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Original Article

PCR-RFLP Analysis of Insulin-Like Growth Factor 2 Gene Polymorphisms in Two Commercial Broiler Chicken Strains (Cobb 500 and Hubbard F-15) and Their Associations with Performance Traits

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Abstract

The present research aimed to study the polymorphisms of the chicken insulin-like growth factor 2 (IGF2) in two commercial broiler breeds (Cobb 500 and Hubbard F-15). In total, 300 avian blood samples were obtained. The genomic DNA was isolated using a fast salt-extraction technique. Moreover, polymerase chain reaction (PCR) was used to amplify 1146 bp fragments of the gene. The amplified fragments were subjected to restriction enzyme digestion using the HinfI endonuclease enzyme, and the digested products were separated on a 2% agarose gel. The findings indicated that there were two alleles T and C for the target locus, with frequencies of 73.3% and 26.7%, respectively. Three distinct genotype variations, TT, TC, and CC, were found, with genotype frequencies of 59.1%, 28.4%, and 12.5%, respectively. A test based on actual and anticipated frequencies of various genotypic variances of the IGF2 gene revealed that the divergence from Hardy-Weinberg equilibrium was not significant ($P \leq 0.01$) in commercial broiler breeds (Cobb 500 and Hubbard F-15) chickens. In addition, it was found that birds with genotype TC had a greater body mass at 8 weeks of age, compared to those with genotypes TT and CC. It was determined that the IGF2 gene exhibited a significant degree of variability and might be regarded as a possible genetic marker in selection and breeding programs for poultry.

Keywords: IGF2; Polymorphism; PCR; Chicken

1. Introduction

Insulin-like growth factor 2 (IGF2) is a mutagenic polypeptide with an insulin-like structure that controls primary chicken growth. The genes for IGFs are situated on chromosome 5, which has two intron and three exon regions (1, 2). The introns and exons of the chicken IGF2 gene resemble those of the mouse and human IGF2 genes (1). The chicken IGF2 is composed of 187 amino acids, 24 signal peptides, 67 IGF2 peptides, and 96 amino acids for its C-terminal portion. It has 33 amino acids in common with its rat counterpart and 82 amino acids with its human counterpart (1).

Numerous studies on various mammalian species have indicated that IGF1 significantly affects growth (3). Function of the IGF system in chickens is similar to that in mammals (3, 4). Chicken tissues are stimulated to develop and differentiate by IGFs. These variables primarily influence the rate of protein synthesis, DNA synthesis, and substrate transition. In addition to altering chicken body and muscle development, insulin-like growth hormones may also impact

ovulation rates and ovarian follicle extension (4). Besides acting on insulin-like receptors, this gene decreases blood glucose as well (5).

Compared to embryonic IGF2 gene transcripts, there are significantly fewer IGF2 peptides in chicken embryos, as shown by gene expression (6). In mammals, 2 out of the 10 kinds of IGF1-binding proteins are significantly expressed in chickens. *In vitro* testing has shown that myoblasts and satellite cells in chicken embryos release binding proteins (7). The chicken IGF1, IGF2, and IGF3 are polypeptide hormones that contribute to their function by binding to particular type 1 receptors (8). There is evidence that IGF2 gene inheritance is paternal in several placental animals (9, 10). The IGF2 influences the development rate, body composition, and lipid metabolism of chickens (3, 11, 12).

The IGFs significantly influence the embryonic development and differentiation of several animal species. In addition, previous research has shown that IGF is the primary gene responsible for overweight in chickens (4, 13). It works in accordance with the paracrine system of the body (14). The IGF system is complicated due to peptide hormones, cell surface receptors, and binding proteins. The IGF1 and IGF2 hormones bind to insulin-like growth factor receptor 1 and insulin receptor, respectively, and activate intrinsic and main actions of tyrosine kinase (15). The IGF2 has also been shown to affect muscle atrophy in rats, pigs, and cows as well as the lipid metabolism in poultry (16). The IGF2 is a molecular marker for the selection of chickens with low abdominal fat (13) since it is the primary gene influencing chicken obesity.

In this study, the polymorphism of the promoter region of IGF in commercial broiler breeds (Cobb 500 and Hubbard F-15) of chickens was analyzed using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique. Moreover, this study aimed to determine the allele and genotype frequencies, as well as the association between these polymorphisms and chicken growth traits.

2. Materials and Methods

In total, 300 chicks from the commercial broiler breeds (Cobb 500 and Hubbard F-15) were randomly selected. The breeding facility provided the characteristic performance data, including their weight at 1 to 42 days of age. Blood was extracted from the wing veins of chickens and preserved in ethylenediamine tetraacetic acid-coated sterile tubes containing 1.5 ml. The blood samples were transported to the Central Laboratory of the College of Veterinary Medicine.

It should be mentioned that 200 ml of blood was used for genomic DNA extraction. The DNA was extracted using the rapid salt-extraction technique (17). A primer combination consisting of (IGF2-F) 5'-CCA GTG GGA CGA AAT AAC AGG AGG A and (IGF2-R) 5'-TTC CTG GGG GCC GGT CGC TTC A was used to amplify the 1146 bp fragment of the IGF2 gene (Amills et al. 2003). The PCR was performed in 25 l reaction volume comprising 50 mM of dATP, dTTP, dCTP, and dGTP, 0.5 mM of each primer, 2.5 l of 10X PCR buffer, 2 mM magnesium chloride, 2.5 U of Taq DNA polymerase, and 50 ng of extracted DNA as a template. The amplification procedure consisted of 35 cycles of denaturation at 94 °C for 1 min, annealing at 67 °C for 3 min, elongation at 72 °C for 3 min, and final extension at 72 °C for 5 min. The PCR products were separated on an agarose gel containing 1.5% agarose, and the gel was imaged using UV transillumination.

The PCR products were digested with HinfI restriction endonuclease. The digestion process was carried out in 15 l mixtures comprising 5 l of PCR product, 5U Hinf I endonuclease, and 1.5 l Hinf I buffer. The mixes were incubated for 2 h at 37 °C. The fragments of digested DNA were then run on a 1.5% agarose gel and imaged using UV transillumination. PopGene32 (version 1.23) (18), was used to calculate the allelic and genotypic frequencies as well as the observed and anticipated heterozygosities. The Hardy-Weinberg equilibrium test was also conducted using PopGene32. The percentages of homozygosity and heterozygosity were also determined.

3. Results and Discussion

The PCR products of size 1146 bp were produced satisfactorily using the given primers. All extracted genomic DNA from chicken blood samples generated a PCR result with a single, specific band and no nonspecific bands. Consequently, the PCR results were used immediately for RFLP analysis. Figure 1 depicts the RFLP patterns generated by HinfI digestion of PCR products derived from the IGF2 gene. Two alleles, T and C, with frequencies of 73.3% and 26.7%, respectively, and three genotypes, TT, TC, and CC, with frequencies of 59.1%, 28.4%, and 12.5%, respectively, were found (Table 1). The TT homozygous genotype was discovered to be the most common genotype.

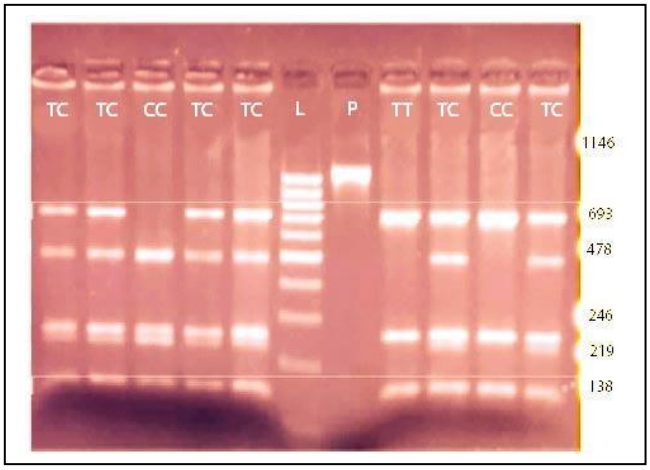

Figure 1. Depicts RFLP patterns generated by Hinf1 enzyme cutting and electrophoresed on 2% agarose gel. L is the molecular marker for German Ferments (50 bp or more); there are three types of genotype, TT, TC, and CC

Table 1. Displays the genetic variability of IGF-2 exons 2 and 3 in commercial broiler breeds (Cobb 500 and Hubbard F-15)

Genetic diversity statistics	Value	Allele frequencies	Value	Genotype Frequencies	Value
NA	2	T	0.733	TT	0.591
NE	1.4	C	0.260	TC	0.284
Observed homozygosity	0.7159	CC	0.125		
Observed heterozygosity	0.2841				
Expected homozygosity	0.6063				
Expected heterozygosity	0.3937				
Average heterozygosity	0.3915				
Nei Heterozygosity	0.3915				

NA = the observed number of alleles, and NE = the effective number of alleles (Kimura and Crow, 1964

The allele heterozygosity and allele effective size values were 0.39 and 1.64, respectively. The chi-squared test revealed the absence of the Hardy-Weinberg equation ($P \leq 0.01$), showing the existence of allele and genotype frequency transformers between generations. The CC genotype considerably outperformed the TT and TC genotypes in terms of eighth-week weight and puberty weight among the genotypes of the study group. Growth qualities are among the most significant economic features of poultry; hence, identification of genetic data of growth-related genes in domestic animals is helpful for genetic selection and improvement through marker-assisted selection.

The IGF system induces liver glycogenesis in chickens and enhances DNA synthesis and tissue growth (3). The IGF2 is a polypeptide hormone that regulates the division and differentiation of embryonic cells and is crucial to embryo development. In several animals, the gene is expressed throughout development. Until puberty, the transcriptional activity of the IGF2 gene is constant throughout the main embryonic phase, but it diminishes in many organs, which influences growth (19). Usage of candidate genes is an effective strategy for the examination of connections between gene variation and economically significant characteristics of domestic animals (20).

This research investigated the polymorphism of the IGF2 gene and its connection with growth parameters in commercial broiler chickens. It was demonstrated that the IGF2 gene may be a candidate gene for growth and body characteristics, and its genotype is connected to phenotype, confirming its substantial effect on growth and development (19). Findings of the present study corroborate those of a study performed by Amills, Jimenez (21), who found no connection between the single nucleotide polymorphism of the IGF2 gene and growth and feeding parameters. In contrast, they discovered a substantial correlation between these polymorphisms and average daily growth at a certain age.

In another investigation conducted by Li et al., PCR-RFLP and DNA sequencing were used to find polymorphism in the exon 2 region of the chicken IGF2 gene (13). Results of the current research showed that the CC genotype had superiority regarding eighth-week and puberty weight growth records, compared to the TT and TC genotypes. Consequently, the results of the current study and prior research indicated that IGF2 might be a major gene influencing chicken obesity and could be employed as a genetic marker during the selection process.

Exons 2 and 3 of the IGF2 gene were determined to be very distinct from one another using the PCR-RFLP technique. Although there is no link between the discovered SNPs and development characteristics, there is a considerable correlation between these polymorphisms and daily weight increase at puberty. Consequently, the IGF2 gene may be exploited as an appropriate selection marker to enhance carcass economic features.

Authors' Contribution

Study concept and design: A. S. A.
Acquisition of data: A. S. A.
Analysis and interpretation of data: D. H. A.
Drafting of the manuscript: D. H. A.
Critical revision of the manuscript for important intellectual content: I. A. A.
Statistical analysis: I. A. A.
Administrative, technical, and material support: A. S. A.

Ethics

We hereby declare all ethical standards have been respected in preparation of the submitted article according to the ethics committee of the University of Baghdad, Baghdad, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

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