International Journal of
Science and Engineering Investigations vol. 2, issue 15, April 2013
ISSN: 2251-8843

Investigation of IGF-1 Gene Polymorphism by PCR-RFLP in Crossbreed Cow Population in Khouzestan Province

Ahmad Ebrahimzadeh[1], Kamal Hasanpoor[2], Morteza Asghari Moghadam[3]
[1]M.Sc in Animal Sciences, University of Zabol, Zabol, Iran
[2]M.Sc in Animal Sciences, University of Ahvaz, Ahvaz, Iran
[3]Department of Animal science, University of Zabol, Zabol, Iran
([1]a_ebrahimzadeh2010@yahoo.com, [2]hasanpoor.kamal@gmail.com, [3]m.asghari.moghadam@gmail.com)

Abstract- The insulin-like growth factor I gene is a candidate gene for growth and production rate of meat and milk. This gene is located on chromosome 5 in the cow. The IGF-I hormone is secreted by most of tissues, but it is mainly secreted from the liver and after releasing, it is transferred to other tissues. The IGF-I hormone plays a key role in various physiological and metabolic processes. Factors such as growth hormone, nutrition, insulin and temperature will influence on serum IGF-I concentration. In order to study the IGF-I gene polymorphism, blood samples were taken randomly from 93 hybrid cows in Khuzestan province. DNA extraction from blood samples and the polymerase chain reaction (PCR) for amplification of 249-bp fragment of Exon 1 of the gene were performed. The cutting SnaBI enzyme was used for enzymatic digestion. The results showed that the genotype frequencies of AA, AB and BB, respectively are as 0.06, 0.53 and 0.41. AB genotype and AA genotype had the highest and the lowest frequencies in the herd, respectively. The frequencies of allele A and allele B in the population were estimated respectively as 0.33 and 0.67. Due to the selection, the Hardy Weinberg balance did not exist in the cattle.

Keywords- *IGF-1, Polymorphism, Gene, PCR-RFLP, Sna BI.*

I. INTRODUCTION

Research showed that marker-assisted selection rapidly increases rate of genetic progress annually. One of these markers is IGF-I gene that plays an important physiological role in milk growth and production as well as reproductive activities (Grochowska et al. 2001).

IGF-I hormone (somatomedin C) is a member of the IGF family. This family consists of three peptides namely IGF-I ، IGF-II and insulin, cognate receptors with them and at least 6 binding proteins with them (Roite et al. 2001).

This hormone is a single chain peptide with a molecular weight of approximately 7000 Daltons and contains 700 amino acids (Weber et al. 1999) and is secreted by many tissues but mainly in the liver which is reached to other tissues after secretion (Elgin et al. 1987).

IGF-I gene in bovine is located on Chromosome No. 5. This gene in bovine contains 3 introns and 4 exons and has a total of 72495 nucleotide pairs. Access number to the gene in the gene bank is AF017143 (Ge et al. 2001).

Many studies have been done on the polymorphism of this gene which was observed polymorphism in most studies. Since the gene has not been evaluated on hybrid ovine in Khuzestan, the aim of this study was to in investigate the polymorphism of this gene in hybrid cattle in Khuzestan.

II. MATERIALS AND METHODS

To review the polymorphism of IGF-I, phlebotomy from the jugular vein at a rate of 5 ml hybridization was performed on 93 cows and then was poured in the vacuum tubes containing EDTA anticoagulant material. After the phlebotomy process was finished, the samples were transferred to the laboratory of Ramin University at -4 degree and were maintained at a temperature of - 20 ° C until DNA extraction.

DNA was extracted using Kit diatom based on BOOM et.al method (Boom et al. 1989). Primers sequences for PCR were designed based on Ge et al, 2001 ، Siadkowska et al, 2006 ، Li et al, 2004و et al, 2009 Laureano primers sequences (Ge et al. 2001, Siadkowska et al. 2006, Laureano et al. 2009 and Li et al. 2004). Primers sequences used here include:

Inward primer sequence:
5′- ATTACAAAGCTGCCTGCCCC-3′
Outward primer sequence:
5′-ACCTTACCCGTATGAAAGGAATATACGT-3′

After experimenting various concentrations of PCR components, the optimal condition of PCR with final volume of 15 microliters as PCR 1X buffer, two $MgCl_2$ mm, 0.25 primers mm, 200 dNTP mm, a single polymerase enzyme and a sample DNA in 150 Ng were obtained in each PCR reaction. The appropriate thermal program for the primers was as follows:

TABLE I. THERMAL PARAMETERS OF THE POLYMERASE CHAIN

Stages	*Denaturing*	*Connection*	*reproduction*	*Number of cycles*
Stage 1	94 ° C, 5 minutes	---	---	1
Stage 2	94 ° C, 30 seconds	61 ° C., 30 seconds	61 ° C., 40 seconds	35
Stage 3	---	---	72° C., 4 minutes	1

After reproducing the regarded position of fragment, 249 game pairs were obtained. Digestion of fragment 249 of the game pair with (SnaBI) Eco1051 outlines three genotypes of AA, BB and AB. Eco105I enzyme recognizes 6 sequence of nucleotide including TACGTA bases and cuts them in the junction of G and C bases.

This enzyme does not cut BB genotype and fragment 249 of the play remains unchanged while both strands are cut in AA genotype and each strand is cut into two fragments of 226 and 23 games.

In AB genotype, a strand is cut into and is transformed into two 226 and 23 games fragments and the other strand remains unchanged. For a statistical analysis, the data obtained from PG 32 software were analyzed. In this study, allele and genotype frequency and amount of heterozygosity of the regarded community was determined.

III. RESULTS AND DISCUSSION

Results achieved from surveying extracted DNA on agarose gels showed that the extracted DNA lacks any protein and RNA contamination. Also, studying DNA concentration using spectrophotometry showed that the DNA is 30 to 50 Ng/ml. DNA extraction in this method provided suitable concentration of DNA for polymerase chain reaction. After doing polymerase chain reaction, reproduction of fragment 249 of game pair from reproduced exon 1 out of IGF-I gene was digested by SnaBI cutting enzyme and three AB, AA and BB genotype were identified.

To measure the size of fragments, 100 bp size markers was used. Results achieved from studying PCR markers on 1.25 agarose gel confirmed the reproduction of regarded fragment.

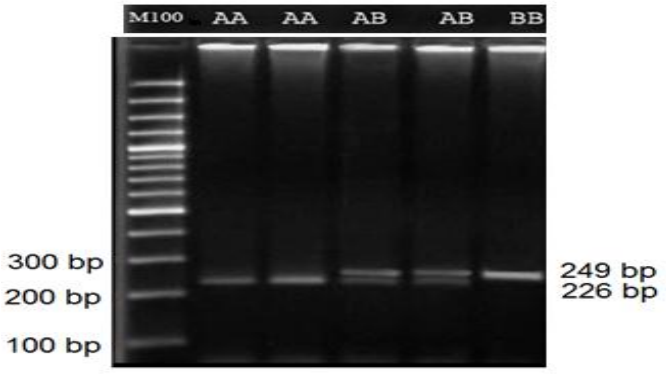

Figure 1. PCR-RFLP pattern of digested fragments of IGF-I gel using Sna BI cutting enzyme

Genotype frequencies of AA, AB and BB are 0.06, 0.53 and 0.41, respectively (Figure 2), allele A frequency in this population equals to 0.33 and allele B equals to 0.67, respectively (Figure 3).

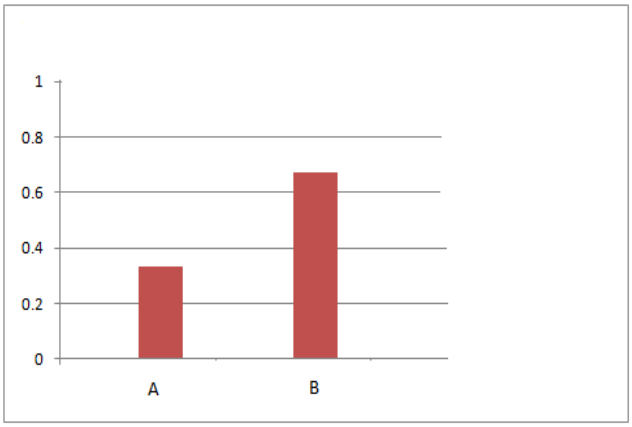

Figure 2. Allele frequencies of IGF-I gel

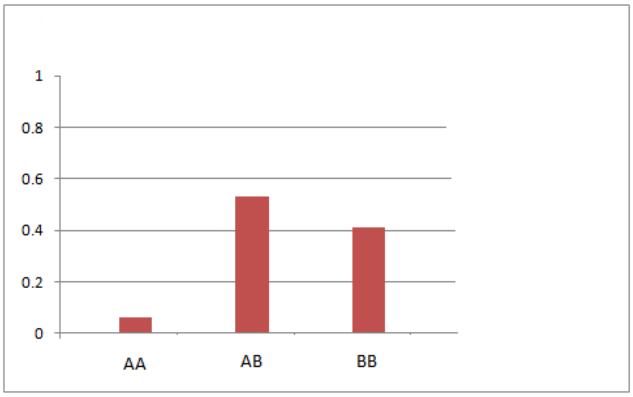

Figure 3. genotypic frequencies of IGF-I gene

The amount of heterozygosity observed in the IGF-I gene in cattle hybrid is 0.526 and the expected heterozygosity is 0.441. Thus, the IGF-I gene diversity was relatively high in this population (Table 2).

TABLE II. OBSERVED HETEROZYGOSITY AND EXPECTED HETEROZYGOSITY OF IGF-I EXPECTED GENE IN HYBRID COWS

Gene position	*observed heterozygosity*	*expected heterozygosity*	*Average heterozygosity*
IGF-I	0.526	0.441	0.483

AB genotype had the most frequency and AA genotype had the least frequency in the cattle. Allele B frequency was more than that of allele A which was compatible with the results obtained by Reyna et al. 2010, Curi et al. 2005, Laurano et al. 2009 and Kim et al. 2004 and were not compatible with those of Li et al. 2004, Siadkowska et al. 2005 and Ge et al. 2001.

According to the study conducted on 84 Najdi cows, Yazdanpanah et al (2010) reported the AA, AB and BB genotype frequency as 0.02, 0.14 and 0.84, respectively, and alleles A and B frequency as 0.09 and 0.91 and also the heterozygosity frequency as 0.142 (Yazdanpanah et al. 2010).

Reyna et al, 2010 reported a single SNP polymorphic nucleotide of IGF-I gene which is associated with production traits in cattle. Allele A frequency in Sharolize population was 0.46 and allele B frequency was 0.54. In Beef Mister population, allele B had the most frequency which was 0.97 (Reyna et al. 2010).

Increasing the amount of heterozygosity is led to increasing IGF-I concentration in bold that can be used in improving reproductive function in that IGF-I increases follicular diameter and subsequently those of ovulation (Reyna et al. 2010 and Zulu et al. 2002). Increasing the amount of heterozygosity reduces the expression of harmful recessive alleles. When heterozygosity is increased due to crossbreeding, the capability of heterozygosity of recessive alleles is transformed as a genotype in the absence of their emergence (Yazdanpanah et al. 2010). By conducting a study on 148 Holstein-Freezin cows, Zych et al, 2007 obtained the allele A and B frequency as 0.84 and 0.16 and genotype AA, AB and BB frequency as 0.723, 0.236 and 0.041, respectively (Zych et al. 2007).

According to an experiment conducted on 348 oxen belonging to four genetic groups containing 70 Nelore cow breeds, 30 Kanshim cow breeds (by the crossbreeding of Charolais and zebu and 275 hyb rid cattle of Semintal with Nelore, Curi et al, 2005 found out that genotype BB had the highest frequency (Curi et al. 2005).

REFERENCES

[1] Boom, R., sol, C. J. A., Salimans, M. M. M.,Jansen, c. l. and Wherteim Van Dillen, P. M. E. 1989. Rapid and simpl method for purification of nucleic acid. J. Clin. Microbiol. 28: 495-503.

[2] Curi, R. A., Oliveirb, H. N., silveir, A. C. and Lopes, C . R. 2005. Association Between IGF-I, IGF-IR and GHRH Gene polymorphisms and Growth and Carcass Traits in Beef Cattle. J. Livest. prod. Sci. 94: 159 -167.

[3] Elgin, R .G., busby, W. H. and Clemmons, D . R. 1987. An Insulin-like Growth Factor Binding Protein Enhances the Biologic Response to IGF-I. J. Cell Biol. 84: 3254-3258.

[4] Ge, W., Davis, M. E., Hines, H. C., Irvin, K. M. and Simmen, R. C. M. 2001. Association of a genetic marker with blood serum insulin-like growth factor-I concentration and growth traits in Angus cattle. j. Anim. sci. 79: 1757-1762.

[5] Grochowska, R., Sqrensen, P., Zwierzchowski, L., Snochowski, M. and Lqvendahl, P. 2001. Genetic variation in stimulated GH release and in IGF-I of young dairy cattle and their associations with the leucine/valine polymorphism in the GH gene. J. Anim. Scin. 79: 450- 476.

[6] Kim, M. H., Seo, D. S. and Ko, Y. 2004. Relationship between Egg productivity and Insulin-Like growth factor-1 genotypes in Korean native Ogol chickens. Poultry Scin. 83: 1203-1208.

[7] Laureano, M. M. M., Otaviano, A. R., Lima, A. L. F., Costa, R. B., Salman, A . K. D., Sena, J. A. D., Tonhati. H. and Albuqurque, L. G. D. 2009. Characterization and polymorphism screening of IGF-I and prolactin genes in Nelore heifers. Ital. J. Anim. Sci. vol. 8. 277-283.

[8] Li, C., Basarad, J., Snelling, W. M., Bwnkel, B., Murdoch, B,. Hansen, C. And Moore, S .S. 2004. Assessment of positional candidate genes myf5 and igf1 for growth on bovine chromosome 5 in commercial lines of Bos taurus. J. Anim Sci. 82:1-7.

[9] Reyna, X. F., Montoya, H. M., Castrellin, V.V., Rincon, A. M. S., Bracamonte, M. P. and Vera, W. A. 2010. Polymorphisms in the IGF1 gene and their effect on growth traits in Mexican beef cattle. Genet. Mol. Res. 9(2) 875-883.

[10] Roite, D., Bondy, C., Yakar, S., Liu, J. L. and Butle, A. 2001. The somatomedin hypothesis. Endocr. Rev. 22: 53: 74.

[11] Siadkowska, E., Zwierzchowski, L ., Oprzadek, J., Strzalkowska, N., Bagnicka, E. and Krzyxewski, J. 2006. Effect of polymorphism in *IGF-1* gene on production traits in Polish Holstein-Friesian cattle. Anim. Sci. Papers and Reports vol. 24 no. 3. 225-237.

[12] Weber, M. S., Purup, S., Vestergaard, M., Ellis, S. E., Andersen, J. S., Akers, R. M. and Sejrsen, K. 1999. Contribution of Insulin-like Growth Factor (IGF)-I and IGF-Binding Protein-3 to mitogenic activity in Bovine Mammary Extracts and Serum. J. Endocr. 161: 365-373.

[13] Zulu, V. C,. Nakao, T. and Sawamuka, Y. 2002. Insulin-like growth factor-I as a possible hormonal mediator of nutritional regulation of reproduction in cattle. J. Med. Sci. 64: 657-665.

[14] Zych, S., Szewczuk, M., Piatkowska, W. C. and Szatkowska, I. 2007. A new ACRS-SNP in the 5' flanking region of the bovine insulin-like growth factor 1 (*IGF1*) gene (Brief report). Arch. Tierz. Dummerstorf 50.5.531-532.

[15] Yazdanpanah, A. 2010. Investigation of IGF-1 gene polymorphism by PCR-RFLP in najdi cow population in Khuzestan province. Department of Animal and Food Sciences, University of Khuzestan Ramin. 57 p.