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GROWTH HORMONE GENE POLYMORPHISM IN TWO KINDS OF IRAQ NATIVE FOWLS

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ABSTRACT

Molecular genetic selection on individual genes is a promising method to genetically improve economically important traits in chickens. The polymorphism in the intron 1 of chicken growth hormone (cGH) gene was investigated in the Iraq native fowls by using polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) method. The genomic DNA was extracted from 95 samples (47 from the Black feather native fowls and 48 from the Nicked neck native fowls) by using modified salting out technique. The DNA fragment of the growth hormone gene with 770 bp was amplified by PCR using specific primers. Then the PCR products were digested with MspI restriction enzyme and analyzed on 1.5% agarose gel. The allelic frequency of intron 1 locus for A and B alleles in Iraq native fowls (Black feather) were 0.47 and 0.53 and those in Nicked nick native fowls were 0.46 and 0.54, respectively. The results of current study indicated that the intron 1 of cGH is polymorphic in Iraq native fowls and could be exploited as a candidate gene for marker-assisted selection for growth-related traits.

Key words: Fowls, growth hormone, genes, polymorphism.

INTRODUCTION

Polymorphism was defined as the co-occurrence of two or more varieties in the same population at the same time in such proportions that the rarest of them cannot be maintained by mutation alone (Osterhoff, 1964). Moreover the biochemical polymorphism is the occurrence of varieties attributed to the biochemical differences, under genetic control. It has created a way for the genetic improvement in farm animals. This kind of diversity can be used as a useful tool for characterization of farm animals. Moreover, it is possible to determine the degree of differences or similarities between and within breeds and therefore they are very useful raw materials for genetic improvement of farm animals. The cGH is a polypeptide hormone that is involved in a wide variety of physiological functions, such as growth, egg production, aging and reproduction. The cGH gene is located on the tip of the long arm of the chromosome 27 and carrying ID 378781 in NCBI. It consists of 5 exons and 4 introns with an overall length of 4.1 kb (Ip *et al.*, 2001; Kansaku *et al*., 2008). This gene encodes a mature growth hormone protein with 191 amino acids and a signal peptide with 25 amino acids (Tanaka *et al.*, 1992). Chicken growth hormone is an important hormone which is secreted from the anterior pituitary gland lobe and plays a crucial role in growth and development of the chickens. PCR-RFLP sere also investigated in various populations of Chinese native chickens and it was suggested that an allele present in the intron 1 might be linked to laying performance (Ip *et al.*, 2001). Su *et al.* (2014) confirmed that polymorphism of the GH gene and its haplo types is related to chicken egg production traits. RFLPs have been identified in the cGH gene showing that these polymorphisms are associated with egg production traits, resistance to Marek's disease and avian leukosis (Feng *et al*., 1997; Kuhnlein *et al.*, 1997). It has been reported that the RFLPs of cGH gene are significantly related to the chicken abdominal fat content (Fotouhi *et al.*,

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1993). A significant correlation was reported between GH and meat quality in Anka and Rugao hens (Sheng-Long *et al.*, 2008). Moreover researches on the intron 1 of GH showed that this gene can affect some body composition traits in Arian broiler chickens (Ghelghachi *et al.*, 2013). The objective of the present study was to investigate the cGH gene polymorphism in two Iraq native fowls by using PCR-RFLP technique.

MATERIALS AND METHODS

Animal and Blood Samples

The blood samples were collected randomly from two indigenous Iraq local chicken flocks: 95 blood samples (2 ml in EDTA containing tubes) collected from wing vain using disposable syringes in all birds and stored at -20 C° until used at hematology laboratory. The genomic DNA was extracted from the blood samples by using modified salting out technique (Miller *et al*., 1988). The quantity and quality of the extracted DNA was checked by spectrophotometer and agarose gel electrophoreses. The intron 1 region of chicken growth hormone gene was amplified by a set of primers (5'-ATCCCCAGGCAAAC ATCCTC-3' forward and 5'-CCTCGACATCCA GCTCACAT-3' reverse) earlier used by Ip *et al.* (2001). The specificity of primer pairs was confirmed by BLAST with all the nucleotide sequences available for chicken at the National Center for Biotechnology Information (NCBI). The reaction mixture was subjected to initial denaturation of 95°C for 2 min followed by 35 cycles of 95°C for 30 sec., annealing at 60°C for 30 sec., and extension at 70°C for 1.20 sec. Final extension was given for 5 min at 95°C. The PCR products were separated on 1.5% agarose gels containing 1X Tris-Borate-EDTA (TBE). The gels were stained with ethidium bromide and the images were obtained in UV gel documentation systems (Ez-Capture MG Japan). RFLPs were used for analyses of cGH gene polymorphisms. The PCR products (8 µl) of GH gene were digested with 0.5 µl of MspI restriction nzyme and 2 µL buffers 10X, Acetylated BSA 0.2 µl, D.w 4.3 µl in a final reaction volume of 15 µL. The reaction mixture was incubated at 37°C for 4 hrs. The resulting fragments were separated by horizontal electrophoresis (50 V, 2 hrs) on 1.5% agarose gel, stained with ethidium bromide and were visualized under UV light. The observed number of alleles and genotypes, the observed and expected heterozigosity for each locus and the average of heterozigosity over all loci were used to assess the genetic variability of two investigated populations (Krasnopiorova *et al*., 2012). The Hardy Weinberg equilibrium was evaluated in studied populations.

Results

DNA extraction and quality determination with electrophoresis and spectrophotometer done attainded result was acceptable (Fig. 1).

The PCR products with 770 bp length which run on 1.5% agarose gel were presented in Figure 2.

The PCR products were digested with MspI restriction enzyme (5U), which recognizes the 5'- C↓C G G -3' sequence. In total, three restriction digestion profiles were found in the intron 1. There were two hmozygous and one heterozygous genotypes (Fig. 2).

With electrophoresing of digested samples, 3 types of genotype were diagnosed. In AA genotype 529 and 241 bp bands were registered. In AB genotype 529,373,241and 156 bp and in BB genotype 373, 241 and 156. The genotypic frequencies were estimated by considering the presence of various RFLP patterns and the results were presented in Table 1. The differences among genotypes for Black native fowls and Nicked neck native fowls were found to be not significant. It indicated that both populations were in Hardy-Weinberg equilibrium, which is probably because of the genetic selection for growth related traits in these populations. Based on the genotypic frequencies, the allelic frequencies were calculated for each variety (Table 2).

DISCUSSION

The PCR-RFLP analysis of the GH gene is effective in selection programs of poultry and also to estimate the genetic similarities and differences among different poultry breeds. The molecular data emerged as a useful tool to investigate birds with the great phenotypic plasticity.

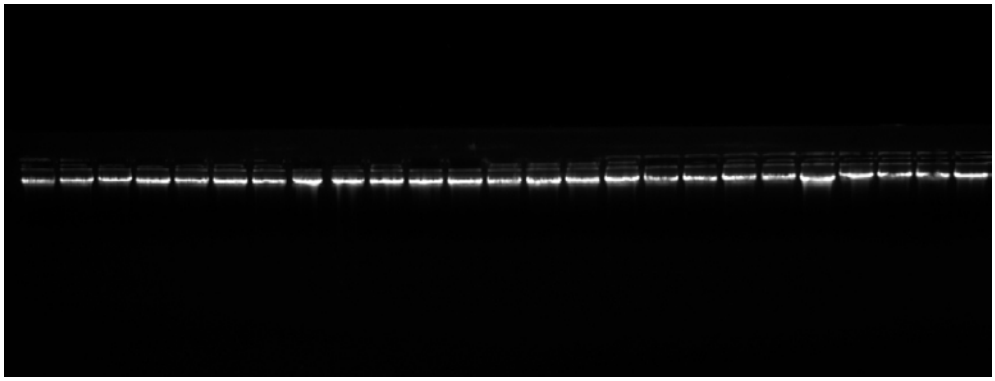

Fig. 1: DNA extracted samples

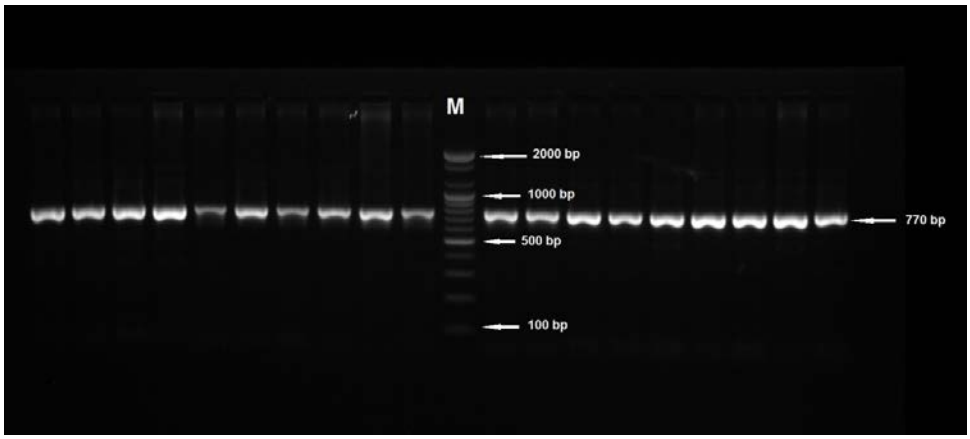

Fig. 2. Representative result of Agarose gel electrophoresis of PCR products of intron 1 chicken growth hormone gene. A commercial DNA marker was used for size analysis

M: Molecular weight marker.

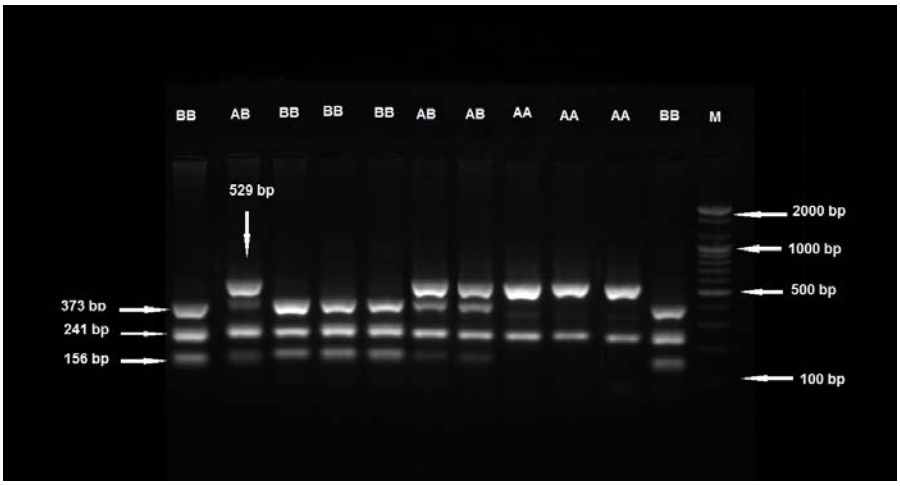

Fig. 3. The electrophoresis result of MSPI PCR-RFLP of chicken GH gene. Lane M: Molecular weight marker genotype

Table 1. Genotypic frequencies for Hardy-Weinberg equilibrium from intron 1of the cGH gene in Iraq native fowls

Population	Black native fowls		Nicked neck native fowls	
Genotype	**No. of birds**	**Frequency**	**No. of birds**	**Frequency**
AA	12	25.5	11	0.23
AB	20	42.5	22	0.46
BB	15	32.0	15	0.31
Chi-Sq.	4.23 NS		4.59 NS	

Table 2. Allelic frequencies in intron 1 of the cGH gene in Iraq native fowls

Breeding station	No. of birds	A	B
Black native fowls	47	0.47	0.53
Nicked neck native fowls	48	0.46	0.54

In this research, a 770 bp fragment was amplified in the GH locus. After digestion of this fragment with MspI restriction enzyme, AA (529, 241 bp), AB (529, 373, 241, 154 bp) and BB (373, 241, 154 bp) alleles were revealed. Our findings were concordance to those of Pipalia (2003) and Thakur *et al*. (2009). Data obtained on gene and genotypic frequencies through the polymorphism study makes it not only possible to compare the gene stocks of animals, the possible effects of the genes on reproductive and performance traits, but also to study genetic variability under different environmental conditions of selection (Egena and Alao, 2014). The allele frequencies in the Iraq local chicken flocks (Black native fowls and Nicked neck fowls) are shown in Table 2. In Black native fowls, allele frequency for A and B (0.47, 0.53), respectively and in Nicked neck fowls allele frequency for A and B showed (0.46, 54), respectively. However the data which indicated non-significant differences between them. Thus, it can be concluded that the two varieties belongs to the Iraq local chicken having almost similar genetic base. The results of this study indicated that the cGH gene could be exploited as a candidate gene for marker-assisted selection (MAS) in Iraq native fowls.

REFERENCE

Egena, S.S.A. and R.O. Alao (2014). Haemoglobin polymorphism in selected farm animal: A review. Biotechnol. in Anim. Husbandry, 30: 377-390.

Feng, X.P., U. Kuhnlein, S.E. Aggrey, J.S. Gavora and D. Zadworny (1997). Trait association of genetic markers in the growth hormone and the growth hormone receptor gene in a White Leghorn strain. Poult. Sci., 76: 1770-1775.

Fotouhi, N., C.N. Karatzas, U. Kuhlein and D. Zadworny (1993). Identification of growth hormone DNA polymorphisms which response to divergent selection for abdominal fat content in chickens. Theoretical and Appl. Genet., 85: 931–936.

Ghelghachi, A.A., H.R. Seyedabadi and A. Lak (2013). Association of growth hormone gene polymorphism with growth and fatness traits in Arian broilers. Int. J. Biosci., 3 : 216-220.

Ip, S.C., X. Zhang and F.C. Leung (2001). Genomic growth hormone gene polymorphisms in native Chinese chickens. Exp. Biol. and Med., 226: 458-462.

Kansaku, N., G. Hiyama, T. Sasanami and D. Zadworny (2008). Prolactin and growth hormone in birds: Protein structure, gene structure and genetic variation J. Poult. Sci., 45: 1-6.

Krasnopiorova, N., L. Baltrenaite and I. Miceikien (2012). Growth hormone polymorphism and its influence on milk traits in cattle bred in Lithuania.Veterinarija ir Zootecknika, 58: 42-46.

Kuhnlein, U, L. Ni, D. Zadworny, S. Weigend, J.S. Gavora and W. Fairfull (1997). DNA polymorphisms in the chicken growth hormone gene: response to selection for disease resistance and association with egg production. Anim. Genet., 28: 116-123.

Miller, S.A., D.D. Dykes and H.F. Polesky (1988). A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res., 16: 1215.

Osterhoff, D.R. (1964). Recent research on biochemical polymorphism in livestock. J. South Afr. Vet. Med. Assoc., 35 : 363-380.

Pipalia, D.L. (2003). Growth hormone gene polymorphism in Bantam, White Leghorn and Bantamised White Leghorn. Indian J. Poult. Sci., 38 206-211.

Sheng-Long, W., H.H. Musa, B. Wen-Bin, W. Ke-Hua, Z. Guo-Qian and C. Guo-Hong (2008). Mutation in exon 4 apo VLDL-II gene is a candidate for meat tenderness in chickens. J. Anim. and Vet. Adv., 7 : 1624-1627.

Su, Y.J., J.T. Shu, M. Zhang, X.Y. Zhang, Y.J. Shan, G.H. Li, J.M. Yin, W.T. Song, G.P. Li HFZhao (2014). Association of chicken growth hormone polymorphisms with egg production. Genet. and Molecular Res., 13: 4893- 4903.

Tanaka, M., Y. Hosokawa, M. Watahiki and K. Nakashima (1992). Structure of the chicken growth hormone-encoding gene and its promoter region. Gene., 112:235–239

Thakur, M.S, S.N.S. Parmar, M.V. Chaudhari and J.K. Bhardwaj (2009). Growth hormone gene polymorphism and its association with egg production in Kadaknath chicken. Livestock Res. for Rural Develop., 21 : 8.

Yazdanpanah, A., H. Roshanfekr, K. Mirzadeh, M. Mamouei and S. Khederzadeh (2013). Polymorphism of insulinlike growth factor 1 gene in najdi cattle population. Am. J. Biochem. Biotechnol., 9 (3): 300- 306.

المظـــاهـــر المتعـــددة لجيـــن هـــرمـــون النمـــو فـي نـــوعيـــن مـــن الدجـــاج المحلـــي العـــراقـــي

مهند منذر جواد - إيمان حسن كافي - رأفت رؤوف الغدير - حميد عبد كريم - يوسف عكاب علوان

وزارة العلوم والتكنولوجيا - العراق

الإنتخاب في الوراثة الجزيئية على أساس الجينات المفردة هو وسيلة في التحسين الوراثي للصفات الاقتصادية المهمة في الدجاج، تم تحديد المظاهر المتعددة في الإنترون رقم ١ في جين هرمون النمو في الدجاج المحلي العراقي بواسطة تفاعل الكوثرة المتسلسل باستخدام تقنية المظاهر المتعددة لأطوال القطع المقننة، تم استخلاص الحامض النووي من ٩٥ عينة (٤٧ من طيور محلية سوداء الريش و ٤٨ من طيور عارية الرقبة) بتقنية الفصل بالأملاح، ضمت قطعة الدنا لجين هرمون النمو البالغ حجمها ٧٧٠ زوجاً قاعدياً بواسطة بادئة متخصصة، هضم ناتج تفاعل الكوثرة المتسلسل باستخدام إنزيم القطع MSPI وتم الكشف عن نتائج الهضم باستخدام ١.٥% من هلام الأكاروز، كانت قيمتي التكرارات الأليلية للأنترون رقم ١ لكل من الاليلين A وB في الدجاج المحلي العراقي (أسود الريش) ٠.٤٤ و ٠.٥٣ في حين سجلت القيمتين في الدجاج عاري الرقبة ٠.٤٦ و ٠.٥٤ على التوالي، تشير نتائج هذه الدراسة إلى أن المظاهر المتعددة لجين هرمون النمو في الانترون رقم ١ في الدجاج المحلي العراقي يمكن أن تستغل لجينات مرشحة تستخدم كمؤثرات مساعدة في الإنتخاب للصفات المرتبطة بالنمو.

الكلمات الاسترشادية: الدجاج المحلي العراقي ، المظاهرة المتعددة ، جين هرمون النمو.

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