Original Research

Effect of Intron 3 and Exon 5 Polymorphism in GH1 Gene on Milk Production and Milk Composition Traits in Karan Fries (Holstein Friesian Crossbred) Cattle

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Abstract

The present study was undertaken to study the effect of SNPs in intron 3 and exon 5 region of GH1 gene on milk production and milk composition traits in Karan Fries (HF crossbred) cattle. DNA was isolated from the blood samples of 78 pedigreed animals and PCR amplification was done, followed by PCR-RFLP. The phenotypic data was adjusted for non- genetic factors and regression analysis was done. Multiple regression of SNPs in intron 3 and exon 5 region of GH1 gene revealed that on an average, test day milk yield increased by 1.199 kg, test day fat yield by 51 gms and test day SNF yield increased by 113 gms. Both of these SNPs could be considered as potential genetic markers for early selection of animals based on test day milk production and milk composition traits and could be incorporated in the Marker Assisted Selection strategy in future.

Key words: GH1 Gene, Intron 3 and Exon 5, Milk Production and Milk Composition Traits, Karan Fries, Polymorphism

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Introduction

Crossbreeding programme in dairy cattle ushered in an era of increased milk production and productivity in India. According to BAHS (2017), the country produced 163.7 million tonnes of milk in 2016-17 and crossbred cattle contributed a major portion of this produce. Over the years, in organised herds, genetic improvement of crossbred populations has been done, following selection and random mating by progeny testing programme. The integration of molecular genetics approach in conventional selection methods could increase the genetic selection response (Wakchaure *et al*., 2015). Marker Assisted Selection (MAS) is indirect selection process where a trait of interest is selected, not only based on the trait itself but also on marker(s) linked to it (Ribaut, 2007). MAS can be employed to select the superior animals at a young age based on molecular markers associated with particular traits like milk yield, fat yield, protein yield and SNF yield.

Growth hormone gene (*GH1*) has effect on almost all the important biological functions associated with livestock economics including growth, body composition and development of mammary cell, lactogenesis and proliferation of mammary cells (Horvat and Medrano, 1995; Nielsen *et al*., 1995 and Lagziel *et al*., 1999). Bovine growth hormone (bGH) plays a very important role in many physiological actions (Kratochvilová, 2000). The bGH/AluI genotypes have association with several production traits in cattle, such as milk-fat percentage, milk production and quality (de Mattos *et al.*, 2004; Kovacs *et al.*, 2006). Zhou *et al.*, 2005 analysed association of various variants/polymorphisms of the growth hormone gene (localized in intron 3 using *GH-MspI*) and milk production traits of Beijing Holstein cows. Ozdemir *et al.* (2018) studied AluI polymorphism in exon 5 of the *GH1* gene related to performance traits.

However, literature regarding the quantification of SNP effect of *GH1* gene in Karan Fries (KF) or HF crossbred cattle in relation to milk production and milk composition traits is lacking. This is required to deduce the contribution of individual SNPs in the total trait breeding value which will help in Marker Assisted Selection of animals. So, this study was taken up to estimate the effect of SNPs found in the intron 3 and exon 5 region of *GH1* gene in KF cattle.

Materials and Methods

Sample Collection and DNA Isolation

The study was conducted in 39 KF families, comprising of 39 adult female dams, 22 male calves and 17 female calves, maintained at Livestock Research Centre (LRC) of ICAR-National Dairy Research Institute, Karnal. Blood samples were collected from the animals and DNA was isolated by Phenol-chloroform method (Sambrook and Russel, 2001). The quality and concentration of DNA was checked using Agarose Gel and Nanodrop Spectrophotometer, respectively.

PCR Amplification and RFLP

PCR amplification was carried out for the intron 3 and exon 5 region of *GH1* gene using primers designed by Primer3 software. The primers for intron 3 region of *GH1* gene were: F-CCCACGGGCAAGAATGAGGC (20) and R- TGAGGAACTGCAGGGGCCCA (20). The primers for exon 5 region of *GH1* gene were: 5' F-GCTGCTCCTGAGGGCCCTTCG 3' (21) and 5'F-GCGGCGGCACTTCATGACCCT 3' (21). The amplified PCR product was checked on 1.7% agarose gel

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to verify the amplification of the targeted region. Restriction endonucleases designed using NEB cutter and cleaver were used to cleave the targeted regions in PCR-RFLP.

Statistical Analysis

The frequency of genotypes and gene were calculated by Gene Counting method as suggested by Falconer and Mackay (1996) as: Genotype frequency = Total number of KF cattle of a particular genotype / Total number of KF cattle of all genotypes and Gene frequency = (2D + H) / 2N, where, D = Number of homozygote, H = Number of heterozygote and N = Total number of Karan Fries cattle. The phenotypic data of Karan Fries animals' viz. first and second lactation 305 day milk yield (kg), ten monthly test day milk yield (TDMY, in kg), test day fat percentage and SNF percentage of first and second lactation was collected. The test day fat yield (TDFY, in kg) and test day SNF yield (TDSNFY, in kg) were generated for both the lactations. The data was normalised (Snedecor and Cochran, 1994) and the effect of significant non-genetic factors were adjusted using least-squares analysis (Harvey, 1990), employing the following model: $Y_{ijk} = \mu + S_i + P_j + e_{ijk}$, where Y_{ijk} = observation of k^th 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 $(0, \sigma^2_e)$. The joint effect of SNPs in the targeted region was estimated using the following multiple regression model: $Y_{ij} = a + b_1SNP_1 + b_2SNP_2 + e_{ij}$, where Y_{ij} = test day milk yield or milk composition traits, a= intercept, b_1= partial regression coefficient for SNP_1 in intron 3 region, SNP_1=effect of SNP_1 in intron 3 region, b_2= partial regression coefficient for SNP_2 in exon 5 region, SNP_2=effect of SNP_2 in exon 5 region, and e_{ij} = random residual, NID $(0, \sigma^2_e)$ (Wang *et al*., 2011).

Result and Discussions

PCR amplification of intron 3 region of *GH1* gene revealed a 329 bp PCR product at an annealing temperature of 58° C for 30 sec and for exon 5 region of *GH1* gene, it revealed a 223 bp PCR product at an annealing temperature of 59.4° C for 40 sec. Restriction cleavage of 329 bp PCR product of intron 3 region of *GH1* gene was done using MspI and two genotypes- BB (329 bp) and AB (329, 224 and 105 bp) were observed. Exon 5 region of *GH1* gene was restricted using AluI and two genotypes- LL (171 and 52 bp) and LV (223,171 and 52 bp) were seen. A number of workers have reported genetic polymorphisms in the intron 3 and exon 5 region of *GH1* gene in HF and HF crosses. Zhou *et al.* (2005) found three genotypes A/A, A/B and B/B on PCR-RFLP using GH-MspI in Beijing Holstein cows and deduced that cows with A/A genotype had higher milk fat content than those with A/B genotype. Nam *et al*. (2014) reported that the best milch animals with respect to somatotropin gene are those possessing the complex genotype LL(-/-) using Alu I and Msp I enzymes in local and Holstein cattle breeds of Iran. The estimated gene and genotype frequencies for intron 3 and exon 5 regions of *GH1* gene are depicted in Table 1 and Table 2, respectively. Mohammadabadi *et al*. (2010) identified that the frequency of L and V alleles in the exon 5

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region of *GH1* gene in the local herds were 0.23 and 0.77 and in the Holstein herds were 0.63 and 0.37 respectively.

Table 1: Estimated gene and genotype frequency of intron 3 region of *GH1* gene

Allele	Frequency	Genotype	Frequency
B	0.59	BB (7)	0.18
A	0.41	AB(32)	0.82

Table 2: Estimated gene and genotype frequency of exon 5 region of *GH1* gene

Allele	Frequency	Genotype	Frequency
L	0.73	LL (18)	0.46
V	0.27	LV (32)	0.54

The test day mean values of milk yield, fat yield and SNF yield for pooled first and second lactations was calculated and is depicted in Fig.1, 2 and 3, respectively.

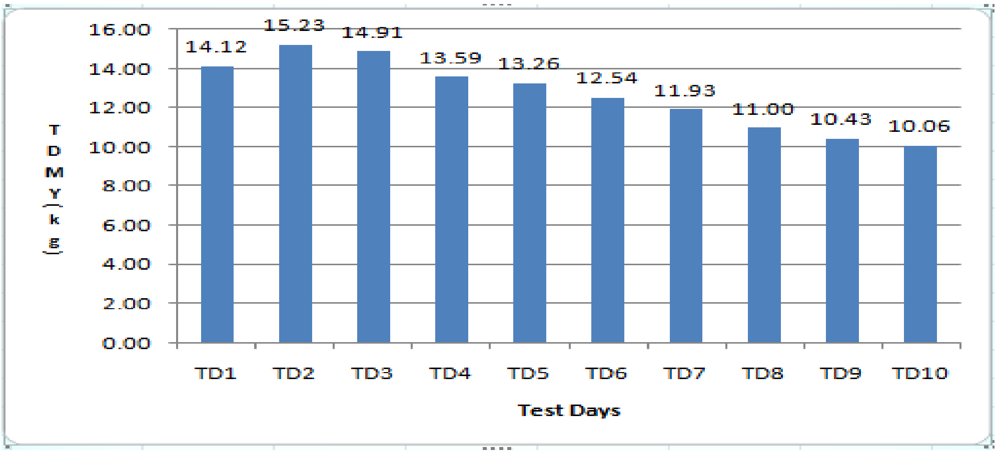

Fig. 1: Mean test day milk yield of pooled first and second lactations in Karan Fries cattle

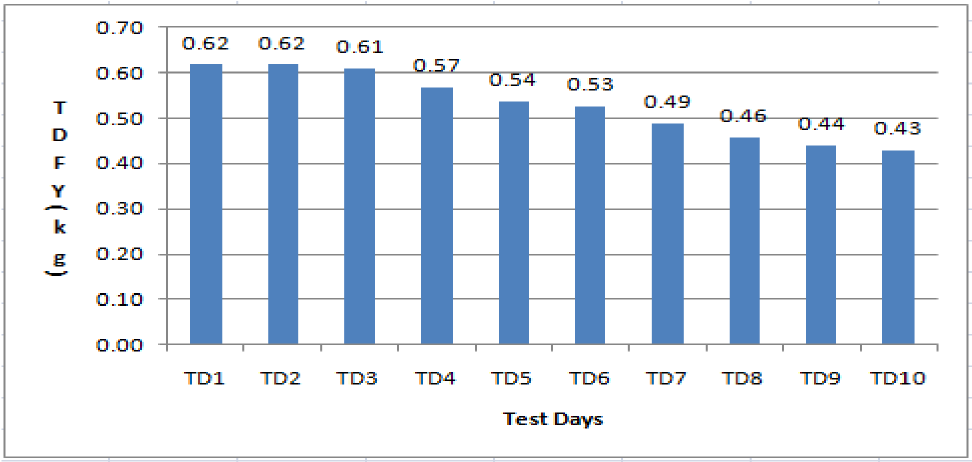

Fig. 2: Mean test day fat yield of pooled first and second lactations in Karan Fries cattle

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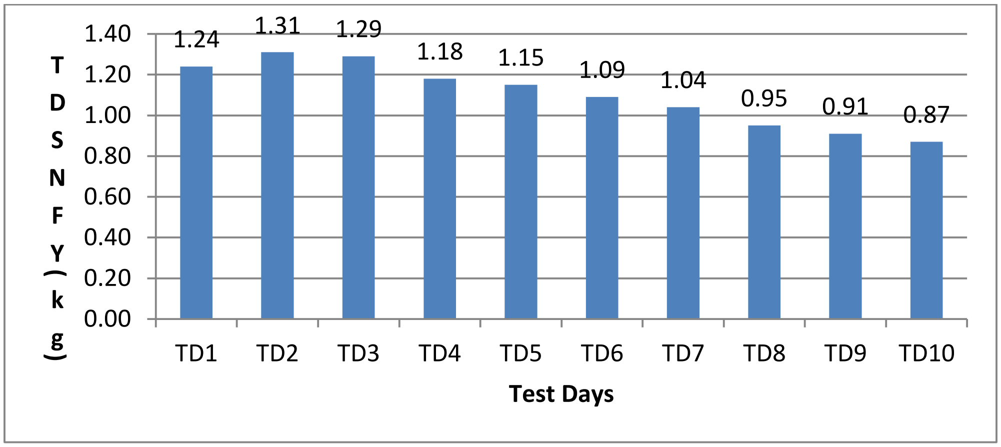

Fig. 3: Mean Test day SNF yield of pooled first and second lactations in Karan Fries cattle

The average test day milk yield (ATDMY) was found to be 12.69± 0.49 kg, the average test day fat yield (ATDFY) was found to be 0.53 ± 0.02 kg and the average test day SNF yield (ATDSNFY) was found to be 1.11 ± 0.04 kg. Sarkar et al. (2006) reported ATDMY in Karan Fries cattle as 11.19 ± 0.70 kg. Tripathy (2015) estimated ATDMY in Karan Fries cattle as 12.21 ± 0.22 kg and ATDFY as 520.10 ± 9.21 gm.

The overall 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. Season of calving was found significant ($p \leq 0.05$) for fat yield of TD3, TD6 and TD10. This was in line with the results obtained by Tripathy (2015) which showed that the period of calving had highly significant effect and season of calving had significant effect on first lactation average test day fat yield in KF cattle. Kumar (2016) also reported significant effect of period of first calving and non-significant effect of season of first calving, genetic group and age at first calving group on first lactation average test day fat percentage in KF cattle.

The joint effect of SNPs in intron 3 and exon 5 region of *GH1* was analysed for test day milk yield, test day fat yield and test day SNF yield and partial regression coefficients were estimated for the different test days and are depicted in Table 3. On an average, the SNP in intron 3 region of *GH1* gene contributed an increase of 0.119 kg in ATDMY, 0.007 kg in ATDFY and 0.008 kg in ATDSNFY. The SNP in exon 5 region of *GH1* gene contributed an increase of 1.080 kg in ATDMY, 0.044 kg in ATDFY and 0.105 kg in ATDSNFY. So, the joint contribution of the two SNPs is an increase in ATDMY by 1.199 kg, in ATDFY by 51 gms and in ATDSNFY by 113 gms. Moreover, maximum effect of both the SNPs was seen in Test day 3, 4 and 5 and these test days were good for early selection of animals as they were having high correlation with the average lactation milk yield of pooled first and second lactations. So, these SNPs could be incorporated as

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potential markers for selection of young animals at an early age for milk production and milk composition traits.

Table 3: Joint effect of SNPs in intron 3 (b_1) and exon 5 (b_2) region of *GH1* gene on Test day milk yield, Test day fat yield and Test day SNF yield

Test Day	**TDMY**		**TDFY**		**TDSNFY**	
(TD)	**b_1**	**b_2**	**b_1**	**b_2**	**b_1**	**b_2**
TD1	1.864	-0.266	0.083	-0.069	0.179	-0.037
TD2	0.174	1.561	-0.044	0.023	0.067	1.275
TD3	0.587	2.601	0.076	0.08	0.061	0.224
TD4	0.114	2.087	0.011	0.112	0.02	0.186
TD5	0.994	0.201	0.015	0.014	0.098	0.014
TD6	-0.135	0.055	0.073	-0.02	-0.007	0.006
TD7	-0.041	2.53	-0.007	0.11	-0.003	0.219
TD8	1.335	-0.153	0.077	-0.025	0.115	-0.026
TD9	0.691	0.693	0.024	0.013	0.059	0.053
TD10	-0.419	1.379	-0.024	0.034	-0.045	0.118
Overall.	0.119	1.08	0.007	0.044	0.008	0.105

All the 'b' values are in kg

Kour *et al.* (2017) quantified the effect of SNP in exon 5 region of *GH1* gene in Karan Fries cattle and found that the overall test day milk yield, fat yield and SNF yield increased by 1.05 kg, 43.2 gm and 103 gm, respectively.

Conclusion

The joint effect of SNPs in intron 3 and exon 5 region of *GH1* gene is an increase of 1.199 kg in ATDMY, 51 gms in ATDFY and 113 gms in ATDSNFY. So, these SNPs could be considered as potential markers for these traits and could be incorporated in any future Marker Assisted Selection strategy for selection of young KF animals for these traits.

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