

Association of insulin-like growth factor 1 (IGF1) gene polymorphism with the reproductive performance of three dual-purpose chicken breeds

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ABSTRACT: The study was designed to investigate the association of Insulin-like growth factor 1 (IGF1) gene polymorphism with the reproductive performance of FUNAAB-Alpha, Sasso, and Kuroiler dual-purpose chicken breeds. To achieve this, a total of 250 healthy hens were selected at 12 wk of age and were intensively managed in cages for 52 wk. Blood sample was taken from each chicken at the 34th week and genomic DNA was extracted using Qiagentm DNA extraction kit, PCR was used to amplify the DNA fragments, and the PCR products were electrophoresed. Amplicons obtained were digested with restriction enzyme *hinf*I, and were further electrophoresed on 1.5% agarose gel. Data obtained were analyzed using the General linear model of SAS

(2002) version 9.0 to determine the effect of IGF1 gene polymorphism and the distribution of alleles within the breeds. Results show polymorphism of the IGF1 gene and the restriction analysis indicated two alleles; A 58% and C 42% with the identification of genotypes AA, AC, and CC, and genotypic frequency of 22%, 43%, and 35%, respectively. Significant associations were observed between the polymorphism of the IGF1 gene, age of the bird at first lay, and weight of the hen at first lay. Chickens with haplotype CC came earlier into lay compared to those with the other two haplotypes (AA and AC). Therefore, the study suggests that haplotype CC could be used as a genetic marker to select for an improved laying performance in chickens.

Key words: allele, DNA, IGF1 gene, polymorphism, reproduction

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INTRODUCTION

In recent years, various researches have been carried out on the association of Insulin-like growth factor 1 (IGF1) gene polymorphism with the growth performance traits of different

animals (El-Magd et al., 2017). Few experiments have been conducted on the relationship that exists between IGF1 gene polymorphism and the reproductive performance of the three breeds of chickens here studied, that is, the FUNAAB-Alpha, Sasso, and Kuroiler dual-purpose chicken breeds. The FUNAAB-Alpha dual-purpose breed comprises three chicken genotypes and is; the Normal Feathered, Naked Neck, and the Frizzle Feathered genotypes. The three genotypes were

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considered in this study. IGF1 gene consists of six exons (Fotsis et al., 1990; Grosse et al., 1999; Sahana et al., 2010) and IGF1 gene is one of the most important candidate genes because of its relationship with most developmental and productive activities in chickens (McMurtry et al., 1997; Hosnedlova, et al., 2020). McMurtry (1998), Yan et al. (2017), and Jia et al. (2018) further reported that the IGF1 gene can influence growth rate, body composition, and lipid metabolism in poultry. Furthermore, Yakar and Adamo (2012), Promwatee et al. (2012), Shanmugalingam et al. (2016), and Ipsa et al. (2019) emphasized that the IGF1 gene is known to play an important role in growth, proliferation and cell differentiation whereas Scanes et al. (1989) and EL-Attrouny et al. (2020) reported that the plasma concentration of chicken IGF1 gene is higher in the genetic line selected for high growth rate than in slower-growing lines. As the age of the chicken increases, the plasma concentration of the IGF1 gene increases (Jia et al., 2018). It should be noted that the IGF1 gene in animals is found in the liver and some other tissues including muscle, brain, and kidney (Barton, 2006). Meanwhile, IGF1 gene has been described as a ligand that has a continuous function throughout cell development (Liu et al., 1993), thereby stimulating the proliferation, differentiation, and metabolism of myogenic cell lines from different species. According to Soe et al. (2001), the chicken IGF1 gene is structurally similar to mammalian IGF1, but there are some fundamental differences in the IGF1 gene physiologically between mammals and poultry. Nevertheless, the molecular selection of individual genes has been discovered to be a promising method to genetically improve economically important traits in chickens (Zhou et al., 2005) and the selection based on this candidate gene can pave way for a more productive flock. Thus, the experiment was undertaken to evaluate the relationship between IGF1 gene polymorphism and the reproductive performance of different genotypes of FUNAAB-Alpha, Sasso, and Kuroiler dual-purpose chicken breeds.

MATERIALS AND METHODS

Location of the Study

The research was carried out at the Federal University of Agriculture, Abeokuta's Programme for Emerging Agricultural Research Leaders (FUNAAB-PEARL) Breeding Centre. Alabata road, Abeokuta, Nigeria. Alabata is in Odeda Local Government Area of Ogun State, Nigeria. Alabata

is Located on latitude 7°15'N, longitude 3°26'E and its 76 m above sea level (Google Earth, 2006). The research site was located in the derived savannah zone of South-West Nigeria with relative humidity in the rainy season (late March–October) and dry season (November–early March) that ranged between 63 to 96% and 55 to 84%, respectively. It has a mean annual precipitation of 1,037 mm with a mean annual temperature of 34.7 °C (Google Earth, 2018).

Experimental Birds

The handling of the experimental birds was done following the rules and regulations of the Animal welfare committee of the Federal University of Agriculture, Abeokuta, Nigeria.

Reproductive Performance

250 healthy pullets of FUNAAB-Alpha, Sasso, and Kuroiler dual-purpose birds were selected from the total of 600 female chickens that were reared from day old till 12 wk of age, and were managed intensively on a deep litter system. Each of the selected birds was placed in an individual cell of cages for efficient monitoring and proper recording for another 52 wk of lay. However, the FUNAAB-Alpha chicken breed consists of three genotypes, at 50 birds per genotype, the sample size of the FUNAAB-Alpha breed is 150, Kuroiler 50, and Sasso 50 making 250 birds altogether.

Blood Sample Collection

Blood samples of 250 birds of three chicken breeds were collected at 34th week of age through the wing vein with the aid of a 5 ml disposable syringe as reported by Li et al. (2008) and, Bian et al. (2008), as revised thus. The blood samples were transferred into 15 × 55 mm tube of Ethylene Di-amine Tetra-Acetic Acid (EDTA) bottles manufactured by Agary Pharmaceuticals Limited, China, which served as the anticoagulant agent and were subsequently transported into the Department of Animal Breeding and Genetics, FUNAAB Laboratory for genomic DNA extraction.

Genomic DNA Extraction and Quantification

Genomic DNA was extracted from chicken erythrocytes following the manufacturer's guide of Qiagen DNA easy blood kit. The purity and

concentration of the extracted DNA were determined using a Nano-drop spectrophotometer.

Optimization of Polymerase Chain Reaction (PCR) conditions

PCR was carried out in a final reaction volume of 25 μ L. The reaction mixture was subjected to initial denaturation of 940 °C for 2 min followed by 30 cycles of 980 °C for 10 s, 550 °C for 30 s, and extension at 680 °C for 40 s. The reaction was carried out in 200 μ L thin-walled PCR tubes. PCR tubes containing reaction mixture were tapped gently and then centrifuged at 1,000 rpm for few seconds to mix the volume.

PCR Amplification (Gel Electrophoresis)

The quality of PCR product obtained was checked by taking 5 μ L of PCR product (631 bp) with 1 μ L gel loading dye from each tube and electrophoresed in 1.5% agarose gel at a constant voltage of 100 V for 30 min using 1 \times TAE buffer. The 100 bp ladder was used as a marker to analyze the molecular size of the migrated bands. The amplified product was visualized under UV and documented.

IGF1 Gene Primers and Dilution

The PCR primers specific for the chicken IGF1 gene were used, that is; Forward: 5-GACTATACAGAAAGAACCAC-3; Reverse: 5-TATCACTCAAGTGGCTCAAGT-3 (Nagaraja et al., 2000).

PCR-Restriction Fragment Length Polymorphism Analysis

The DNA amplified fragment was digested by HinfI restriction enzyme for detecting the polymorphism. The digested products were subjected to 2% agarose gel electrophoresis and were allowed to run at 100 V for 1 h and the resulting fragments were viewed using the gel documentation system and genotyping was done manually following the scoring procedure of Darabi et al. (2010). Allelic and genotypic frequencies were calculated using the following formula

Allelic frequency:

$$p = \frac{2(AA) + (AC)}{2N}$$

$$q = \frac{2(CC) + (AC)}{2N}$$

where p = gene frequency of allele A, q = gene frequency of allele C, and N = total number of birds tested

Genotype frequency:

$$AA = A^2$$

$$AC = 2(A * C)$$

$$CC = C^2$$

where AA = genotype frequency for genotype AA, AC = genotype frequency for genotype AC, and CC = genotype frequency for genotype CC.

Test for Hardy–Weinberg equilibrium (HWE) and population differentiation measures were determined by chi-square (χ^2) analysis.

Statistical Analysis

Data obtained on the laying performance was analyzed using General Linear Model (SAS, 2002) version 9.0. The frequency of distribution of alleles within the lines was compared with the model as specified below:

$$Y_{ijk} = \mu + G_i + B_j + (GB)_{ij} + \varepsilon_{ijk}$$

where Y_{ijk} = observed performance of individual's i th breed, μ = overall mean, G_i = fixed effect of the i th breed ($i = 1 - 3$), B_j = fixed effect of the IGF1 gene on chicken breeds; $(GB)_{ij}$ = interaction effect between the genotype and the IGF1 marker, and ε_{ijk} = random error.

RESULTS

Identification of IGF1 Gene Polymorphism

The electrophoretic pattern of the PCR-RFLP that was performed for the chicken IGF1 gene using HinfI as its restriction enzyme is presented in Figure 1. The PCR-RFLP analysis of exon 3 revealed the existence of one polymorphism (A > C) in all chicken groups. Three genotypes were obtained from the combination of the A and C; these were the AA (129 bp), AC (297 bp) CC (215 bp).

The gel picture of the PCR product for the identified IGF1 gene in the three genotypes of FUNAAB-Alpha, Kuroiler, and Sasso dual-purpose chicken breeds is shown in Figure 2.

Genotype Frequencies Based on IGF1 Gene Polymorphism

The number and percentages of IGF1 gene polymorphism among the chicken breeds are presented in Table 1.

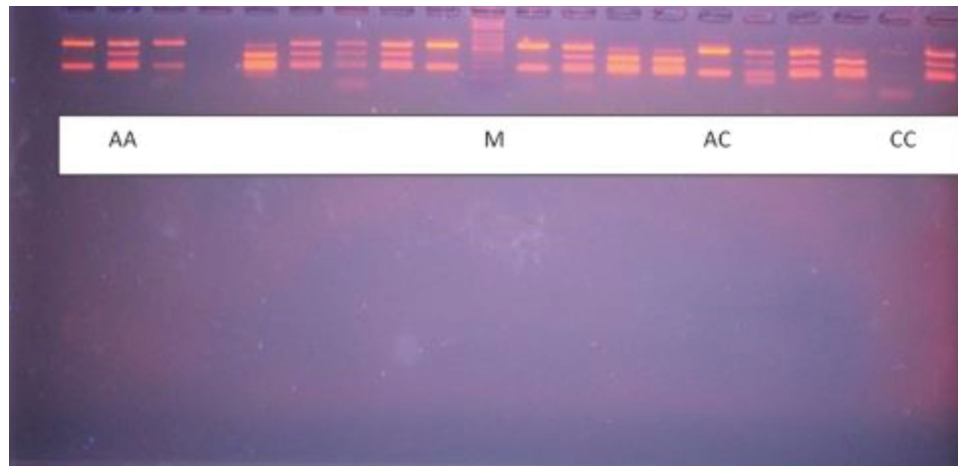


Figure 1. Result of PCR-RFLP analysis of IGF1 gene using HinfI.

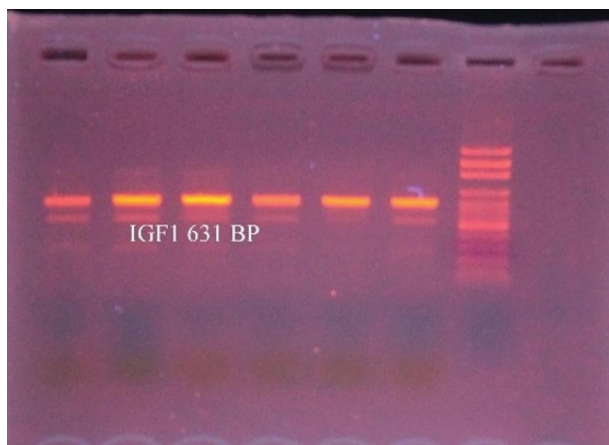


Figure 2. IGF1 gene optimization on agarose gel.

Allelic and Genotypic Frequencies of IGF1 Gene Within Chicken Populations

Table 2 shows the allelic frequency of IGF1 gene polymorphism as observed in the chicken populations. Allele A had the highest frequency in the chicken population when compared to the C allele. Considering the three genotypes resulting from the combination of A and C alleles (AA, AC, and CC). The heterozygote genotype (AC) had the highest frequency followed by AA and CC genotype, respectively.

Effect of IGF1 Gene Polymorphism on the Laying Performance of Kuroiler, Sasso, and Different Genotypes of FUNAAB-Alpha Dual-Purpose Chickens

The least-square means for the weight of bird at first lay, age of bird at first lay, and weight of the first egg as affected by IGF1 gene polymorphism of the three dual-purpose chickens are presented in Table 3. It was found that two alleles (A and C) exist for

the IGF1 gene polymorphism and their genotypes of AA, AC, and CC significantly ($P < 0.05$) affected the weight of the bird and age of the bird at first lay but show no significant ($P > 0.05$) influence on the weight of the first eggs. In this study, it was found that the Kuroiler chicken with genotype AA had $1,780.58 \pm 35.60$ g as their weight at first lay, 60.24 ± 3.48 g as the weight of their first egg and came into the first lay at an average of 147.35 ± 5.83 d of age. Those with band CC showed $1,700.50 \pm 25.45$ g as the weight of the bird at first lay, 58.37 ± 4.32 g as the weight of the first egg and came into lay at 140.60 ± 2.56 d while the weight of chickens with genotype AC was $1,690.69 \pm 18.21$ g at first lay, 53.58 ± 7.28 g as the weight of the first egg and they laid their first eggs at 147.35 ± 5.83 d.

However, Sasso chicken with haplotype AC recorded an average of $1,805.22 \pm 12.76$ g as their weight at first lay, 58.45 ± 6.60 g as the weight of the first eggs, and 154.30 ± 6.30 d was reported as their average period of the first lay. The value of $1,750.53 \pm 50.48$ g was reported as the weight of the bird at first lay for band CC, followed by 55.00 ± 9.90 g as the weight of their first eggs and 150.26 ± 8.80 d as the average period of first lay. Those with haplotype AA had $1,850.24 \pm 35.35$ g as average value for the bird at first lay, 60.45 ± 2.48 g as the average weight for first egg and 157.40 ± 4.90 d as their average age of first lay.

The Normal Feathered chicken with genotype CC had weights of $1,508.50 \pm 20.80$ g, AC ($1,550.40 \pm 25.70$ g) and AA ($1,650.45 \pm 25.68$ g) at first lay. The chickens with haplotype AA were reported to have 58.50 ± 4.35 g for weight of the first eggs followed by haplotypes CC and AC with corresponding values of 54.75 ± 6.04 g, and 50.60 ± 5.50 g, respectively. Those with genotype AC showed 137.64 ± 3.84 d, followed by AA ($133.43 \pm$

Table 1. Genotype frequencies of different chicken breeds based on IGF1 gene polymorphism

IGF1 genotype	FUNAAB-Alpha (%)	Sasso (%)	Kuroiler (%)	Total (%)
AA	11.35	8.80	1.65	21.80
AC	42.24	11.29	7.45	60.98
CC	10.55	6.35	0.32	17.22

Table 2. Allelic and genotypic frequencies of *IGF1* gene within each chicken's locus

IGF1	Allele		Genotype		
	A	C	AA	AC	CC
	0.58	0.42	0.22	0.43	0.35

8.68 d) and CC (126.50 ± 4.40 d) as their respective ages at first lay.

Similarly, the naked neck chicken with band CC was observed to have $1,580.69 \pm 18.21$ g as the weight of bird at first lay followed by those with bands AA and AC that showed the values of $1,600.55 \pm 20.95$ g and $1,440.25 \pm 26.10$ g, respectively. For the weight of the egg at first lay, AC haplotype had 58.00 ± 5.55 g while those with haplotype CC and AA displayed the corresponding values of 60.22 ± 3.25 g and 62.42 ± 2.45 g. On the other hand, chickens with band AA showed 135.44 ± 4.48 d, whereas genotypes CC and AC had corresponding values of 130.48 ± 4.28 d and 132.50 ± 4.80 d as their ages at first lay of eggs.

Nevertheless, the frizzle-feathered chickens with genotype AC showed $1,665.64 \pm 10.44$ g as their weight at first lay followed by genotypes AA and CC with corresponding values of $1,700.45 \pm 60.18$ g and $1,640.55 \pm 20.26$ g, respectively. Similarly, chickens with haplotype AC had 59.48 ± 7.28 g for the weight of egg at first lay and the values of 55.03 ± 4.30 g were observed for haplotype AA and 50.12 ± 8.18 g for chickens with band CC. The corresponding ages at first lay for genotypes AA, AC, and CC are 158.35 ± 6.80 , 160.55 ± 7.70 , and 157.57 ± 4.28 d, respectively.

DISCUSSION

Several studies have shown that the IGF1 gene is related to some reproductive traits in chickens. In the current study, the significance of IGF1 gene polymorphism on the reproductive performance of three dual-purpose chicken breeds was determined and it was found that three genotypes of IGF1 that is, AA, AC, and CC of different fragments were detected. However, the candidate gene approach has been reported by [Rothschild and Soller \(1997\)](#) and [Zhou et al. \(2005\)](#) to be an important method of estimating the relationship between genetic polymorphism and economically important

traits in farm animals. Also, polymorphism within the chicken IGF1 gene has been reported by many authors like ([Nagaraja et al., 2000](#); [Amills et al., 2003](#); [Bennet et al., 2006](#); [Moe et al., 2009](#); [Zhou et al., 2009](#); [Wheto et al., 2016](#); [Ilori et al., 2016](#)) but few reports have been given on the influence of IGF1 gene polymorphism on the reproductive performance of FUNAAB-Alpha chicken which is a Nigerian Improved indigenous chicken breed developed at the Federal University of Agriculture, Abeokuta, Nigeria ([Udoh, 2014](#); [Wheto et al., 2017](#); [Ogunpaimo et al., 2020](#)) and two other exotic breeds (Sasso and Kuroiler) that were imported into the country for comparison with the FUNAAB-Alpha breed in the establishment of dual-purpose chicken breeds for rural households in Nigeria.

This experiment affirms that the allele frequencies of IGF1 gene polymorphism in the three dual-purpose chicken breeds (FUNAAB-Alpha, Sasso, and Kuroiler) are in HWE, which shows that IGF1 gene of the chicken populations remains in equilibrium and the existence of genotypic variation in the reproductive performance of the three different chicken breeds. The variations in the body weight and age at the first lay of the birds can be linked to the difference in genetic background. This reason may be attributed to why the birds with CC genotype came earlier into lay compared to the other homozygote AA and their heterozygous counterpart, AC.

In addition to this, although, polymorphism of IGF1 shows that it may be a potential candidate gene associated with growth ([Zhou et al., 2005](#)). Among the three dual-purpose chicken breeds, the allele frequency for A was higher than C. It was also observed that the FUNAAB-Alpha breed had the highest frequency for allele A when compared to others, suggesting that the FUNAAB-Alpha chickens are genetically similar. This observation may be attributed to the fact that the FUNAAB-Alpha breeding chickens are mated through artificial insemination and within a specific location.

Table 3. Effect of IGF1 gene polymorphism on the laying performance of Kuroiler, Sasso, and different genotypes of FUNAAB-Alpha chicken breeds

Chicken breed	IGF1	Weight of bird at 1st lay (g)	Weight of 1st egg (g)	Age of bird at 1st lay (d+)
Kuroiler	AA	1,780.58 ± 35.60 ^a	60.24 ± 3.48	147.35 ± 5.83 ^a
	AC	1,690.69 ± 18.21 ^c	53.58 ± 7.28	145.60 ± 2.56 ^b
	CC	1,700.50 ± 25.45 ^b	58.37 ± 4.32	140.26 ± 6.36 ^c
Sasso	AA	1,850.24 ± 35.35 ^a	58.45 ± 6.60	157.40 ± 4.90 ^a
	AC	1,805.22 ± 12.76 ^b	60.45 ± 2.48	154.30 ± 6.30 ^b
	CC	1,750.53 ± 50.48 ^c	55.00 ± 9.90	150.26 ± 8.80 ^c
Normal feathered	AA	1,650.45 ± 25.68 ^a	58.50 ± 4.35	133.43 ± 8.68 ^b
	AC	1,550.40 ± 25.70 ^b	50.60 ± 5.50	137.50 ± 4.40 ^a
	CC	1,508.50 ± 20.80 ^c	54.75 ± 6.04	126.64 ± 3.84 ^c
Naked neck	AA	1,600.55 ± 20.95 ^a	62.42 ± 2.45	135.44 ± 4.48 ^a
	AC	1,440.25 ± 26.10 ^c	58.00 ± 5.55	132.50 ± 4.80 ^b
	CC	1,580.69 ± 18.21 ^b	60.22 ± 3.25	130.48 ± 4.28 ^c
Frizzle feathered	AA	1,700.45 ± 60.18 ^a	55.03 ± 4.30	158.35 ± 6.80 ^b
	AC	1,665.64 ± 10.44 ^b	59.48 ± 7.28	160.55 ± 7.70 ^a
	CC	1,640.55 ± 20.26 ^c	50.12 ± 8.18	157.57 ± 4.28 ^c

a, b, and c on the same column are significantly ($P < 0.05$) different.

Also, the Sasso chicken had a higher frequency for allele C than the Kuroiler chicken breed, hence the better laying performance of Sasso over the Kuroiler chicken breed. Moreover, it was discovered that IGF1 gene polymorphism had no significant association with the weight of first egg which contradicts the reports of Nagaraja et al. (2000) who found that the IGF1 gene has a significant influence on the weight of egg and the specific gravity. Although, it was observed that most of the chickens with higher body weight laid eggs with higher mean values which confirms a significant relationship between body weight and egg size. It must therefore be noted that chickens with genotype AA had the highest mean value for all the chickens used and this observation may also signify the need for further research to unravel the association between genotype AA and the bodyweight of these laying birds.

CONCLUSION

The study confirms that a significant relationship exists between IGF1 gene polymorphism and the chicken reproductive traits. However, further studies can be carried out to examine the effect of IGF1 genotype AA on the weight of first egg laid by the chickens.

Conflict of interest statement. The authors declare no conflict of interest.

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