VOL. 9, NO. 10, OCTOBER 2014 ISSN 1990-6145
ARPN Journal of Agricultural and Biological Science

www.arpnjournals.com

GENETIC POLYMORPHISM AND ALLELIC FREQUENCY OF GHRH GENE LOCUS IN IRANIAN SARABI BREED OF CATTLE

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ABSTRACT

Animal selection based on molecular markers is one of the latest breeding methods that can improve the correctness of predictions and of response to selection. The GHRH (growth hormone-releasing hormone) gene is one of the candidate genes for selection strategies based on markers. According to available reports, gene polymorphism is significantly related to the traits of milk constituents and milk production. Blood samples were taken from 112 head of Sarabi breed cattle to study polymorphism in the GHRH gene locus. The genomic DNA of the blood samples was extracted and a 297-bp fragment of this gene was amplified using the polymerase chain reaction. The amplified fragment was cut by the HaeIII restriction enzyme and electrophoresed on 2% agarose gel. Results showed that the two alleles $GHRH^A$ and $GHRH^B$ with the respective frequencies of 0.19 and 0.81 in the whole population are in this locus. Three genotypes $GHRH^A\ GHRH^A, GHRH^A GHRH^B, GHRH^B\ GHRH^B$) were identified the calculated genotype frequencies of which were 0.0357, 0.3037, and 0.6607. The Chi-square test indicated the Hardy-Weinberg Equilibrium existed in the population. Results of this research show that the genetic variety in the Sarabi breed of cattle can help future selection programs, especially the MAS (marker-assisted selection) programs.

Keywords: GHRH, cattle, sarabi breed, allelic frequency, genetic polymorphism.

INTRODUCTION

Advances in molecular genetics have resulted in the identification of many genetic markers. These genetic markers have enabled us to study genomic regions related to markers and will eventually allow the identification of QTLs (quantitative trait loci) effective on economically important quantitative trait deviations. Markers related to QTL can be used for selection, and this information will augment the number of accuracy criteria for selection and, hence, will increase responses to selection (Beuzen *et al*., 2000). The hypothalamus plays a very important role in secreting hormones. It is connected to the pituitary gland by a stalk in which there is a portal venous system and axons of the nerve cells of the hypothalamus that connect these two glands. The anterior portion of the hypothalamus, which is an endocrine gland, secretes the growth hormone somatotropin and the hypothalamus controls the secretion of the growth hormone by the anterior pituitary gland via the GHRH (growth hormone-releasing hormone) and the SRIF inhibiting hormone (somatostatin). The growth hormone- releasing hormone GRHR (somatoliberin) is a polypeptide that releases hormones and is secreted by the hypothalamus gland and reaches the pituitary gland via the portal venous system in the pituitary stalk. The GHRH hormone stimulates the secretion of the growth hormone in and its release from the pituitary gland. This hormone affects the anterior portion of the pituitary gland (i.e., the anterior pituitary) and causes the secretion of the growth hormone in this gland (Frohman *et al*., 1992). The GHRH hormone bonds to a series of special receptors that are located in the anterior pituitary and causes the secretion of the growth hormone (Frohman *et al.*, 1992). The somatoliberin hormone is a polypeptide consisting of 44 amino acids.

The bovine GHRH gene is located on chromosome 13 (Barendse *et al*., 1994) and contains five exons (Zhou *et al*., 2000). Moody *et al*. were the first to study the GHRH gene locus by using the PCR-RFLP method and the enzyme HaeIII. They investigated the relationship between the alleles produced in this locus and the traits of milk production in Holsteins. Results showed that the genotypes $GHRH^A\ GHRH^A$ has a significant relationship with increases in milk production and in fat content of milk (Moody *et al.*, 1995). The purpose of conducting this research was to identify polymorphs of the growth- hormone-releasing factor and to estimate the frequency of its various alleles and genotypes for this locus in the Sarabi breed cattle.

MATERIALS AND METHODS

Blood samples were taken from 112 head of the Sarabi breed cattle at the Sarabi Breed Cattle Support Center. Volumes of EDTA solution (0.5 molar at pH 8) equal to one- tenth of those of the blood samples were added them to the blood samples to prevent blood clotting. The guanidine - silica gel method was used to extract DNA from 100 microliters of each blood sample. This extraction method is based on the use of guanidine isothiocyanate as a lytic agent of blood cells and on collection of released DNA with the help of silica particles (Boom *et al*., 1989). The PCR-RFLP method was employed to determine polymorphism. A 297-bp fragment of the GHRH locus was amplified using the following specific primers (Moody *et al*., 1995):

GHRHF- $5' - TTCCCAAGCCTCTCAGGTAA - 3'$

GHRHR – $5' - GCGTACCGTGGAATCCTAGT - 3'$

The PCR program included 35 amplification cycles at the initial denaturation temperature of 94 C° (for 5 minutes), secondary denaturation temperature of 94 C° (for one minute), annealing temperature of 60 C° (for 50 seconds), amplification temperature of 72 C° (for 50 seconds), and final amplification temperature of 72 C° (for 5 minutes). The PCR products were electrophorosed on 1.5% agarose gel and stained with ethidium bromide. The PopGen32 software was used to calculate the frequency of alleles, heterozygosity, and the number of effective alleles.

Enzymatic digestion of PCR products was performed using the restriction enzyme HaeIII for three hours at 37 degrees centigrade. The reaction volume was 20 microliters, the reagents included five microliters of PCR products, two microliters of buffer 10 X buffer, five units of the restriction enzyme, and 12 microliters of distilled water. After enzymatic digestion, the digestion products were electrophorosed by using 2% agarose gel and stained with ethidium bromide to be visualized.

RESULTS AND DISCUSSIONS

Extraction of DNA with the thiocyanate guanidine - silica gel method yielded a large quantity (about 70 ng) of genomic DNA that was evaluated by using a spectrophotometer. Results of the polymerase reaction confirm amplification of the 297- bp fragment of the GHRH gene (Figure-1). Comparison of the bands present in the samples digested by the enzyme HaeIII with the M, 50- bp weight marker on agarose gel is presented in Figure-2.

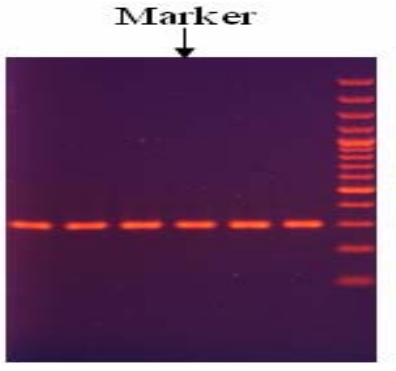

Figure-1. PCR products of the GHRH gene (the 297-pb fragment) on 1.5% agarose gel. The molecular marker used is M 100.

Examining the fragments obtained from the digestion led to the identification of the three genotypes of $GHRH^A\ GHRH^A$, $GHRH^A\ GHRH^B$, and $GHRH^B\ GHRH^B$ (Figure-2)

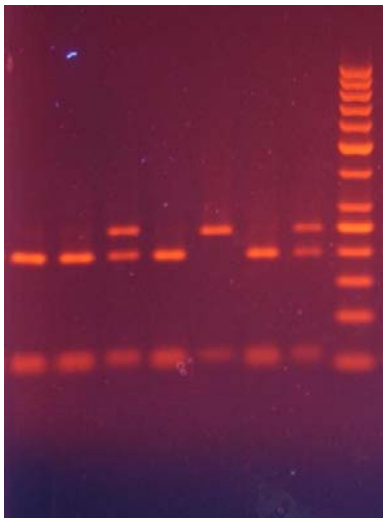

Figure-2. Band patterns obtained from enzymatic digestion on 2% agarose gel.

There is one restriction site in allele $GHRH^A$ and two restriction sites in allele $GHRH^B$ of the amplified fragment for the enzyme HaeIII. Therefore, enzymatic digestion of allele $GHRH^A$ produces the 55- bp and 242- bp digestion products and the enzymatic products of allele $GHRH^B$ include 48-, 55-, and 195- bp bands. Consequently, the genotype $GHRH^A\ GHRH^B$ has two bands of 55-, 242-bp, the genotype $GHRH^B\ GHRH^B$ has three $48-, 55-,$ and $242-$ bp bands, and the genotype $GHRH^A\ GHRH^B$ has four 48-, 55-, 195-, and 242-bp bands. The genotype $GHRH^B\ GHRH^B$ exhibited the highest number and frequency among the genotypes in the herd. The allelic frequencies obtained for the alleles of $GHRH^A$ and $GHRH^B$ were 0.19 and 0.82, respectively (Table-1).

In 2006, a study was carried out on 881 head of Polish black- and- white cattle in which the calculated frequencies of the three genotypes $GHRH^A\ GHRH^A$,

$GHRH^A$ $GHRH^B$,and $GHRH^B$ $GHRH^B$ in the whole population were 0.0545, 0.313, and 0.632, respectively. The researchers did not find any significant differences between polymorphism of the GHRH gene locus and milk production (Dybus *et al*., 2006).

In another study conducted in 2003 on 130 head of Limousine cattle, the genotypic frequencies found for the genotypes $GHRH^A GHRH^A$, $GHRH^A GHRH^B$, and $GHRH^B GHRH^B$ were 0.154, 0.692, and 0.154, Respectively (Dybus *et al*., 2003). The Chi-square test showed that the Hardy- Weinberg Equilibrium existed in the herd, which suggests that no selection had taken place in the population for increasing or decreasing the genotypes of the GHRH gene (Table-1).

Table-1. Genotypic and allelic frequencies of the GHRH gene and Chi-square tests.

Genotype	Number	Percentage of genotypic frequency	Allelic frequencies		X^2
			$GHRH^A$	$GHRH^B$	
$GHRH^A GHRH^A$	4	3.57			0.007 (Not significant)
$GHRH^A GHRH^B$	34	30.36	0.19	0.81	
$GHRH^B GHRH^B$	74	66.07			

The extent of genetic changes within a population is determined by measuring heterozygosity and the number of effective alleles in the gene locus studied. In this research, the observed heterozygosity was 0.303 and the expected heterozygosity 0.306. These Figures are very close to each other and indicate a high level of genetic diversity in this gene locus in the Sarabi breed of cattle. This may suggest that the population studied is maintained in a structure by almost random mating and that no selective and organized program has been used in this population (Table-2). In a study conducted on Polish black-and-white cattle, a high level of heterozygosity similar to that of this research was reported (Dybus *et al*., 2006). However, in the research carried out on Holsteins, a lower level of heterozygosity has been reported indicating a low level of genetic diversity in the population of Holsteins studied (Kmiec *et al*., 2007). The number of effective alleles is the number of alleles that create identical heterozygosities. This parameter is shown in Table-3. The number of effective alleles for found for the GHRH gene in the Sarabi breed of cattle was 1.43.

Table-2. Observed and expected heterozygosities and the average heterozygosity of the GHRH gene locus.

Gene locus	Expected heterozygosity	Observed heterozygosity
GHRH	0.306	0.303

Table-3. The effective allelic size of the GHRH gene locus.

Gene locus	The effective allele size	The number of samples
GHRH	1.43	112

Results of this research showed that there is genetic polymorphism at the GHRH gene locus for the Sarabi breed of cattle. According to available reports, these polymorphs have a meaningful relationship with the traits of milk constituents and milk production. These polymorphs can be used as genetic markers to study the relationship between genetic diversity and production traits such as milk composition and milk production. Moody *et al*. studied the relationship between alleles produced at this locus and the trait of milk production in Holsteins. They found that the genotype $GBRH^A$ $GHRH^A$ has a significant relationship with increased milk production and percent fat content of milk (Moody *et al*., 1995). Results of the research conducted by Kmiec *et al*. on 719 head of head of Holsteins indicated that the genotype $GHRH^A$ $GHRH^A$ has a significant relationship with milk production (Kmiec *et al*., 2007). As can be seen, this locus has been identified in many studies as the selected marker for increasing milk production and for milk composition. Therefore, this marker can be used to raise the quality and improve the accuracy of breeding programs. Results of this research show that the genetic diversity in this breed can help future selection programs, especially the marker - assisted selection programs.

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