

The relationship between the genetic polymorphism of the *IGF-1* gene and some production traits in Ross 308 broiler chickens has been studied

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Abstract

To determine the genetic variations of the Insulin-like Growth Factor-1 *IGF-1* gene and study its relationship with certain production traits, including weekly weight, weekly weight gain rate, bird length, back width, carcass length, Chest fullness, relative weights of consumed organs, and abdominal fat percentage, in Ross 308 broiler chickens, a total of 150 chicks were bred from day-old to 35 days under good rearing conditions based on the rearing guide and followed a free feeding system. At the end of the rearing period, blood samples were collected from the birds through the wing vein to extract the genetic material, DNA. Three genetic structures were identified using sequencing technology: GG, GT, and TT, with frequencies of 0.34, 0.58, and 0.08, respectively. The allele frequencies for G and T were 0.63 and 0.37, respectively. The results showed a significant superiority ($P \leq 0.05$) in weight and weight gain for the TT genetic structure in the third, fourth, and fifth weeks compared to the other genetic structures GT and GG. Similarly, the TT structure showed superiority in bird length measurement in the third and fourth weeks, as well as in carcass length measurement. The GG genetic structure exhibited superiority in back width measurement in the second week of rearing compared to the other genetic structures. However, no significant differences were observed among the genetic structures in the relative weights of consumed organs (liver, heart, and gizzard), abdominal fat percentage, and Chest fullness.

Keyword: *IGF-1* · Ross 308 · SNP

I. Introduction

Undoubtedly, global consumption of poultry products is steadily increasing, necessitating intensive and rapid poultry production (Abdulwahid *et al.*, 2021). This requires the poultry industry to find suitable ways to enhance the production value to meet the growing consumer demands (Abdul-kareem and Falh, 2022). The current trend is towards incorporating molecular genetics applications, including information technology and genetic techniques, into breeding and genetic improvement programs (Thbit *et al.*, 2021). Among these, Single Nucleotide Polymorphisms (SNPs) have gained significant popularity (Li *et al.*, 2008). They are considered as markers for detecting chromosomal regions containing genes that influence important production traits in animal production (Salim and Abdulkareem, 2019). The recent advancements in molecular genetics have introduced various genetic markers that have assisted researchers in analyzing and evaluating genetic diversity and differentiating strains for conservation purposes (Abdulkareem, 2020). This has provided an opportunity to enhance selection response, particularly for traits that are difficult to improve through conventional selection (Al-Omer, 2022), by providing molecular-level information on different genomic regions (Jaffer and Abdulkareem, 2022).

Numerous studies have indicated the potential for genetic variations in several genes directly involved in growth and development processes in chickens (Abdul-kareem and Raysan, 2022). Insulin-like growth factor *IGF-1* gene has been identified as one of the important genes with multiple Single Nucleotide Polymorphisms (SNPs) that can be utilized in genetic improvement applications for growth traits in



agricultural animals (Estany *et al.*, 2007). Insulin-like growth factor 1 (IGF-1), also known as Somatomedin C, is a multifunctional polypeptide hormone structurally similar to insulin. It is located on the first chromosome in poultry and consists of six exons (Boschiero *et al.*, 2013). It is primarily secreted by Kupffer cells in the liver under the stimulation of growth hormone (GH) (Ohlsson *et al.*, 2009). Its main function includes cell specialization and differentiation, growth regulation, amino acid and glucose absorption, as well as involvement in the stimulation of thyroid hormone, insulin, DNA and mRNA synthesis, protein synthesis, and it is considered the key mediator of growth hormone effectiveness (Nakae *et al.*, 2001).

Given the importance of the *IGF-1* gene and its close relationship with growth and production traits, our study aimed to identify the genetic variations of the *IGF1* gene and explore its relationship with some production traits in Ross 308 broiler chickens.

II. Materials and Methods

Field work was conducted in the poultry field affiliated with the Scientific Research Station at the College of Agriculture and Marshlands, Thi-Qar University. A total of 150 hybrid (Ross 308) meat chicken chicks were raised. These chicks were obtained from the modern hatchery of Al-Dair Company in Al-Kut. The rearing period lasted for 35 days, from November 30, 2022, to January 3, 2023. The chicks were reared using a floor rearing system with a bedding of wood shavings with a thickness of 3-5 cm. Upon arrival, the chicks were immediately provided with water containing 5% sugar as a quick energy source. Gas heaters, as well as electrical heating, were used to heat the facility. All chicks were fed ad libitum, and their diet was formulated freely.

The birds were individually numbered using plastic rings that were numbered and colored, which were attached to their legs. These rings were replaced with larger-sized numbers in the second week as the birds advanced in age.

Body Measurements:

1. Live body weight: All birds were individually weighed after numbering. The initial weight was measured on the first day of age, followed by weekly weight measurements until the end of the 5-week experimental period. A sensitive electronic scale was used for weighing.
2. Weight gain rate: The weekly weight gain was calculated individually for each bird using the following equation: Weekly weight gain (g) = Final live body weight (g) - Initial live body weight (g). (Al-Fayadh *et al.*, 2011)
3. Length of the bird and back width: These measurements were taken weekly at the end of each week throughout the experiment.
4. Measurement of carcass length and calculation of breast fill score according to the following equation: (Al-Alwani, 2002)

$$\text{Breast fill score} = \text{Chest circumference (cm)} / \text{Carcass length (cm)}$$

5. Relative weight of edible internal organs: The edible internal organs (giblets), including the heart, liver, and gizzard, were separated from the rest of the internal organs of the carcass and weighed using a sensitive scale. The weights of these parts were expressed as percentages using the following equation: (Al-Fayadh *et al.*, 1989)

$$\text{Relative weight of organ} = \text{Organ weight (g)} / \text{Live body weight (g)} * 100$$

The abdominal fat percentage was calculated using the equation developed by (Griffiths *et al.*, 1978).

$$\text{Abdominal fat percentage} = \text{Abdominal fat weight (g)} / \text{Carcass weight (g)} * 100$$



Blood Sample Collection and Molecular Analysis:

Blood samples were collected randomly from 50 birds after the rearing period. The samples were placed in tubes containing an anticoagulant substance (EDTA) to prevent blood clotting. Molecular genetic analyses were conducted at the Research Center for Wetlands, Thi-Qar University. DNA was extracted from the bird blood samples following the instructions provided by the Geneaid kit, prepared by the Korean company Geneaid. The extracted DNA samples were analyzed using 1% agarose gel electrophoresis and subjected to polymerase chain reaction (PCR) using the following primers:

5'- GACTATACAGAAAGAACCAC - 3'
5'- TATCACTCAAGTGGCTCAAGT - 3'

After confirming the size of the PCR product for the studied gene by comparison with a DNA ladder standard, 20 microliters of each PCR product were sent to the Korean company Macrogen. The samples underwent purification and then underwent nucleotide base sequencing using Sanger sequencing technology. The obtained base sequence results were analyzed using BLAST tools on the NCBI international gene bank website, along with some bioinformatics tools.

III. Results and Discussion

Figure 1 shows the successful extraction of DNA using 1% agarose gel electrophoresis with a voltage of 70 volts and a current of 85 milliamperes. This step was crucial for isolating the *IGF_1* gene.

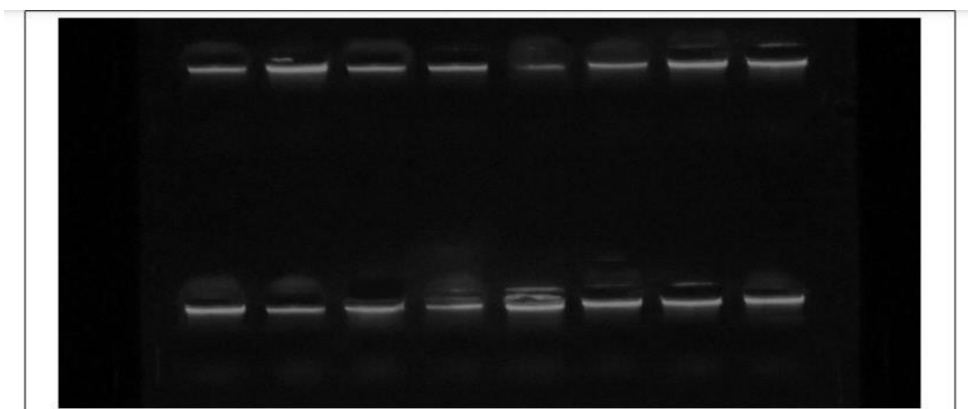


Figure 1: Agarose gel electrophoresis of DNA

Using PCR technology, the amplification of the targeted segment of the *IGF_1* gene was successful, as indicated by its electrophoretic migration on a 1.5% agarose gel with a voltage of 70 volts and a current of 85 milliamperes. The desired segment of the *IGF_1* gene was obtained with a size of 631 base pairs, as shown in Figure 2.

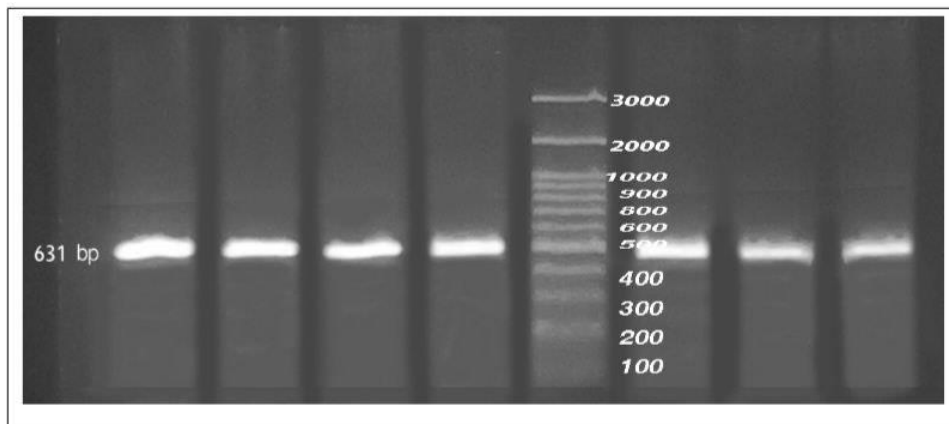


Figure 2: Electrophoretic migration of the PCR product of the *IGF-1* gene (631 base pairs)

After successfully amplifying and isolating the targeted segment of the *IGF-1* gene, 50 samples were sent to Macrogen for nucleotide base sequencing. The obtained sequences were partially analyzed to identify variations in the targeted segment of the *IGF-1* gene. It was found that the mutation occurred at position 102 in the promoter region, indicating that it did not result in amino acid changes in the IGF-1 protein.

Table 1 presents the genetic structures based on the variation site G>T. Three genetic structures were identified based on the sequence analysis results, showing highly significant differences ($P < 0.01$) for the genetic structures GG, GT, and TT, with frequencies of 0.34, 0.58, and 0.08, respectively. This indicates a clear prevalence of individuals carrying the hybrid genetic structure GT for the identified site, followed by the homozygous genetic structure GG, with a lower frequency of individuals carrying the homozygous genetic structure TT in the studied samples. The allele frequencies of G and T were 0.63 and 0.37, respectively, with the G allele having a higher frequency compared to the T allele. This suggests that the G allele can be utilized for selection due to its high frequency in the studied gene. Our study did not agree with (Ogunpaimo *et al.*, 2021) in their study on the *IGF-1* gene in dual-purpose FUNAAB_Alpha, Sasso, and Kuroiler breeds, where the AC genetic structure had a frequency of 43%, followed by the CC genetic structure with a frequency of 35%, and the AA genetic structure with the lowest frequency of 22%. The allele frequency for allele A was 58%, while the allele frequency for allele C was 42%. This difference may be attributed to the variation between the breeds.

Table 1: Frequency of Genotypes and Allele Frequencies for the *IGF-1* Gene Mutation (G>T.102)

Mutation	Genotypes	Number	Frequency of Genetic	Allele	Frequency	Chi-square (X^2) Value
(102.G>T)	GG	17	0.34 AB	G	0.63 A	2.97
	GT	29	0.58 A	T	0.37 B	
	TT	4	0.08 B			
Total	50					

From tables (2) and (3), it is evident that there were no significant differences between the genetic variants (GT, GG, TT) of the *IGF-1* gene in body weight and weight gain during the first and second weeks of rearing. However, in the third week, there was a significant superiority ($P \leq 0.05$) in favor of the TT genetic variant. This superiority continued in the fourth and fifth weeks over the GT and GG genetic

variants. These results differ from the findings of (AL_Hassani and AL_Suhail, 2017), where a significant superiority ($P \leq 0.05$) was observed in body weight for the TT genetic variant in the first week, surpassing the TC and CC genetic variants. No significant differences were observed in weight gain for all weeks of the experiment in the Coob 500 strain.

The variation in previous results regarding the different phenotypic manifestations of the *IGF-1* gene and body weight, as well as weight gain, may be attributed to the different experimental strains used in previous studies, relative to prevailing environmental management conditions. The superiority observed in our study in terms of weight and weight gain, accompanied by the same weeks, may be due to the physiological role of the T allele, as well as the impact of the single mutation and its physiological effects, in addition to the close correlation between the studied gene and growth hormone.

Table 2: Relationship between Genotypes of *IGF-1* Gene and Body Weights in Ross 308 Broiler Chickens

Weight (g)	Genotypes	Number	Mean \pm Standard Error	Significance
Week 1	GT	29	212.55 \pm 15.42	N.S
	GG	17	220.59 \pm 13.96	
	TT	4	227.25 \pm 9.54	
	Total	50	216.46 \pm 15.15	
Week 2	GT	29	610.62 \pm 54.97	N.S
	GG	17	626.29 \pm 49.07	
	TT	4	660.25 \pm 35.05	
	Total	50	619.92 \pm 52.78	
Week 3	GT	29	1236.90 \pm 132.46b	*
	GG	17	1266.24 \pm 132.69ab	
	TT	4	1405.75 \pm 86.07 a	
	Total	50	1260.38 \pm 135.25	
Week 4	GT	29	1957.93 \pm 244.09 b	*
	GG	17	2005.88 \pm 216.49ab	
	TT	4	2233.75 \pm 130.18a	
	Total	50	1996.30 \pm 236.42	
Week 5	GT	29	2544.34 \pm 290.25 b	*
	GG	17	2588.18 \pm 269.76 ab	
	TT	4	2873.00 \pm 189.88a	
	Total	50	2585.54 \pm 286.12	

Significantly different at a significance level of ($P < 0.05$)*

N.S: Indicates no significant differences between means

Table 3: Relationship between Genotypes of *IGF-1* Gene and Weight Gain in Ross 308 Broiler Chickens

Weekly weight gain (g)	Genotypes	Number	Mean \pm Standard Error	Significance
Week 1	GT	29	172.55 \pm 15.42	N.S
	GG	17	180.59 \pm 13.96	
	TT	4	187.25 \pm 9.54	
	Total	50	176.46 \pm 15.15	
Week 2	GT	29	444.90 \pm 110.90	N.S
	GG	17	461.24 \pm 104.85	
	TT	4	486.25 \pm 84.17	

Week 3	Total	50	453.76 ± 105.85	*
	GT	29	626.59 ± 83.64b	
	GG	17	639.94 ± 91.98ab	
	TT	4	745.50 ± 57.09a	
Week 4	Total	50	640.64 ± 89.29	*
	GT	29	721.03 ± 136.76 b	
	GG	17	739.65 ± 93.81ab	
	TT	4	828.00 ± 76.92a	
Week 5	Total	50	735.92 ± 121.46	*
	GT	29	586.41 ± 102.97 ab	
	GG	17	582.29 ± 104.20b	
	TT	4	639.25 ± 73.71a	
	Total	50	589.24 ± 100.81	

* Significantly different at a significance level of ($P < 0.05$)

N.S: Indicates no significant differences between means

Table (4) shows no significant differences for the genetic variants GG, GT, and TT of the *IGF_1* gene with regards to bird length in the first, second, and fifth weeks. However, there was a significant superiority ($P \leq 0.05$) for the genetic variant TT compared to the other genetic variants in the third and fourth weeks of the breeding process. These results do not align with the findings of (AL_Hassani *et al.*, 2018), where the TC variant in the Coob500 meat chicken breed outperformed the TT and CC variants significantly ($P \leq 0.05$). The increase in bird length in our study may be attributed to higher growth rates and weight gain, which consequently contributed to an increase in bird length.

Table (4): Relationship between the Genotypes of the *IGF_1* Gene and Bird length for Ross 308 meat chickens

Bird length (CM)	Genotypes	Number	Mean ± Standard Error	Significance
Week 1	GT	29	11.29 ± 0.30	N.S
	GG	17	11.42 ± 0.38	
	TT	4	11.85 ± 0.24	
	Total	50	11.38 ± 0.35	
Week 2	GT	29	14.49 ± 0.63	N.S
	GG	17	14.97 ± 0.87	
	TT	4	14.85 ± 0.76	
	Total	50	14.68 ± 0.75	
Week 3	GT	29	18.97 ± 0.92 b	*
	GG	17	19.11 ± 1.11 ab	
	TT	4	20.00 ± 1.22 a	
	Total	50	19.10 ± 1.02	
Week 4	GT	29	21.81 ± 1.18b	*
	GG	17	21.87 ± 0.97 ab	
	TT	4	22.48 ± 0.42 a	
	Total	50	21.88 ± 1.07	
Week 5	GT	29	26.01 ± 1.07	N.S
	GG	17	26.22 ± 0.80	
	TT	4	26.48 ± 0.45	
	Total	50	26.12 ± 0.95	

Significantly different at a significance level of ($P < 0.05$) *

N.S: Indicates no significant differences between means

The results from Table (5) indicate no significant differences among the genetic variants GG, GT, and TT in back width in the first, third, fourth, and fifth weeks. However, there was a significant superiority ($P \leq 0.05$) of the genetic variant GG over GT and TT in back width in the second week of breeding. This could be attributed to the increased weight of the back portion for this genetic variant (GG).

Table (5): Relationship between the Genotypes of the *IGF_1* Gene and Back width in Ross 308 broilers

Back Width (cm)	Genotypes	Number	Mean \pm Standard Error	Significance
Week 1	GT	29	6.30 ± 0.13	N.S
	GG	17	6.29 ± 0.14	
	TT	4	6.35 ± 0.10	
	Total	50	6.30 ± 0.13	
Week 2	GT	29	$7.79 \pm 0.46b$	*
	GG	17	$8.18 \pm 0.47a$	
	TT	4	$7.83 \pm 0.17ab$	
	Total	50	7.93 ± 0.48	
Week 3	GT	29	10.40 ± 0.30	N.S
	GG	17	10.54 ± 0.27	
	TT	4	10.60 ± 0.18	
	Total	50	10.47 ± 0.29	
Week 4	GT	29	12.27 ± 0.21	N.S
	GG	17	12.42 ± 0.23	
	TT	4	12.40 ± 0.14	
	Total	50	12.33 ± 0.22	
Week 5	GT	29	13.10 ± 0.17	N.S
	GG	17	13.18 ± 0.10	
	TT	4	13.08 ± 0.10	
	Total	50	13.12 ± 0.15	

* Significantly different at a significance level of ($P < 0.05$)

N.S: Indicates no significant differences between means

Table (6) shows no significant differences between the genetic variations GT, GG, and TT of the *IGF_1* gene in chest fullness. However, the results indicate significant differences ($P \leq 0.05$) among the genetic variations in carcass length. The TT genetic variation outperforms the GT and GG variations, suggesting that the increase in carcass length may be attributed to the superiority of individuals with the TT genetic variation in bird length.

Table (6): Relationship between the Genotypes of the *IGF_1* Gene and the Chest fullness and Carcass length of Ross 308 broiler chickens

Adjective	Genotypes	Number	Mean \pm Standard Error	Significance
Chest fullness	GT	29	1.22 ± 0.07	N.S
	GG	17	1.24 ± 0.06	
	TT	4	1.24 ± 0.05	
	Total	50	1.23 ± 0.06	
Carcass length (CM)	GT	29	$20.84 \pm 1.14b$	*
	GG	17	$20.85 \pm 0.84ab$	
	TT	4	$21.23 \pm 0.35a$	
	Total	50	20.87 ± 0.99	

Significantly different at a significance level of ($P < 0.05$)*

N.S: Indicates no significant differences between means

Table (7) did not show any significant differences between the genetic structures GT, GG, and TT of the *IGF_1* gene in the relative weight of the consumed internal organs (liver, heart, gizzard). These results were not consistent with the findings of (AL_Hassani *et al.*, 2018) who observed a significant difference ($P \leq 0.05$) in the weight of the gizzard in favor of the genetic structure TC compared to the genetic structures TT and CC in the Cobb500 strain. Additionally, they found that male individuals with the genetic structure TC significantly outperformed other genetic structures in heart weight, while female individuals with the genetic structure TT showed a significant increase ($P \leq 0.05$) in liver weight compared to other genetic structures.

On the other hand, the current study did not reveal any significant differences between the genetic structures GT, GG, and TT of the *IGF_1* gene in the abdominal fat ratio. These findings were not in line with (Kadlec *et al.*, 2011) who observed that the genetic structure AC had a lower fat ratio compared to the genetic structure AA, but the differences were not significant in the absence of the genetic structure CC.

Table (7): Relationship between the Genotypes of the *IGF_1* Gene and the relative weight of consumed internal organs and abdominal fat ratio in Ross 308 broiler chickens

Adjective	Genotypes	Number	Mean \pm Standard Error	Significance
Liver Weight (g)	GT	29	2.76 ± 0.21	N.S
	GG	17	2.73 ± 0.16	
	TT	4	2.59 ± 0.10	
	Total	50	2.74 ± 0.19	
Heart Weight (g)	GT	29	0.55 ± 0.09	N.S
	GG	17	0.53 ± 0.09	
	TT	4	0.51 ± 0.04	
	Total	50	0.54 ± 0.09	
Gizzard Weight (g)	GT	29	1.62 ± 0.20	N.S
	GG	17	1.59 ± 0.19	
	TT	4	1.40 ± 0.09	
	Total	50	1.59 ± 0.19	
Abdominal Fat Ratio (g)	GT	29	1.30 ± 0.17	N.S
	GG	17	1.26 ± 0.13	
	TT	4	1.11 ± 0.16	
	Total	50	1.27 ± 0.16	

N.S: Indicates no significant differences between means

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