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RESEARCH ARTICLE

IDENTIFICATION OF HINDIII POLYMORPHISMS IN THE TWO INTRON OF GHR GENE IN NATIVE IRAQI CHICKEN

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ABSTRACT

The gene of the growth hormone receptor has played a great role in the chicken growing and reproduction. Polymorphisms for this gene were several variations in the animal genome, could be used as genetic marker for growth selection and economic traits. The purpose of this study was to identify polymorphisms in Growth hormone receptor in Iraqi local chicken .we collected 95 blood samples form Iraqi Native chicken (47 samples from the Black feather native fowls and 48 samples from the Nicked neck native fowls) to inspect growth hormone receptor gene polymorphism using PCR-RFLP technique. As stated to the data, *HindIII* enzyme produced 2 final alleles R and G. the R allele was placed in 290 and 428 bp while G allele placed in 170, 258 and 290 bp. Also, RR genotype stracture was in 290 and 428 bp while GG placed in 170, 258 and 290 bp, respectively. Besides, in this research no heterozygote identified. As it was seated in the data, there was significant difference (P>0.01) among polymorphism in intron 2 gene in growth hormone receptor. The consequences of current study showed that the intron 2of GHR is polymorphic in Iraq native fowls and could be exploited as a genetic marker for selection programs of growth-related traits.

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INTRODUCTION

The Iraqi native chicken are a special homegrown breed. They have a small body conformation and a double intent, one is for meat and other for egg production. This type of chicken meat and eggs suit Iraqis consumers' tastes. therefore, necessary to study the Iraqi chicken by the molecular technologies as role in any research effort to make efficient its reproductive traits, As a result of these effort , production will increase and meet the market demand. Somatotropic play a vital role in the regulation of growth and development. Former studies showed that difference of these genes impact gene expression at the transcription and translation levels in most animals (Lo *et al*., 2003 and Wyszynska *et al*., 2006). Variant in the genes of somatotropic axis could work as candidates for the evaluation of their effects on chicken growth and production traits. The chicken growth hormone gene is considered as one of the most significant candidate genes that can effect chicken performance traits because of its fundamental function in metabolism and growth (Vasilatos *et al*., 2000).

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The chicken growth hormone gene has four exons and five introns with an overall length of 4.1 kb (Kansaku *et al*., 2008). The cGH is amain hormone which is released from the anterior pituitary gland, also has effected on disease resistance and egg production. The chicken growth hormone receptor are most vital candidate genes that can affect on chicken economic traits including growth, body measurement, carcass and reproduction., Thus current study was to determine growth hormone receptor Gene Polymorphism in two Iraq native fowls by using PCR-RFLP technique.

MATERIALS AND METHODS

Animal and Blood Samples

The blood samples were taken haphazardly from two indigenous Iraq local chicken flocks: 95 blood samples (2ml in EDTA containing tubes) collected from via wing vein using disposable syringes in all birds and stored at -20 C° until used at hematology laboratory. The total DNA was removed from the blood samples by using adapted salting out technique (Miller *et al*., 1988). The extracted DNA was tested by agarose gel electrophorus and spectrophotometer. The intron 2 region

of chicken GHR gene was amplified by primers (5'GGCTCTCCATGGGTATTAGGA3' forward and 5'GCTGGTGAACCAATCTCGGTT3' reverse). The specificity of primer was established by BLAST with all the nucleotide sequences available for chicken at the (NCBI) National Center for Biotechnology Information. The mixture was exposed to initial denaturation of 95°C for 120 s followed by 35 cycles of 95°C for 30 s, annealing at 60°C for 30 s and extension at 70°C for 1.20 s. Final extension was given for 5 min at 95°C. The polymorphism chain reaction products were separated on 1.5% agarose gels containing 1X Tris-Borate-EDTA (TBE). The gels were marked by ethidium bromide and the images were obtained in UV gel documentation systems (Ez – Capture MG Japan). RFLPs were used for analysis of cGHR gene polymorphisms. The products (8 µl) of GHR gene were digested with 0.5 µl of HindIII restriction enzyme and 2 µL buffers 10X , Acetylated BSA 0.2 µl , D.w 4.3 µl in a finishing reaction volume of 15µL. The reaction product mixture was incubated at 37°C for 4 hrs.The resulting fragments were detached by horizontal electrophoresis (50 V, 2 hrs) on 1.5% agarose gel were stained by ethidium bromide and were seen under ultra violet light to observe number of alleles and genotypes, the observed and expected heterozigosity of each locus and the average of heterozigosity were used to assess the genetic diversity of two investigated populations. The Hardy Weinberg equilibrium was evaluated in studied populations. All data were analyzed using SAS (2012).

RESULTS AND DISCUSSION

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

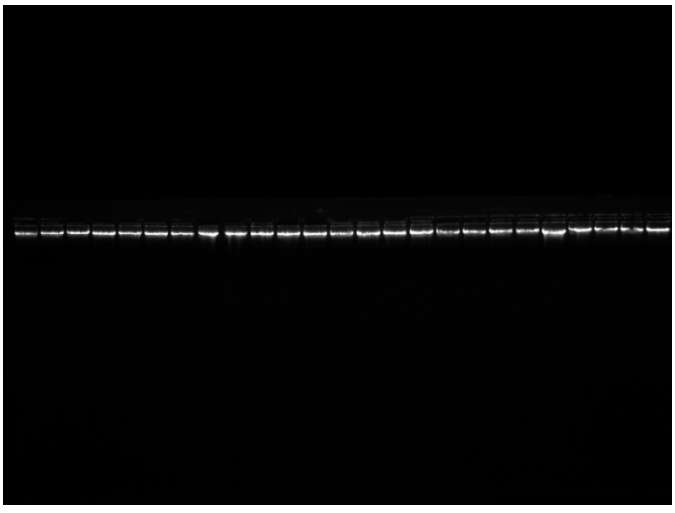

Fig. 1. DNA extracted samples

The products of PCR were digested with *HindIII* restriction enzyme (5U), which where recognized the 5'- **A ↓A A↑A** -3' sequence. In this research 718 bp in intron 2 was propagated Figure 2. The index for DNA was RFLPs of terminated by *HindIII*. *HindIII* produced two final alleles: R and G. The R allele was placed in 290 and 428 bp while G allele placed in 170, 258 and 290 bp. In addition, RR genotype was in 290 and 428 bp while GG placed in 170, 258 and 290 bp, Moreover, in this research no heterozygote identified figure 3. In this regard, Li *et al*., (2008) on native Chinese poultry A1A1 and A2A2 were detected. In similar study, Enayati *et al*., 2011 reported that these were a polymorphism in intron 2 of GHR gene. Our result was similar to their observations on many of segments but not in segment sizes.

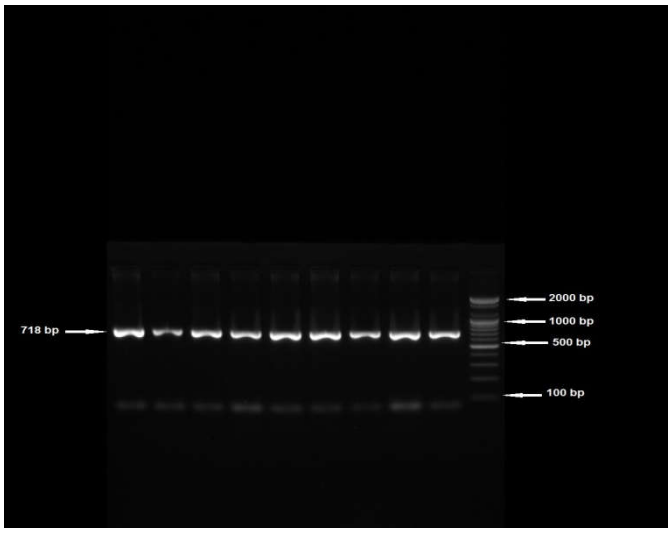

Figure 2. Representative result of Agarose gel electrophoresis of PCR products of intron 2 chicken growth hormone receptor gene

Table 1. Genotypic frequencies for Hardy-Weinberg equilibrium from intron 2of the GHR gene in Iraq native fowl

Population	Black native fowls		Nicked nick native fowls	
Genotypes	No. of birds	Frequency	No. of birds	Frequency
RR	17	34.7	14	29.8
GG	32	65.3	33	70.2
Chi-Sq.	20.33**		11.46**	

** significantly (P < 0.01).

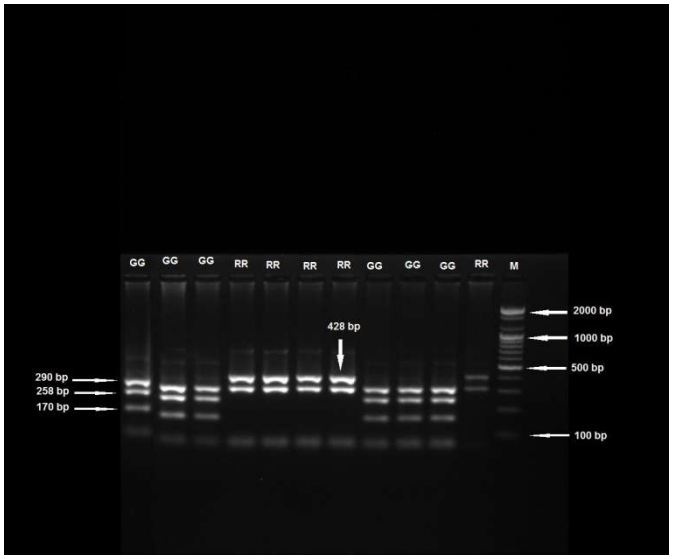

Figure 3. PCR RFLP pattern for GHR introne 2 with HindIII digestion

Table 2. Allelic frequencies in intron 2 of the GHR gene in Iraq native fowl

Breeding station	No. of birds	R	G
Black native fowls	49	34.7	65.3
Nicked nick native fowls	47	29.8	70.2

Genotype +/+ with 3 segment (157, 247 and 314) and genotype -/- with 314 and 404 bases. The allele frequencies in the Iraq local chicken flocks (Black native fowls and Nicked nick fowls) are shown in Table 1. In Black native fowls, allele frequency for genotype RR and GG (34.7, 65.3) respectively and in Nicked nick fowls allele frequency for genotype RR and GG showed (29.8,70.2) respectively .However the data which was indicated important variance between them. In Black native fowls, allele frequency for R and G (34.7, 65.3) respectively and in Nicked nick fowls allele frequency for R and G showed (29.8,70.2) respectively Table 2. In this regard, frequencies for allele + was 0.9935 while for allele – 0.0065 as reported by Enayati *et al*., (2011). In addition, Similarly genotype frequencies for +/+ and - /- were reported 0.52 and 0.48 Feng (1996). They stated that growth hormone receptor gene (GHR) was out of Hardy-Weinberg equilibrium. Data were gained on gene and genotypic frequencies through the polymorphism study makes it possible to differentiate the gene stocks of animals, which reflect on performance and reproductive traits, The genetic variation study under several environmental conditions of selection (Egena and Alao, 2014). Allele frequency for R and G showed (0.46 ,54) respectively. However the data which where indicated significant variations between them. Thus, it can be concluded that the two varieties belong to the Iraq local chicken, having almost different genetic base. The obtained results of the aim study indicated the GHR gene could be exploited as a genetic marker for selection programs in Iraq native fowls.

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