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### Key Words

Secondary structure, phylogenetic, IGF-1, gene sequence, local chicken, strains

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**Received:** 20<sup>th</sup> June 2025

**Accepted:** 18<sup>th</sup> July 2025

**Published:** 31<sup>st</sup> August 2025

**Citation:** V L.J. Isaac, B. Okon, L.A. Ibom and A. Dauda, 2025. Secondary Structures and Phylogenetic Relationship in the IGF 1 Gene Sequence of Three Local Chicken Strains. Res. J. Anim. Sci., 19: 1-6, doi: 10.36478/makrjans.2025.1.1.6

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## Secondary Structures and Phylogenetic Relationship in the IGF 1 Gene Sequence of Three Local Chicken Strains

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### ABSTRACT

Secondary structure prediction is vital for determining protein's structure and functions, whereas phylogenetic analysis provides an understanding of how species evolve due to genetic alterations. Since the IGF-1 gene is a major participant in the developmental and reproductive abilities of poultry and other animals, its potential as a molecular marker was thus assessed. Sixty local chicken strains-normal feathered, frizzle feathered and naked neck-comprising twenty from each strain, from which fifteen-five was sampled per strain for blood collection and DNA extraction) were involved in the work. Jena Bioscience GmbH preparation kit was used in extracting DNA, while the Shine Gene Primers given by: GTCGGGCTACTTGAGTTACTAC-Forward. TTGCGCAGGCTCTATCT GCTC-Reverse, was used to identify genomic DNA for sequencing of the gene (IGF-1). About 2% agarose gel was used to assess the DNA purity. Results showed variation in the secondary structure of IGF-1 gene in these strains with the naked neck having a higher percentage of alpha helix, beta turn and random coil than the others and the mingling of the strains with other Gallus species in the phylogenetic tree. This is an indication of its ability to withstand mutation which makes it stable. Thus, making IGF-1 a marker of interest in the genomic selection of chicken for development and improvement.

## INTRODUCTION

The Nigerian local chicken varies both genetically and phenotypically, which accounts for varying performances noticed among them<sup>[1-3]</sup>.

Animal breeding and selection is based on genetic diversity which is the bedrock of genetic improvement. Genetic resources are better utilized based on this knowledge. Economic traits in animals exhibit a complex genetic nature and thus show continuous variation<sup>[4]</sup>.

Secondary structures are non-canonical arrangements of nucleic acids as a result of intra-strand interactions, including base pairing, stacking or other higher-order features that deviate from the standard double-helical conformation. They are formed when DNA becomes single stranded, as such creating topological road blocks which impact essential DNA-based processes such as replication, transcription and repair, ultimately affecting genome stability<sup>[5]</sup>.

DNA secondary structures plays an important role in recognizing proteins, defining the origin of replication in many single stranded DNA as well as in the survival and propagation of retroviruses, as they participate in activities like strand jumping. DNA based transactions can unwind the double helix, promoting the formation of secondary structures through intra-strand interactions and canonical folding<sup>[5]</sup>.

Phylogenomic, the use of large datasets to examine phylogeny, has revolutionized the study of evolutionary relationships. Understanding the constraints due to protein structure will improve phylogenetic estimation<sup>[6]</sup>.

Insulin-like Growth Factor (IGF) has a similar structure like insulin, inducing insulin like metabolic effects in adipose tissues and muscles<sup>[7]</sup>. Insulin-like growth factor-1 (IGF-1) protein, is a potent mitogen, altered significantly by genotype is essential for stimulating the differentiation of adipocytes, proliferation, differentiation and metabolism of myogenic cell lines in chickens<sup>[8]</sup>. It is a candidate gene for selection programmes that can be done in terms of meat production efficiency, affecting the pattern of muscle and bone mass formation as well as stimulating growth of skeletal muscle by increasing protein synthesis rate thereby, increasing level of IGF-1 to increase broiler chickens' body weight<sup>[7,9,10]</sup>.

Molecular marker assisted selection has been seen to be efficient in improving both productive and reproductive abilities. As a result of the importance of the IGF-1 gene and its close relationship with growth and production trait it is used as a molecular marker in genomic selection<sup>[11]</sup>.

IGF-1 gene can therefore be used as a molecular marker in genomic selection, development and improvement of chicken strains, since it can be used to identify, manipulate and cross-breed for improving genetic potential and in livestock species.

This study was therefore carried out to further evaluate the secondary structures and the phylogenetic estimation of the IGF-1 gene sequence with a view to ascertain its suitability as a genetic marker.

## MATERIALS AND METHODS

**Experimental animals:** Three hundred local chickens comprising of one hundred (100) Frizzle Feathered (FR), one hundred Naked Neck (NN) and one hundred (100) Normal feathered (NM) strains sourced from local markets in Uyo, Uruan, Ibesikpo Asutan, Ibiono Ibom, Ikono and Ikot Ekpene Local Government Areas were used in this study. They were replicated ten times with ten birds in each replicate. They were kept in deep litter system with wood shaving litter material for ten weeks and fed commercial growers mash and water *ad libitum*.

The study was conducted in the poultry unit of the Department of Animal Science, University of Uyo where birds were raised.

Blood samples were collected from sixty birds-twenty from each variety and two from each replicate at the end of ten weeks and used for molecular analysis which took place in the molecular laboratory at Covenant University, Ota in Ogun State.

Jena Bioscience Gmbh preparation kit was used in extracting DNA, while the Shine Gene Primers given by:

- GTCGGGCTACTTGAGTTACTAC-Forward
- TTGCGCAGGCTCTATCTGCTC-Reverse

This was used to identify genomic DNA for sequencing of the gene (IGF-1). 2% agarose gel was used to assess the DNA purity.

**Bioinformatics analysis:** The secondary structure of the amino acid sequence of IGF-1 was predicted with ExPASy's SOPMA tool. It predicts for 3 state description<sup>[12]</sup>, while the phylogenetic trees were constructed by Neighbor-Joining analysis using IGF-1 gene sequence while Poisson correction method computed evolutionary distances and bootstrap confidence values used to calculate the reliability of the tree<sup>[13]</sup>.

## RESULTS AND DISCUSSION

The prediction of IGF-1 gene secondary structure in the three strains is shown in Table 1. The naked neck strain had a higher percentage of random coils (32.22), normal feathered strain (31.11) and frizzle feathered strain (30.02), it equally had a higher alpha helix (46.12), compare to the frizzle feathered strain (40.42) and the normal feathered strain (39.03). Similarly, it had a higher beta turn (10.44), compare to the normal feathered strain (10.25) and frizzle feathered strain

Table 1: Prediction of Secondary Structure of insulin growth factor-1 the three strains of chicken

Strains	Alpha helix (%)	Extended strand (%)	Beta turn (%)	Random coil (%)
Frizzled	40.42	17.21	9.62	30.02
Normal	39.03	27.00	10.25	31.11
Naked	46.12	17.21	10.44	32.22

Window width: 17, Number of states: 4, Similarity threshold: 8

(9.62). The normal feathered strain had a higher percentage (%) of extended strand (27.00), naked neck strain (17.21) and the frizzle feathered strain (17.21). Secondary structure prediction is very vital for determining protein's function and structure so as to enable easy classification by their structure. The alpha helix is associated with aliphatic proteins which save as a factor of increasing thermal stability therefore the naked neck with a higher alpha helix will a more stable sequence and as such will withstand mutation. Protein helix functions in binding with substrates while loop determines the proteins stability. Consequently, protein with high helix tends to bind better with substrates and those with high loop tend to be less stable<sup>[14]</sup>. The naked neck strain with a higher alpha index in this study tends to be thermally stable. This, according to Dauda *et al.*<sup>[15]</sup> indicates resistance to mutation.

A higher proportion of the random coils signifies increased protein unfolding, which is attributed to the higher net negative charges possessed by protein molecules when they were extracted at higher alkaline pH. The larger net charges led to increased repulsive forces between the deprotonated amino acid chains in the protein, which in turn triggered protein unfolding. In addition, intramolecular hydrogen bonds within the protein molecules, which contributed to the stability of the protein secondary structures, were disrupted at a higher alkaline pH mainly due to deprotonation of the amino groups, resulting in reduced numbers of hydrogen bond donors and acceptors<sup>[16]</sup>. This, according to Wang *et al.*<sup>[14]</sup>, indicates a less stable condition and the tendency to easily change form and function, thus will be susceptible to mutation.

The primary structure determines the formation of secondary, tertiary and quaternary structures, which as a whole will determine the shape of the molecule and the existence of hydrophilic zones and hydrophobic patches on the protein surface<sup>[17]</sup>.

The secondary structure of proteins plays an important role in understanding its functional properties<sup>[18]</sup>. Secondary structures with relatively high proportions of charged (negative or positive) amino acid residues are related to the electrostatic repulsion and ionic hydration, which promote the solubilization of protein and influence functional properties<sup>[17]</sup>. The three-dimensional structure and properties of proteins are changed by amino acid substitutions due to non-synonymous mutations affecting the binding ability of proteins with other molecules in the process<sup>[14]</sup>. This is capable of affecting fitness<sup>[19,20]</sup>.

The amino-acid side chains influence the three-dimensional shape and overall hydrophobicity of the protein. A relatively high proportion of amino acids with hydrophilic side chains tend to result rod-like shape of the protein molecules, which causes an increase in water holding and thus enhances its hydrophilic properties. The  $\beta$  conformation is associated with the protein globular structure which may cause a lower digestibility and improved functional properties, thus enhancing its hydrophilic properties<sup>[17]</sup>.

Secondary structures, amino acid compositions and the variations in the molecular weight distribution accounts for the thermal stability of proteins<sup>[16]</sup>. Predicting the secondary structure of proteins is critical to understanding protein folding in three dimensions<sup>[21]</sup>.

Secondary structures have a direct impact on how proteins fold and deform, the way various amino acid sequences of a DNA form helices, sheets and turns in the molecule. Positive charged proteins help in the folding of DNA thus affecting the structure. This also affects the functions of such a DNA due to the fact that bonds are broken and new ones formed in the process. The fold of a protein is the overall three-dimensional arrangement of secondary structures (architecture) and the path of the polypeptide chain through the structure (topology). However, according to Zhang *et al.*<sup>[22]</sup>, some proteins do not follow the classic "one sequence, one-fold" paradigm but can switch between different folds reversibly under physiological conditions.

Secondary structures such as  $\alpha$ -helices and  $\beta$ -strands are often conserved among protein super families even in distantly related species and because amino acids differ in their propensity to form  $\alpha$ -helices and  $\beta$ -strands, they could influence how often they are used in proteomes, depending on their role in secondary structures. Amino acid frequencies in the proteome reflected their propensity to appear in protein secondary structures, such as  $\alpha$ -helices and  $\beta$ -strands, which could explain the edge effect and why few amino acids appear in most and least frequent ranks in proteomes in all domains of life<sup>[23]</sup>.

The phylogenetic tree is presented in Fig. 1. It shows that the local strains of chicken (frizzle and normal feathered as well as the naked neck) are closely related.

The genetic association shown by IGF-1 proteins shown in the phylogenetic tree aligned with the Gallus subfamily evolution. There is a mingling of the Gallus

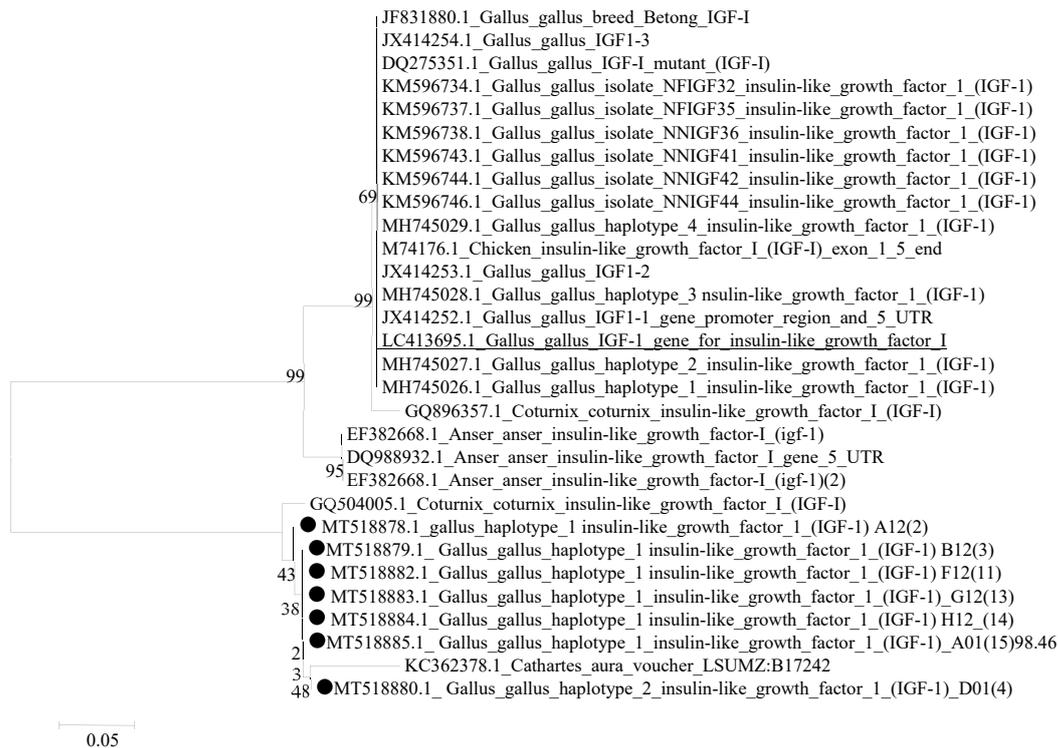


Fig. 1: Phylogenetic tree of the IGF-1 gene of the three strains of local chicken from the sequence of the extracted gene

species following the phylogenetic tree. Phylogenetic trees have depicted evolutionary relationships among organisms, viruses, genes and other evolving entities, enabling an understanding of shared ancestry and tracing the events that led to the observable extant diversity. In contrast, the fold of proteins is often conserved well past sequence signal saturation. Furthermore, because 3D structure determines function, protein structures have long been studied to gain insight into their biological role within the cell whether it be catalyzing reactions, interacting with other proteins to form complexes or regulating the expression of genes among a myriad of other functions<sup>[24]</sup>. Proteins perform a wide variety of vital roles which depend on their structure and ultimately, their amino acid composition. Amino acids are the building blocks of proteins and enzymes which are essential for life<sup>[23]</sup>.

Evolutionary information on protein sequences is crucial for various purposes, including homologue detection, protein design and drug target selection. Phylogenetic analysis is a widely used method for extracting this information that provides valuable insights into the early evolution of proteins, such as identifying ancestral peptide motifs and key sites for protein conformation shifts. However, an unbiased phylogenetic analysis remains a challenge because of high sequence divergence and a large data scale<sup>[25]</sup>.

The frequency of, or changes to, amino acid profiles in the proteome provide insights into evolutionary mechanisms shaping genomes and their products and can be used in disease diagnostics and synthetic biology. Understanding amino acid usage offers insights into protein function and molecular mechanisms underlying life histories<sup>[23]</sup>.

The phylogenetic analysis provides a depth understanding of how species evolve due to genetic alterations. Scientists can use phylogenetics to examine the path that connects a modern organism to its ancestral origin and anticipate future genetic divergence. It can also be helpful in comparative genomics, which analyzes the relationship between genomes of different species by gene prediction or discovery, locating specific genetic regions along a genome.

Such information is useful in synthesizing a specific catalase probe or primer that would serve as a marker to remove putative genes from sequenced strains. The advancement in the comparative genomic study of proteins provides a detailed understanding of functional genes within and between plant species, providing clear evidence for evolution research and gene function hypotheses<sup>[21]</sup>.

The alpha helix percentage for all the strains showed that the IGF-1 gene is stable and will be resistant to mutation. It is therefore an indication of

the fact that IGF-1 gene will be suitable as a molecular marker for genomic selection in the genetic development and improvement of poultry and indeed other livestock species.

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