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Genetic Diversity in the IGF-1 Gene Sequence of Three Local Chicken Strains

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Abstract
Genetic diversity being the basis of animal breeding forms the bedrock of animal improvement. The IGF-1 gene is involved in the developmental and reproductive abilities of poultry and other animals. Its potential as a molecular marker was thus assessed. Local chicken strains-normal feathered, frizzle feathered and naked neck-were used in this study to evaluate the diversity of insulin like growth factor-1 (IGF-1) gene sequence. A total of sixty (60) chickens (twenty (20) from each strain, from which fifteen (15)-five (5) 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. TTGCGCAGGCTCTASTCTGCTC-Reverse. was used to identify genomic DNA for sequencing of the gene (IGF-1). 2% agarose gel was used to assess the DNA purity. Results showed diversity in the amino acid composition as well as the chemical and physical properties of the IGF-1 gene in these strains thus pointing to its stability and thus ability to withstand mutation. Thus, making IGF-1 a marker of interest in the genomic selection of chicken for development and improvement.

INTRODUCTION

Genetic diversity is the basis of animal breeding and selection and the bedrock of genetic improvement. Its knowledge is a prerequisite for better utilization of genetic resources Isaac[1]. Information on genetic diversity is necessary to optimize conservation and breeding programmes of animal genetic resources to ensure food security Ajibike[2]. Economic traits in animals exhibit a complex genetic nature and thus show continuous variation. The Nigerian local chicken shows a lot of variations both genetic and phenotypic Eda[3] Lawal and Hanotte[4], which accounts for varying performances noticed among them (Okafor *et al.,* 2019., Van and Dekkers[5] Lawal and Hanotte[4]. Insulin-Like growth factor 1 (IGF-1) gene is one of the most important candidate genes because of its relationship with most developmental and productive activities in chickens. IGF-1 protein is a potent mitogen and an essential stimulus for the differentiation of adipocytes, this protein plays an important role in the proliferation, differentiation and metabolism of myogenic cell lines in chickens. IGF-1 is significantly altered by genotype, suggesting a pivotal role in the control of growth rate in broiler chickens Hosnedlova[6]. It is a mitogenic polypeptide with similarities to insulin and it plays a major role for cellular growth, assisting in mediating growth hormone actions and affects biological processes such as growth and reproductive differentiation in poultry Wheto[7]. IGF-I belongs to polypeptide hormones family, prepro-insulin, which is comprised of proinsulin, IGF-I, IGF-II and C peptide with multiple metabolic and anabolic functions. It has beneficial effects during postdelivery growth and its production in the liver is under the effect of growth hormone and nutritional conditions. IGF-I can stimulate endocrine, autocrine and/or paracrine growth functions. Fouda[8]. Insulin-Like growth factor (IGF-1), also known as Somatomedin C, is a multi-peptide hormone similar in molecular structure to insulin. It was first discovered in 1970 and consists of 70 basic amino acid peptides. It is located on the first chromosome in poultry and is composed of six exons. It is primarily secreted from the liver by Kupffer cells under the stimulation of growth hormone (GH). Its main function is cell specialization and differentiation, regulation of growth and the absorption of amino acids and glucose, as well as other roles such as stimulating thyroid and insulin hormones. It also plays an important role in DNA and mRNA synthesis reactions and protein synthesis and it is considered essential for the effectiveness of growth hormone Mohsin and AbdulKareem[9]. There is no doubt that the global consumption of poultry products is increasing, which requires the poultry industry to find suitable ways to produce birds that are healthy, have good growth rates and have a good body muscle size to meet the increasing demands of consumers. Modern technology in molecular genetics has recently introduced a number of genetic markers that have helped researchers analyze and evaluate genetic diversity, distinguish breed types to preserve them as sources of diversity AbdulKareem[10], by providing information at the molecular level for different regions of the genome Jaffar and AbdulKareem[11]. As a result, it has become necessary to follow several programs to achieve high and good production. The current trend is towards incorporating applications of molecular genetics and including them in breeding and genetic improvement programs, especially the well-known aspect of genetic marker. Molecular marker assisted selection has proven to be efficient in helping to improve both productive and reproductive abilities Wheto[7]. Due to 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 Mohsin and AbdulKareem[9]. This work was thus carried out to further assess the suitability of the IGF-1 gene as a molecular marker for genomic selection, development and improvement of poultry and other livestock species.

MATERIALS AND METHODS

Experimental Animals: Three hundred local chickens comprising of one hundred (100) Frizzle feathered (FR), one hundred (100) 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 (10) times with ten (10) birds in each replicate. They were kept in deep litter system with wood shaving litter material for ten (10) 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 (60) birds-twenty (20) from each variety and two (2) from each replicate at the end of ten (10) 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

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:

Physical and Chemical Properties: The properties for the IGF-1 gene were computed on the amino acid sequences using Protparam tool. The following parameters were computed: amino acid composition,

molecular weight, grand average of hydropathicity (GRAVY), instability and aliphatic indexes, extinction coefficient, estimated half-life, index and theoretical pI (isoelectric point) Gasteiger[12].

RESULTS AND DISCUSSIONS: The percentage composition of the IGF-1 gene amino acid is presented on Table 1. It shows varying composition of the various amino acids with the normal feathered strain having a higher percentage (%) composition of alanine (9.50), isoluecine (9.60), phenylalanine (8.80), serine 6.60), threonine (8.00) and valine (9.30). The naked neck had a higher percentage (%) of glutamate (7.00), methionine (9.80), proline (7.60) and trypsin (7.00) while the frizzle feathered had a higher percentage (%)

Table 1: Amino Acids Composition of Insulin-Like Growth Factor-1 Protein Sequence of three Strains of Chicken

Amino acids (%)	Frizzled	Normal	Naked
Alanine	4.6	9.5	1.5
Arginine	5.2	5.2	1.5
Asparagine	9.3	9.3	7.3
Aspartate	9.2	9.2	5.1
Cysteine	5.0	5.0	3.7
Glutamine	0.0	0.0	0.0
Glutamate	5.1	1.5	7.0
Glycine	3.1	2.1	5.1
Histidine	0.2	1.2	1.8
Isoleucine	4.6	9.6	6.2
Leucine	7.1	6.1	6.9
Lysine	0.0	0.0	0.0
Methionine	4.6	9.6	9.8
Phenylalanine	5.9	8.8	1.8
Proline	4.4	4.6	7.6
Serine	3.8	6.6	1.1
Threonine	4.6	8.0	4.4
Trypsin	5.0	5.0	7.0
Valine	3.9	9.3	1.8
Pyrrolysine	0.0	0.0	0.0
Selenocystein	0.0	0.0	0.0

Table 2: Physical and Chemical Properties of Insulin Growth Factor-1 in the three Strains of Chicken

Strains	No of AA	MolWt (KDa)	pI	Q	EC	Half life	II	AI	GRAVY
Frizzled	205	27451.7	8.81	+ve	29800	1.1	31.75	122.58	0.721
Normal	254	29834.4	6.82	+ve	29800	1.1	24.31	113.71	0.034
Naked	265	28781.7	9.43	+ve	30480	1.1	29.68	98.70	1.210

A- amino acid, pI- isoelectric point, EC-extinction coefficient, AI-aliphatic index, GRAVY-grand average of hydropathcity, II-instability index, Molwt-molecular weight, Q-net charge, +-amino acid resides that positively charge,

of leucine (7.10). The three strains had zero percentage (%) composition of glutamine, lysine, pyrrolysine and selenocystein. The IGF-1 proteins of the three local chicken strains had varying amino acid composition. The normal and frizzle feathered strains had a higher composition of aliphatic amino acids (isoleucine-for normal and leucine-for frizzle) whereas none of the strains had the basic amino acid (lysine). This is an indication of their stability such that they do not easily accept electron and as such cannot easily change form. They had no selenocystein and pyrrolysine that acts like stop codons-Protein that makes it difficult to determine residue identity (Suchanek *et al.,* 2005). The implication for aliphatic amino acid composition percentage being higher is that they are stable thermally whereas that for basic amino acid implies that they are not stable since they can accept electron-an indication of the fact that they can change form and as such function. Results showed IGF-1 protein of the three strains were stable and as such agreed with Dauda[13] as being suitable as a molecular marker since it will not be susceptible to mutation. The physical and chemical properties of IGF-1 for the three strains are as presented in Table 2. There was an increase in molecular weight (KDa) of the three strains (frizzle-27451.70, normal-29834.40 and naked neck-28781.70) as the number of amino acid (frizzle-205, normal-254 and naked neck-265) increases. Isoelectric point (PI) was positive for all the strains with the naked neck strain (9.43) having the highest value compared to the frizzle (8.81) and normal (6.82). The three strains had a net positive charge (Q), a high extinction coefficient (EC) where naked neck (30480) had the highest value, frizzle (29800) coming next and the normal (29800) following. The frizzle feathered strain had the highest instability index (II) (122.58), followed by the normal (113.71) and the naked neck (98.70). Similarly, it had a higher aliphatic index (AI) (31.75) followed by the naked neck (29.68) and the normal (24.31), while they all had a positive grand average of hydropathicity (GRAVY) with the naked neck having highest value (1.210), frizzle (0.721) and normal (0.034) among the strains. The amino acid (AA) number increased with an increase in the molecular weight (Mwt) of the proteins. The isoelectric point (pI): the pH that a gene has zero charge. It is significant in the purification of protein in that solubility is minimal at this point. It is an indication of amount of light that proteins can absorb at any wavelength. PI is the pH value at which proteins carry no charge or the sum of negatively and positively charges is equal. A protein with a pI value of 7 is acidic, whereas protein with a pI value greater than 7 is alkaline. The isoelectric point (pI) is the pH of the protein's surface. The protein purification process relies heavily on pI, which is used in the development of buffers for protein purification Darmawati[14]. The pI of IGF-1 gene for the frizzle and naked neck strains was basic (pI>7), while that of the normal feathered strain was acidic (pI<7). Isoelectric points (pI) of all the strains help in the development of an isoelectric focusing method buffer system of purification Darmawati[14]. It has significance for protein purification since solubility at this pH is minimal, mobility is zero within electro focusing system as such the point for protein accumulation. The implication is that a protein may receive or reject electrons based basically on how it is charged. The charge in proteins showed number of residues of amino acid. The proteins that are charged positively bound to those charged negatively. Whereas those that are neutral don't bond with neither of the two, it thus provides information of where proteins are located. So many of the proteins with a neutral or alkaline pI are usually membrane proteins or have specific functions, such as binding to nucleic acids (DNA or RNA), which are negatively charged. Acidic pI

corresponds to a higher percentage of negatively charged residues; this higher percentage of Glu+Asp residues is one of the different strategies to adapt proteins to work in environments with high salt concentrations Paya[15]. The fraction of negatively charged residues is higher for intracellular proteins whereas the extra cellular proteins contain large fractions of residues charged positively Paya[15]. The protein of the three strains in this study all had positive charges. This agreed with Dauda[16] that a protein that is positively charged can help in regulating gene expression or folding of DNA. Extinction coefficient for the three strains was in the range of 29800-30480 with the naked neck strain having the highest value (30480). It depends on the number of aromatic residues in the gene. Thus, when EC value is high, the quantity of aromatic residues in such a protein will be high thus making the protein to be highly stable (Kaur *et al.,* 2023). Amino acids that are not hydrophobic bring about the binding, thus recognizes lipids which are ligands that are hydrophobic (Betts and Russell, 2003). The IGF-1 proteins for the three strains had no selenocystein and pyrrolysine that acts like stop codons (protein that is unable to identify a residue conclusively) (Suchanek *et al.,* 2005). The EC value of a protein solution is an important parameter based on amount of light absorbed per mole of protein at a certain wavelength, most commonly 280 nm wavelength is used. EC value of protein is calculated from the number of tryptophan, tyrosine and cysteine residues per molecule because these residues contribute significantly to measured optical density of denatured protein at 276-282 nm range Kaur[7]. Since EC is a function of the size of a compound that is aromatic with abnormal firmness with respect to some connective or geometric formations within an array of atoms and being stable, it becomes difficult for the molecules that are aromatic to fall apart or go into reactions Hofmann and Stoffel[18], therefore resisting mutation in many generations. The reason vaccines and drugs meant to control diseases are altered through post translation modification where they will be more effective to destroy organisms that cause diseases. The naked neck strain with a high extinction coefficient for the IGF-1 gene showed that the gene is stable due to the fact that it is associated with aliphatic amino acids and depends on the high aromatic residues. This coupled with the high alpha helix contributed to the stability of the gene as they are not easily broken and therefore suitable as a marker in genomic selection in strains of local chicken. The result of half-life for the IGF-1 gene of all three strains of local chicken showed a ratio of 1:1. Half-life is important for determining proteins stability Bachmair[19]. It is time required for half quantity of cell protein to disappear during cell synthesis. The instability index (II) for IGF-1protein for all the three strains of local chicken were <40 and as such are stable. The reason for the stability and or the instability is dipeptide bonds and the presence of such dipeptide bond makes the significant difference between the stable and unstable proteins. The instability index estimates, in vitro, protein stability. Where the index of instability <40, it is said to be stable. At this point such proteins will not react with any compounds for substrate/recognition role and so can resist mutation. While an index >40 shows it to be unstable Kaur[17]. The frizzle and normal feathered strains had an aliphatic index >100 which makes them stable, while the naked neck strain had an aliphatic index <100 a sign that it is not stable. The aliphatic index (AI), defined as the relative volume of protein occupied by aliphatic side chains, was thought to be a positive factor in globular protein thermal stability. Proteins with a high number of aliphatic side chains resulting in high AI are predicted to be stable over a wide temperature range (thermostable) Darmawati[14]. Lower values of AI indicate that they are less thermostable and have more flexible protein structure, whereas a high AI value indicates that the protein is stable under wide range of temperature conditions Kaur[17]. The Grand average hydropathicity (GRAVY) of IGF-1 proteins of the three local chicken strains were all positive. The positive GRAVY value of a proteins means such are not water soluble (hydrophobic) having their surfaces covered richly with amino acids which are negatively charged (eg glutamate and aspartate) while proteins with negative GRAVY value means such can dissolve in water (hydrophilic) and are covered with amino acids that are positively charged eg lysine and arginine. Hydrophobic amino acids are involved with hydrophobic ligands such as lipids in binding/ recognizing (point of no mutation effect) Paya[15]. The proteins of the IGF-1 gene in the three strains in this study having positive GRAVY is an indication of their being stable and cannot undergo mutation easily. All proteins bound to positively charged DNA are capable of regulating gene expression or folding of DNA. Species are equipped with an array of mechanisms to regulate their metabolic activities Wang[20] and many genes are expressed only when they are needed in survival and reproduction Rivera[21]. Physiochemical changes which accounts for mutations have severe fitness penalty to mutants, which are purged out by natural selection, leading to the conserved evolution observed in the gene Wang[20]. Genetic variation that causes differences between individuals and is responsible for the differences in the appearance and performance of species is a powerful tool for gene regulation studies, rooted in recombination events as well as the generation of new mutations (Taylor *et al.,* 2024).

CONCLUSION

The physical and chemical properties of the IGF-1 gene show it to be stable, can not change form and function easily and as such is suitable as a molecular marker for genomic selection for the development and improvement of chickens as well as other livestock species.

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