Association of milk production traits with genetic variants in exon 5 and intron 3 of bovine growth hormone (bGH) gene in Sahiwal cattle

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Received: 14 September 2020; Accepted: 6 April 2021

ABSTRACT

The main objective of this study was to determine the association of production traits with genetic variants in exon 5 and intron 3 of bovine growth hormone (bGH) gene in Sahiwal cattle. The analyses were based on the detection of single nucleotide polymorphisms (SNPs) in GH-AluI (exon 5) and GH-MspI (intron 3) using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay. The frequency of AluI (L/V) and MspI (T/C) alleles were 86.20/13.70 and 89.80/9.20, respectively. The distribution of the frequency of GH genotypes for LL, LV, and VV were 73.4, 25.5, and 1.0 and for TT, TC, and CC genotypes were 80.9, 17.7, and 13.0, respectively. Season of calving had none but period of calving had significant effect on the studied production traits. The phenotypic data was adjusted for non-genetic factors and regression analysis was done. There was detectable significant effect of the GH-AluI and GH-MspI on the analyzed production traits. The cows with LL genotype had significantly higher milk yield traits than the LV genotype. Similarly, the productive performance of the studied cows with TT genotype was also significantly higher than TC and CC genotypes. Results revealed that the Sahiwal cows with LL and TT genotypes of the bGH locus can be considered to be a favourable genotype for production traits, although these findings need to be confirmed by further research before SNPs can be used in a marker assisted selection program by the animal breeder.

Keywords: Bovine growth hormone, GH-AluI, GH-MspI, Production traits, Sahiwal cows

Functional traits are controlled by major and minor genetic loci therefore; linked candidate genes like growth hormone (GH) gene of the encoding loci are searched to optimize productive performance. GH is a major regulator of metabolism and postnatal growth in mammals, and it plays an important role in nutrient metabolism, tissue growth, milk production and reproduction of cows (Thidar et al. 2008). The metabolic and growth promoting actions of GH are primarily mediated by the insulin-like growth factor-1 (IGF-1) (Ramesha et al. 2015).

Many polymorphisms of the bovine GH (bGH) gene have been described and three polymorphic sites have been discovered within the fifth exon of this gene (Yao et al. 1996). Many researchers have studied the associations of GH gene polymorphisms with lactation traits in dairy cattle (Balogh et al. 2009a, Heidari et al. 2012) owing to the critical role of GH in growth, metabolism regulation, mammary gland development, and production (Mullen et al. 2010). A polymorphic site for GH-AluI restriction endonuclease was identified due to cytosine to guanine transversion at position 2141. This transversion induced a change of leucine (L) amino acid into valine (V) amino acid sequence at position 127 of the bGH protein chain (Lucy et al. 1993). The genotype of GH-AluI polymorphism was reported to be associated with milk yield and milk composition traits (Kovacs et al. 2006). The polymorphism which is digested by AluI restriction endonuclease is located on the exon 5. However, GH-MspI polymorphism is located in intron 3 of the GH gene at position 1547 (Zhang et al. 1993). As a consequence, two alleles occur, and MspI contains a C–G transition at the C837 position and a T insertion at the C837 position (Lee et al. 1994). Until now, limited studies (Amiri et al. 2018) have been identified in association of GH-AluI and GH-MspI polymorphism with milk production traits. Based on the above mentioned evidence, the present investigation was carried out to identify GH-AluI and GH-MspI polymorphisms and uncover their association with productive traits in Sahiwal cattle.

MATERIALS AND METHODS

Experimental design: This study was conducted on the Sahiwal cows maintained at Livestock Research Centre (LRC) of National Dairy Research Institute (NDRI), Karnal, India. The data on first lactation production traits of 451 lactating Sahiwal cows over a period of 24 years (1992–
2016) were recorded and used in this study. The data included in this study was collected from the history-cum-
degree sheets and daily milk recording registers
maintained at Animal Genetics and Breeding (AGB)
Division. Information regarding animal number, date of
calving, sire number, dam number, date of first calving,
first lactation 305-day milk yield (FL305MY), first lactation
total milk yield (FLTMY), and first lactation length (FL)
has been considered during data recording. The colostrum
yield for the first 5 days after calving was not included in
milk yield. The records of the cows with known pedigree
and normal lactation were considered for this study. The
cows with lesser than 500 kg of milk production and 100
days lactational length were excluded from this study. To
ensure the normal distribution, the outliers were removed
and data within the range of mean ± 2SD were only
considered for this study.

Genomic DNA extraction, primers, PCR conditions and
genotyping: Animal care procedures were approved and
conducted under the established standard of the Institutional
Animal Ethics Committee (IAEC). Out of 451 lactating
Sahiwal cows, those Sahiwal cows crosses T6 (n=315) were
used for genotyping. Among theses, genotypes of 226
Sahiwal cows were already present with Molecular Genetics
Laboratory. Genotyping of rest Sahiwal cows were
performed using isolation of genomic DNA and PCR-RFLP
assay. Peripheral blood samples were collected by jugular
venipuncture in the EDTA coated vacuutainer tubes (BD,
Bioscience, India). 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,
Bangalore, India) and nanodrop spectrophotometer (Bio-
Rad Laboratories, India, Pvt. Ltd.), respectively. The ratio
between OD260 and OD280 was calculated for each DNA
sample. Sample with ratio of 1.8 or more was considered
good and used for analysis.

In silico primer designing for target regions of bGH gene
was carried out using Primer3 software available at NCBI
database. Two sets of forward (GH-AluI: GCTGCTCC-
TGAGGGCCCTTCG and GH-MspI: CCCACGGGC-
AAGAATGAGGC) and reverse (GH-AluI: GCGGCGG-
CACTTCATGAACCT and GH-MspI: TGAGGAACCT-
GCAGGGGGCCA) region specific oligonucleotide primers
were designed. Digestion product size (bp) for GH-AluI
and GH-MspI is LL 171, 52; LV 223, 171, 52; VV 223 and
TT 224, 105; CT 329, 224, 105; CC 329, respectively.
BLAST programme was used to check the specificity of
the designed primers.

The used primer of AluI and MspI were designed to
amplify a 223 and 329 bp fragments, respectively using
the published DNA sequence of the bGH gene (Genbank
Accession Number 280804, Hernandez et al. 2016). PCR
reactions were performed in a total volume of 25 µL
consisted of 0.50 µL forward primer, 0.50 µL reverse primer,
13.50 µL PCR master mix, 2.0 µL templates DNA (33.33
ng/µL), and 8.50 µL milli Q water. The PCR amplification
was performed in programmed 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 (GH-AluI) or 68.5°C
(GH-MspI) for 30 sec followed by a final expansion for 10
min at 72°C. The PCR amplification yielded 223 and 329
bp fragments that were revealed using electrophoresis on a
2% agarose gel.

RFLPs and SNP detection: The amplified PCR products
were subjected to RFLP with a selected restriction
endonuclease (AluI and MspI). PCR-RFLP reaction
contained 10 µL of the PCR product, 2 µL of the reaction
buffer, 0.3 µL of the restriction enzyme, 7.7 µL of
autoclaved distilled water in a total volume of 20 µL
incubated at 37°C for at least 16 h. Agarose gel (2.5%)
electrophoresis with ethidium bromide 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 according to band
sizes. 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. Each edited sequence with
corresponding reference sequences were performed with
Clustal W multiple sequence alignment programmes for
DNA to identify SNPs (Larkin et al. 2007).

Calculations and statistical analysis: The significance
of non-genetic factors was identified by least squares
analysis (Harvey 1990). The effect of period of calving and
season of calving was studied using following model and
data were adjusted for significant non-genetic factors.

\[ Y_{ijk} = \mu + S_i + P_j + e_{ijk} \]

where, \( Y_{ijk} \) is the observation on \( k^{th} \) individual belonging
\( j^{th} \) period and \( i^{th} \) season of calving, \( \mu \) is the overall
population mean, \( S_i \) is the fixed effect of \( i^{th} \) season of calving,
\( P_j \) is the fixed effect of \( j^{th} \) period of calving, and \( e_{ijk} \) is the random error associated with each observation
which is assumed to be normally and independently
distributed with mean zero and variance \( \sigma^2_e \). The pairwise
comparison of means was carried out using “Tukey’s
honestly significant difference (HSD) test.” Significance
was determined at P<0.05 and the values are presented in
the tables.

The seasons of calving were classified into \( S_1 \) (winter
season, December to March), \( S_2 \) (summer season, April to June), \( S_3 \) (rainy season, July to September), and \( S_4 \) (autumn
season, October to November). The total span of 24 years
was divided into eight periods (\( P_1 \) to \( P_8 \)). \( P_1 \) and \( P_8 \) have
four consecutive years and \( P_2-P_7 \) have three consecutive
years. Genotypic and allelic frequencies were calculated
by using gene counting method (Falconer and Mackay
1996).

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

\[ Y_{ij} = \mu + G_i + e_{ij} \]

where, \(Y_{ij}\) jth observation on lactation traits of Sahiwal cow having \(i^t\)th SNP genotypes; \(\mu\), overall mean; \(G_i\), effect of \(i^t\)th genotype of SNP and \(e_{ij}\), random error associated with \(Y_{ij}\) observation, and assumed to be NID \((0, \sigma^2 e)\).

**RESULTS AND DISCUSSION**

**Gene and genotypic frequency:** In the present study, 223 and 329 bp fragments from the bhGH gene were characterized and successfully amplified from the DNA of each sample. This indicated a strong conservation of the DNA sequence existing in cattle. The findings of this study showed that the L allele was significantly \((P<0.05)\) more frequent than the V allele (Table 1).

Table 1. Genotypic and allelic frequency of different genotype

<table>
<thead>
<tr>
<th>Restriction Locus</th>
<th>Geno- type</th>
<th>No. of Animal</th>
<th>Geno- typic frequency</th>
<th>Allelic frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Alu I</strong></td>
<td>Exon 5</td>
<td>294</td>
<td>LL (216)</td>
<td>0.734</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>LV (75)</td>
<td>0.255</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>VV (3)</td>
<td>0.010</td>
</tr>
<tr>
<td><strong>Msp I</strong></td>
<td>Intron 3</td>
<td>305</td>
<td>CC (4)</td>
<td>0.013</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CT (54)</td>
<td>0.177</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>TT (247)</td>
<td>0.809</td>
</tr>
</tbody>
</table>

The GH-MspI frequency distribution showed that the occurrence of MspI (TT) genotype was the most abundantly followed by MspI (CT). The least frequently found genotype was MspI (CC). The MspI (T) allele was more frequent than the MspI (C) allele.

The PCR-RFLP results of the present study revealed the polymorphism in exon 5 of bhGH gene with 2 alleles (L and V) and in intron 3 of bhGH gene with 2 alleles (C and T). Similarly, 2 alleles at GH loci were reported by Pawar et al. (2007). However, Ferraz et al. (2006) identified 3 alleles at bGH-AluI locus. In the present study, frequency found for allele L (0.862) was higher than that of allele V (0.137), which was in close agreement to the results of Ozkan et al. (2005) who found 0.84 and 0.16 allele frequencies of allele L and V, respectively in Holstein Friesian cows. Results of Dybus (2002) and Vasconcellos et al. (2003) also observed similar L and V allele frequency that support the GH allele frequency data observed in our study. Allele and genotype frequencies for the GH-AluI polymorphisms in the present study were lesser than those reported in previous studies (Amiri et al. 2018, Ozdemir et al. 2018).

The analysis of the restriction fragments using the MspI enzyme originated two restriction patterns, viz. T and C alleles. The occurrence of TT genotype was more frequent as compared to the CC genotype hence; the presence of allele T was more frequent than the allele C. Genotypic and gene frequencies obtained in this study agree with those reported by Gorbani et al. (2009), who, in a population of 183 Holsteins, found frequencies of 0.787, 0.191, and 0.022 for TT, CT, and CC genotypes and 0.883 and 0.117 for T and C alleles, respectively. 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 Holstein cows.

**Non-genetic factors affecting production traits:** Season of calving had none whereas, period of calving had significant \((P<0.05)\) effect on the studied production traits (Table 2).

Trend followed by production traits during the different periods can be generalized as phases where first they increased then decreased and then again increased. However, the increase in later periods was lesser than that of the earlier period. Results revealed that FL305DMY, FLTMY, and FLL were found highest \((P<0.05)\) during third period.

The findings of the present study revealed that least square mean of FL305DMY and FLTMY was similar to the earlier report of Verma et al. (2016). Contrary to the

Table 2. Least squares mean and standard error of production traits in Sahiwal cattle

<table>
<thead>
<tr>
<th>Code</th>
<th>No. of observations</th>
<th>FL305DMY (kg)</th>
<th>FLTMY (kg)</th>
<th>FLL (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall mean</td>
<td>451</td>
<td>1756.61±</td>
<td>1873.05±</td>
<td>290.77±</td>
</tr>
<tr>
<td></td>
<td></td>
<td>48.89</td>
<td>58.54</td>
<td>5.42</td>
</tr>
<tr>
<td>Season of calving</td>
<td>S1</td>
<td>1754.50±</td>
<td>1864.75±</td>
<td>290.24±</td>
</tr>
<tr>
<td></td>
<td></td>
<td>48.23</td>
<td>57.75</td>
<td>5.34</td>
</tr>
<tr>
<td></td>
<td>S2</td>
<td>1729.57±</td>
<td>1851.88±</td>
<td>284.93±</td>
</tr>
<tr>
<td></td>
<td></td>
<td>54.85</td>
<td>65.67</td>
<td>6.08</td>
</tr>
<tr>
<td></td>
<td>S3</td>
<td>1775.31±</td>
<td>1892.34±</td>
<td>300.59±</td>
</tr>
<tr>
<td></td>
<td></td>
<td>89.27</td>
<td>106.89</td>
<td>9.90</td>
</tr>
<tr>
<td></td>
<td>S4</td>
<td>1767.07±</td>
<td>1883.25±</td>
<td>287.31±</td>
</tr>
<tr>
<td></td>
<td></td>
<td>158.44</td>
<td>189.71</td>
<td>17.57</td>
</tr>
<tr>
<td>Period of calving</td>
<td>P1</td>
<td>1968.95±</td>
<td>2140.43±</td>
<td>315.86±</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100.63</td>
<td>120.49</td>
<td>11.61</td>
</tr>
<tr>
<td></td>
<td>P2</td>
<td>1977.33±</td>
<td>2048.31±</td>
<td>271.76±</td>
</tr>
<tr>
<td></td>
<td></td>
<td>110.70</td>
<td>132.55</td>
<td>12.28</td>
</tr>
<tr>
<td></td>
<td>P3</td>
<td>2164.57±</td>
<td>2372.81±</td>
<td>320.41±</td>
</tr>
<tr>
<td></td>
<td></td>
<td>109.13</td>
<td>130.66</td>
<td>12.10</td>
</tr>
<tr>
<td></td>
<td>P4</td>
<td>1594.30±</td>
<td>1704.54±</td>
<td>288.79±</td>
</tr>
<tr>
<td></td>
<td></td>
<td>110.70</td>
<td>132.54</td>
<td>12.27</td>
</tr>
<tr>
<td></td>
<td>P5</td>
<td>1160.81±</td>
<td>1194.30±</td>
<td>253.58±</td>
</tr>
<tr>
<td></td>
<td></td>
<td>95.86</td>
<td>114.78</td>
<td>10.63</td>
</tr>
<tr>
<td></td>
<td>P6</td>
<td>1678.59±</td>
<td>1814.82±</td>
<td>290.85±</td>
</tr>
<tr>
<td></td>
<td></td>
<td>95.86</td>
<td>105.97</td>
<td>9.81</td>
</tr>
<tr>
<td></td>
<td>P7</td>
<td>1682.77±</td>
<td>1766.10±</td>
<td>293.19±</td>
</tr>
<tr>
<td></td>
<td></td>
<td>99.12</td>
<td>118.68</td>
<td>10.99</td>
</tr>
<tr>
<td></td>
<td>P8</td>
<td>1825.57±</td>
<td>1943.11±</td>
<td>291.69±</td>
</tr>
<tr>
<td></td>
<td></td>
<td>87.98</td>
<td>105.35</td>
<td>9.75</td>
</tr>
</tbody>
</table>

Means bearing different superscript in a column differs significantly \((P<0.05)\).
findings of the present study, FL305DMY was higher in study that reported by Ratwan et al. (2019). In the present study, FLTMY was lower as compared to that reported by Dongre et al. (2013) and it was higher than that reported by Ratwan et al. (2019). The overall least square mean of FLL in our study was closely similar to the findings of Manoj et al. (2012). On the contrary, FLL was lower than that reported by Dahlin et al. (1998) and higher than that reported by Singh and Singh (2016).

Season of calving had non-significant effect on FL305DMY as reported by Pandey et al. (2019), and Ratwan et al. (2019). FLTMY was also found to be non significant and according to the findings reported by Singh and Singh (2016) and Ratwan et al. (2019). The effect of season of calving on FLL was found to be non significant. Similar observation was reported by Singh and Singh (2016), and Ratwan et al. (2019) but significant by Dhawan et al. (2015). The non significant influence of season on the production traits may be due to better management practices throughout the year in all seasons and also because adaptability of Sahiwal cattle is better under local climatic conditions.

The period of calving had significant effect on the studied productive traits. The significant effect of period of calving on FL305DMY was also reported Singh and Singh (2016), and Pandey et al. (2019) in dairy cows. Ratwan et al. (2019) also observed significant effect of period of calving on FLTMY in Sahiwal cows. However, non significant effect of period of calving on FLTMY was reported by Singh and Singh (2016). Alike to the present study, Singh and Singh (2016), and Ratwan et al. (2019) also noted a significant effect of period of calving on FLL in Sahiwal cows. The decrease in the performance in the middle periods might be due to increased focus given to cross breeding as compared to the improvement of indigenous cattle. The significant effect indicated the environmental changes and the change in managerial practices during these periods which caused the variations in production traits.

Association of bGH gene with production traits: As shown in Table 3, GH-AluI had significant (P<0.05) effect on FL305DMY, FLTMY, and FLL.

The replacement of the V allele by the L allele was found to be responsible for an increase in the productive performance. Therefore, cows with the LL genotype had the highest FL305DMY, FLTMY, and FLL compared with cows having LV genotypes. VV was genotype not considered in this study due to their low incidence (n=3) and higher error variance. Few studies have investigated the relationship between GH-AluI and GH-MsplI polymorphisms and productive traits in dairy cattle. Similarly, Dybus (2002) in Black and White cattle also suggested an additive effect of the L allele on milk production traits with LL genotype cows yielding more milk than LV and VV genotype. Contrary to the above mentioned studies and to us, Zwierzchowski et al. (2001) observed that V allele had no effect on milk yield in Holstein cattle. Sommez et al. (2018) also noted non significant relationship between the mean lactational milk yield, peak daily yield, 305 days milk yield, and lactation length in different genotypes Holstein cows.

There was detectable significant (P<0.05) effect of the GH-MsplI on the production traits analyzed. The replacement of the C allele by the T allele was found to lead to an increase in the FL305DMY, FLTMY, and FLL. The productive performance of the cows with TT genotype was significantly higher (P<0.05) compared with similar values of the TC and CC genotypes.

In a study conducted by Hoj et al. (1993) it was observed that cows with T allele showed higher milk production compared to cows with C allele. Accordingly, Yao et al. (1996) in Holstein cows also found better milk yield in T allele than C allele. MsplI cows with TT genotype are considered to yield more milk, protein and fat than TC genotype (Zhou et al. 2006). However, a non-significant association was reported between GH-MsplI and 305-day milk yields in Egyptian Baladi cows (El-Nahas et al. 2018).

In conclusion, AluI and MsplI polymorphisms of the bGH gene were identified in a population of Sahiwal cows which displayed L and T allele dominance. Cows with LL and TT genotypes showed better productive performance than cows with other genotypes. Although the confirmation of our findings needs further analysis on other herds, the association of genotypes with better milk production is a very interesting finding and could be used in marker assisted selection to improve the animals which will ultimately lead to higher milk production.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge assistance of staff members of Animal Genetics and Breeding Division and LRC, NDRI, Karnal, India. Financial assistance to carry out this research work was provided by Indian Council of Agricultural Research (ICAR), New Delhi, India.

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