



Association of lactation persistency with genetic variants of bovine growth hormone gene in Indian Sahiwal cows

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ABSTRACT

The aim of this study was to determine the associations of persistency with genetic variants of bovine growth hormone (bGH) gene in Indian Sahiwal cows. The data pertaining to the monthly test day milk yield (MTDMY) and first lactation length (FLL) of 305 lactating Sahiwal cows over a period of 23 years (1993–2016) were subjected to analysis. Persistency of milk yield was estimated by using test day based ratio method and Wilmink b function. Mean LL was 220.06 ± 8.15 days in all studied Sahiwal cows, however, 276.67 ± 10.25 days in cows with ≥ 100 days of LL and having 500 kg MY. The peak of milk yields was achieved at third TD of the first lactation. *Macrophage stimulating protein 1 (MspI)* and *Arthrobacter luteus I (AluI)* restriction endonucleases were used to digest the amplified regions in intron 3 and exon 5, respectively of bGH gene. The bGH *MspI* restriction fragment length polymorphism (RFLP) yielded TT, CT and CC genotypes, whereas bGH-*AluI* RFLP yielded CC, CG and GG genotypes. Significant association was found between first lactation persistency estimated using test day based ratio method and bGH-*MspI* polymorphism. No significant association was found between first lactation persistency and bGH-*AluI* polymorphism. In summary, results of present study revealed that the TT genotype of bGH-*MspI* gene in Sahiwal cows has better lactation persistency than other genotypes.

Keywords: Growth hormone, Lactation persistency, Ratio method, Sahiwal cow, Wilmink function

The persistency of lactation is the ability of lactating animal to maintain a relatively constant milk yield throughout lactation (Strabel *et al.* 2001) or the potential of a cow to continue producing milk at a high level after attaining the peak of lactation (Cole and Null 2009). More persistent lactations are advantageous since they are associated with higher milk yield, lesser health problems and reduce feeding and reproductive costs (Dekkers *et al.* 1997). Many mathematical models were developed to describe lactation persistency. Among them test day based ratio method and Wilmink model are two of them (Ali and Schaeffer 1987, Wilmink 1987).

The bovine growth hormone (bGH) gene is a member of multiple families having approximately 3396 bp and contains 5 exons which are separated by several introns and assigned with chromosome region 19q26 (Hediger *et al.* 1990). Although, a number of polymorphisms were identified in bGH gene, among them 2 polymorphisms located in the intron 3 and exon 5 were found significant for their effects on lactation persistency and milk production traits (Lucy *et al.* 1993). The polymorphism which is digested by *MspI* restriction endonuclease (RE) is located on the intron 3 and the polymorphism in the exon 5 could be digested by *AluI* restriction enzyme (Zhang *et al.* 1993a). Two alleles occur following digestion with *MspI* restriction

enzymes with T-insertion at +837 position and a C-G transition at +837 position (Lee *et al.* 1994). Two alleles were also found with *AluI* digestion and the found alleles were designated as leucine amino acid (L) and valine amino acid (V).

Significant association of bGH variants with different production traits has been confirmed by various genetic studies. However, association of the effect of the GH gene with lactation persistency in indigenous cattle is not studied. Therefore, this study was designed to investigate the possible association of the bGH gene polymorphism with persistency of lactation in Sahiwal cows, in order to identify potential markers to be used as complementary parameters in the selection of pure-bred Sahiwal cattle with better persistent of lactation.

MATERIALS AND METHODS

Cattle farm and data collection: The data included in this study was collected from the history-cum-pedigree sheets and daily milk recording registers maintained in the data recording section of the Animal Genetics and Breeding Division of National Dairy Research Institute (NDRI), Karnal, India. The data on first lactation production traits of 305 lactating Sahiwal cows over a period of 23 years (1993–2016) were recorded and used in this study. Information regarding animal number, date of calving, sire number, dam number, date of first calving and first lactation

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length (FLL) has been considered during data recording. The records of the animals with known pedigree and normal lactation were considered for this study. Culling, disposal in middle of lactation, abortion, stillbirth and other pathological conditions which affected the lactational yield were considered as abnormalities and hence such data were excluded from the analysis. To ensure the normal distribution, the outliers were removed and data within the range of mean $\pm$ 2 SD were only considered for the present study.

Determination of persistency of lactation: Test day based ratio method (Sollkner and Fuchs 1987) and Wilmink mathematical lactation curve model (Wilmink 1987) were used to determine the persistency of lactation. Wilmink model was the estimated slope of the lactation curve after peak milk yield. Percent persistency calculated by test day ratio method is as follow:

$$\text{Persistency (\%)} = \frac{\text{Milk yield at later test day}}{\text{Milk yield at earlier test day}} \times 100$$

Mean lactation persistency was calculated as sum of persistency between any two points on the lactation curve having 30 days interval divided by number of intervals.

Persistency by using Wilmink b function was determined by using following model:

$$Y_t = a + bt + ce^{-0.05t}$$

where Y, adjusted milk yield on t^{th} day of lactation; a, regression coefficient related to the level of production; b, regression coefficient related to production decrease after peak yield; c, regression coefficient related to production increase towards peak and t is the test day (TD). The value -0.05 is analogous to the approximate day of peak milk yield (Wilmink 1987). Animals reached upto TD6 were only used for persistency determination. Genetic association of persistency of lactation with bGHSNPs was studied using PCR-RFLP method.

Animals and genomic DNA extraction: DNA samples of 305 first lactating Sahiwal cows maintained at Livestock Research Center (LRC) of NDRI, Karnal, India were used in the present study. Peripheral blood samples were collected by jugular venipuncture in the EDTA coated vacutainer tubes (BD, Bioscience, India). Blood samples were stored at -20°C until DNA extraction. Phenol-chloroform method, as guided by Sambrook and Russel (2001) with few modifications was used for DNA isolation. The quality and the quantity of DNA were checked by agarose gel electrophoresis (Maxi-Horizontal gel electrophoresis and power pack, GeNei, Bengaluru, India) and nanodrop spectrophotometer (Bio-Rad, India), respectively.

Primers, PCR conditions and genotyping: Primer sequence of *MspI* and *AluI* bGH genes were adopted from Satyanarayana *et al.* (2006) and Mitra *et al.* (1995), respectively. The set of forward and reverse primer for *MSPI* were CCCACGGGCAAGAATGAGGC and TGAGG-AACTGCAGGGGCCCA respectively. However,

respective primers for *AluI* were GCTGCTCCTG-AGGGCCCTTCG and GCGGCGGCACTTCATGACCCT. The used primer of *MspI* and *AluI* were designed to amplify a 329 and 223 bp fragments, respectively. PCR reactions were performed in a total volume of 25 μl consisting of 0.50 μl (0.10 μM) forward primer, 0.50 μl reverse primer, 13.50 μl PCR master mix (consisted of *Taq* DNA polymerase, dNTPs, MgCl_2 and reaction buffers), 2.0 μl templates DNA (33.33 ng/ μl) and 8.50 μl milli Q water. The PCR amplification was performed in Thermal cycler (Bio-Rad PTC-200, India). The PCR cycling profile consisted of pre-denaturation at 95°C for 5 min, 40 cycles of denaturation at 94°C for 30 sec, annealing at 63°C for 30 sec followed by a final extension at 72°C for 10 min.

RFLPs and SNP detection: PCR products were digested in a total volume of 20 μl , containing 10 μl of PCR products, 0.30 μl of either *MspI* or *AluI*, 2 μl of $10\times$ buffer and 7.70 μl milli Q water at 37°C for 15 h in temperature controlled water bath. NEB cutter and cleaver, two bioinformatics software were utilized to detect the restriction enzyme sites for typing SNPs. Agarose gel (2.5%) was used to check the restricted PCR products. The agarose gels were photographed in the gel documentation system under the UV light for their respective genotypes. The forward and reverse sequences for each PCR fragments were assembled to form complete sequence for the respective region of bGH gene were visualized and edited using Bio Edit software (Burland 2000). Each edited sequence with corresponding reference sequences were performed with Clustal W multiple sequence alignment programme for DNA to identify SNPs (Larkin *et al.* 2007).

Restriction fragments using the *MspI* enzyme originated two restriction patterns; 329 bp, corresponding to the (T) allele and 224 and 105 bp corresponding to the (C) allele. The analysis of the restriction fragments using the *AluI* enzyme originated two restriction patterns; 223 bp, corresponding to the (G) allele and 171 and 52 bp, corresponding to the (C) allele.

Statistical analysis: Genotypic and allelic frequencies were calculated by using gene counting method (Falconer and Mackay 1996).

$$\text{Genotypic frequency} = \frac{\text{No. of Sahiwal cows with specific genotype}}{\text{Total no. of Sahiwal cows in the study}}$$

$$\text{Allelic frequency of A} = AA + \frac{1}{2} AB$$

$$\text{Allelic frequency of B} = BB + \frac{1}{2} AB$$

where AA and BB is the genotypic frequency of homozygote and AB is the genotypic frequency of heterozygote.

Association of bGHSNP genotype with persistency of lactation in Sahiwal cow was analyzed using Fixed Model Least Squares Analysis (Harvey 1990). The significant effect of SNP variants on persistency of lactation in Sahiwal cow were analyzed using the following model:

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

where Y_{ij} , j^{th} observation on persistency of lactation of Sahiwal cow having i^{th} SNP genotypes; μ , overall mean; G_i , effect of i^{th} genotype of SNP and e_{ij} , random error associated with Y_{ij} observation and assumed to be NID (0, σ^2_e).

RESULTS AND DISCUSSION

Descriptive statistics findings: In the present study, among 305 Sahiwal cows, only 163 cows reached to TD6. Average LL of all 305 studied Sahiwal cows was 220.06 ± 8.15 days. Among them, average LL of 180 cows which completed 100 days of LL and 500 kg milk yield was 276.67 ± 10.25 days. About 58.70% of Sahiwal cows completed 185 days or TD6 of first lactation period while only 36.25% cows reached the standard level of 305 days in milk (DIM; TD10) (Table 1). The peak milk yield was achieved at TD3 (95 DIM) followed by gradual decrease. The daily milk yield in studied Sahiwal cows between TD3 and TD6 averaged 8.12 kg/day.

Table 1. Descriptive statistics of Sahiwal cows

Test day (TD)	Class range (days)	Number of observations	Milk yield (kg/day)	% of cows completing each TD
1	5–35	299	4.86	100.00
2	36–65	289	8.15	92.50
3	66–95	241	8.64	77.50
4	96–125	210	8.47	67.78
5	126–155	196	7.88	63.41
6	156–185	181	7.48	58.70
7	186–215	168	7.17	55.62
8	216–245	148	6.59	48.33
9	246–275	132	6.40	43.25
10	276–305	110	5.64	36.25
11	306–335	86	5.49	30.56

Similar to present findings, Pandey *et al.* (2018) also reported 267 days mean LL in Sahiwal cattle. However, records of higher LL were reported by Verma *et al.* (2018) as 311 days in Sahiwal cattle maintained at LRC of NDRI-Karnal, India. Manoj *et al.* (2013) also reported higher estimates of LL (290.41 ± 6.29 days) in the same herd of Sahiwal cattle may be due to exclusion of lactation records having LL <100 days. Bajwa *et al.* (2004) stated that most of the indigenous cattle were found with average LL below 305 days. Similar to the present study, Dematawewa *et al.* (2007) also estimated higher peak milk yield at 93 DIM during the first lactation. However, early peak milk yield at 57.55 DIM during the first lactation was observed in first lactation Canadian Holsteins (Muir *et al.* 2004).

Lactation persistency estimates: In this study, means lactation persistency estimated using test day based ratio method and Wilmink b function were 87.86% and -7.06, respectively. Test day based ratio method is recent method of lactational persistency determination in dairy animals; therefore, limited information is available. DHI Cow Production Monthly Report (2017) stated that persistency

% estimated by test day based ratio method in Holstein Friesian, Ayrshire, Brown Swiss and Jersey cows during first lactation ranges between 95–98%. Similarly, higher lactation persistency compared to the present study (97 vs. 87.86%) was observed by Widyas *et al.* (2018) in Indonesian Friesian Holstein cows.

This trait was chosen as a phenotypic measure of persistency because of its ease of calculation and moderate heritability (Muir 2004). In accordance to the present findings, Daltro *et al.* (2019) found -7.952 and -12.000 values for Wilmink b function in Gyr and Holstein cows, respectively during first lactation. Otwinowska *et al.* (2016) estimated -0.0378 value of Wilmink b parameter in the first lactation of Polish Holstein-Friesian cows. However, Muir *et al.* (2004) estimated -0.03991 Wilmink b parameter in the first lactation of Canadian Holsteins. Negative value of parameters b showed that the lactation curve presents a typical pattern when fitted by Wilmink model (Macciotta *et al.* 2005).

Genotypic and allelic frequency: The PCR-RFLP results of present study revealed the polymorphism in intron 3 of bGH gene with 2 alleles (C and T) and in exon 5 of bGH gene with 2 alleles (C and G) (Table 2). Similar to the findings of the present study, 2 alleles at GH-*MspI* and *AluI* was found by Khatami *et al.* (2005), Zhou *et al.* (2005) and Pawar *et al.* (2007) whereas Ferraz *et al.* (2006) identified 3 alleles at bGH-*AluI* locus.

Table 2. Genotypic and allelic frequency of different genotype

RE	Locus	Genotype	Animal no.	Genotypic frequency	Allele	Allelic frequency
<i>MspI</i>	Int3	CC (4)	305	0.013	C	0.095
		CT (54)		0.177		
		TT (247)		0.809	T	0.898
<i>AluI</i>	Ex 5	CC (216)	294	0.734	C	0.862
		CG (75)		0.255	G	0.137
		GG (3)		0.010		

Genotypic and allelic frequencies obtained in the present study agree with Gorbani *et al.* (2009), who reported genotypic frequencies of 0.787, 0.191 and 0.022 for (TT), (CT) and (CC) genotypes, respectively and 0.883 and 0.117 for (T) and (C) alleles in Iranian Holstein cows. Arango *et al.* (2014) reported 0.91 and 0.09 allelic frequencies for respective (T) and (C) alleles and the genotype frequencies were 0.77, 0.20 and 0.03 for (TT), (CT) and (CC) genotypes, respectively in Colombian Holstein cows.

The result of bGH-*AluI* locus in present study was similar to finding reported by Satyanarayana *et al.* (2006) in Sahiwal and Tharparkar cows. Zhang *et al.* (1993b) observed similar frequency (0.91 for C and 0.09 for G) in a breeding Holstein-Friesian bull population. Results of Sabour and Lin (1996) in Canadian Holstein-Friesian bulls (G: 0.09) also support the GH allele frequency data observed in our study. Ozdemir *et al.* (2018) reported the similar frequencies of the CC, CG and GG genotypes were 0.50,

Table 3. Association of bGH-MspI and bGH-AluI polymorphism with lactation persistency

Locus	Genotype	Mean lactation persistency	
		Test day based ratio method	Wilink b parameter
bGH-MspI	TT	92.095 ^a ±2.249 (43)	-7.669±0.832 (42)
	TC	86.410 ^b ±2.949 (25)	-7.043±1.039 (30)
	CC	86.524 ^b ±1.474 (95)	-8.108±0.562 (91)
bGH-AluI	CC	88.083±1.301 (128)	-6.948±0.495 (120)
	CG	87.886±2.448 (35)	-6.780±0.824 (43)

0.48 and 0.02, respectively and the frequency of the C allele was 0.74 for the bGH gene in 186 studied Holstein cows.

Association analysis of bGH-MspI and bGH-AluI SNPs with persistency of lactation: Significant ($P<0.05$) association was found between bGH-MspI polymorphism and lactation persistency estimated using test day ratio method (Table 3). Sahiwal cows with TT genotype had significantly ($P<0.05$) higher lactation persistency than cows with TC and CC genotypes. However, no association among bGH-MspI polymorphism and lactation persistency observed while using Wilink b function. Accordingly, no significant ($P>0.05$) association was found between lactation persistency and bGH-AluI polymorphism by using either method.

Mean with different superscript in column differs significantly ($P<0.05$), Sahiwal cows reached upto TD6 (163) had been considered for association study.

No information available regarding the association of bGH-MspI and bGH-AluI polymorphism with lactation persistency in dairy cows. However, Kovacs *et al.* (2006) reported that there was no association between lactation persistency and bGH-AluI polymorphism in a Hungarian Holstein-Friesian bull dam population.

In conclusion, the findings of this study revealed that the Sahiwal cows with TT genotype of bGH-MspI gene has better lactation persistency than other genotypes.

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