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Detection of Polymorphism in Insulin-Like Growth Factor I Gene in Fulani and Yoruba Ecotype Chickens

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Abstract

This research examined polymorphism in the insulin-like growth factor I (IGF-1) gene of native Nigerian chickens (Fulani and Yoruba ecotypes) using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis. This study examined 98 chickens (50 Fulani and 48 Yoruba ecotypes) with approximately 2 mL of blood collected from the wing vein for DNA extraction. Genetic variation was tested by digesting PCR products with PstI. Two alleles (C and T) and three genotypes (CC, CT, and TT) were identified in both ecotypes, confirming that the IGF-1 locus was polymorphic. Frequencies of the T allele and TT genotype were higher in the populations. Chi-square test revealed a departure from the Hardy- Weinberg equilibrium in the Fulani ecotype, but not in the Yoruba ecotype. Observed heterozygosity was significantly lower than the expected, implying inbreeding. The genetic diversity was fairly high while the genetic distance was low, suggesting high genetic similarity between the two ecotypes. Additional studies are needed to determine the relationship between IGF-1 polymorphism and productivity traits.

Keywords: PCR-RFLP, Allelic frequency, Genetic diversity, Marker-assisted selection, Indigenous chickens

INTRODUCTION

Insulin-like growth factor 1 (IGF-1) is a significant growth, metabolic, and development factor, including milk production in animals (Eom and Kim, 2024). The rapid development in molecular genetics has improved the ability to identify candidate genes linked with economically significant traits in chicken (Amao et al., 2019; Abdi et al., 2014; Fatemi et al., 2012).

IGF-1 plays a critical role in physiological activities through its involvement in protein synthesis, cell differentiation, and metabolism (Yadav et al., 2023). It is present in various tissues and cells, where it is crucial for organ formation and tissue maintenance. Moreover, IGF-1 has anabolic and mitogenic effects in growth hormone target tissues (El-Tahawy and Abdel-Rahman, 2020; Laron, 2001; Ali et al., 2016; Yadav et al., 2023).

While the liver is the main source of IGF-1 production, it is also synthesized in a tissue-specific manner in extrahepatic tissues (Etherton, 2004). The insulin-like growth factor system is made up of a family of peptide hormones (IGF-1 and IGF-2), receptors, and binding proteins. IGF-1 binds to the IGF-1 receptor and insulin receptor and then activates intrinsic tyrosine kinase activities.

Circulating IGF-1 has been shown to affect growth performance, body composition, and fat metabolism in chickens (Zhou et al., 2005). Candidate genes like IGF-1 have an impact on various traits (phenotypes) through biochemical and regulatory mechanisms by affecting the structure and function of proteins (Eom & Kim, 2024). So this study seeks to identify single nucleotide polymorphisms (SNPs) in the IGF-1 gene and to test the efficacy of these genetic markers for the

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evaluation of genetic diversity and phylogenetic relationships among Fulani and Yoruba ecotype chickens.

MATERIALS AND METHODS

Chicken Population and Blood Sampling

A total of ninety-eight indigenous chickens were used in this study, consisting of 50 Fulani and 48 Yoruba ecotypes. Fulani birds were obtained from pastoral settlements in Osun State, while Yoruba ecotypes were sourced from local farmers in Ibadan.

Blood samples were collected via the wing vein using sterile 1 mm needles and 2 mL syringes. Approximately 1.5 mL of blood from each bird was transferred into EDTA-coated tubes for preservation. Samples were subsequently spotted onto Whatman FTA cards (0.5 mm; 176.83 g/m²) and stored in sealed bags at room temperature until analysis. Laboratory procedures were carried out at the ACUTIG Laboratory, Abeokuta, Ogun State.

Genomic DNA extraction

Genomic DNA was extracted from FTA cards following a modified protocol:

1. Two discs were punched from each sample and placed into 0.2 mL tubes.
2. 0.15 mL of Tris-SDS purification reagent was added and gently agitated for 30 minutes.
3. Samples were washed with distilled water for 10 minutes, and the solution discarded.
4. A second wash was performed without agitation.
5. Tubes were heated at 90°C for 15 minutes.
6. Extracted DNA was stored at −20°C until use.

PCR Amplification

The primer in this study was modified from that published by Nagaraja et al. (2000).
Forward primer:

5'-GACTATACAGAAAGAACCCAC-3'

Reverse primer:
3'-TATCACTCAAGTGGCTCAAGT-5'

The PCR reaction mixture was 6.25 µL, containing 0.5 µL genomic DNA, 1.25 µL Fast Taq, 4 µL nuclease-free water and 0.25 µL of forward (F) and reverse (R) primers. The thermocycling conditions were as follows: 15 minutes at 96°C (initial denaturation) followed by 35 cycles of 1 minute at 94°C (denaturation), 1 minute at 62°C (annealing), 30 seconds at 70°C (extension), and the final extension at 70°C for 6 minutes (Nagaraga et al., 2000).

Digestion of Amplicons (RFLP Analysis)

The amplicons were digested in a total reaction volume of 9.6 µL containing 3 µL of PCR products, 1 µL of 10× NE Buffer, 5.5 µL of nuclease-free water, and 0.1 µL of restriction enzyme (Pst-I). The mixture was incubated at 37°C for 1 hour (Tunnisa et al. (2024).

Gel Preparation and Loading of Samples

Electrophoresis apparatus, conical flask, measuring cylinder, power pack, micropipette and tips, 1X TBE buffer, gel loading dye, ethidium bromide, agarose, microwave, combs, trays, and plates, according to Harisha (2007), were used.

0.5 µl of the ladder and 8 µl of the digested products were slowly pipetted into the well, using a micropipette and tips. The run was performed at 100V for one hour. The digested DNA products were run for 45 minutes at 100V on 1% agarose gel. The sizes of individual PCR-RFLP fragments were measured in each sample using the Gel Doc documentation system (Bio-Rad, USA).

Statistical analysis

Allele frequencies were determined using the direct gene-counting method. The gene frequencies, genetic variation parameters, heterozygosity measures, and fixation index were calculated with the POPGENE 1.32 software package. A chi-squared goodness-of-fit test at a 5% significance level was conducted to assess whether genotype frequencies align with Hardy-Weinberg equilibrium expectations, using the formula provided below.

$$\chi^2 = \frac{\sum(O-E)^2}{E} \quad (1)$$

where:
χ^2 = Chi-squared value
O = observed frequency
E = expected frequency.

Band Scoring

Bands were visually scored according to migration pattern.

CC: shows a single light band, which generally indicates the restriction enzyme has not cut the DNA (or has cut both alleles the same way), resulting in only a single band.

TT: shows a single thick band and represents the other homozygous pattern due to fragment migration.

CT: It has the 'C' and 'T' alleles. This may appear as a fatter band or double band due to the presence of different-sized fragments from the restriction enzyme.

RESULTS AND DISCUSSION

Results

The PCR-RFLP patterns of the Pst 1 digested amplicons of IGF-1 are shown in Figure 1.

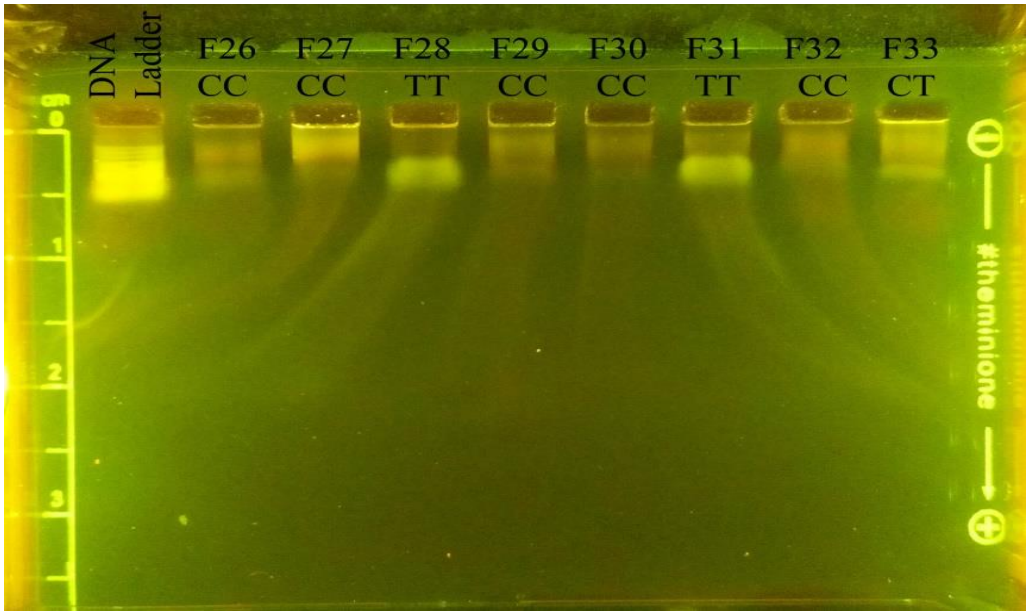

Figure 1. A gel picture showing PCR-RFLP patterns of the IGF-1 gene obtained by polymerase chain reaction (PCR). CC, TT, and CT-observed genotypes.

The allelic and genotypic frequencies of the Insulin-Like Growth Factor 1 (IGF-1) gene in Fulani and Yoruba ecotype chickens are shown in Table 1.

In the Yoruba ecotype, the frequencies of C and T were 0.35 and 0.65, respectively. Genotype frequencies were 8 (CC), 18 (CT), and 22 (TT). The tests for Hardy-Weinberg equilibrium (HWE) using chi-square (χ^2) and likelihood ratio (G^2) were 1.75 and 1.73, respectively, showing the absence of significant departure from HWE.

The Fulani ecotype had C and T allele frequencies of 0.44 and 0.56, respectively, and genotype frequencies of 14 (CC), 16 (CT), and 20 (TT). Tests for HWE showed χ^2 and G^2 values of 6.51 and 6.61, respectively, indicating that this population was not in equilibrium.

When the two ecotypes were combined, the T allele had a higher frequency: 0.60 (60%) in comparison to 0.40 (40%) for the C allele. The pooled genotypic frequencies were 0.43 (42 birds) for TT, 0.35 (34 birds) for CT, and 0.22 (22 birds) for CC.

Table 2 contains the heterozygosity estimates for the insulin-like growth factor 1 gene in Fulani and Yoruba ecotype chickens. The observed homozygosity (ObsHom) (0.65) was greater than the expected homozygosity (ExpHom) (0.52) whereas the observed heterozygosity (ObsHet) (0.35) was less than the expected heterozygosity (ExpHet) (0.48).

Nei's expected heterozygosity (Nei's) (0.48) is equal to the average heterozygosity (AveHet) (0.48).

Table 1. Allelic and Genotypic Frequencies of the Insulin-Like Growth Factor 1 Gene in Fulani and Yoruba Ecotype Chickens.

Ecotype	Allelic Frequency		Genotypic frequency			X^2	G^2	df	*p*-Value
	C	T	CC	CT	TT				
Fulani	0.44	0.56	14	16	20	6.51	6.61	1	0.01
Yoruba	0.35	0.65	8	18	22	1.75	1.73	1	0.19
Overall	0.40	0.60	22	34	42	7.75	7.75	1	0.01

X^2: Chi-square test for Hardy-Weinberg equilibrium, G^2: Likelihood ratio test for Hardy-Weinberg equilibrium, df: degree of freedom, Probability level: 0.05.

Table 2. Heterozygosity estimates of the Insulin-Like Growth Factor 1 gene in Fulani and Yoruba ecotype chickens.

Ecotype	N	Obs_{Hom}	Obs_{Het}	ExpHom	Exp_{Het}	Nei	Ave_{Het}
Fulani	50	0.68	0.32	0.50	0.50	0.49	0.48
Yoruba	48	0.63	0.38	0.54	0.46	0.46	0.48
Overall	98	0.65	0.35	0.52	0.48	0.48	0.48

N: Sample size, Obs_{Hom}: Observed homozygote, $Obs_{Het:}$ Observed heterozygote, Exp_{Hom}: Expected homozygote, Exp_{Het}: Expected heterozygote, Nei: Nei expected heterozygosity, Ave_{Het}: Average heterozygosity

Table 3. Summary of genetic variation statistics of the insulin-like growth factor I gene in Fulani and Yoruba ecotype chickens.

Ecotype	Na	Ne	I
Fulani	2.00	1.97	0.69
Yoruba	2.00	1.84	0.65
Overall	2.00	1.92	0.67

Table 3 shows genetic variation of the insulin-like growth factor I gene in Fulani and Yoruba ecotypes. The number of alleles (Na) in both ecotypes is 2.00. The effective number of alleles (Ne) in Fulani and Yoruba ecotypes is 1.97 and 1.84, respectively, and Shannon's information index (I) in Fulani and Yoruba ecotypes is 0.69 and 0.65, respectivel.

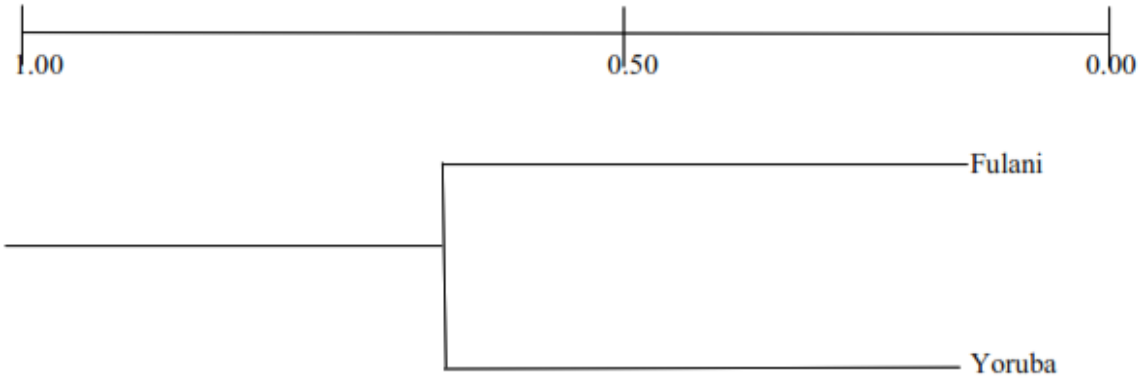

Figure 2. Genetic distance between Fulani and Yoruba ecotypes of Nigerians Indigenous chicken.

DISCUSSION OF FINDINGS

Polymorphism analysis is carried out to identify quantitative trait loci that may influence productivity and to optimise the breeding strategy (Edea *et al*., 2017). One important hormone essential for the growth and development of muscles in chickens is insulin-like growth factor I (IGF-I) (Scanes, 2009; Boschiero et al., 2013).

In this study, IGF-1 was polymorphic, and the allelic variants were C and T, and three genotypic classes (CC, CT, and TT) were observed. This is in line with the Alope chickens studied by Tunnisa *et al*. (2024). Three allelic variants of IGF-1 were also reported by Nguyen et al. (2015) and Al Hassani *et al*. (2015) in Thai and broiler chickens, respectively.

The frequency of the T allele was higher than the frequency of the C allele in both Fulani and Yoruba populations. The genotypic frequency of TT was greater in the ecotypes than CC and CT. However, the frequency of CT was significantly (P 0.05) greater than the frequency of CC in both ecotypes. This was also true for the frequency of CC in the Yoruba ecotype, which was significantly lower than the frequency of CC in the Fulani ecotype.

Differences in the genotypic and allelic frequencies may be due to the difference in the ecotypes. Li *et al*. (2010) found genotypic frequencies of IGF-1 in Wancheng chicken as TT = 0.27, TC = 0.41, and CC = 0.32, with allelic frequencies of T = 0.47 and C = 0.53. Khadem *et al*. (2010) reported frequencies of TT, TC, and CC as 0.18, 0.42, and 0.40 and frequencies of T and C as 0.39 and 0.61 in Mazanderan native fowls. Tunnisa *et al.* (2024) reported the frequency of the IGF-1 genotype was found to be homozygous (AA) in cocks and hens (0.10 and 0.08), heterozygous (BB) in cocks and hens (0.63 and 0.63), and (AB) in cocks and hens (0.27 and 0.30). The most prevalent was BB (0.63), followed by the lowest AA (0.10), which is similar in Alope hens. This is reflected in the fact that the frequency of the B allele is higher than the frequency of the A allele in both cocks and hens. This result has also been reported by others. Ali et al. (2016) observed allele B to be more prevalent (55%) than allele A (44%). An SNP is considered to be polymorphic when its allele frequency is less than or equal to 0.99 in large populations and 0.95 in small populations (Allendorf *et al*., 2013; Pandey *et al*., 2013).

Native poultry of West Azerbaijan did not have the TT homozygous genotype (Babayi et al.,

2014). The frequency of the C allele was 0.83, while that of the T allele was 0.17. Nguyen et al. (2015) reported that all Thai broilers had the CC genotype at a low frequency (0.13 - 0.15) compared to other genotypes. Nei (1987) speculated that the frequency of an allele below 0.99 is polymorphic. Nei and Kumar (2000) also described genetic diversity as a case where two or more alleles are present in a population (usually more than 1%).

In this study, the number of alleles was 2 in both the ecotypes. The effective numbers of alleles (Ne) and Shannon information index (I) also showed that the Fulani ecotype had a greater number of alleles than the Yoruba ecotype. This may be due to the sampling size of the ecotypes, which is an important factor of the precision of population-genetic diversity parameters (Bashalkhanov et al., 2009).

The observed heterozygosity (ObsHet) of the Fulani and Yoruba ecotypes were 0.32; 0.38 while the expected heterozygosity (ExpHet) was 0.50 and 0.46, respectively. This shows that ObsHet is greater than 0, and the IGF-1 gene displays high diversity of the ecotypes. Tunnisa et al., 2024 reported an ObsHet of 0.27 and ExpHet of 0.73 in Alope cocks while ObsHet and ExpHet in Alope hens were 0.30 and 0.70 respectively. The heterozygosity observed in the whole population was 0.28 (in agreement with the genotype frequencies) and the expected heterozygosity is 0.72. Deviation of ObsHet in a population from the ExpHet is a negative measure which reflects inbreeding (Nassiry et al., 2009). In the indigenous chicken populations of Cameroon, the ObsHet was higher than ExpHet (Keambou et al., 2014), which indicated a deficit of heterozygotes. The lack of fit of expected heterozygosity may be due to selection, null alleles, or population substructure due to genetic drift.

The p-value of the likelihood-ratio test in this case was 7.75, which suggests that there is a non-conformity to Hardy-Weinberg equilibrium, which is in agreement with the chi-square (χ^2) result. The results of chi-square (χ^2) and likelihood-ratio tests of this study support the Hardy-Weinberg law, which states that allele frequencies in a large population do not change as the population grows without selection, migration, mutation, or drift (Graffelman, 2022). The critical χ^2 of the total population (3.84) was less than the calculated χ^2 value (4.44) in Alope chickens (Tunisa et al., 2024), which means that this population does not follow Hardy-Weinberg equilibrium.

The χ^2 value of this study is also consistent with the study of Wheto et al. (2016), who reported Hardy-Weinberg proportions of the IGF-1 gene polymorphism in carcass traits of the improved indigenous chickens from Nigeria. In the study of Abbasi and Kazemi (2013), it was reported that there was no significant difference in the genotypic frequencies of the native population of Mazandaran fowls in the χ^2 test on the populations that were in Hardy-Weinberg equilibrium. There was no difference in the genotypic frequencies from the Hardy-Weinberg proportions except RIFF birds. This was in line with Jafari et al. (2015) who reported that there are differences between the genotypes of Isfahan and Mazandaran native fowls, and it is possible that this population is not on the Hardy-Weinberg equilibrium because it may undergo selective breeding.

Nei's unbiased estimates of genetic identity and genetic distance were used to calculate the genetic distance (Nei, 1978) between Yoruba and Fulani ecotype chickens, and the genetic distance (0.0089) and genetic identity (0.9911) were very low and high, respectively. According to Abou-Emera et al. (2017), genetic distance based on allele frequency is related to the genetic diversity of studied genotypes, so such methods are suitable to reveal the domestication history of chicken. The genetic distance among the populations revealed no significant differences, which indicates that the two ecotypes are very close at the investigated locus. These findings are in line with Amao et al. (2023) who suggested that the sub-populations are not genetically distinct. Moreover, the genetic distances in the current study, from the lowest to the highest, can be compared to those of four Indonesian native and broiler chickens (Marian Dayani et al., 2013). Fosta et al. (2011) also reported the lowest genetic distance values of the Cameroonian indigenous chickens. The results were, however, lower than those of the five ecotypes of local chickens in Egypt (Abou-Emera et al., 2017). However, there were higher genetic distances among three local varieties of chicken in Nigeria (Ohwojakpor et al., 2012).

CONCLUSION

The study confirms the polymorphism of the IGF-1 gene in the local chicken populations of Nigeria, with the T allele as the most frequent (0.60) in the populations studied. The genetic diversity of the Fulani ecotype is greater than that of the Yoruba ecotype by the fact that the allelic frequencies of the former (C: 0.44; T: 0.56) are more balanced. Moreover, the observed deviation from Hardy-Weinberg equilibrium in the Fulani and pooled populations ($P < 0.005$) reveals that this gene is likely to be under selection or non-random mating is occurring. Based on these findings, the IGF-1 gene can be used as a good marker to study genetic distance and serves as a molecular basis for future selection and improvement of growth traits in Nigerian indigenous chicken ecotypes.

Moreover, the reduction in heterozygosity observed in this study points to the presence of some inbreeding in the studied populations. However, the two ecotypes were moderately diverse with very low genetic distances, underscoring that the two ecotypes were more similar at the studied locus. The results support the prospect of the IGF-1 gene as a biological marker for genetic improvement programs to enhance the growth performance of indigenous chickens of Nigeria. It is therefore recommended that additional studies incorporating phenotypic and larger sample sizes be used to validate its use as a marker-assisted selection tool.

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