African Journal of Biotechnology Vol. 9(41), pp. 6848-6852, 11 October, 2010
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.799
ISSN 1684–5315 © 2010 Academic Journals

Full Length Research Paper

Analysis of bovine growth hormone gene polymorphism of local and Holstein cattle breeds in Kerman province of Iran using polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP)

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Accepted 18 August, 2010

Bovine growth hormone (bGH) gene is a part of the multiple gene family that contains prolactin and placental lactogens. Also, variations in introns have potential usefulness as genetic markers and could help in the genetic improvement of populations. Genomic DNA was isolated from blood samples of two local herds (53 animals) and two Holstein herds (50 animals). Genomic DNA samples were genotyped for the GHI-*Alu*I polymorphism by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP). A 211 bp (bGH) gene exon 5 segment was amplified by PCR using bovine specific primers. RFLPs in this segment were studied using *Alu*I restriction enzyme. The frequencies of V and L alleles in the local and Holstein herds were 0.2 and 0.65, respectively. For both herds, significant difference from the Hardy-Weinberg equilibrium was observed.

Key words: Growth hormone, polymorphism, polymerase chain reaction restriction fragment length polymorphism, local herds, Holstein herds.

INTRODUCTION

The bovine growth hormone (bGH) gene, in addition to the milk protein genes, has been intensively studied in farm animals owing to its defined key role in growth, body composition, metabolism regulation, lactation and mammary gland development (Horvat and Medrano, 1995; Nielsen et al., 1995; Bauman, 1999; Lagziel and Soller, 1999; Cornicella et al., 2003).

Bovine growth hormone is a single peptide of about 22-KDa molecular weight. It is composed of 190 or 191 amino acids, containing Ala or Phe at the N terminus, due to alternative processing of bGH precursors (Dybus, 2002a; Vukasinovic et al., 1999). bGH gene is approximately 1800 bp in size and contains 5 exons and 4 introns. It is located on the 19th chromosome in q26-qter band region (Hediger et al., 1990). Although, a number of polymorphisms have been determined in the growth hormone (GH) gene of cattle up to date, 2 polymorphisms located in the intron 3 and exon 5 were found significant for their effects on milk and meat yield parameters by

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Abbreviations: bGH, Bovine growth hormone; **RFLP,** restriction fragment length polymorphisms; **GH,** growth hormone; **ETA,** estimated transmitting ability; **PTA,** predicted transmitting ability; **ADG,** average daily gains.

restriction fragment length polymorphisms (RFLP) (Hoej et al., 1993; Lucy et al., 1993). The polymorphism which is digested by MspI restriction enzyme is located on the intron 3 (Zhang et al., 1993a). As a result of digestion with this enzyme, 2 alleles occur and MspI (-) contains a T-insertion at +837 position and a C-G transition at +837 position (Lee et al., 1994). Yardibi et al. (2009) reported an association between bGH-AluI polymorphism and milk fat percentage in EAR and SAR cow, 2 of the native cattle breeds of Turkey. This hormone was shown to be polymorphic in many breeds, being that the distribution of GH variants (LL, LV and VV) and their frequencies differ among each breed. Dario et al. (2008) reported that daily milk yield in the LL genotype was higher than in the LV genotype. The Leu/Val locus (AluI-/AluI+) was significantly associated with fat and protein estimated transmitting ability (ETA) of the selected Holstein groups and approached significance for milk predicted transmitting ability (PTA), with the Val allele being more frequent in the top than in the bottom group of bulls. Estimates of transmitting ability for milk production tended to be greater for Holstein cows that were homozygous for Leu (Sabour et al., 1997). Shariflou et al. (2000) investigated the effect of Leu/Val locus on milk production by lactation and test-day data. Results from the two data sets consistently showed that the Leu allele is associated with higher production of milk, fat and protein. The average effects of the gene substitution were 95 L for milk, 7 kg for fat and 3 kg for protein yield per lactation. Dybus (2002b) estimated the frequency of Val and Leu alleles of exon 5 in Danish Black-and-White cattle. The associations between Val/Leu polymorphism and milk production traits were found only in the first lactation. In the first 305-day lactation, the cows of Leu/Leu genotypes produced more milk (+225 kg), fat (+7 kg) and protein (+7 kg) than the Leu/Val individuals. Lee et al. (1993) reported that PTA-milk was not associated with genotype for average and high-milk production cows.

Presence of Val allele was associated with decrease of PTA-milk in high-milk production cows. Local cattle in Kerman province are excellent gene pool of genetic information for studying genetic characteristics, because these animals have resulted from a natural selection process. They were not under selection for production traits, hence must have high genetic variation that allowed them to adapt to harsh environmental conditions. In comparison to local cattle, the Holstein cattle, which have been selected for high milk production, are less adapted to the environment of the Kerman province. On the other hand, performed molecular researches on local cattle in Kerman are rather very low; until now animals have not been molecularly studied for bGH gene. The aim of this study is to estimate the allelic frequencies in polymorphic sites of exon 5 of bGH gene in two Iranian native and Holstein cattle. The present study is the first report on GH genotyping based on polymerase chain reaction (PCR)-RFLP in four bovine herds and can be considered as a preliminary study.

MATERIALS AND METHODS

A total of 103 genomic DNA samples were used for this study from four Iranian herds including two local herds (25 animals from herd C and 28 animals from herd D) and two Holstein herds (25 animals from A and 25 animals from herd B). DNA was extracted from whole blood by samples using DIAtom DNA preb kit (Iso Gene Moscow). 200 µl of whole blood was added to 800 µl "lysing reagent" (5 M guanidinethiocyanate, 20 mm EDTA, 100 mM tris-HCl, pH 6.4 and 1% triton of the X-100), intermixed and thermostatically controlledat a temperature of 65°C for 30 min. After thermostating test tube with the mixture, centrifugation was carried out for 10 s at 5000 g using a table centrifuge. Supernatant were transferred into clean test tube, and 40 µl of the suspension of the sorbent "nucleos™" (specially processed glass beads of the specific size) were added, intermixed and centrifuged for 10 s with 5000 g. Supernatant was removed. To the precipitate, 400 µl "lysing reagent" was added and were intermixed to the homogeneous state. Beside the test tube, 800 µl of the working solution "salt buffer" was added and was prepared from the initial 10-X "salt buffer" (1 M NaCl and 1 M KCl) by breeding 10 times with distilled water and 96% ethanol was added). These were intermixed and centrifuged for 10 s with 5000 g. Supernatant was removed, precipitate was washed with centrifuging 1 ml "salt buffer" and partially dried at 65°C for 3 - 4 min. 50 - 100 µl of the suspension Of extraGene™ (10% mixture of ion exchangers (type Of chelex), 0.02% dye of orange and 0.01% triton of the X-100) were added to the precipitate and then thoroughly suspended on the vortex for 5 - 10 s. Incubation was carried out for 10 min at 65°C. Contents of test tube were suspended again on the vortex and centrifuged with 10000 g for 2 min at 25°C. The supernatant were transferred to the clean test tube which contains DNA, and stored at -20°C. DNA was amplified in a total volume of 25 µl containing 100 ng genomic DNA, 0.5 µM of each primer, 0.2 mM dNTP, 2 - 2.5 µl 10XPCR buffer (750 mM Tris HCl (pH 8.0)), 200 mM NH4SO4 0.1% Tween 20, l.5 mM $MgCL_2$, 2.5 mM $MgC1_2$ and 1 unit Taq DNA polymerase. The Primer sequence used for the GH AluI site was CGH1 5' GCTGCTCCTGAGGGCCCTTC3' and CGH2 5'CATGACCC TCAGGTACTCCG3' (Dario et al., 2008). PCR conditions were 5 min at 94°C, 60 s at 94°C, 60 s at 60°C, 60 s at 72°C, 32 cycles and 10 min at 72°C. The amplified bands were subjected to digestion with 5U AluI (AG|CT) at 37°C for 12 h. The resulting products were loaded to 2% agarose gel within IX TBE and then run with 120 V for 30 min for separation of the DNA fragments. The bands were stained with ethidium bromide prior to visualization by UV light. PopGen and Smouse software were used to estimate the allelic and genotypic frequencies as well as Hardy-Weinberg equilibrium and diversity index was calculated.

RESULTS

The fragment of bGH gene (211 bp) has been characterized and successfully amplified from the DNA of each sample (103 samples) used in the present study. DNA had good quality. Digestion of 211 bp target region of GH gene exon 5 was carried out with *Alu*I endonuclease enzyme. The samples with 211 bp fragment (uncut) were taken as VV (+/+) homozygous genotype, those with 211, 159 and 52 bp fragments as VL (+/-) heterozygous genotype and those with 159 and 52 bp were evaluated as LL (-/-) homozygous genotype (Figure 1). Genotype of 15 animals was VV (+/+), 32 animals was VL (+/-) and 56 animals was LL (-/-).

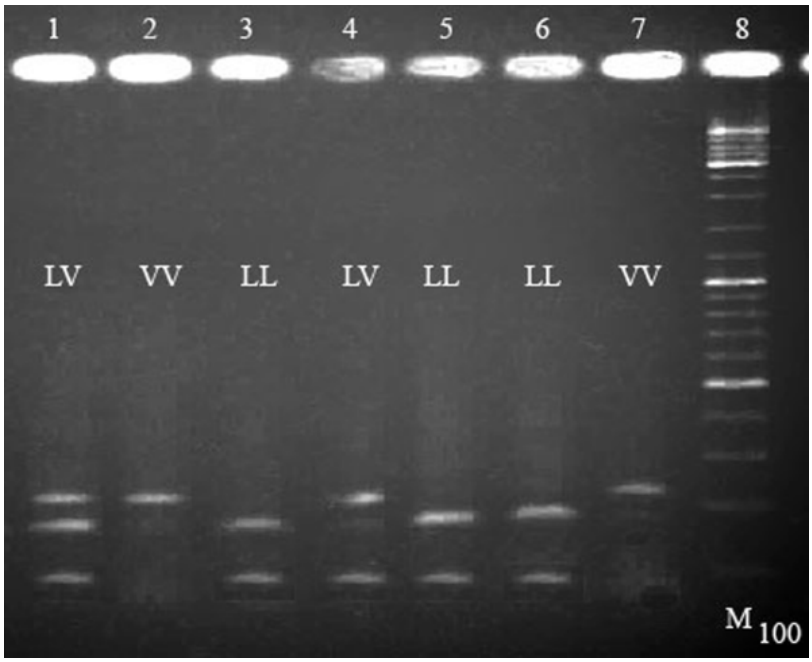

Figure 1. Different genotypes resulted from *Alu*I endonuclease. Column 8 is DNA marker; columns 2 and 7 are homozygous *Alu*I (+/+); columns 4 and 1 are heterozygous *Alu*I (+/-); column 3, 5 and 6 are homozygous *Alu*I (-/-) RFLP-genotyping of the bGH-exon 5 gene.

Table 1. Genotype and gene frequencies of the *Alu*I polymorphism in the bGH gene in the herds investigated.

Herd	n	GH genotype				Allele frequency (%)	
		+/+	+/-	-/-		F(+)	F(-)
A	25	0	10	15		0.2	0.80
B	25	3	7	15		0.26	0.74
C	25	6	7	12		0.38	0.62
D	28	6	8	14		0.36	0.64

The gene frequencies for each breed and the summary of Chi-square, G-square test, number of actual alleles, effective alleles for Shannon and Nei Indexes for genotypic frequencies are presented in Tables 1, 2 and 3.

Heterozygosity as a variation criterion was calculated and Hardy-Weinberg proportions were 0.35 and 0.47 for local and Holstein herds, respectively. The results of likelihood ratio test (G^2 and X^2 test) for the Hardy-Weinberg equilibrium showed significant departures from equilibrium for two herds loci ($p < 0.01$).

DISCUSSION

Statistical analysis showed no significant difference between local and Holstein herds in bGH gene. Comparison of the observed with expected numbers of GH genotypes showed a deficit of the V/V homozygote and an excess of both the L/L homozygote and the V/L heterozygous ($P < 0.05$). Allele frequencies for the Leu and the Val variants were 0.77 and 0.23, and 0.63 to 0.37 in the local and Holstein herds, respectively. Homozygous AluI (V/V) genotype was not observed in Holstein herds. Frequency of the V allele was interestingly lower than L allele. The results of the present study were in agreement with the previous observations achieved by Lucy et al. (l993) who reported that the dairy breeds with the largest mature size (Holstein and Brown Swiss) had the highest frequency of L, whereas smaller breeds (Ayrshire and Jersey) had the highest frequency of V. Yardibi et al. (2009) reported that in EAR and SAR cow, an association between LL genotype of AluI polymorphism has significantly, higher fat level when compared to VV genotype. Our results showed that, local cattle are similar to Holstein cattle based on allele frequency. We expected these results because breeders and farmers use Holstein sperm for artificial insemination of local cattle in Kerman province. Chrenek (1998) reported that

Table 2. Chi-square, g-square test, number of actual (Na), effective allele (Ne) and Shannon (I) Nei index of genotypic frequencies of *AluI* locus between and within herds.

Herd	GH genotype	Observed heterozygocity (H_O)	Expected heterozygocity (H_E)	Mean heterozygocity (H_T)	Expected Frequency	χ^2	G^2
A	VV				0.918	0.918	
	LV				8.163	0.413	
	LL				15.918	0.053	
	Total	0.400	0.327	0.352	24.999	1.384	2.276
B	VV				2.591	1.246	
	LV				9.816	0.808	
	LL				13.591	0.146	
	Total	0.280	0.393	0.352	25.998	2.2	2.026
Holstein total	0.340	0.358	0.354				
C	VV				3.489	1.806	
	LV				12.020	2.097	
	LL				9.489	0.664	
	Total	0.280	0.481	0.465	24.998	4.567	4.566
D	VV				3.455	1.876	
	LV				13.091	1.979	
	LL				11.455	0.566	
	Total	0.286	0.468	0.465	28.001	4.421	4.364
Local total	0.283	0.469	0.465				

Table 3. The number of actual allele (Na), effective allele (Ne), Shannon Index (I) and Nei index for animals based on *AluI* locus.

Herd	*AluI* locus	I	Ne	Na	Nei
Holstein	A	0.500	1.470	2.000	0.3200
	B	0.573	1.626	2.000	0.385
	Total	0.537	1.549	2.000	0.354
Local	C	0.664	1.891	2.000	0.471
	D	0.652	1.849	2.000	0.459
	Total	0.658	1.869	2.000	0.465

genotype VV was associated with lower body weight and lower average daily gains (ADG) in Slovak Simmental bulls in comparison with the bulls with genotypes LL or LV. Higher frequency of LL and LV genotypes of cattle in Kerman province suggest that these animals are suitable cases for studying the association between body weight and ADG and bGH genotypes. The AluI (-) allele frequency has been reported to be 0.04 in Sahiwal Zebu (Mitra et al., 1995), 0.19 in Polish Black and White (Dybus, 2002b), 0.32 in Simmental, 0.24 and 0.29 in Jersey and Ayrshire (Masoudi et al., 2002), 0.0 in Iranian Sistani and Dashtyari breeds (Masoudi et al., 2002) and 0.18 in Holstein (Shariflou et al., 2000). In our studied animals, the frequency of AluI (-) allele was 0.7 which is very high in comparison with other breeds. The low frequency of AluI (+) allele in the studied cattle populations can be due to low number of samples, low actual allele frequency or the effect of reverse natural selection at this locus. Sabour et al. (l997) reported GH gene AluI polymorphism in Holstein cows and reported that V/V genotypes had better milk traits, especially higher milk protein, when compared to the other genotypes. In contrast, Chung et al. (1996) reported that cows with LL genotype had higher milk protein level. Dybus (2002a) reported that LL genotype cows had higher milk yield and milk protein. In addition, Lucy et al. (1991) reported that in L/L genotype, German Black-White cows GH releases more milk than the V/V genotypes. Therefore, next studies on these animals, especially on local cattle should aim to determine crucial relationship between bGH genotypes and production and physiological traits, as selection could be enhanced by the inclusion of

genetic markers in selection decisions.

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