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ANIMAL GENETICS

Effect of Polymorphic Variants of *GH*, *Pit-1*, and β-*LG* Genes on Milk Production of Holstein Cows[1]

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Abstract—Effect of polymorphic variants of growth hormone (*GH*), β-lactoglobulin (β-*LG*), and *Pit-1* genes on milk yield was analyzed in a Holstein herd. Genotypes of the cows for these genes were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. Allele frequencies were 0.884 and 0.116 for *L* and *V* variants of *GH*, 0.170 and 0.830 for *A* and *B* variants of *Pit-1*, and 0.529 and 0.471 for *A* and *B* variants of β-*LG*, respectively. GLM procedure of SAS software was used to test the effects of these genes on milk yield. Results indicated significant effects of these genes on milk yield ($P < 0.05$). Cows with *LL* genotype of *GH* produced more milk than cows with *LV* genotype ($P < 0.05$). Also, for *Pit-1* gene, animals with *AB* genotype produced more milk than *BB* genotype ($P < 0.05$). In the case of β-*LG* gene, milk yield of animals with *AA* genotype was more than *BB* genotype ($P < 0.01$). Therefore, it might be concluded that homozygote genotypes of *GH* (*LL*) and β-*LG* (*AA*) were superior compared to heterozygote genotypes, whereas, the heterozygote genotype of *Pit-1* gene (*AB*) was desirable.

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INTRODUCTION

Growth hormone gene is a member of multi-gene family approximately 1800 bp in length with 4 intervening sequences [1] and assigned to chromosome region 19q26 in bovine genome [2]. This hormone plays an important role in biological processes such as mammary development, lactation, growth, and metabolism regulation [3].

Association of *GH* gene polymorphism with production traits in dairy cattle have been studied by many researchers [4–9].

Pit-1 is a pituitary-specific transcription factor that is responsible for pituitary development and hormone secreting gene expression in mammals [10] and it has been mapped by linkage analysis of bovine chromosome 1 [11]. There are a few reports on the relationships of *Pit-1* genotypes with productive traits in dairy cattle [12–15].

β-*LG* is the major whey protein of ruminant species, and also presents in the milk of many, but not all, other species [16], and it accounts for approximately 10 to 15% of total milk proteins [17]. β-*LG* gene is located on the chromosome 11 in cattle, and has a 4.7 kb transcriptional unit, including seven exons and six introns [18].

The effects of bovine β-*LG* variants on milk components, milk production, and cheese manufacturing have been extensively studied [19–26].

So these genes were considered promising candidate markers for economically important quantitative traits, and the purpose of this study was to investigate their effect on milk yield in an industrial Holstein herd.

MATERIALS AND METHODS

The current study was carried out from July 2008 to May 2009. About 100 Holstein cows were genotyped for *GH*, *Pit-1,* and β-*LG* loci. The blood samples were collected randomly from dairy cows with at least one lactation milk production record. The animals belonged to Behin Talise farm in Golestan Province. DNA was extracted using modified salting out extraction protocol [27]. Polymorphism of the genes was determined by PCR-RFLP method. The primers sequences, region, and size of the amplified fragments for these genes are shown in Table 1.

PCR for all of the genes was carried out using Personal Cycler™ amplificator (Biometra, Germany) and PCR Master Kit (CinnaGen Inc., Iran). The Kit contained master mix including 0.04 u/μL *Taq* DNA polymerase, PCR buffer, 3mM $MgCl_2$, and 0.04 mM of each dNTP. In each reaction, 12.5 μL master mix, 1 μL of DNA (50 to 100 ng/μL), 2–4 μL primers (5 pmol/μL), and some deionized water till final vol-

[1] The article is published in the original.

Table 1. Sequence of the primers, size and region of the amplified fragments in PCR

Gene	Primer sequence	Size (bp)	Amplified region	Reference
GH	5'-GTGGGCTTGGGGAGACAGAT-3' 5'-GTCGTCACTGCGCATGTTTG-3'	282	intron 4, exon 5	[30]
Pit-1	5'-CAA TGA GAA AGT TGG TGC-3' 5'-TCT GCA TTC GAG ATG CTC-3'	1355	intron 5, exon 5 and exon 6	[31]
β-*LG*	5'-TGTGCTGGACACCGACTACAAAAAG-3' 5'-GCTCCCGGTATATGACCACCCTCT-3'	247	exon 4 , intron 4	[32]

Table 2. Genotypes and size of the fragments of digestion reaction for the studied genes [8, 15, 18]

Gene	Endonuclease	Genotypes and size of the fragments (bp)		
GH	*Alu*I	$\frac{LL}{150, 82, 50}$	$\frac{VV}{150, 132}$	$\frac{LV}{150, 132, 82, 50}$
Pit-1	*Hinf*I	$\frac{AA}{660, 425, 270}$	$\frac{BB}{660, 385, 270, 40}$	$\frac{AB}{660, 425, 385, 270, 40}$
β-*LG*	*Hae*III	$\frac{AA}{148, 99}$	$\frac{BB}{99, 74}$	$\frac{AB}{148, 99, 74}$

ume of 25 μL were used. Amplification programs for amplificating the investigated genes were as follow:

GH. The amplification program consisted of an initial denaturation at 94°C for 2 min, then 30 cycles of 94°C for 45 s, 62°C for 1 min and 72°C for 1 min, and a final extension of 72°C was maintained for 3 min.

Pit-1. For this gene conditions were 95°C for 2 min and an initial annealing of 55°C for 1 min, then an initial extension of 72°C for 2 min, followed by 29 cycles of 94°C for 45 s, 55°C for 1 min, and 72°C for 1 min. The final step was at 72°C for 2 min.

β-*LG*. PCR condition consisted of an initial denaturation at 94°C for 5 min, then 35 cycles of 94°C for 1 min, 60°C for 1 min and 72°C for 1 min, and a final extension of 72°C was maintained for 5 min.

The PCR products were digested by restriction enzymes following their protocol: four units of *Alu*I for *GH* gene, five units of *Hinf*I for *Pit-1* gene, and ten units of *Hae*III for β-*LG* gene. After digestion, the fragments were separated on 3% (for *GH* and *Pit-1* genes) and 2.5% (for β-*LG* gene) agarose gel electrophoresis. Determination of gene and genotype frequencies was carried out using POP Gene 1.31 software [28].

A fixed model was used to test the effect of *GH*, *Pit-1*, and β-*LG* genotypes on mature equivalent milk yield records. Statistical analysis was performed using GLM procedure of SAS software, 2002 [29], and comparisons of least squares means were carried out using Tukey-Kramer test at 5% probability level. The following linear model was used for analysis:

$y_{ijkLm} = \mu + A_i + B_j + C_k + D_L + e_{ijkLm}$, where

y_{ijkLm} – milk record of the cow;

μ – population mean;

A_i – fixed effect of calving year ($i = 1,..., 3$);

B_j – fixed effect of calving season ($j = 1,..., 4$);

C_k – fixed effect of parity ($k = 1,..., 8$);

D_L – fixed effect of genotype ($L = 1,..., 3$, *GH*, *Pit*-1, β-*LG*);

e_{ijkLm} – random residual error with 0 mean and σ_e^2 variance.

RESULTS

PCR of the investigated genes was without contamination and resulted in clear bands. Digestion of the PCR products with *Alu*I, *Hinf*I, and *Hae*III showed three genotypes for each locus. Genotypes of the investigated loci accompanied by size of the fragments are shown in Table 2.

The frequencies of *L* and *V* variants of *GH* gene were 0.884 and 0.116, respectively. Also, allele frequencies were 0.170 and 0.830 for *A* and *B* variants of *Pit-1* gene, and 0.529 and 0.471 for *A* and *B* variants of β-*LG*, respectively.

Table 3 presents results of comparison of milk yield squares means for the *GH*, *Pit-1*, and β-*LG* genotypes.

As Table 3 shows cows with the *LL* genotype of *GH* gene produced more milk than cows with the *LV* genotype ($P < 0.05$). Although *VV* genotype presents more milk than other genotypes of this locus, but it's differences with them was not significant. Cows with the *AB* genotype of *Pit-1* gene had more milk than the cows with *BB* genotype ($P < 0.05$). There were not any sig-

nificant differences between other genotypes of these genes. In the case of β-*LG* gene, the effect of *AA* and *BB* genotypes on milk yield was significant ($P < 0.01$) but it was not significant with *AB* genotype ($P > 0.05$).

DISCUSSION

In present study, the frequency of *GH L* allele (0.884) was more than *V* allele (0.116) that was in agreement with other studies. For example, the frequency of *L* allele was reported 0.93 in Danish Holstein; 0.85 in Danish Red; 0.51 in Danish Jersey [30], 0.909 in Hungarian Holstein Friesian [6], 0.936 in Iranian Holstein bulls [8], 0.85 in Holstein breed [33], 0.52 in Jersey breed [7]. Studies on Gyr breed showed that the breed was monomorphic for this allele [15]. Based on the reports, the Holstein breed have had the maximum frequency for *L* allele [8] and the minimum frequency for this allele have been observed in Jersey breed [7].

Because of the important effects of *GH* gene in growth and lactation, association of *GH* polymorphism with productive traits has been considered by many researches. In present investigation, the cows with *LL* genotype had significantly more milk yield than *LV* genotype (Table 3) and it confirmed the results of other researchers [7, 8]. Many authors found significant association between *LL* genotype and higher fat yield [4, 5, 8, 9].

There are some contradictory reports on association between *LV* genotype and milk composition. Some authors reported significant association between *LV* genotype and milk yield [4–6], While Dario et al. [7] reported higher fat yield with this genotype. Some researchers observed the superiority of this genotype in protein yield [4, 7]. Differences of least squares means of *VV* genotype with *LL* and *LV* were not significant in our study (Table 3).

Generally, for *Pit-1* gene, in previous studies on Holstein breed and other breeds, the frequency of *B* allele was more than *A* allele. *A* allele frequency in different breeds was as follows: in Holstein breed, 0.15 [34], in Polish Black and White cows, 0.25 [13, 31], in dairy Gyr breed, 0.05 [15], and in Italian Holstein cows, 0.188 [12]. These results were in agreement with the findings of current study.

Regarding to importance of *Pit-1* gene in transcription of growth hormone and prolactin genes, association between this gene and productive traits has been studied by some researchers. In present study, it was recognized that the cows with *Pit-1 AB* genotype had significantly more milk compared to *BB* genotype (Table 3) and most of the authors reported association of *A* allele with increasing the milk yield [12–14]. Renaville et al. [12] reported high protein yield with *Pit-1 A* allele, but this allele was recognized undesirable for fat percent. However, Mattos et al. [15] found the superiority of the heterozygote cows compared to the *BB* homozygote for milk fat yield. Our results did not show significant difference between *AB* and *AA* genotypes (Table 3).

Table 3. Comparison of least squares means (± standard errors) of milk yield for the genotypes

Gene	Genotypes	LSM ± SE
GH	*LL*	$11393.67^{a} \pm 1246.24$
	LV	$8897.63^{b} \pm 1404.37$
	VV	$13968.94^{ab} \pm 3087.64$
Pit-1	*AA*	$12730.21^{ab} \pm 3336.82$
	AB	$12571.40^{a} \pm 1452.56$
	BB	$10165.32^{b} \pm 1418.45$
β-*LG*	*AA*	$12979.27^{a} \pm 837.78$
	AB	$12032.57^{ab} \pm 796.91$
	BB	$10788.65^{b} \pm 922.84$

Note: Least squares means with dissimilar letters (a, b) were differed significantly ($P < 0.05$).

In current study, the estimated frequency of β-*LG A* allele was more than *B* allele. Our results were similar to some other studies [23, 24, 26, 35]. Most of the studies have reported the higher frequency of *B* allele than *A* allele.

The estimated frequency of *A* allele of β-*LG* gene in Holstein breed has been reported by other authors as 0.27 [36], 0.516 [23], 0.52 [24], 0.498 [37], 0.57 [26], 0.43 [38], and 0.52 [35]. However, in other breeds the frequency of this allele was: 0.44 in Brown Swiss [36], 0.37 in Polish Black and White [32], 0.0159 in Kangayam breed [39], 0.41 in Ayrshire, and 0.68 in Jersey [40], 0.17 in Sahiwal, and 0.36 in Tharparkar [41], and 0.41 in Italian Friesian [42]. Among different breeds, Kangayam and Sahiwal breeds had the least frequency of *A* allele.

Many studies have been conducted on association of β-*LG* gene with milk production and milk composition traits. In present research, the Holstein cows with β-*LG AA* genotype had significantly higher milk yield compared to *BB* genotype (Table 3) that was in agreement with other studies [20–22]. Some authors reported the association of *AB* genotype with higher milk yield [23, 24]. While, there are some reports on dissociation of β-*LG* genotypes with milk yield and some other traits concern to milk production and composition [19, 25, 26].

Totally, it might be concluded that homozygote genotypes of growth hormone (*LL*) and β-*LG* (*AA*) genes are superior compared to heterozygote genotypes. On the contrary, in *Pit-1* gene the heterozygote genotype (*AB*) was desirable.

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