried out using *MspI* restriction endonuclease revealed two genotypes namely BB and AB in the population with frequencies as 0.18 and 0.82, respectively and the frequency of B and A allele as 0.59 and 0.41, respectively. Season of calving was found to be significantly (P≤0.05) affecting test day fat yield of TD3, TD6 and TD10. High association was found between test day milk production traits and average lactation milk yield from TD3 onwards. For selection at an early age, TD1 and TD5 were found to be suitable as SNP of GH1 gene (intron 3) increased the test day milk yield by 1.9352 kg in TD1 (b=1.9352) and 0.9271 kg in TD5 (b=0.9271) Similarly, test day fat yield improved by 101.2 g in TD1 (b=0.1012), and 10.6 g in TD5 (b=0.0106) and test day SNF yield improved by 188.8 g in TD1 (b=0.1888) and 93.7 g in TD5 (b=0.0937). Based on the phenotypic data analysis and identified genetic marker, adult KF animals can be selected as early as on test days 1 and 5. This genetic marker information for GH1 gene could be used for early selection of young KF cattle for test day milk production traits and could be further employed in the Marker Assisted Selection strategy for Karan Fries cattle.

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