

## BROILER GENOTYPING AND ALLILIC FREQUENCY ANALYSIS BY PCR-RFLP FOR IGF1 AND IGF2 GENES

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### ABSTRACT

This study was aimed to investigate Broiler chickens using PCR-RFLP (IGF1 and IGF2 genes). *HinfI* and *Hsp92II* restriction enzymes were used to amplify and digest 793-bp and 1146-bp fragments of the IGF1 gene, respectively. TT, TC, and CC genotypes were found in both genes in two different T and C allele subgroups. IGF1 had a higher prevalence of the C-allele (0.86) than IGF2, whereas IGF2 had a higher prevalence of the T-allele (0.5611).

Key Wards: *HinfI*, *HinfI*, Amino Acids.

الحسني

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تعدد المظاهر الجينية لفروج اللحم وتحليل التكرار الايلي باستخدام التفاعل المتسلسل لجني عامل النمو الشبيهة بالانسولين

1 و 2

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المستخلص

تمت دراسة ترددات الأليل لعوامل النمو الشبيهة بالانسولين (ILGFs) في فروج اللحم باستخدام PCR-RFLP جينات (IGF1 او IGF2) تم استخدام إنزيمات الهضم *HinfI* و *Hsp92II* لتضخيم وهضم 793 bp و 1146 bp من جين IGF1 او IGF2، على التوالي. تم العثور على الأنماط الجينية TT و TC و CC في كلا الجينين في مجموعتين فرعيتين مختلفتين من أليل C, T لل IGF1 كان له انتشار أعلى للأليل (0.86) من IGF2، في حين أن IGF2 كان له انتشار أعلى للأليل (0.5611). T

الكلمات المفتاحية: *HinfI*, *HinfI*, الاحماض الامينية.

## INTRODUCTION

In animals, IGF-1 regulates growth, development, and metabolism. There are several neuroendocrine processes that must be coordinated to control the growth process. In this biological process, Influenced by IGF and its receptor, intermediary metabolic processes and cell development are influenced by IGF and its receptors. There are two kinds of polypeptide hormones, one of which is IGF1 and the other is IGF2 (25). Enhanced intake of amino acids and glucose, as well as increased DNA and protein synthesis, are all examples of the biochemical responses that birds have to IGFs (13). A decrease in IGF1 and IGF2 levels is associated with fasting, but an increase in levels is associated with aging (2). IGF-1 has been shown to alter chicken development, metabolism of fats and carbohydrates (13), (2, 21). IGF1 and IGF2 regulation and expression in chickens has provided us with a wealth of information. The chicken IGF1 gene is 50 kilo bases long on chromosome 1. It has been reported (8,9). Multiple promoter and splicing variation variations have been identified in genomes (8). mRNA for IGF1 may also be found in the heart, ovaries, brain, and digestive tract (13,19). In the brain, chromosome 5 includes all three exons of the gene. The IGF2 gene is passed down from father to son in mammals through paternal ancestry (3,24). For further information, please see (4) and (10). A balletic expression pattern was found for the chicken IGF2 gene (16,17,24) in a number of studies. A link was found between phenotypic variation in various growths and carcass characteristics and a polymorphism in chicken IGF2 gene exon 2, according to (23). This study aimed to identify IGF1 and IGF2 gene polymorphisms.

## MATERIAL AND METHODS

There were 300 broiler chickens used in the current experiment (150 Cobb and 150 Hubbard F-15). EDTA-containing vacutainer tubes were used to collect blood samples of about 0.5–1 mL. Ice and gel cold packs were used to keep the vials cool. After the DNA extraction, the samples were kept at -20 degrees Celsius until further processing.

**DNA extraction:** The (7) phenol-chloroform extraction method was shown to be susceptible to improvement. Reverse lysis buffer and

buffy coat separation are not necessary in this method. Following centrifugation, 400 liters of extraction buffer containing 10 mg/mL RNase, 20 mg/mL proteinase K, and 50 mg/mL proteinase K was added to the blood. The solution was agitated every 10 minutes for an additional hour after being incubated for two hours at 55 °C. Phenol chloroform isoamyl alcohol (25:24:1) was completely combined in an inverted mixer. At 10,000 rpm for 10 minutes, the supernatant was collected from the sample. After chloroform was added, the liquid was thoroughly mixed using the inversion method. Ten minutes at 4 degrees Celsius and 10,000 revolutions per minute were used to spin down the mixture in the first micro-centrifuge tube. When the mixture was heated to room temperature, it was diluted with 2 L cold isopropanol and 10 L 3 M sodium acetate (pH 7.2). After the treatment, it was kept at -20 degrees Celsius for the remainder of the night. A 15-minute spinning process at 12,000 rpm separated the various components. However, a single particle was left in the tube for further study after it was rinsed away. First, the pellets were to be sanitized with 70% alcohol and then dried in the open air. To safeguard the DNA, the solution was maintained at -20°C in 100 L of TE buffer. In order to determine the purity of the extracted DNA, a gel documentation system, horizontal electrophoresis, and UV trans-illumination were used. A Nano drop spectrophotometer was used to verify the DNA's purity. In order to conduct additional research, only DNA samples with OD260/OD280 ratios of 1.7 to 1.9 were considered.

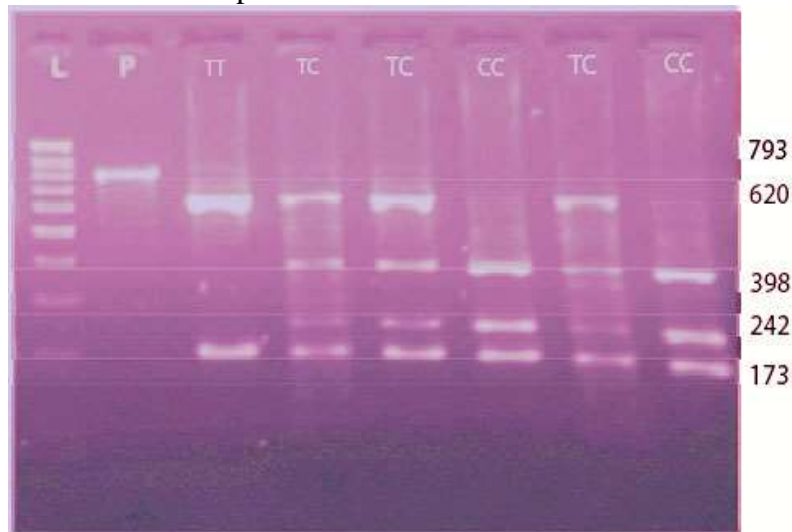
## Restricted Fragment Length Polymorphism and Polymerase Chain Reaction (RFLP)

The polymerase chain reaction, according to (1), amplified a 793-bp DNA fragment. Software from SAS was used to figure out the gene and allele frequencies. Amplification of the IGF2 gene product was carried out using primers GALIGF2 5'-CCA GTG GGA CGA AAT AAC AGG and 5'-GALIGF2-TTC CTG GGG GCC GGT CGC TTC T-3. (1). used the same method to compute the frequency of genes and alleles as in the past.

**RESULTS AND DISCUSSION**

Genomic DNA samples were used to successfully amplify the IGF1 and IGF2 gene sequences. Restrictive digestion examination of 793 IGF1 PCR findings indicated the presence of two distinct restriction patterns.

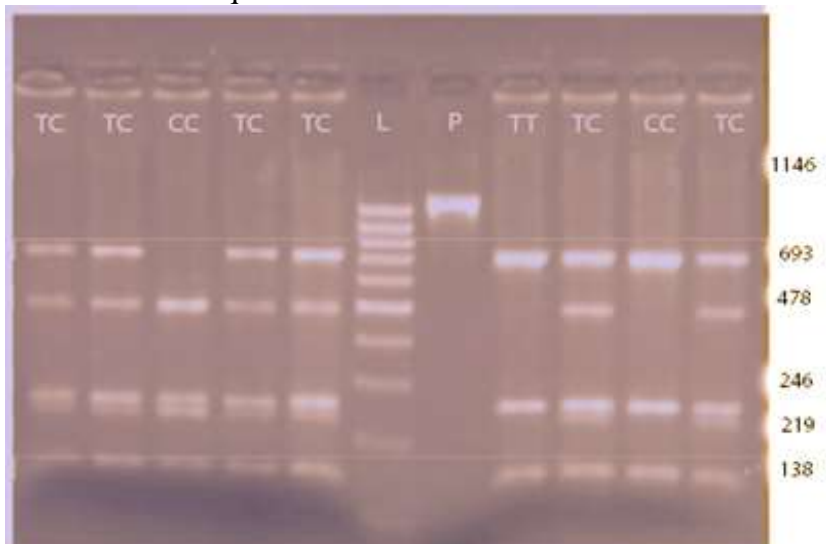
There were 620/173 bp fragments in the first pattern, whereas 378/242/173 bp fragments were found in the second pattern (Figure 1). Genotype CC was allocated to the first pattern, whereas genotype TT was given to the second.



**Fig.1. Genotyping patterns for IGF1 genes were discovered using the PCR-RFLP method on a 2 percent Agarose gel, One hundred milligrams of L Ladder of DNA**

The IFG2 PCR product was digested to yield T and C restriction patterns (246/39/138/30/693 bp and 478/215 bp, respectively) (Figure 1). Gene frequency and allele frequency estimates for the two human insulin-like growth factors are shown in Table 1. Allele frequencies of

0.16 and 0.86 were found in IGF1 samples with genotype frequencies of 0.066 and 0.166, respectively (Table 1). IGF2 samples had genotype and allelic frequencies of 0.5862, 0.4436, and 0.2882 respectively (Table 1).



**Fig. 2. Two percent Agarose gel electrophoresis was used to identify IGF2 gene genotypes using the PCR-RFLP method. One hundred milligrams of sodium Ladders of DNA**

Chickens strain was similarly affected. The sample's high Chi square score indicated that

neither of the population's two genes was in Hard Weinberg equilibrium (Table 1).

**Table 1. *Hinf*I and *Hin*II restriction enzyme genotypes and allele frequencies for 793 bp and 1146 bp segments, respectively**

Genotype	Frequency	
	IGF1	IGF2
TT	0.1122	0.3788
TC	0.1827	0.3522
CC	0.6552	0.2822
	Alleles	
T	0.1625	0.5862
C	0.8622	0.4436
Chi-square value	10.8234	8.1023
Heterozygosity	0.1827	0.3522
Allelic diversity	0.2652	0.4839

Three SNPs were found in the alignment of chicken IGF sequences published by Amills M. et.al. (1). A single TC substitution was identified in the IGF1 gene's 5'UTR region, and this was designated as IGF1-SNP1. A *Hinf*I RFLP was shown to be linked to this mutation. Two SNPs in the IGF2 gene were also discovered. The *Hsp92*II restriction enzyme was able to identify an IGF2 neutral substitution TC in exon 3 (IGF2-SNP2). Intron 2's second SNP was swapped with a GA (IGF2-SNP3). Nagaraja S.C. et al (15) reported three SNPs (IGF1-SNP1, IGF2-SNP2, and IGF2-SNP3) with genotype frequencies identical to our own. Allele frequencies and genotypes of chickens are described here. There are more research showing an association between IGF1 and post-hatchling development than IGF2, according to these studies. While IGF1 was shown to significantly increase growth rate in three different chicken breeds, IGF2 was found to have no effect at all. Additionally, higher quantities of hepatic IGF1 mRNA Beccavin C. et al (2) are present in both the high- and low-growth strains Wu. G. et al (22) is expressed sooner than in the low-growth strain. The IGF-I gene polymorphisms of (5) have an enormous influence on chicken development features, according to their findings. The expression levels and SNPs of IGF2 and *STAT5b* are critical for IGF protein action because they operate as modulators of the biological activity of IGF genes in multiple signaling pathways. Mice raised for low body weight were shown to have increased IGF2 mRNA expression and higher blood IGF2 levels, which were associated to lower growth in mice (6). The C1996A single nucleotide polymorphism of the IGF2 gene was discovered to be linked to abdominal fat weight and percentage (SNP).

A C/T SNP in the IGF2 gene has been shown to alter a number of hen characteristics, including body weight, metatarsal and femoral lengths and weights, metatarsal claw, and abdominal fat weights. In protein-starved chickens, supplementing them with methionine and glycine reduced their weight loss, but this effect was not impacted by changes in IGF1 plasma levels (14). According to research, IGF1 has been shown to have a major role in the formation of fat and the metabolism of lipids (7),(20),(18) In order to understand the molecular basis of these relationships, it is essential to look for mutations in IGF1 and IGF2 as well as their modulators (*IGFBP2* and *STAT5b*). An investigation of IGF gene mRNA expression profiles may be useful in determining the association between body weight and feed conservation in light of recent scientific results.

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