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Research Paper

Variation In The Pit 1 Gene Sequence Of Two Nigerian Local Chicken Strains.

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ABSTRACT
This study was carried out to investigate the variation in the PIT 1 gene sequence of two Nigerian local chicken strains in a bid to assess its potential as a molecular marker. A total of 15 chickens – 5 normal feathered (NF) and 5 frizzle feathered (FF) strains of Nigerian chickens as well as 5 broiler strains (BS) were used for this study. Genomic DNA was extracted from the blood of the chickens and was used to amplify the PIT 1 gene, sequenced, aligned and then analyzed by Bioinformatic analysis. Results showed that single nucleotide polymorphisms (SNPs) 3 and 4 had the highest allelic and genotypic frequencies for C (0.75) and the lowest allelic and genotypic frequencies for A (0.25), while SNPs 1,2 and 5 had the same allelic and genotypic frequencies (0.50). SNPs 3 and 4 had lowest polymorphic information content (PIC) value (0.3046), Hardy-Weinberg Equilibrium (HWE) value (0.0820), heterozygosity (HE) value (0.375) and effective number of alleles (Ae) value (1.6). The highest estimates of average evolutionary divergence over sequence pairs within chicken strains was observed between NF, FF and NF 1 (0.0246), the least was between FF 1 and 2, NF 1 and 2 (0.0146). The result obtained from this study showed the NF and FF are related in their genetic makeup, revealing a clearer understanding of the genetic diversity in PIT 1 across the different strains and thus make PIT 1 gene an excellent molecular marker in determining genetic diversity between any populations.

Keywords: *PIT 1, sequence, variation, local chickens, strains*

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I. INTRODUCTION

The Nigerian local chicken shows a lot of variations both genetic and phenotypic (Eda, 2021; Lawal and Hanotte, 2021), which accounts for varying performances noticed among them (Okafor *et al*., 2019; Van and Dekkers,2020; Lawal and Hanotte, 2021). Local chicken genetic resources which serve as excellent source of animal protein and income to people in the rural and semi urban areas (Okafor *et al*., 2019) as well as represent a valuable animal genetic resources for the development of the livestock value chain due to their broad genetic diversity which allows for poultry rearing under different environmental conditions are waiting to be fully exploited in developing locally adapted strains to the ever- changing production environments and breeding objectives to the benefit of poultry farmers (Ajibike *et al*.,2017).

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. Information on genetic diversity is necessary to optimize conservation and breeding programmes of animal genetic resources to ensure food security (Ajibike *et al*.,2017). The present alarming global challenges such as climate, emerging diseases, population growth and rising consumer demands, makes it imperative that new genotypes (strains) will be required in the future to meet the ever changing environmental and production conditions (Liang Ke *et al*., 2019).

Growth performance is a very significant part of economic trait in poultry production which is controlled, and regulated, by a set of complex genes among which is the pituitary transcription factor 1 (PIT 1). It plays a role in transcription factor for growth hormone, prolactin and transforming growth factor-□ genes that play the most pivotal role in controlling growth in chickens. It has been used in genetic studies in terms of its expression and association with growth parameters (Bello *et al*.,2020). The use of molecular tools has facilitated biodiversity studies particularly microsatellite markers because of their sufficient number, easy identification, ubiquitous presence throughout the genome, high polymorphism and co-dominant nature (Lim et al.,2019), thus making it a

marker of choice in the estimation within and between-breed genetic diversity, genetic admixture among breeds, determination of parentage, the establishment of genetic linkage maps and reconstruction of phylogenetic relationships among populations. Advances in molecular genetics have led to identification of variation in genes that influence growth and carcass composition in farm animals. Genetic markers, unlike morphological markers (which are mostly visible mutations), can detect and analyze genetic differences between individuals, populations and species at the level of the organism's DNA.

PIT1 is involved in the development of the anterior pituitary gland, silencing adrenarche, inducing differentiation of hepatic progenitor cells into prolactin-producing cells. It is auto regulated, influencing growth rate, carcass parameters and feed efficiency in poultry birds (Reshman and Das, 2021). Information on the selective advantage which the polymorphic types of PIT 1 confers on the body weight of local chickens in Nigeria is presently scanty. This study was therefore carried out to assess the variation in the PIT1 gene sequence of Nigerian local chickens with a view to understanding its role in their genetic improvement.

MATERIALS AND METHOD

A total fifteen (15) chickens (birds) - five (5) broilers, five (5) local Nigerian frizzle feathered chickens and five local Nigerian normal feathered chickens were bought from a local market in Akwa Ibom State, Nigeria.

Each of the chickens were tagged and 10ml of blood was collected was the neck region from each of them using a syringe. The blood was immediately discharged into a heparin blood tube and refrigerated to avoid clotting. DNA Extraction – Genomic DNA extraction was carried out with solution-based JENA Bioscience blood DNA preparation (extraction) kit following manufacturer's instruction using the primer sequence: PIT 1 – F: 5' AGCCTGACCCCTTGCCT 3'

PIT 1 – R: 5' CCAGCTTAATTCTCCGCAG 3'

which was synthesized by Macrogen, South Korea.

GEL Electrophoresis – Amplicon was viewed on a 20% (wt/vol) agarose gel dissolved in 0.5x Tris-borate buffer, stained with meastrosafe stain and visualized under blue light trans- illumination, New English BioGroup,USA.

Bioinformatic Analysis – Using the gene sequence, bioinformatic analysis was carried out to determine genetic distance between, as well as the allelic and genotypic frequencies of the strains.

II. RESULT AND DISCUSSION

The genetic similarity between two (2) Nigerian local chicken is presented in table 1. It reveals the highest sequence identity matrix to be between normal feathered 2 and normal feathered (1.00), followed by Frizzle feathered 2 and Normal feathered (0.985), Frizzle feathered2 and Normal feathered 1 (0.985) as well as Frizzle feathered 2 and Frizzle feathered 1 (0.985). the least sequence identity matrix is between Frizzle feathered 1 and Normal feathered (0.976) as well as between Frizzle feathered 1 and Normal feathered 1 (0.976).

The Normal feathered and Normal feathered 2 showed the greatest genetic similarity, while frizzle feathered and Normal feathered 1 were similar genetically. The low diversity but high genetic similarity could be a function of the absence of major changes in the genome of these strains (Nweke Okorocha *et al*., 2022). This also revealed the potential of PIT 1 in evaluating sequence identity matrix between any populations (Bello *et al*., 2020).

The sequence distance matrix between the two Nigerian local chicken strains is presented in table 2. It shows that Normal feathered 2 and Normal feathered had the same genetic match (0.0000). Frizzle feathered 1, Normal feathered and Normal feathered 1 had the highest sequence distance (0.0246) between them while the least sequence distance was recorded between Frizzle feathered 2, Normal feathered, Normal feathered 1 and frizzled feathered 1 (0.0146).

The Normal feathered and Normal feathered 2 had an exact genetic match between them while frizzle feathered 1, Normal feathered and Normal feathered 2 had the highest genetic distance between them with Frizzle feathered 2, Normal feathered, Normal feathered 1 and frizzled feathered 1having the least distance between them.

The result revealed that Normal feathered strain had the greatest genetic makeup of its ancestral parents and a genetic distance that is similar to the one observed by Agaviezor *et al*, (2020) between poultry breeds, giving a clearer understanding of the genetic diversity in PIT1 gene across the different strains and breeds. Significant variations among strains and breeds is an indication of genetic improvement that has taken place over time (Agaviezor *et al*., 2020). Genetic variation is a major cause of differences between individuals and it represents a powerful tool to study gene regulation, accounting for a large proportion of phenotypic differences within and between specie (Taylor *et al*.,2024). It has deep roots in recombination events which is a mitotic recombination event that converts heterozygous loci to homozygous loci changing both genotypic and phenotypic expressions (Heil,2023), as well as the generation of new mutations that will disrupt biochemical reactions which can result in disease conditions (Jin *et al*.,2021; Yan *et al*., 2021).

The single nucleotide polymorphic indices are presented in table 3. The results indicate the allelic frequency for SNP 1 (A>G), SNP 2 (C>G) and SNP 5 (T>C) to be (0.5 0.5) but for SNP 3 (C>A) and SNP 4 (T>G) to be (0.75 0.25) while genotypic frequency for SNP 1 (AA AG GG), SNP 2 (CC CG GG) and SNP 5 (TT TC CC)

to be (0.5 0 0.5) but for SNP 3 (CC CA AA) and SNP 4 (TT TG GG) to be (0.75 0 0.75). Allelic and genotypic frequency indices for the various SNPs are the same across the gene sequence between Frizzle feathered and Normal feathered chickens. SNPs 1,2 and 5 had a higher polymorphic information content (PIC) indices (0.375), while SNPs 3 and 4 had the lowest (0.3046).

SNPs 1,2 and 5 had the highest Hardy-Weinberg equilibrium (HWE) indices (0.1353), Heterozygosity (HE) indices (0.5) and Effective number of Alleles (AE) indices (2) while SNPs 3 and 4 had the lowest HWE indices (0.0820), HE indices (0.375) and AE indices (1.6).

The mean observed heterozygosity (the percentage of loci heterozygous per individual) for all populations across loci in this study was less than the expected heterozygosity (gene diversity). This could be as a result of selection against heterozygosity which is in agreement with Thobela *et al*. (2018) and Yu *et al*. (2018) that selection against heterozygosity would cause the observed heterozygosity to be lower than the expected. The observed heterozygosity obtained in this study falls within the range of 0.39 – 0.56 reported by Ozdemir and Cassandro (2017), 0.47 reported by Zhang *et al* (2019) and 0.45 – 0.67 by Liang *et al*, (2019), but differed from the range of 0.64 – 0.70 by Kim et al, (2018).

The variation in the expected heterozygosity may be adduced to differences in location, sample size and population structure (Bello *et al*., 2020). This variation across strains of a species forms the genetic basis for differences in their behavior showing different expressions in different environment (Catoiua *et al*., 2023). More so variations from mutations will affect the expression of traits as well as disrupt biochemical reactions that can result in disease conditions (Jin *et al*., 2021; Yan *et al.,* 2021), resulting in the appearance of genetic variants which is a key point in the evolution of genomes as well as in the adaptation of a species to environmental changes (*Dutta et al*., 2021), allowing scientists find target genes for applications in trait improvement and development (Lovell *et al*.,2022).

The informativeness of molecular markers is better determined by calculating the polymorphic information content (PIC) (Thobella *et al.,* 2018) and a marker is said to be highly informative when the PIC value is 0.50 and above (Fan et al., 2017). SNPs 1,2 and 3 had the highest mean value of PIC indicating that this locus is the most informative locus among the set of loci used in this study.

The overall mean PIC value calculated based on the number and frequency of alleles per marker at a specific locus across populations obtained in this study was lower than the value of 0.8010 reported by Wolc (2018) in chicken populations in the South- South region of Nigeria. However, the values of PIC in this study though below the threshold value of 0.50 showed that the PIT 1 as a molecular marker is fairly polymorphic and quite informative for genetic diversity studies.

The frizzle and normal feathered chickens in this study are closely related as shown by the bioinformatic detail an indication of the fact that they share a common ancestor.

PIT 1 gene is involved in growth and other physiological metabolic process. It is polymorphic and has association with body weight (Bello *et al*., 2020) and is known to be associated with body weight and carcass parameters (Agaviesor *et al*., 2020). These associations, according to Agaviesor *et al*, (2020), are responsible for variations in performance which should be harnessed for improvement and conservation.

Genetic variation which results in gene expression whose variability is caused by genetic and environmental exposures (Einarsson *et al*., 2022), is an important molecular step that translates genotypes into phenotypes (Tsouris et al., 2024). Cellular adaptation, physiology, and development is affected by changes in gene expression which is guided by transcription factors (TFs) that bind DNA at sequence motifs allowing activation or repression of gene transcription, whose understanding is central in how gene regulation evolves (Krieger *et al*., 2022). The understanding of how transcriptional regulation influences gene expression variability is of fundamental importance to understand how organisms are capable of generating proper phenotypes in the face of stochastic, environmental, and genetic variation (Timshel et al., 2020; Einarsson *et al*., 2022). Molecular phenotypes like gene expression remains key in understanding physiology, disease, and evolutionary adaptations and thus can directly be involved in determining fitness (Wolf *et al*., 2023), drive phenotypic variation (Hansen and Pe labon,2021), and the genetic architecture of variance itself can evolve (Bruijning *et al*., 2020), and thus furthering our understanding of complex traits and diseases (Wolf et al.,2023).

III. CONCLUSION

The analysis of the sequence of PIT 1 gene revealed that frizzle feathered and normal feathered strains are closely related to each other, expressing the possibility of revealing relationships among individuals in a population. It is therefore plausible to postulate that the PIT 1 gene can be used as a molecular marker for genomic selection, conservation and breeding programmes for the development and improvement of the local chicken population.

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Table 1: Sequence Identity Matrix Between Normal feathered and Frizzle feathered chickens.

Strain	NF	NF 1	FF 1
NF			
NF 2	1		
FF 1	0.976	0.976	
FF 2	0.985	0.985	0.985

NF = Normal feathered, FF = Frizzle feathered

Table 2: Sequence Distance Matrix Between Normal feathered and Frizzle feathered chickens.

Strain	NF	NF 1	FF 1
NF			
NF 2	0.0000		
FF 1	0.0246	0.0246	
FF 2	0.0146	0.0146	0.0146

NF = Normal feathered, FF = Frizzle feathered

Table 3: Single Nucleotide Polymorphism Indices for PIT 1 Gene in Normal feathered and Frizzle feathered chickens.

SNPs	AF		GF			PIC	HWE	HE	AE
1 (A>G	A	G	AA	AG	GG	0.375	0.1353	0.50	2
	0.50	0.50	0.50	0	0.50				
2 (C>G)	C	G	CC	CG	GG	0.375	0.1353	0.50	2
	0.50	0.50	0.50	0	0.50				
3 (C>A)	C	A	CC	CA	AA	0.3046	0.0820	0.375	1.6
	0.75	0.25	0.75	0	0.25				
4 (T>G)	T	G	TT	TG	GG	0.3046	0.0820	0.375	1.6
	0.75	0.25	0.75	0	0.25				
5 (T>C)	T	C	TT	TC	CC	0.375	0.1353	0.50	2
	0.50	0.50	0.50	0	0.50				

SNP = Short Nucleotide Polymorphism, AF = Allelic Frequency, GF = Genetic Frequency, PIC = Polymorphic Information Content, HWE = Hardy – Weinberg Equilibrium, HE = Heterozygosity, AE = Effective number of alleles.