RESEARCH ARTICLE Indian Journal of Animal Research, Volume 59 Issue 12: 1982-1987 (December 2025)

Identification of Genes Related to Growth (Growth Hormones and Insulin-like Growth Factor-I) for Crossbred of Produced from Commercial Broilers Breeder and Local Breeds

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10.18805/IJAR.BF-2015

ABSTRACT

Background: Growth hormone (GH) and insulin-like growth factor I (IGF-I) play a critical role in animal growth rates. We aimed to investigate the effect of GH and IGF-I genotypes on body weight (BW), dominanceand gene expression in slow-growing chickens at different ages. Where selection molecular marker has a tool in the acceleration of the genetic response of desired traits to improve production performance in chickens.

Methods: In this experiment, it was used the crossbred from commercial parent broiler (Arbor acres) with two local breeds were used to study the association among chicken growth hormones (cGH) and the insulin-like growth factor (IGF-I) genes for growth, with the aim of developing the local breed in terms of weight at marketing age. Two hundred chicks of local breeds were used in the restriction fragment length polymorphism and polymerase chain reaction techniques.

Result: Based on the results of this study, there was a substantial correlation between the GH gene and body weight at nine weeks. In both crossbreds, there were noteworthy correlations between body weight and the polymorphism of the IGF-I gene. Thus, GH gene and IGF-I may be used as a candidate gene, to improve growth traits of crossbred.

Key words: Broilers breeder, Crossbred, GH, IGF-I genes, Local breeds, Marker-assisted selection.

INTRODUCTION

Customers are preferred Meat from local strain chickens more from commercial broilers because of their high protein content, low fat and cholesterol, meat textureand excellent flavor (Promwatee and Duangjinda, 2010). However, when com-pared to commercial breed production, the native chickens' low growth rates result in lower productivity. Furthermore, native chickens are almost more expensive on the market than commercial broilers. The modern trait market is interested in the crossbreeding of Egyptian local strains with broiler parental strains in order to produce a final hybrid that is 25% local and 75% broiler. Compared to commercial broilers, the products have lower price, taste betterand have superior meat texture. To satisfy customer expectations, local strains' genetic advances should be investigated. As livestock output has increased, crossbred of carefully particular genetic strains that exhibit extremely favorable productive traits and feed conversion have replaced native varieties. Moula *et al.* (2009) reported that the poultry business is particularly affected by the well-known decline in biodiversity in farmed animal species. Actually, International corporations who supply the chicks for the manufacturing chain are in charge of managing the genotype selection of chickens (Phocas *et al.*, 2016).

According to Hartcher and Lum (2020), numerous health and welfare problems, including decreased locomotor ability, weakened immunity, heightened susceptibility to stressors and illnessesand poor meat quality, are frequently observed in high-performance chicken genotypes. Local breeds, on the other hand, an essential genetic resource in guaranteeing food security for nations worldwide and can be seen as both a vital genetic heritage and a part of the history of human communities (Boonkum *et al.*, 2021).

According to Zhang *et al.* (2008), sets of intricate genes govern growth performance parameters, which are highly important economic factors in broiler production. Because growth is a complex process that is controlled by numerous neuroendocrine pathways, it is exceedingly challenging to make quick progress within breeds using traditional genetic selection techniques. New methods for assessing genetic

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How to cite this article: Rayan, G.N. and Rezk, H.H. (2025). Identification of Genes Related to Growth (Growth Hormones and Insulin-like Growth Factor-I) for Crossbred of Produced from Commercial Broilers Breeder and Local Breeds. *Indian Journal of Animal Research*. **59(12):** 1982-1987. doi: 10.18805/IJAR.BF-2015.

Submitted: 04-05-2025 **Accepted:** 11-07-2025 **Online:** 01-08-2025

variety at the DNA level have been made possible by recent developments in molecular technology (Kaya and Yıldız, 2008). Consequently, the candidate gene technique has become a powerful tool for genetic advancement in the chicken breeding program. Using a candidate gene could increase the effectiveness of identifying the desirable characteristics required to enhance manufacturing performance. Among the most interesting potential genes for variables linked to chicken growth performance and carcass quality are those that encode the growth hormone (GH) and insulin-like growth factor-I (IGF-I).

Bijna and Karthiayini (2020) reported that the insulin-like growth factor-I is a pleotropic growth factor that modulates cell replication. It plays a crucial role in animal fertility acting as a monitoring signal. The study by Okafor *et al*. (2019) demonstrated that the chicken growth hormone gene (cGH) plays a significant role in chicken production. This gene may serve as a useful molecular marker in selective breeding programs aimed at improving body weight in SB chickens and shank length in NLC chickens.

Animal growth rates are significantly influenced by (IGF-I) gene and growth hormone (GH). In chickens that grow slowly at different ages, we sought to examine the impact of Genotypes of GH and IGF-I on gene expression and body weight (Sinpru *et al.*, 2021). The cGH is a 22-kDa protein, containing 191 amino acid residues (Hrabia *et al*., 2008). According to Kansaku *et al.* (2008), cGH in poultry has four introns and five exons, totaling 4,101 base pairs. The pituitary gland produces and secretes cGH, a poly-peptide hormone that influences several physiological processes related to growth performance (Apa *et al*., 1994). The GH gene is one of the most significant genes influencing the performance characteristics of chickens, according to several publications. It is essential for growth and metabolism rates (Vasilatos-Younken, 2000).

Thinh *et al*. (2020) reported that the SNP T3737C INS gene was associated with BW at 10 to 12 and ADG at 6-8 weeks of age. Also, the GG genotype corresponds to higher BW and ADG than the other genotypes.

One of the more important hormones required to promote the proper growth of chickens is IGF-I (Boschiero *et al.*, 2013). Moreover, IGF-I affects the release and control of growth hormones (Rousseau and Dufour, 2007). According to earlier research, up to 70 amino acids are involved in chicken IGF-I (Ballard *et al.*, 1990). IGF-I, a complex system of pep-tide hormones, activates the intrinsic tyrosine kinase domain of the insulin-like growth factor I receptor (IGFIR) by binding to it (Denley *et al.*, 2005).

While numerous genes are involved in growth aspects, the primary hormones required to maintain proper growth are (GH) and IGF-I (Kita *et al.*, 2005). The GH is involved in numerous physiological processes, such as body composition, development, aging, reproductionand egg production (Su *et al.*, 2014) and it is essential for both growth and metabolic rates (Anh *et al.*, 2015). A hormone with several metabolic and anabolic functions, IGF-I shares structural similarities with insulin (McMurtry *et al.*, 1997). For chickens to grow normally and generate bone and fat tissue, this hormone is essential (Boschiero *et al.*, 2013). However, the liver's intermediary pathway between pituitary gland and one of its target tissues, muscle, is where GH functions (Soendergaard *et al.*, 2017). GH in the liver is probably responsible for controlling the synthesis of many proteins by controlling the expression of their genes (Rastegar *et al.*, 2000). The GH controls the paracrine synthesis of IGF-I in numerous different tissues and promotes IGF-I synthesis in the liver (Laron, 2001). The main objective of the research is to study the association among chicken growth hormones (cGH) and the insulin-like growth factor (IGF-I) genes for growth, with the aim of deve-loping the local breed in terms of weight at marketing age.

MATERIALS AND METHODS

Animals and experimental design

To study the effect of genotypes on growth performance of crossbred, the GH and IGF-I genotypes from male broiler and both female fayoumi and Gimmazzh local strains chickens were determined. A total of 200 chicks for both mixed-sex crossbred of local strains were produced by mating pairs of parents based on their genotype. A completely randomized experimental design was applied. At one day, then from one to nine weeks of age, each crossbred's individual BW was noted (Image 1). To extract genomic DNA and the IGF-I and GH genes, blood samples from parent stock, local strains and crossbred chickens were gathered. Three samples from each replication were chosen at random for the gene expression analysis after the crossbred was nine weeks old. The chickens were fed three feed combinations from tube feeders ad libitum and were watered by automatic dropper drinking basins. This experiment was conducted in January 2025 at the



Image 1: The crossbred produced from commercial broilers breeder and local breeds female.

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Additionally, part of the work was conducted at Gene Tech Laboratory.

Gene expression

Material used for cDNA synthesis

Maxima First Strand cDNA Synthesis Kit for RT-qPCR #K1641 from Thermo Scientific One practical method designed for cDNA synthesis in two-step real-time quantitative RT-PCR (RT-qPCR) applications is the Maxima® First Strand cDNA Synthesis Kit. The complex enzyme Maxima Reverse Transcriptase, which is produced by the in vitro synthesis of M-MuLV RT, is used in the kit. Compared to wild type M-MuLV RT, the enzyme is substantially more thermostable, robustand has a greater rate of cDNA synthesis. Reproducible cDNA synthesis from a variety of starting total RNA levels (1 pg-5 µg) at high temperatures (50-65°C) is possible using the Maxima First Strand cDNA Synthesis Kit. The synthesis reaction can be completed in 15 to 30 minutes.

Material used for RT-PCR

Kit number K0251 for Thermo Scientific's Maxima SYBR Green qPCR Master Mix (2X) Following reverse transcription procedures (RT-qPCR) with varying initial RNA transcript dosages (5 ng to 0.5 fg), amplification was carried out using the Maxima SYBR Green/ROX qPCR Master Mix. The slope varies from -3.09 to -3.58, the correlation coefficient is more than 0.99 and the reaction efficiency falls between 90 and 110 per cent. The genotypes of GH and IGF-I were determined by the polymerase chain reaction (PCR) utilizing restriction fragment length polymorphism (RFLP). Table 1 lists the forward and reverse primers, enzymesand annealing temperatures for the GH and IGF-I gene PCR-RFLP. About the gene IGF-I .

Statistical analysis

According to the methods outlined by (Falconer and Mackay, 2001). A genotype was ex-cluded from the analysis if its frequency was less than 2%. The following model was used to assess the relationship between candidate genes and characteristics using pooled data from two crossbreds and the fixed effect of the altered line effect:

$$yij = \mu + Gi + HI + HI*GJ + eij$$

Gi = Fixed effect of the gene.
HI = Different hybrid cross effect.
HI×Gi = Interac-tion effect between the examined breed and gene.
eijk = Residual random errorand is trait observation (BW).

RESULTS AND DISCUSSION

Body weight for crossbred

Results show in Table (2) that the mean body weight crosses 1 and 2 was 25 and 27 gm, respectively. In this study, crossbred 2 showed a heavier body weight (1020 gm/ bird) at 6 weeks and 9 weeks (2111.25) in comparison with other native chicken genotypes. As stated to (Gharib *et al.*, 2006), hatchability percentage of heavier Fayoumi chicks decreased significantly. It has been documented that hatchability is impacted by the female parent's body weight (Fasenko *et al.*, 1992). Additionally, Gimmizah chickens chosen based on body weight exhibit a notable increase in body weight for the chosen first generation as compared to the base generation (Boutrous, 2020).

Allele frequencies and genotype of GH gene

The genotype of the GH gene were ascertained after the crossbred line populations were genotyped. According to Table 3, that in crossbreds 1 and 2, The GH gene for a crossbred 1 was inherited at a rate of 2.29 from the broiler

Table 1: Primer sequences and their applications.

Gene	Primer (Forward/Reverse)	AT (°C)	PCRproduct (bp)	Enzyme
cGH	5′- TCCCAGGCTGCGTTTTGTTACTC-3′	65	429	EcoRV
	5′- ACGGGGGTGAGCCAGGACTG-3′			
IGF-I	5′- TCAAGAGAAGCCCTTCAAGC-3′	60	813	HinfI
	5′- CATTGCGCAGGCTCTATCTG-3′			

PCR: Polymerase chain reaction; GH: Chicken growth hormone gene; IGF-I: Insulin-like growth factor gene (Anh *et al.*, 2015).

Table 2: Body weight of crossbred.

	Initial wt.(g)	BW1	BW2	BW3	BW4	BW5	BW6	BW7	BW8	BW9
Fayoumi × broiler	25	40	137.5	273.75^{b}	445^{b}	635^{b}	898.75^{b}	1227.5^{b}	1602.5^{b}	1642^{b}
gimmizh × broiler	27	38.33	136.25	290^{a}	462.5^{a}	705^{a}	1020^{a}	1465^{a}	1815^{a}	2111.25^{a}
P value	NS	NS	NS	0.0001	0.001	0.001	0.001	0.001	0.001	0.0001

^{a,b} Significant differences exist between the means in the columns with different superscripts. Significant when P≤0.01. NS= Non significant.

breeder. Also, in (Table 4) that crossbred 1 inherited at rate 0.44 from female Fayoumi. In Table (5) that crossbred 2 was inherited at a rate of 1.97 from the broiler breeder. Also, in Table (6) that crossbred 2 inherited at rate 4.57 from female Gimmazh. 191 amino acid residues make up the 22 kDa protein known as cGH (Hrabia *et al.*, 2008). According to (Kansaku *et al.*, 2008), cGH in poultry has four introns and five exons, totaling 4,101 base pairs. The pituitary gland produces and secretes cGH, a polypeptide hormone that influences several physiological processes related to growth performance (Apa *et al.*, 1994). The cGH gene is one of the most significant genes influencing the performance characteristics of chickens, according to several publications. It is essential for growth and metabolism rates (Vasilatos-Younken, 2000).

IGF-I gene and allele frequencies

Following the genotyping of the crossbred line populations, the IGF-I gene was computed, as shownin Table 7. The gene IGF-I for a crossbred 1 was inherited at a rate of 0.24 from the broiler breeder. Also, in Table (8) that crossbred 1 inherited at rate 0.36 from female Fayoumi. In Table 9 that crossbred 2 was inherited at a rate of 1.26 from the broiler breeder. One of the more important hormones required to promote the proper growth of chickens is IGF-I (Boschiero *et al.*, 2013). Additionally, growth hormone

Table 3: Genotype and allele frequencies of GH genes in crossbred and broiler male.

	b-actin=B	cGH=A	Δ ct=A-B	Avg	ΔΔ ct	Fold change
crossbred 1	26.32	25.39	-0.93	-2.96667	-1.19667	2.292094724
♂ Broiler	30.15	29.14	-1.01	-1.77	0	1

Table 4: Genotype and allele frequencies of GH genes in cross bred and Fayoumi female.

	b-actin=B	cGH=A	Δ ct=A-B	Avg	ΔΔ ct	Fold change
Crossbred 1	26.32	25.39	-0.93	-	1.163333	0.446479756
♀ Fayoumi	28.18	25.19	-2.99	2.96667	0	1

Table 5: Genotype and allele frequencies of GH genes in crossbred 2 and broiler male.

	b-actin=B	cGH=A	Δ ct=A-B	Avg	ΔΔ ct	Fold change
Crossbred 2	27.37	24.81	-2.56	-2.75	-0.98	1.972465409
♂ Broiler	30.15	29.14	-1.01	-1.77	0	1

Table 6: GH gene genotype and allele frequencies in crossbreds with Gimmazh.

	b-actin=B	cGH=A	Δ ct=A-B	Avg	ΔΔ ct	Fold change
Crossbred 2	27.37	24.81	-2.56	-2.75	2.19333	4.573609948
♀ Gimmazh	25.64	25.45	-0.19	-	0	1

Table 7: IGF-I gene and allele frequencies in broiler males and crossbred 1.

	b-actin=B	cGH=A	Δ ct=A-B	Avg	ΔΔ ct	Fold change
Crossbred 1	26.32	25.64	-0.68	0.76667	2.02	0.246558176
♂ Broiler	30.15	27.18	-2.97	2.78667	0	1

Table 8: IGF-I gene genotype and allele frequencies in crossbred 2 and broiler male.

	b-actin=B	cGH=A	Δ ct=A-B	Avg	ΔΔ ct	Fold change
Crossbred 2	31.27	29.21	-2.06	3.12333	0.33667	1.262835451
♂ Broiler	30.15	27.18	-2.97	2.78667	0	1

Table 9: IGF-I gene genotype and allele frequencies in crossbred 2 and female Gimmazh.

	b-actin=B	cGH=A	Δ ct=A-B	Avg	ΔΔ ct	Fold change
Crossbred 2	27.37	24.33	-3.04	1.97333	-0.5	1.414213562
♂ Gimmazh	25.64	22.78	-2.86	1.47333	0	1

release and regulation are influenced by IGF-I (Rousseau and Dufour, 2007). According to earlier research, up to 70 amino acids are involved in chicken IGF-I (Ballard *et al.*, 1990). The complex system of peptide hormones known as IGF-I binds to activate its intrinsic tyrosine kinase domain capabilities (Denley *et al.*, 2005).

CONCLUSION

In conclusion, the GH gene polymorphisms may be used markers gene in commercial hybrid chicken breeding operations to improve growth characteristics. The findings demonstrated that we may create a local hybrid by combining the immunological traits and environmental adaptation of commercial broilers with their productive traits in terms of body weight.

ACKNOWLEDGEMENT

This work was supported by the Deanship of Scientific Research, Vice Presidency for Graduate Studies and Scientific Research, King Faisal University, Saudi Arabia [Grant No. KFU250272]'.

Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal Care and Handling Techniques were approved by the University of Animal Care Committee.

Funding

This work was supported by the Deanship of Scientific Research, Vice Presidency for Graduate Studies and Scientific Research, King Faisal University, Saudi Arabia [Grant No. KFU250272].

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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