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Allelic Polymorphism of Insulin Like Growth Factor 1 Gene and its Effect on Growth Performance of FUNAAB Alpha Chickens

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Abstract:- Insulin like Growth Factor 1 gene is a biological and positional candidate gene known to play an important role in growth, proliferation and differentiation, body composition, metabolism, skeletal characteristics, growth of adipose tissue and fat deposition in chicken. The polymorphisms of Insulin like Growth Factor 1 gene were investigated in the FUNAAB Alpha and Marshall Broiler chicken populations. A total of 642 FUNAAB Alpha dual purpose, 67 Marshall and 118 FUNAAB Alpha Broiler chickens were used. Blood samples were collected through the wing vein of each chicken with the use of a 5ml disposable syringe. Genomic Deoxyribonucleic acid was extracted from the blood samples using Zymobead™ Deoxyribonucleic acid extraction kit which was amplified using Polymerase Chain Reaction. The Polymerase Chain Reaction products were electrophoresed and amplicons obtained were digested with *HinfI* which was then electrophoresed on 1.5% agarose gel. Results obtained indicated that there was no observed polymorphism in the exons of the Insulin like Growth Factor 1 gene. However, the restriction analysis revealed two alleles; *A* and *C* in the promoter region. Within the chicken population, Allele *A* 0.57 and *C* 0.43 and genotypes *AA, AC* and *CC* were identified to have a genotypic frequency of 0.32, 0.49 and 0.19 respectively. The Chi square test showed significant difference among the three chicken populations. Significant association was also observed between the polymorphism of the Insulin like Growth Factor 1 gene promoter region and chicken breast girth. Higher mean values for body weight and breast girth were observed in the chicken with genotype *CC* and this confirmed that the effective allele in the chicken population is allele *C*. The study concluded that polymorphism of Insulin Like Growth Factor 1 gene in the promoter region is significantly associated with growth performance of FUNAAB alpha chickens. Insulin like Growth Factor 1 gene can therefore be used as a genetic marker to select for a higher body weight and wider breast girth in the chicken population.

Keywords:- *FUNAAB Alpha.*

I. INTRODUCTION

Local chicken still represents an appropriate system for supply of quality protein and production of adequate income for the increasing human population (Gueye, 2003). Their meat is being preferred widely by consumers because of their taste, leanness and suitability for special dishes (Horst, 1989). This ever increasing demand for poultry product has poised the poultry industry into the use of molecular approach in poultry development, both in growth and better reproductive potentials, which has led to the development of indigenously developed chicken breed; FUNAAB Alpha (Dual-Purpose and Broiler). This broiler line of indigenous origin was selected between 2014 and 2016 for distribution across rural household in Nigeria (Adebambo *et al.*2018). The breed was developed using the Naked neck and Frizzled feather chicken genotypes at the Federal University of Agriculture, Abeokuta, Nigeria over 6 generations of selection and inbreeding, followed by 4 generations of crossbreeding with some exotic lines to improve its growth and productive performance (Adebambo, *et al.*, 2018). However, the use of selection and cross breeding offered a means through which this genetic improvement in Nigeria indigenous chicken has been systematically leveraged and sustained for improved productivity.

Growth is regulated by various factors such as genes, hormones, nutrition and environment (Sato *et al.*, 2011). Massive improvements in chicken production trait and health have been achieved simultaneously through the use of molecular approach, using markers associated with two or more characteristics (Ilori *et al.*, 2016). Insulin like Growth Factors (*IGFs)* regulates muscle growth development in chickens (Duclos *et al.*, 1999), having a greater proportion existing in plasma as free peptides compared with the situation in mammals. Moreso, it is similar to some other species and several differences in Insulin Like Growth Factor 1 (*IGF1)* physiology does not exist between birds and mammals (McMurtry, 1998). Notably, *IGF*1 gene is a candidate gene for growth, body composition, metabolism, skeletal characteristics, growth of adipose tissue and fat deposition (Zhou *et al.*, 2005) in chickens. McMurtry (1998) reported that GH-*IGF1* axis in chickens seems to vary in sensitivity to alteration in the

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plane of nutrition. For example, feed deprivation for five (5) days depresses circulating *IGF1* concentration and upon feeding; the concentration returns to near its initial concentration, which suggests that the extent of nutrient deprivation determines the degree to which *IGF1* synthesis and secretion returns to normal following periods of nutrient modification (Zhou *et al.,* 2005).

The existence of different strains of chickens with different growth rate (Gouda and Essawy, 2009) was thought to have resulted from the complexes of both Growth Hormone Receptor (GHR) and Insulin like Growth Factor 1 (*GH-IGF1*) system controls a number of focciles in animals that are recruited towards rapid growth phase (Monget *et al.*, 2004) which might have been modified as a result of selection towards enhanced growth rate (Ge *et al.,* 2001). This existing variations between and among strains has brought to light evidence indicating that genetic polymorphism of *IGF1* gene is associated with body weight, breast girth, and height characteristics (Ballard *et al.*, 1990). Thus, an indication that the *IGF1*gene is a very suitable target gene for genetic manipulations (Goddard and Boswell, 1991). The aim of this study was to assess the genetic variability amongst FUNAAB Alpha chickens based on the *IGF1* gene polymorphism and it effect on their growth performance.

II. MATERIALS AND METHODS

A. Study Location

The experiment was carried out at the Poultry Breeding Unit of Directorate of University Farms, Federal University of Agriculture Abeokuta, Nigeria. The University is located within latitude 7^{o} 10'N and longitude 3^{o} 2'E and lies in the south western part of Nigeria. It has an average temperature of 33.7oC and relative humidity of 80% with rainfall of about 1037mm. the vegetation in the University represents an interphase between the tropical rainforest and the derived savannah (Goggle Earth, 2018).

B. Experimental Birds and Management

A total of 827 birds were used for this experiment. The experimental birds comprises of three FUNAAB Alpha dual-purpose genotypes (Normal feather, Frizzled feather and Naked neck) in comparison with the Marshall exotic and FUNAAB Alpha Broiler chickens and this consisted of 642 FUNAAB Alpha dual-purpose chickens (Normal feather 443, 178 Naked neck and 21 Frizzled feather), 67 Marshall exotic and 118 FUNAAB Alpha Broiler chicken. Pure mating between sires and dams of each genotype was carried out to generate the progenies used for this study through Artificial Insemination. (A I). Eggs collected from inseminated female chickens were pedigreed along sire and dam lines. Excellent shape and sound eggs were selected and stored for a period of one (1) week in a cold room at a temperature between 20^{o}C and 25^{o}C and relative humidity of 75%. Proper cleaning, disinfection and fumigation were done before setting of eggs in the incubator along genotype lines.

Chicks produced from the mating of each genotype were properly identified and wing tagged then transferred to a previously disinfected brooder pen. Each genotype was brooded for a period of four weeks. Necessary medications and vaccination were administered in addition to proper management practices. The chicks were fed at *ad libitum* with chick's starter marsh which supplied 21% crude protein and 2800Kcal/Kg metabolizable from 0-8 weeks of age and grower marsh that supplied 19% crude protein and 2700 Kcal/kg metabolizable energy for the remaining 10 weeks. The birds had free access to clean water throughout the period of experiment.

III. DATA COLLECTION

Growth data which included the body weight and breast girth of the birds were taken on a weekly basis from day-old till 10 weeks of age as described below:

- Body weight: This was measured using a sensitive scale having a maximum calibration of 5kg and sensitivity of 0.01.
- Breast girth: This was measured as the circumference of the breast from the deepest region of the breast using a flexible tailor's tape rule.

A. Blood Collection

Blood samples of the different chicken genotypes were collected at 10th week of age. This was done via the wing vein using 5ml disposable syringe. The area of skin was disinfected with alcohol which made the vein visible as 2ml of blood was taken into the syringe and transferred into a labeled test tube containing an anticoagulant Ethylene Diamine tetracetic Acid (EDTA).

B. DNA Extraction and Genotyping

Genomic DNA was extracted from whole blood using Zymobead™ DNA extraction kit following the manufacturer's protocol. The purity and concentration of the extracted DNA was carried out using Nanodrop™ spectrophotometer.

The genomic sequence and primer characteristics as reported by Sato *et al.*, (2012) were used for the research Table (1). The Polymerase Chain Reaction (PCR) mixture contain a 15µl reaction volume; 2µl gDNA, 3µl Master mix and 8µl Free water. The reaction mixture was subject to initial temperature for 2 minutes of denaturation at 94˚C, followed by 30 cycles of denaturation of 98˚C for 10 sec, annealing temperature at 55 ˚c for 30 seconds, extension of 72˚C for 10 minutes. The amplicons were digested with *Hinf1* restriction enzyme. 17µl of Nuclease free water, 5µl of 10X FastDigest Green Buffer, 10µl of PCR product and 1µl of FastDigest enzyme were subjected to digestion for 20 minutes at 80^{0}C. The restriction digests were separated using 1.5% agarose gel in 1×TBE at a constant current of 100v for 1hour. The gel was stained with ethidium bromide and the fragments were visualized using a UV trans illuminator. Genotyping was carried out manually following the scoring procedure of Darabi *et al.* (2010).

ISSN No:-2456-2165

IV. STATISTICAL ANALYSIS

Growth data collected were analyzed using the General Linear Model (GLM) procedure of Statistical Analysis System (SAS, 2009). Duncan's Multiple Range Test was used to separate the means that differed significantly.

Allelic and genotypic frequencies were calculated using the following formula:

A. Allelic Frequency

$$p = \frac{2AA+AC}{2N} \quad (1)$$

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

Where:
p = Gene frequency of allele A,
q = Gene frequency of allele C
N = Total number of birds tested

B. Genotype Frequency
$AA = A^2$ $AC = 2(A*C)$
$CC = C^2$
AA= Genotype frequency for Genotype AA
AC= Genotype frequency for genotype AC
CC= Genotype frequency for genotype CC
Test for Hardy Weinberg Equilibrium (HWE) and population differentiation measures were determined by Chi square (χ^2) analysis.

V. RESULTS

Sequences of PCR primers for amplification of *IGF1* Chicken gene			
Fragment name	**Primer Sequence (5'-3') (Forward/Reverse)**	**Region**	**Product Size (bp)**
IGF1- proF	CTCTGCCACGAATGAAATGTGC	Promoter	361
IGF1- proR	GGGAGCATTTGCCTTCTCTC		
IGF1- ex1F	TGACATTGCCAACATCTCA	Exon 1	231
IGF1- ex1R	TCAAAGCAGAAGCAGACAACA		
IGF1- ex2F	CTCTGTTGGGAAACTGACACAG	Exon 2	225
IGF1 - ex2R	GCAGTTGAATGAAAGGGTTGA		
IGF1- ex3F	CGTGAAAACTTTCCATTTGTCTT	Exon 3	247
IGF1- ex3R	AAAATAGAGCTTTTTGTCTTTTGG		
IGF1- ex4F	CAGTGATCTGGCTGAAGAGC	Exon 4	234
IGF1- ex4R	CTGCAGATGGCACATTCATT		

Table 1

F: Forward Primer R: Reverse Primer

The gene sequence of the primers for the IGF1 gene amplification are listed in Table (1). The electrophoretic pattern of the PCR-RFLP performed for the chicken *IGF1* gene using *Hinf1* as its restriction enzyme is presented in Fig. (1). The PCR-RFLP analysis of the four exons (1, 2, 3 and 4) and promoter region of the different chicken genotypes revealed the existence of one polymorphism (*A* > *C*) in all chicken groups. This was located in the promoter region which produced an *A* to *C* transverse substitution of the base. Three genotypes were obtained from the combination of the *A* and *C*; these were the *AA* (117bp), *AC* (244bp) *CC* (361bp).

The allelic and genotypic frequencies of the *IGF1* gene of the various chicken populations are represented in Tables (2) and (3). The Allele *A* had the highest frequency (0.57) in the chicken population when compared to the *C* allele (0.43). The three genotypes which resulted from the combination of *A* and *C* alleles (*AA*, *AC* and *CC*), the heterozygote genotype (*AC*) had the highest frequency (0.49) followed by *AA* (0.32) and *CC* genotype (0.19) respectively. The AC genotype of the *IGF1* gene genotypes (AA, AC and CC) had the highest proportion (64.33%) between and within the chicken populations. The improved Nigerian indigenous chicken (47.88%) had the highest proportion, followed by FUNAAB Alpha Broiler (10.16%) and Marshall (6.29%). Similarly, the *AA* genotype had a higher proportion (24.55%) than the *CC* (11.12%) among and within chicken populations.

The allelic and genotypic frequencies of the *IGF1* gene among the chickens are presented in Table (3). The FUNAAB Alpha dual-purpose chicken had the highest frequency of *A* allele (0.58) and the least observed in the FUNAAB Alpha broiler (0.52). FUNAAB Alpha Broiler had the highest frequency for the *C* allele (0.48) and the least was found in the FUNAAB Alpha dual-purpose chicken (0.42), while the Marshall has its allele frequency for *A* and *C* to be 0.54 and 0.46 respectively. The frequency of the heterozygote genotype; *AC* was higher than its homozygote counterparts (*AA* and *CC*) in all chicken genotypes. The frequency of the *AC* genotypes among the different chicken populations was in close margin, such that proportion of the genotype (*AC*) among the chicken population is not different.

ISSN No:-2456-2165

The allele and genotype frequencies of *IGF1* gene in the FUNAAB Alpha dual-purpose genotypes is presented in Table (4). Within the FUNAAB Alpha dual-purpose chicken population; the allele frequency of *A* was higher (0.62) than that of *C* allele (0.42). Frizzled feather had the highest frequency for the *A* allele (0.62), while both Naked neck and Normal Feather had similar but lesser frequencies (0.42), although possessing a higher frequency of the *C* allele (0.58) when compared to the d feather (0.38).

The genotype frequencies of *AA*, *AC*, and *CC* was observed not to be significantly different for the chicken groups. However, the frizzled feather had the highest genotype frequency for the *AA* genotype (0.38), while the least was observed in the Normal feather genotype (0.33). The Normal feather had a higher frequency of *AC* genotype (0.49) than the other chicken groups while the least frequency was observed among the frizzled feather (0.47). The Naked neck had the highest frequency (0.18) for *CC* genotype.

The effect of *IGF1* gene polymorphisms on chicken body weight and breast girth at 10 weeks of age is presented in Table 5. The effect of the *IGF1* genotype was not significant ($p<0.05$) on body weights but on breast girth of the chicken populations. The homozygote mutant genotype *CC* had the highest mean body weight (692.96±27.31) when compared to the AC (668.75±11.36 cm) and AA (627±18.43 cm) genotypes.

There was significant difference between breast girth of *AC* and the *AA* genotype, while there was no significant difference ($p<0.05$) between the *AC* and *CC* genotypes. However, the *CC* genotypes had the highest breast girth values (21.35±0.29) and the least was observed in the *AA* genotype (20.64±0.20), while the AC genotype had a value of (21.28±0.12).

There was consistency in the order of ranking of the genotypes, both for body weight and breast girth. The observed order of supremacy in this present study was *CC* > *AC* and *AA*.

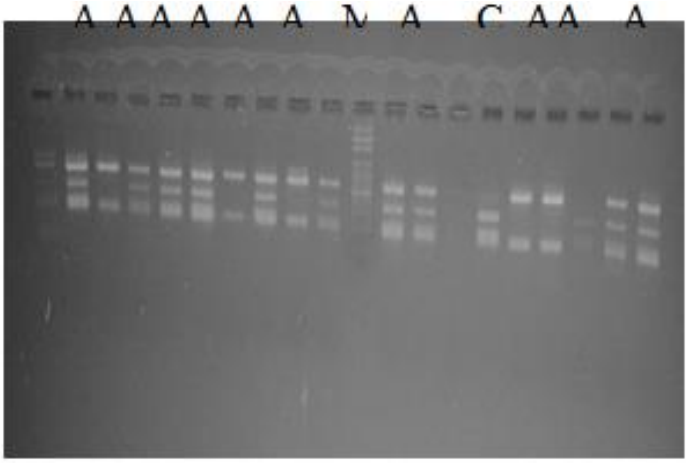

Fig 1:- Result of PCR-RFLP analysis of *IGF1* gene promoter region using *Hinf1* (restriction enzyme).

Allele and genotype frequency of *IGF1* gene within chicken population					
Locus Allele Frequency			Genotype Frequency		
IGF1	**A**	**C**	**AA**	**AC**	**CC**
	0.57	0.43	0.32	0.49	0.19

Table 2

***IGF1* Genotype**	**Genotype frequencies of individuals within chicken populations based on *IGF1* gene polymorphism**			
	FUNAAB Alpha Dual purpose Chicken (%)	FUNAAB Alpha Broiler (%)	Marshall (%)	Total (%)
A A	174 (21.04)	19 **(2.30)**	10 **(1.21)**	**24.55**
AC	396 **(47.88)**	84 **(10.16)**	52 **(6.29)**	**64.33**
CC	72 **(8.71)**	15 **(1.81)**	5 **(0.60)**	**11.12**

Table 3

Allele and genotype frequency of *IGF1* gene among FUNAAB Alpha dual- purpose chicken genotypes					
Chicken Genotype (No.)	**Allele Frequency**	**Genotype frequency**			
	A	**C**	**AA**	**AC**	**CC**
FF (21)	0.62	0.38	0.38	0.47	0.15
NN (178)	0.58	0.42	0.34	0.48	0.18
NF (443)	0.58	0.42	0.33	0.49	0.17

Table 4

FF:Frizzled Feather, *NN:* Naked Neck, *NF:* Normal Feather.

Effect of *IGF1* gene polymorphism on body weight (g) and breast girth (cm) at 10 weeks of age		
IGF1 polymorphisms	Body Weight (g)	Breast Girth (cm)
AA	627.179±18.43	20.64±0.20 [b]
AC	668.75±11.36	21.28±0.12[a]
CC	692.96±27.31	21.35±0.29[a]

Table 5

[a,b] Means in the same column with different superscript are significantly different ($p < 0.05$).

VI. DISCUSSION

Marker assisted selection on an individual gene having major phenotypic effect on chicken's growth is the basis of this study. There was no identifiable changes (Polymorphism) detected in the *IGF1* gene exons of all three chicken populations analyzed. This was also discovered in the studies of Amills *et al.* (2003), Zhou *et al.* (2005) and Sato *et al.* (2011). The allele frequencies of *IGF1*gene polymorphism in the three different chicken populations (FUNAAB Alpha dual-purpose chicken, FUNAAB Alpha broiler and Marshall) are in HardyWeinberg equilibrium, which suggests that the chicken populations remain in equilibrium and no change in genetic constituent was possible. Among the three chicken populations, the Allele frequency for *A* was higher than *C*. The FUNAAB Alpha dual-purpose chicken had the highest frequency for *A* allele when compared to its broiler counterparts, suggesting that the FUNAAB Alpha dual-purpose chicken are genetically similar; maintaining its ancestral allele. This may be due to the fact that breeder birds are managed in a specific location and being mated through artificial insemination. The FUNAAB Alpha broiler and Marshall Chicken had higher frequency for *C* allele than the *A* allele when compared to the FUNAAB Alpha Dual-purpose chickens, hence their better performance; most especially the FUNAAB Alpha chicken. The progressive improvement of this developed crossbred using exotic sire line is to capture the additive effects for growth performance in the tri-hybrids, while the indigenous dam line involved is very useful in incorporating the adaptive potential in the newly developed strain (Adeleke *et al*., 2011). The variation in the phenotypic and genetic constituent of the strains involved in the development of this chicken has led to the better performing ability of the crossbred which confirmed that the effective allele observed in the study is *C*, because the broiler birds (Exotic sire line) had greater percentage of allele *C*, which is being referred to as the broiler allele (Zhou *et al.*, 2005). This further agrees with reports that the *IGF1* gene broiler allele (allele *C)* produced heavier body weight at all ages to market weight (Ilori *et al.*, 2016) when compared to the slower growing allele *A*, in the local chickens (Zhou *et al.*, 2005).

Allele frequencies of detected polymorphism depicts that chickens of the improved Nigerian Indigenous origin, except for the frizzled feather that had the *CC* genotype, indicating that both the Naked neck and Normal feather had all the three polymorphism. This is in agreement with discovery of Ilori *et al.* (2016) that the frizzled feather *IGF1* gene region is relatively conserved for a particular allele. Also, Moe *et al.* (2009) revealed that only Chabo chicken of the Japanese native chicken breed lack the *CC* genotype. The fixation of the *CC* genotype in this chicken group is dependent on the long-term maintenance of the chicken group in small flock as closed colony (Moe *et al.*, 2009) before its present day improvement programme. However, Nigerian indigenous chicken might not be fixed in one allele and or genotype because for centuries, they have been maintained in open back yard, within homes and villages and inter-mating among different chicken groups. Thus, suggesting high diversity in *IGF1* gene among the different chicken populations of Nigerian indigenous chicken (Ilori *et al.*, 2016). Furthermore, the FUNAAB Alpha dual-purpose chicken was not in Hardy-Weinberg equilibrium, for it is very difficult to accurately estimate the probability of these gene and genotypes frequencies among Nigerian indigenous chicken in strict compliance with Hardy-Weinberg principle since both man and nature

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continually selects against genes in local population and both migration and mutation do occur in any natural population (Adebambo, 2010).

Genes of the growth axis are known to play vital role in regulation of growth, development and cell differentiation (Gouda and Essawy, 2009). Many studies have revealed that *IGF1*, a candidate gene of the growth axis and its polymorphisms are related to some growth traits in chicken. From the result obtained, allele *C* showed significant ($p<0.05$) effect on breast girth, while its effect on body weight was not statistically different for the three genotypes.

The mean body weight of birds with of *AA* and *AC* were lower than that of *CC* genotype, while the C*C* and A*C* genotype were significantly higher than *AA* for breast girth. This corroborates with the result obtained by Wheto *et al.* (2016) and Mu"in *et al.* (2010) who found that chicken with genotype *AA* and *AC* had lower body weights than their homozygote *CC* variant. Zhou *et al.* (2009) discovered that *IGF1* gene genotype had significant effect on body weight and carcass composition. Lie *et al.* (2007) also revealed that `high mean values were obtained for body weight of chicken with genotype *CC*, and that the *IGF1* polymorphism is significantly associated with breast yield and leg muscles of chicken. Moreover, the study carried out by Amills *et al.* (2003) revealed that the association between *IGF1* promoter polymorphism found in two genetically diverse Black Penedsenca chicken is associated with average daily gain, while Promwatee and Duangjinda (2010) found that this polymorphism of the *IGF1* promoter region was associated with body weight.

In addition, the reports by several authors (Scheuermann *et al.*, 2003, 2004, Gouda and Essawy 2009) shows that the *IGF1* gene is associated with body weight, breast weight and breast yield; as myofiber number and densities are related to body weight, breast weight and yield. As a result, the *IGF1* could be used as a candidate gene for growth performance traits; for its effect on growth traits is well established in poultry.

VII. CONCLUSION

The promoter region of the chicken *IGF1* gene region was polymorphic. The identifiable polymorphism was the transversion of the *A* to *C*, having three identifiable fragments; *AA, AC and CC* (117, 244 and 361 bp respectively). The identified allele (A and *C*) were in different proportions of Allele and Genotype frequencies. Hence, the three populations were tested to be in Hardy-Weinberg equilibrium and the polymorphism of the *IGF1* gene promoter region was significantly associated with breast girth but not with the body weight.

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