



**COMPARATIVE SEQUENCE ANALYSIS AND FUNCTIONAL ANNOTATION OF
THE INSULIN-LIKE GROWTH FACTOR-1 (IGF-1) GENE IN RED JUNGLEFOWL
(*GALLUS GALLUS*) AND SELECTED AVIAN SPECIES**

**Busari I. O¹., Shittu A. O¹, Akaka A. O¹, Akorchio, J. D¹, Mohammed A. K¹, Rahmah
A.G¹, Egena S. S.A¹, Otu, B. O¹, Sikiru, A. B²**

¹Department of animal production, Federal University of Technology, Minna

²Federal University of Agriculture, Zuru, Kebbi State

busariibrahim012@gmail.com

ABSTRACT

Insulin-like Growth Factor 1 (IGF-1) is a major anabolic peptide hormone that mediates the biological actions of growth hormone and plays essential roles in myoblast proliferation, satellite cell activation, skeletal muscle growth, and overall development in poultry. This study aimed to characterize the genetic variation, sequence conservation, and functional attributes of the gene in Gallus gallus using bioinformatics approaches. The reference mRNA sequence of IGF-1 (NM_001004384.3) was retrieved from the GenBank database together with twelve homologous avian sequences representing 12 species, with nucleotide identity values ranging from 98.78% to 100%. BLAST, MEGA12, and SIFT were used for sequence similarity assessment, phylogenetic analysis, and prediction of the biological effects of non-synonymous single nucleotide polymorphisms (nsSNPs), respectively. Sequence analyses were conducted to evaluate genetic variation, infer evolutionary relationships, and predict the biological effects of non-synonymous single nucleotide polymorphisms (nsSNPs). Pairwise genetic distances ranged from 0.00 to 0.03, indicating low genetic divergence among the sequences examined. A total of five unique missense variants, represented by ten transcript-specific annotations, were identified and assessed using the SIFT algorithm, of which three variants (rs735167637, rs734429485, and rs737895725) were predicted to be deleterious, indicating their potential to alter IGF-1 protein's function and warrant further investigation in relation to growth-related traits in red junglefowl. Phylogenetic analysis revealed that Gallus gallus clustered closely with other members of the family Phasianidae, especially Excalfactoria chinensis and related land-fowl species. This relationship was strongly supported by a bootstrap value of 66%, indicating high confidence in the inferred evolutionary grouping, while the low nucleotide substitution rate

(0.001) reflected high sequence conservation among the species examined. These findings demonstrate that the gene is highly conserved across Phasianidae species and highlight specific deleterious variants that may serve as potential molecular markers for future studies on growth performance and genetic improvement in red junglefowl.

Keywords: comparative genomics; phylogeny; SNP prediction; poultry genetics; bioinformatics

INTRODUCTION

One of the primary growth-influencing genes affecting economically important traits in livestock is insulin-like growth factor 1 (*IGF-1*), which plays an indispensable role in regulating cell growth, proliferation, differentiation, and apoptosis (Hossner *et al.*, 2010; Agrogiannis *et al.*, 2014). *IGF-1* is a naturally occurring protein member of the *IGF* system and possesses a structure that is highly similar to insulin (Meyts and Whittaker, 2002). The red junglefowl *IGF-1* gene, located on the short arm of chromosome 1, is synthesized as a precursor protein, and acts as a mediator for several biological effects, including: stimulating myogenesis, regulating somatic growth, skeletal integrity, muscle development, increasing glucose absorption, promoting the synthesis of lipids, DNA, RNA, and proteins (Klein *et al.*, 1996; Etherton, 2004; Philippou *et al.*, 2007; Hergaty *et al.*, 2006; Yu *et al.*, 2015). Variations in this gene have been associated with significant phenotypes across species, including mice (Fukunaga *et al.*, 2018), pigs (Ojeda *et al.*, 2008), and sheep (Li *et al.*, 2021). Mutations in *IGF-1* regulatory regions, particularly biologically significant variants in the promoter region, are associated with changes in growth and carcass traits, such as abdominal fat deposition in poultry species (Bian *et al.*, 2008). The *IGF-1* gene plays a pivotal role in embryonic and postnatal development; therefore, elucidating its evolutionary history and mutation effects is imperative to support marker-assisted selection in poultry species (Guo *et al.*, 2023). While traditional wet laboratory experiments to illustrate mutations in such growth-regulating genes are essential, they are often laborious, expensive, and time-consuming, particularly when measurements require slaughtering birds to assess traits like abdominal fat.

To overcome these limitations, modern biological research has shifted toward bioinformatics (Baxevanis *et al.*, 2020), which involves discovering, developing, and implementing computational algorithms and software tools that deepen understanding of complex biological

processes. In a developing country like Nigeria, bioinformatics will play a pivotal role in agriculture, where it can be utilized to analyze livestock genomics and proteomics data to drive significant genetic improvements. Advances in these computational methods can improve livestock productivity far more efficiently than laboratory methods (Rude *et al.*, 2018). These *in silico* approaches allow biologists to analyze hereditary information using robust databases such as GenBank (NCBI), Swiss-Prot, and the Protein Information Resource (PIR).

A major challenge in animal breeding remains the understanding of the genetic basis of phenotypic diversity within and among species (Woolliams and Oldenbroek 2017). In Nigeria, livestock selection has often been carried out with limited knowledge of molecular and cellular reactions (Mauki *et al.*, 2022). To pinpoint the precise impact of point mutations on gene functions or products, advanced computational tools like the Variant Effect Predictor (VEP) in Ensembl are highly effective, as they provide a high-resolution platform to identify variants that alter amino acid sequences and distort protein folding. Despite the recognized importance of *IGF-1* in growth regulation, information on the sequence variation, evolutionary relationships, and potential effects of genetic variants within the red junglefowl *IGF-1* gene remains limited. Information on variants that may influence growth-phenotypic traits in *Gallus gallus* remains limited.

Addressing this gap requires a systematic *in silico* characterisation of the gene across avian species, particularly *Gallus gallus*, to understand better its sequence conservation, evolutionary relationships, and the potential functional consequences of naturally occurring variants. This study therefore aims to characterise the *IGF-1* gene in *Gallus gallus* using computational approaches. The study will specifically seek to

1. Compare the nucleotide sequences of the gene across selected avian species.
2. Perform multiple sequence alignment and calculate genetic distances among the retrieved avian *IGF1* sequences.
3. Construct a phylogenetic tree to determine the evolutionary relationships of the gene across the selected avian species.
4. Predict the potential impact of deleterious variants within the gene using variant effect prediction tools.

5. Analyze the protein-protein interaction network of the protein.
6. Design specific PCR primers for the amplification of the *IGF1* gene.

MATERIALS AND METHODS

Sequence of Avian species

A total of twelve (12) *IGF-1* sequences were selected from the GenBank of the National Center for Biotechnology Information to provide a representative yet manageable set of closely related avian homologues that met a predefined BLAST criteria of high query coverage (85–100%), ensuring that alignments spanned the majority of the gene region, including conserved coding sequences and functional domains, and percentage identity (88–100%) to ensure high-confidence homolog selection, and minimize inclusion of distantly related or non-orthologous sequences. Species were selected based on the availability of complete, high-quality *IGF-1* sequences meeting these criteria in GenBank. The GenBank accession numbers of the sequences selected are: NM_001004384.3 (Chicken), XM_072326435.1 (King quail), XM_052668584.1 (Lesser prairie-chicken), XM_048960856.1 (Rock-ptarmigan), XM_042837643.1 (Greater sage-grouse), XM_065847240.2 (Band tailed pigeon), XM_071728313.1 (Tourmaline-sunangel), XM_009913912.2 (White tailed eagle), XM_021387400.1 (Helmeted guinea fowl), XM_052790424.1 (Harpy eagle), XM_010566305.1 (Bald eagle), and XM_009708171.1 (Red-legged seriema).

Multiple Sequence Alignment

Sequence alignment and comparative analyses were performed using the MUSCLE algorithm implemented in the Molecular Evolutionary Genetics Analysis (MEGA) version 12 software. The aligned nucleotide sequences were then used to identify sequence variations and perform phylogenetic analysis.

Variant Effect Prediction

In silico biological analysis of identified missense mutations was performed using the Variant Effect Predictor (VEP) available through the Ensembl website. The potential impact of amino acid substitutions on protein function was assessed using the Sorting Intolerant From Tolerant (SIFT) algorithm integrated within VEP. SIFT predicts the potential effect of an amino acid

substitution on protein function. SIFT was selected because it is integrated within the Ensembl VEP framework and provides functional predictions based on evolutionary conservation of amino acid residues. Variants with a SIFT score less than 0.05 were classified as deleterious, indicating a potential deleterious effect on protein function, whereas variants with a SIFT score greater than or equal to 0.05 were considered tolerated, suggesting little or no functional impact on the protein.

Evolutionary and Phylogenetic Analysis

The evolutionary relationships among the 12 selected avian *IGF1* nucleotide sequences were inferred using the pairwise distance method based on the p-distance model implemented in MEGA software (version 12). Ambiguous positions, gaps, and missing data were handled using pairwise deletion to maximize data retention while minimizing alignment bias. To assess the confidence and reliability of the resulting phylogenetic tree branching patterns, we performed bootstrap testing with 1,000 replicates.

Protein-Protein Interaction

Protein-Protein Interaction (PPI) analysis of the *IGF-1* gene was performed using the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database v12. The analysis predicted functional associations and interaction networks involving *IGF-1* in *Gallus gallus*. The protein sequence was queried against the STRING database, and interactions were retrieved based on known and predicted associations derived from the experimental data, curated databases, gene co-expression, and computational prediction methods. A high-confidence interaction score threshold of 0.700 was applied to ensure reliability of the predicted network. The resulting interaction network was used to infer potential functional partners of *IGF-1* and to provide insight into its biological role in growth and developmental pathways in poultry.

Primer Design

Gene-specific primers for the *IGF-1* gene of *Gallus gallus* were designed *in silico* using the Primer-BLAST tool available in the NCBI platform. The reference transcript used for this study was the *Gallus gallus IGF-1* mRNA sequence (Accession No. NM_001004384.3). Primer pairs were generated based on default Primer-BLAST parameters, with specificity checked against the NCBI database to minimize off-target amplification. Primers were selected based on melting

temperature (T_m) values ranging from 55–60°C, GC content between 40–60%, and amplicon sizes appropriate for efficient amplification of the target gene region for downstream molecular analyses. Primer pairs exhibiting significant self-complementarity, hairpin formation, primer-dimer tendencies, or non-specific binding sites were excluded.

Limitation of the In Silico Approach

The present study relied exclusively on computational analyses and publicly available sequence databases. Consequently, the predicted functional effects of identified variants and inferred evolutionary relationships were not validated through laboratory-based experiments. While this study used SIFT for functional prediction, incorporating additional *in silico* prediction algorithms in future work could further strengthen variant annotation. PROVEAN predictions could not be obtained because the PROVEAN web interface was inaccessible during the analysis period. Other commonly used predictors, including PolyPhen-2 and MutationAssessor, were also considered; however, their application to the present *Gallus gallus* transcript-specific variants was limited by input and annotation compatibility. Therefore, SIFT was retained for the functional prediction analysis. Reliance on a single prediction algorithm is a limitation of the present study, and future analyses should incorporate multiple complementary prediction methods where species-appropriate inputs are available. Therefore, interpret the findings as predictive evidence that requires further confirmation through molecular, functional, and gene expression studies.

RESULTS

The *IGF-1* nucleotide sequences retrieved for this study exhibited considerable variation in sequence length among the selected avian species. A total of twelve (12) reference sequences were obtained from the NCBI GenBank database, with sequence lengths ranging from 736 bp to 9,045 bp (Table 1).

The shortest sequence was observed in *Gallus gallus* (NM_001004384.3) with a length of 736 bp, representing the red junglefowl *IGF-1* gene reference sequence used in the study. In contrast, the longest sequence was recorded in *Tourmaline sunangel* (XM_071728313.1), at 9,045 bp, indicating substantial variation in gene annotation and transcript length among the selected

species. Among the remaining sequences, intermediate lengths were observed, including White-tailed eagle (1,098 bp), Harpy eagle (1,014 bp), Bald eagle (1,068 bp), and Red-legged seriema (1,070 bp), while Helmeted guinea fowl showed a comparatively longer sequence of 2,817 bp. Other species such as the King quail, Lesser prairie-chicken, Rock ptarmigan, Greater sage-grouse, and Band-tailed pigeon all exhibited substantially longer transcript lengths ranging from 5,155 bp to 8,469 bp. These differences in sequence length reflect variability in transcript annotation and gene structure across avian species, and they may influence untranslated regions (UTRs), alternative splicing, and genome assembly completeness.

Table 1: Accession number and base pair of insulin like growth factor-1 gene of *Gallus gallus* and other avian species

Species	Common name	Accession number	Base pairs
<i>Gallus gallus</i>	red junglefowl	NM_001004384.3	736
<i>Excalfactoria chinensis</i>	King quail	XM_072326435.1	5155
<i>Tympanuchus pallidicinctus</i>	Lesser prairie-chicken	XM_052668584.1	8438
<i>Lagopus muta</i>	Rock ptarmigan	XM_048960856.1	8469
<i>Centrocercus urophasianus</i>	Greater sage-grouse	XM_042837643.1	7748
<i>Patagioenas fasciata</i>	Band tailed pigeon	XM_065847240.2	8390
<i>Heliangelus exortis</i>	Tourmaline sunangel	XM_071728313.1	9045
<i>Haliaeetus albicilla</i>	White tailed eagle	XM_009913912.2	1098
<i>Numida meleagris</i>	Helmeted guinea fowl	XM_021387400.1	2817
<i>Harpia harpyja</i>	Harpy eagle	XM_052790424.1	1014
<i>Haliaeetus leucocephalus</i>	Bald eagle	XM_010566305.1	1068
<i>Cariama cristata</i>	Red-legged seriema	XM_009708171.1	1070

Figure 1 presents the multiple sequence alignment of the IGF-1 gene sequences from *Gallus gallus* and selected avian species. The alignment revealed high sequence conservation across species, with several regions showing identical nucleotide residues. However, a few nucleotide substitutions were observed at specific positions, indicating genetic variation among the avian species. The high level of conservation suggests that the IGF-1 gene is evolutionarily conserved and may play essential biological roles across birds.

The phylogenetic analysis of IGF-1 nucleotide sequences (Figures 2 and 3) revealed a clear pattern of inter-clustering among the selected poultry species. Table 2 presents the resulting genetic-distance matrix. It shows the number of base differences per site between sequences. The analysis included 12 nucleotide sequences. The pairwise deletion option was applied to all ambiguous positions for each sequence pair, resulting in a final data set comprising 831 positions.

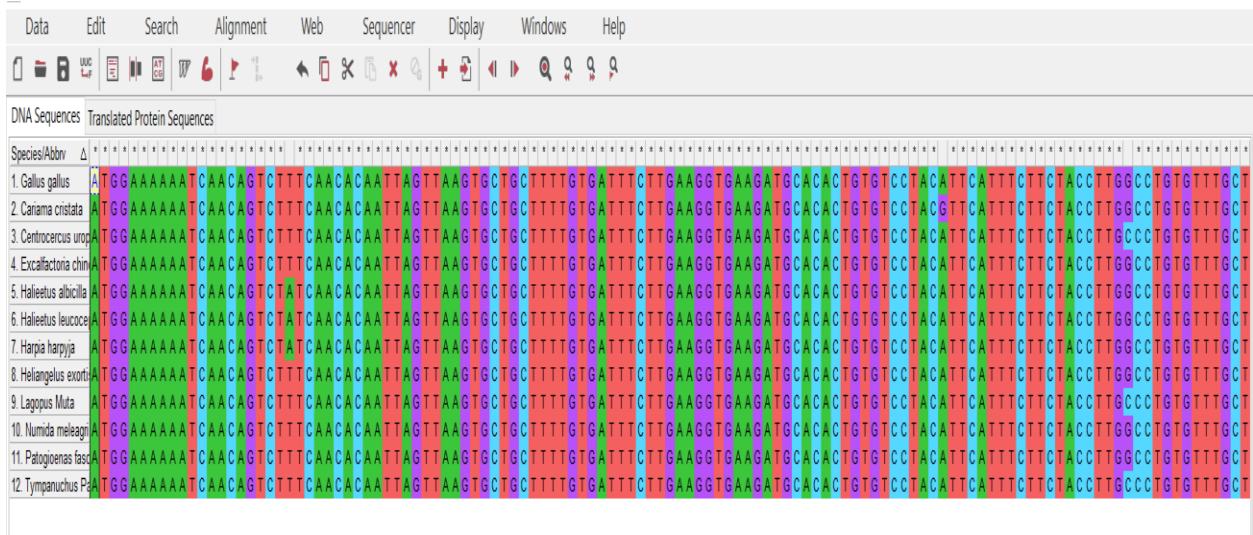


Table 2: Pairwise Genetic Distance Estimates among Avian *IGF-1* Sequences Based on Nucleotide Substitutions

	1	2	3	4	5	6	7	8	9	10	11	12
1												
2	0.02											
3	0.01	0.03										
4	0.01	0.03	0.02									
5	0.02	0.01	0.03	0.02								
6	0.02	0.01	0.03	0.03	0.02							
7	0.02	0.01	0.03	0.2	0.00	0.00						
8	0.02	0.02	0.03	0.03	0.01	0.01	0.01					
9	0.01	0.03	0.00	0.02	0.03	0.03	0.03	0.03				
10	0.02	0.02	0.03	0.02	0.02	0.03	0.02	0.03	0.03			
11	0.02	0.01	0.03	0.03	0.01	0.01	0.01	0.01	0.03	0.03		
12	0.01	0.03	0.00	0.02	0.03	0.03	0.03	0.03	0.00	0.03	0.03	

Where 1 = *Gallus gallus* 2 = *Cariama cristata*, 3 = *Centrocercus urophasianus* 4 = *Excalfactoria chinensis*, 5 = *Haliaeetus albicilla*, 6 = *Haliaeetus leucocephalus*, 7 = *Harpia harpyja*, 8 = *Helianthus exortis*, 9 = *Lagopus muta*, 10 = *Numida meleagris*, 11 = *Patagioenas fasciata*, 12 = *Tympanuchus pallidicinctus*

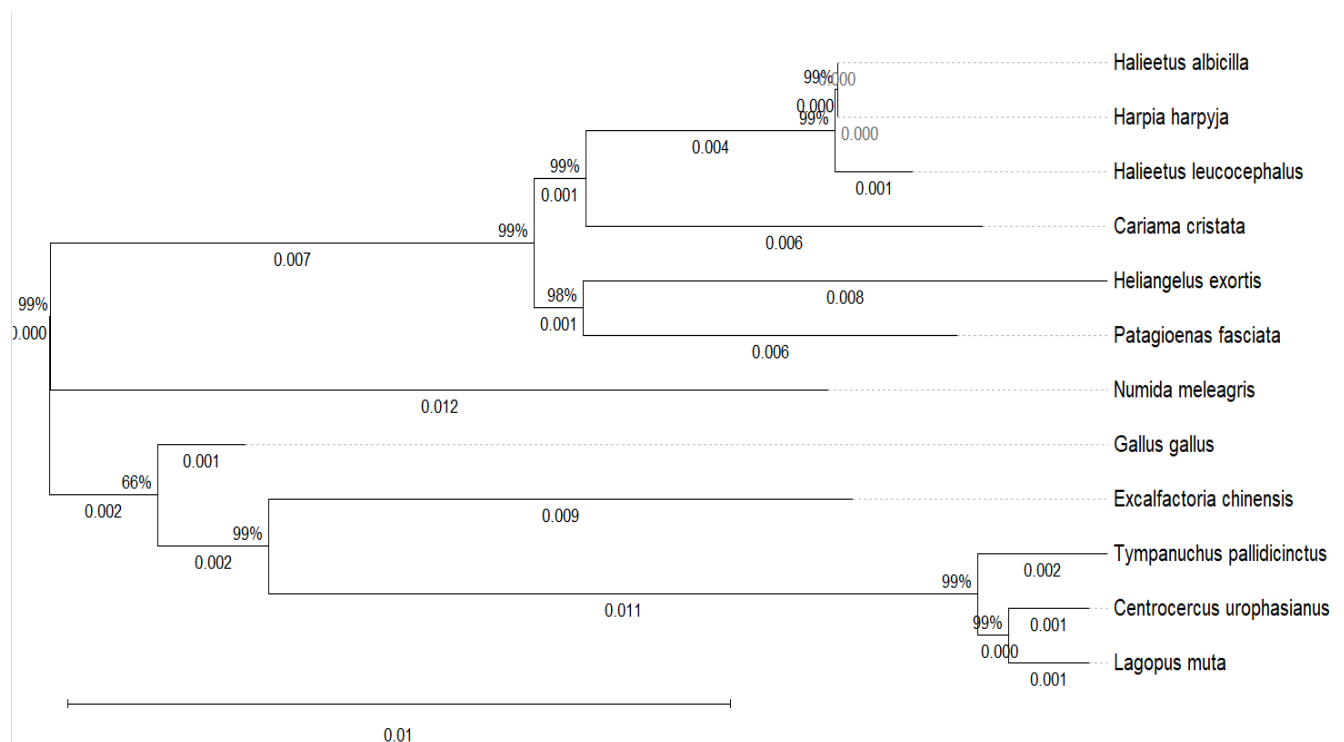


Fig 2: Phylogenetic tree of *Gallus gallus* IGF-1 and the other avian species nucleotide sequences

The results of the *in silico* functional prediction of missense mutations identified in the *IGF-1* gene using the Ensembl Variant Effect Predictor (VEP), based on SIFT scores, are presented in Table 3. The VEP analysis of the *IGF-1* gene in *Gallus gallus* revealed multiple missense mutations with potential functional consequences. The amino acid substitutions observed included H/P, Y/S, A/P, Y/F, and M/V changes at different loci within the gene. SIFT-based functional prediction classified these variants as deleterious or tolerated, depending on their scores relative to the 0.05 threshold. The presence of deleterious variants suggests that certain positions within the *IGF-1* protein are evolutionarily conserved and functionally important, while tolerated substitutions may represent neutral evolutionary changes with limited impact on protein structure or function. Five missense variants were identified in the *IGF-1* gene, comprising three predicted deleterious variants and two tolerated variants based on SIFT scores. Duplicate variant entries represent the same genomic variants annotated against different *IGF-1* transcript isoforms; differences in amino acid coordinates and SIFT scores reflect transcript-specific consequences.

Table 3: *In silico* functional analysis of *Gallus gallus* IGF-1 missense mutations

Variant ID	Chr: bp	Alleles	Transcript/Isoform ID	AA Change	SIFT Score	Effect
rs735167637	1:55285734	A/C	ENSGALT00000020816.4	H55P	0.00	Deleterious
			ENSGALT00000152628.1	H31P	0.00	
rs737280459	1:55285743	A/C	ENSGALT00000020816.4	Y58S	0.15	Tolerated
			ENSGALT00000152628.1	Y34S	0.43	
rs732345984	1:55285781	G/C	ENSGALT00000020816.4	A71P	0.08	Tolerated
			ENSGALT00000152628.1	A47P	0.26	
rs734429485	1:55285857	A/T	ENSGALT00000020816.4	Y69F	0.03	Deleterious
			ENSGALT00000152628.1	Y72F	0.03	
rs737895725	1:55320097	A/G	ENSGALT00000020816.4	M131V	0.00	Deleterious
			ENSGALT00000152628.1	M107V	0.04	

A = adenine, T = Thymine, C = Cytosine, G = Guanine, H = Histidine, P = Proline, Y = Tyrosine, S = Serine, F = Phenylalanine, V = Valine, and M = Methionine.

Furthermore, protein–protein interaction (PPI) analysis of *IGF-1*, as shown in the interaction network figure (Fig 3), highlighted its functional connectivity with key regulatory proteins involved in muscle growth and developmental signalling pathways. The interaction network indicates that *IGF-1* does not function in isolation, but is embedded within a broader regulatory framework involving growth factors and signaling mediators. The resulting interactome stabilized at a remarkably high density, consisting of exactly 11 nodes and 36 edges. An evaluation of the interaction metrics (Table 4) revealed the interactions’ confidence scores.

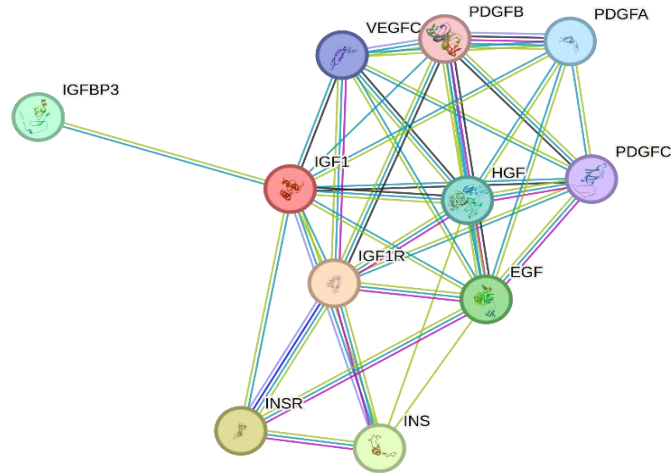


Fig 3: Protein-Protein Interaction (Ppi) network of insulin like growth factor-1 (*IGF-1*) gene of *Gallus gallus*

IGF1 =Insulin-Like Growth Factor 1, *IGF1R* =Insulin-Like Growth Factor 1 Receptor, *IGFBP3* =Insulin-Like Growth Factor Binding Protein 3, *INS*=Insulin, *INSR* =Insulin Receptor, *EGF*=Epidermal Growth Factor, *HGF*=Hepatocyte Growth Factor, *PDGFA*=Platelet-Derived Growth Factor Subunit A, *PDGFB*=Platelet-Derived Growth Factor Subunit B, *PDGFC*=Platelet-Derived Growth Factor Subunit C, *VEGFC*=Vascular Endothelial Growth Factor C

Table 4: Protein-Protein Interaction partners of IGF-1 gene

Interacting protein	Interaction Score
Insulin-like Growth Factor 1Receptor	0.961
Insulin Receptor	0.952
Insulin	0.944
Epidermal Growth Factor	0.934
Insulin-like Growth Factor Binding Protein 3	0.930
Hepatocyte Growth Factor	0.926
Platelet-derived Growth Factor A	0.908
Vascular Endothelial Growth Factor C	0.903
Platelet-derived Growth Factor C	0.901
Platelet-derived Growth Factor	0.900

The primers designed to amplify the *IGF-1* gene (Table 5) were highly specific to the conserved regions identified in the multiple sequence alignment. The primer pairs were selected to target stable regions with minimal sequence variation across the species, ensuring efficient amplification and reducing the likelihood of non-specific binding.

Table 5: Primers designed for *IGF-1* gene of *Gallus gallus*

Primer	Tm	GC (%)	At (C)
Forward: TGCTGCTTCCAGAGTTGTGACC	63.02	54.55	56
Reverse: TGGCATATCAGTGTGGCGCT	62.26	55.00	56

Tm = Melting Temperature, GC = Guanine-Cytosine, At= Annealing Temperature

DISCUSSION

The insulin-like growth factor 1 (*IGF1*) gene is a key candidate gene involved in regulating growth, development, metabolism, and muscle accretion in poultry. Reference sequence records from the National Center for Biotechnology Information (NCBI, 2026) show that the chicken *IGF1* mRNA (NM_001004384.3) exhibits a four-exon structure spanning a length of 736 bp, which aligns with the structural characterization of the red junglefowl *IGF1* gene previously reported by Kajimoto and Rotwein (1991). As established in previous literature, this organization differs from the six-exon structure documented for *IGF1* orthologs in humans and murines (Zhao *et al.*, 2023). Previous studies established that avian *IGF1* lacks the promoter 2 and exon 2 regions observed in mammals, evidence that while the red junglefowl system remains responsive to growth hormone, its transcriptional mechanisms have diverged over evolutionary time (NCBI, 2026). The observed sequence length further supports the limited sequence divergence nature of *IGF-1* among avian species. Rotwein (2018) demonstrated extensive conservation of *IGF-1* exons among vertebrates and reported nucleotide sequence identities ranging from 93–100% between the domestic red junglefowl and other avian species, including turkey, highlighting the

evolutionary stability of this growth-regulating gene. Despite the conserved nature of the *IGF-1* gene, sequence length may vary within and among species. These differences often stem from the genomic regions analysed, the use of complete or partial coding sequences, alternative transcript variants, and evolutionary divergence among taxa. Structural genomic events such as insertions, deletions, gene duplications, and sequence rearrangements may also contribute to differences in nucleotide length and organization. These evolutionary processes promote genetic differentiation while preserving functionally important domains required for normal gene activity. The sequence variation observed among species may therefore reflect evolutionary adaptation and divergence accumulated over time, whereas the overall conservation of the *IGF-1* sequence suggests strong purifying selection on this gene. Such conservation is expected given *IGF-1*'s central role in growth signalling pathways, where substantial alterations in coding regions may impair function and are therefore less likely to be retained during evolution. Biologically, the high degree of conservation observed in this study indicates that *IGF-1* is likely under strong evolutionary constraint (Guo *et al.*, 2023). This matters because *IGF-1* is a major mediator of growth hormone signalling and participates in cellular processes associated with proliferation, differentiation, protein synthesis, and muscle development (Yoshida and Delafontaine, 2020). Conserved regions of the gene represent functionally important regions in which substantial sequence changes are less likely to be tolerated (Guo *et al.*, 2023), whereas the comparatively variable regions may provide opportunities for genetic differentiation among avian species.

The high homology (up to 99%) observed with other species such as *Excalfactoria chinensis* (King quail) emphasizes the important role of *IGF-1* as a stable, conserved downstream mediator of growth-promoting signals. This stability was also supported by Wheto *et al.* (2021), who declared that there were only slight genetic variations among genetic varieties of red junglefowl at the *IGF-1* gene locus. The multiple sequence alignment showed extensive conservation of nucleotide/amino acid residues across the studied species, with only a few variable sites observed. This high conservation suggests the gene has maintained its functional integrity throughout evolution. The conserved residues may be critical for the gene's biological activity, whereas the variable regions could contribute to species-specific genetic diversity. The phylogenetic tree grouped species by evolutionary lineage, with galliform birds forming one cluster and accipitriform birds forming another. The low substitution rate observed in *Gallus*

gallus (0.001) and the short branch lengths observed among land fowl species suggest that there is strong purifying selection and close evolutionary relationships among the species. These results agree with the low genetic variation observed by Khan *et al.* (2025) and Wheto *et al.* (2021) in the *IGF-1* gene. Consistent with Ewuola (2018), high bootstrap support for clustering closely related species further corroborates their shared ancestry. Similarly, Wainaina (2017) reported that the highly homologous *IGF-1* proteins of avian species have conserved structural conformations, indicating functional conservation across species. This evolutionary conservation is biologically relevant to poultry production because genes involved in fundamental growth pathways are expected to retain functional characteristics across closely related species (Zhang *et al.*, 2025). The clustering of the galliform species also supports Guo *et al.* (2023) on the usefulness of *IGF-1* sequence information for understanding evolutionary relationships among poultry and related avian species. The overall conservation observed in this study corroborates the significance of the *IGF-1* gene in regulating growth and development, and its potential as a marker gene in marker-assisted selection programmes (Bhattacharya *et al.*, 2022). Selection pressure analyses such as Ka/Ks (dN/dS) estimation were not performed in the present study. This is an important limitation for the evolutionary interpretation, and future studies incorporating Ka/Ks analysis across a larger, more taxonomically diverse dataset would provide stronger evidence on the relative contributions of purifying and positive selection to the observed patterns of variation.

Missense variant analysis of the *IGF-1* gene identified 5 non-synonymous substitutions, of which 3 were predicted to be deleterious, indicating potential functional constraints on the protein. Although these constraints could be negative, they also provide a basis for selection in farm animals. Previous studies have similarly demonstrated that variation within the *IGF-1* gene locus contributes to economically important traits in red junglefowl. For instance, a promoter SNP (c.-366A>C) has been associated with abdominal fat deposition and body weight across multiple red junglefowl breeds (Wang *et al.*, 2021), while intronic variants such as c.47673G>A have been linked to body weight at 41 days and haematocrit levels (Boschiero *et al.*, 2013). Identifying non-synonymous variation is important because amino-acid substitutions can alter protein structure, stability, or biological activity (Das *et al.*, 2025). The three variants predicted to be deleterious should be regarded as candidate functional variants that warrant further investigation, rather than as confirmed causal mutations. Their potential relevance to growth traits is

noteworthy because *IGF-1* sits at the center of pathways regulating growth and muscle development (Werner, 2023). The proportion of deleterious missense mutations observed in this study suggests that structural alterations in the *IGF-1* gene may contribute to phenotypic variation in growth and production traits, consistent with its established role as a key regulator of growth performance in meat-type red junglefowl. The tolerated variants are not expected to affect the red junglefowl in question negatively. Computational prediction alone cannot establish that these variants cause phenotypic differences (Spielmann and Kircher, 2022). Population-based association studies should validate the predicted functional effects using economically important traits such as body weight, growth rate, breast muscle yield, carcass characteristics, and feed efficiency. From a poultry breeding perspective, the identified *IGF-1* variants provide potential candidate markers for investigating genetic differences in growth performance. Variants that show consistent associations with desirable phenotypes across independent populations could then be considered for marker-assisted selection (MAS). A practical breeding application would involve genotyping the identified variants in a sufficiently large breeding population, evaluating their association with target traits, and determining the favourable alleles and their estimated effects before their incorporation into a selection programme.

The protein–protein interaction (PPI) network of the *IGF-1* gene revealed a highly interconnected interactome comprising 11 nodes and 36 edges. The strongest interactions were observed between *IGF-1* and *IGF1R* (0.961), *INSR* (0.952), and *INS* (0.944), showing the close functional relationship between the insulin and IGF signalling systems and their shared downstream pathways, including PI3K/Akt/mTOR and Ras-MAPK (O'Neill *et al.*, 2015). The interaction with *GHR* supports the role of the *IGF-1* gene in growth hormone-mediated signalling (Gan *et al.*, 2014), while the association with *IGFBP3* confirms the importance of binding proteins in regulating *IGF-1* gene bioavailability and physiological activity (Wheto *et al.*, 2021). Interactions with HGF, EGF, *PDGF*, and *VEGF* family members suggest that the *IGF-1* gene coordinates mitogenic (stimulation of mitosis), angiogenic (biological process of forming new capillary blood vessels from pre-existing ones), and tissue repair processes required for growth and muscle development.

CONCLUSION

The study demonstrates the effectiveness of *in silico* approaches for the molecular characterization of growth-related genes in *Gallus gallus*. Phylogenetic analysis of the *IGF-1* gene revealed high sequence conservation among avian species, with strong clustering patterns and low genetic divergence, indicating purifying selection at this locus. Functional analyses also identified deleterious missense variants predicted to affect protein function and may therefore represent candidate functional variants requiring further investigation. In addition, protein–protein interaction analysis showed that the *IGF-1* gene functions as a central regulatory hub within the somatotrophic axis, interacting with key metabolic, mitogenic, and angiogenic pathways. The designed *IGF-1* gene primers also met the optimal bioinformatic standards for specificity, GC content, melting temperature, and amplicon size, supporting their suitability for downstream molecular applications. Overall, the results highlight the *IGF-1* gene as a conserved and functionally important candidate gene, with potential relevance to growth and production genetic studies in poultry. However, the identified variants cannot yet be considered validated markers for breeding programmes because this study did not include experimental validation, genotype–phenotype association analysis, or population-level evaluation. Further studies involving larger poultry populations, experimental validation and association analyses with economically important growth traits are therefore required to determine whether the identified variants can be reliably used in marker-assisted selection and genetic improvement programmes.

REFERENCES

- Agrogiannis, G. D., Sifakis, S., Patsouris, E. S. and Konstantinidou, A. E. (2014). Insulin-like growth factors in embryonic and fetal growth and skeletal development (review). *Molecular Medicine Reports*, 10(2), 579–584.
- Baxevanis, A. D., Bader, G. D., & Wishart, D. S. (Eds.). (2020). *Bioinformatics* (4th ed.). John Wiley & Sons
- Bhattacharya, T. K., Rajith Reddy, B., Chatterjee, R. N. and Ashwini, R. (2022). Myostatin (GDF8) gene and its intriguing role in regulating growth in poultry. *Indian Journal of Animal Sciences*, 92(10), 1141-1148.
- Bian, L. H., Wang, S. Z., Wang, Q. G., Zhang, S., Wang, Y. X., & Li, H. (2008). Variation at the insulin-like growth factor 1 gene and its association with body weight traits in the chicken. *Journal of Animal Breeding and Genetics*, 125(4), 265–270.

- Boschiero, C., Jorge, E. C., Ninov, K., Nones, K., do Rosário, M. F., Coutinho, L. L., Ledur, M. C., Burt, D. W., & Moura, A. S. A. M. T. (2013). Association of *IGF1* and *KDM5A* polymorphisms with performance, fatness and carcass traits in chickens. *Journal of Applied Genetics*, 54(1), 103–112.
- Das, M., Duodu, S. O., Santra, S., Lalsangi, S. B., Naduviledathu, A., Bharati, R., Das, S., & Banerjee, R. (2025). An inclusive insight on dietary resistant protein and protein hydrolysates: Structural intricacy, bioactivity and benefits on human health. *Food Biomacromolecules*, 2(2), 157-169.
- Etherton, T. D. (2004). Somatotrophic function: the somatomedin hypothesis revisited. *Journal of Animal Science*, 82, E239-E244.
- Ewuola, E. O. (2018). *In silico* analysis of myostatin gene of some selected poultry species. *Nigerian Journal of Animal Science*, 20(1), 1–11.
- Fukunaga, K., Imachi, H., Lyu, J., Dong, T., Sato, S., Ibata, T., Kobayashi, T., Yoshimoto, T., Yonezaki, K., Matsunaga, T., & Murao, K. (2018). *IGF1* suppresses cholesterol accumulation in the liver of growth hormone-deficient mice via the activation of ABCA1. *American Journal of Physiology-Endocrinology and Metabolism*, 315(6), E1232–E1241.
- Gan, Y., Buckels, A., Liu, Y., Zhang, Y., Paterson, A. J., Jiang, J., Zinn, K. R., & Frank, S. J. (2014). Human GH receptor-*IGF-1* receptor interaction: Implications for GH signaling. *Molecular Endocrinology*, 28(11), 1841–1854.
- Guo, Y., Zhang, K., Geng, W., Chen, B., Wang, D., Wang, Z., Tian, W., Li, H., Zhang, Y., Jiang, R. & Liu, X. (2023). Evolutionary analysis and functional characterization reveal the role of the insulin-like growth factor system in a diversified selection of chickens (*Gallus gallus*). *Poultry Science*, 102(3), 102411.
- Hegarty, R., Macfarlane, S., Banks, R. and Harden, S. (2006) Association of Plasma metabolites and hormone with the growth and composition of lambs as affected by nutrition and size genetics. *Austrian Journal of Agricultural Resource* 57: 683-690.
- Hossner, K. L., McCusker, R. H., & Dodson, M. V. (2010). Insulin-like growth factors and their binding proteins in domestic animals. *Journal of Animal Science*, 64(1), 1–15.
- Kajimoto, Y., & Rotwein, P. (1991). Structure of the chicken insulin-like growth factor I gene reveals conserved promoter elements. *Journal of Biological Chemistry*, 266(15), 9724–9731
- Khan, A., Ramesh, K. M., & Raja, K. N. (2025). Genetic variability and phylogenetic analysis of the Insulin Like Growth Factor gene in ten goat breeds. *Indian Agricultural Research Journals*, 70-74.
- Klein, S., Morrice, D. A., Sang, H., Crittenden, L. B., & Burt, D. W. (1996). Genetic and physical mapping of the chicken *IGF1* gene to chromosome 1 and conservation of synteny with other vertebrate genomes. *Journal of Heredity*, 87(1), 10-14.

- Li, S., Zhou, H., Zhao, F., Fang, Q., Wang, J., Liu, X., Luo, Y., & Hickford, J. G. H. (2021). Nucleotide sequence variation in the insulin-like growth factor 1 gene affects growth and carcass traits in New Zealand Romney sheep. *DNA and Cell Biology*, 40(2), 265–271.
- Mauki, D.H, Abdulfatai Tijjani, Cheng Ma, Said I. Ng'ang'a, Akanbi I. Mark, Oscar J. Sanke, Abdussamad M. Abdussamad, Sunday C. Olaogun, Jebi Ibrahim, Philip M. Dawuda, Godwin F. Mangbon, Rudovick R. Kazwala, Paul S. Gwakisa, Ting-Ting Yin, Yan Li, Min-Sheng Peng, Adeniyi C. Adeola & Ya-Ping Zhang (2022). Genome-wide investigations reveal the population structure and selection signatures of Nigerian cattle adaptation in the sub-Saharan tropics. *BMC genomics*, 23(1), 306.
- Meyts, P. D., & Whittaker, J. (2002). Structural biology of insulin and *IGF1* receptors: implications for drug design. *Nature Reviews Drug Discovery*, 1(10), 769–783.
- National Center for Biotechnology Information. (2026). *GH growth hormone [Gallus gallus (chicken)]* (Gene ID: 378781). National Library of Medicine
- Ojeda, A., Huang, L. S., Ren, J., Angiolillo, A., Cho, I. C., Soto, H., Lemús-Flores, C., Makuza, S. M., Folch, J. M., & Pérez-Encizo, M. (2008). Selection in the making: A worldwide survey of haplotypic diversity around a causative mutation in porcine *IGF2*. *Genetics*, 178(3), 1639–1652.
- O'Neill, B. T., Lauritzen, H. P., Hirshman, M. F., Smyth, G., Goodyear, L. J., & Kahn, C. R. (2015). Differential role of insulin/*IGF-1* receptor signaling in muscle growth and glucose homeostasis. *Cell Reports*, 11, 1220–1235.
- Philippou, A., Maridaki, M., Halapas, A., & Koutsilieris, M. (2007). The role of the insulin-like growth factor 1 (*IGF-1*) in skeletal muscle physiology. *in vivo*, 21(1), 45-54.
- Rotwein P. (2018). Insulinlike Growth Factor 1 Gene Variation in Vertebrates. *Endocrinology*, 159(6), 2288–2305.
- Rude, U., Willcox, K., McInnes, L. C., & Sterck, H. D. (2018). Research and education in computational science and engineering. *Siam Review*, 60(3), 707-754.
- Spielmann, M., & Kircher, M. (2022). Computational and experimental methods for classifying variants of unknown clinical significance. *Molecular Case Studies*, 8(3), a006196.
- Wainaina, E. W. (2017). *Computational analysis of signatures of selection in genes for egg production and growth in poultry* [Master's thesis, Jomo Kenyatta University of Agriculture and Technology]. JKUAT Institutional Repository.
- Wang, W. J., Guo, Y. Q., Xie, K. J., Li, Y. D., Li, Z. W., Wang, N., Xiao, F., Guo, H. S., Li, H., & Wang, S. Z. (2021). A functional variant in the promoter region of *IGF1* gene is associated with chicken abdominal fat deposition. *Domestic Animal Endocrinology*, 75, 106584
- Werner, H. (2023). The *IGF1* signaling pathway: from basic concepts to therapeutic opportunities. *International journal of molecular sciences*, 24(19), 14882.

- Wheto, M., Ismaila, O. O., Adeleke, M. A., Adenaike, A. S., Peters, S. O., Yakubu, A., Adebambo, A. O., Ikeobi, C. O. N., & Adebambo, O. A. (2021). Sequence analyses of insulin-like growth factor 1 gene in Nigerian indigenous and Arbor acre chickens. *Genetika*, 53(1), 271-282.
- Woolliams, J. A., & Oldenbroek, J. K. (2017). Genetic diversity issues in animal populations in the genomic era. In J. K. Oldenbroek (Ed.), *Genomic management of animal genetic diversity* (pp. 1–24). Wageningen Academic Publishers.
- Yoshida, T., & Delafontaine, P. (2020). Mechanisms of IGF-1-mediated regulation of skeletal muscle hypertrophy and atrophy. *Cells*, 9(9), 1970.
- Yu, M., Wang, H., Xu, Y., Yu, D., Li, D., Liu, X., & Du, W. (2015). Insulin-like growth factor-1 (IGF-1) promotes myoblast proliferation and skeletal muscle growth of embryonic chickens via the PI3K/Akt signalling pathway. *Cell biology international*, 39(8), 910-922.
- Zhang, F., Chen, H., Chang, C., Zhou, J., & Zhang, H. (2025). Comparative genomic analysis across multiple species to identify candidate genes associated with important traits in chickens. *Genes*, 16(6), 627.
- Zhao, F., Yan, Y., Wang, Y., Liu, Y., & Yang, R. (2023). Splicing complexity as a pivotal feature of alternative exons in mammalian species. *BMC Genomics*, 24(1), 198.