



Heritability of Body Weight, Growth Rate Indices and Their Association with Insulin-Like Growth Factor-2 Gene Polymorphism in Purebred Nigerian Indigenous Chickens and their Crosses with Marshal Chickens

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RESEARCH ARTICLE

Abstract

This research was conducted to estimate heritability of body weight and growth rate indices and also to determine their association with insulin-like growth factor-2 genes (*IGF-2*) polymorphism in purebred Nigerian indigenous chickens and their crosses with Marshal chickens. A total of 300 progenies were generated from the mating of three genotypes of F5 generation of Nigerian indigenous chickens (Normal feather (N), Frizzle feather (F), Naked neck (NN)) and Marshal (M) chickens. Blood samples were collected at twenty weeks for DNA extraction and *IGF-2* gene amplification. *IGF-2* gene was digested by restriction enzyme (Pst1) and genotyped using restriction fragment length polymorphism method. Growth rate indices (absolute (AGR) and relative growth rates (RGR)) were estimated and associated with *IGF-2* gene polymorphism. The results show that *IGF-2* allelic variants had no effect on the body weight (BW), AGR and RGR while chicken's genotype effect was significant ($p < 0.01$) on BW and AGR. Two crossbred genotypes (NxM, 1524.23 ± 137.20 and 1894.92 ± 122.81), (FxM, 1445.19 ± 177.24 and 1840.88 ± 177.86) had better performance for AGR and BW, respectively, compared to the purebred of Nigerian indigenous chickens. The heritability values ranged from low ($.06 \pm .021$) to high ($.77 \pm .24$).

Keywords: Marshal, Purebred, Crossbred, *IGF-2*, Heritability.

INTRODUCTION

Growth is a trait of economic importance in poultry production and it is influenced by genetics and environmental factors. It is an increase in body size per unit of time (Lawrence and Fowler, 2002). Growth is a sigmoidal function during the animal's life from embryonic stages up to adult age and it is mathematically explained by growth models that have parameters with biological meaning (Fitzhugh, 1976). These parameters are used to describe growth over time and to estimate the expected weight of individuals at a specific age (Yakupoglu and Atil, 2001). Mathematical models are used to identify better strategies to improve livestock production and also in estimating the daily nutrient requirements of animals at different ages and genetic groups (Pomar et al., 2009). Growth curves are mathematical parameters that are biologically interpretable (Tzeng and Becker, 1981; Aggrey, 2002; Yang et al., 2006). Moreso, growth curve models are of great importance for animal production because they provide an opportunity for practical interpretations of farmer decisions (Akbas and Oguz, 1998). These growth curve parameters are highly heritable and widely used in selection studies

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(Grossman and Bohren, 1985; Mignon-Grasteau et al., 2000). Mathematical growth functions can be used to predict daily energy, protein, and mineral dietary requirements, suitable slaughter age and age of sexual maturity (Darmani-Kuhi et al., 2010). A lot of reports are available on growth curves in animals such as chickens (Adenaike et al., 2017, Mignon-Grasteau et al., 2000; Peters et al., 2005; Norris et al., 2007) rabbits (Blasco et al., 2003) and turkey (Seng and Kuraz, 2005).

Several non-linear models have been compared and used to evaluate the growth curves of different chicken breeds (Roush and Branton, 2005). These models include Gompertz, Richards, Logistic, Weibull, von Bertalanffy, Exponential and other type of models (Yang et al., 2006; Topal and Bolukbasi, 2008; Eleroglu et al., 2014; Moharrery and Mirzaei, 2014; Mohammed, 2015; Durosaro et al., 2016; Adenaike et al., 2017; Nariç et al., 2017).

A quantitative trait such as growth in poultry is influenced by genetic factors, environmental factors and their interactions (Lawrence and Fowler, 2012, Adenaike et al., 2019a). Growth is a biological function tightly regulated by multiple neuroendocrine factors. Insulin-like growth factor-2 (*IGF-2*) is a peptide hormone that plays a crucial role in the growth and metabolic regulations in vertebrates (Cornish et al., 2007). It is a candidate gene for growth in livestock because it affects development and growth, which regulates somatic growth including muscles, bones, epithelium and fibroblast cells (Abbasi and Kazemi, 2011). It plays significant roles in embryonic growth in avian and also is expressed in liver, kidney, heart, and muscle in juvenile chickens bi-allelically, with no imprinting (Wang et al., 2005). The *IGF-2* gene contains three exons and two intron maps to chromosome 5 (Darling and Brickell, 1996; Yokomine et al., 2001). Various studies revealed associations between *IGF-2* gene polymorphism and economic traits of pigs, cattle, buffalo and chicken (Li et al., 2004; Yan et al., 2015; Wheto et al., 2016).

The Nigerian indigenous chickens are known to be dual-purpose birds that are used both in meat and egg production in the rural and urban areas of the country (Sonaiya and Olori, 1990). The indigenous chicken represents valuable resources for livestock development because their extensive genetic diversity allows rearing under varied environmental conditions (Sonaiya et al., 1999, Adenaike et al., 2023). They have been subjected to purposive selective breeding for particular traits (e.g resistance to diseases) or to the adaptation to particular environments (Uswege et al., 1996, Adenaike et al., 2018, 2019b). The evaluation of the genetic ability and variation of indigenous chickens is vital and forms the basis for modeling breeding programs and making a reasonable decision on the sustainable use of animal genetic resources (Egena et al., 2014).

MATERIALS AND METHODS

The experiment was carried out at the Poultry Unit, Directorate of University Farms (DUFARMS) of the Federal University of Agriculture Abeokuta (FUNAAB), Nigeria. The Federal University of Agriculture, Abeokuta is located on latitude 7°10' 42" N and longitude 3°22' 01" E (GoogleEarth, 2021) and lies in the southwestern part of Nigeria with a prevailing tropical climate with a mean annual rainfall of 1037mm. The mean monthly ambient temperature ranges from 28 °C in December to 36 °C in February with a yearly average humidity of about 82 %. The vegetation in the University represents an interphase between the tropical rainforest and the derived savannah (www.climatedata.com). The laboratory analysis was carried out at the Department of Animal Breeding and Genetics Biotechnology Laboratory, FUNAAB.

Experimental animals

A total number of 300 crossbred progenies were generated from the mating of three (3) genotypes of F5 generation of Nigerian indigenous chickens (Normal feather, Frizzle feather and Naked neck) and Marshal chickens. Chicks from each genetic group were differentiated and individually identified by wing tagging. Vaccinations and medications were administered. The chicks were transferred to a disinfected brooder house where a standard management procedure was strictly adhered to as described by Peters et al. (2005). Brooding was done for 4 weeks. The chicks were fed with a commercial starter ration (23-25% CP and 2850-3000 kcal/kgME) from day old to 8 weeks of age and also with a commercial grower ration (20 %CP and 2850 kcal/kgME) from 8 weeks to 20 weeks of age which was the end of the experiment. Feed and water were provided ad libitum throughout the experiment period.

Data collection

Data were collected on the body weight of birds using a sensitive scale with a sensitivity of 0.01 g at 2 weeks intervals for the period of 20 weeks.

Blood sample collection

A total of 200 chicks were selected at random from the total of 300 chicks hatched from each genotype at sixteen (16) weeks of age, blood samples were collected from 200 birds that were selected across the six (6) genotypes. Samples of blood were collected from each bird through their brachial (wing) vein using a 2 ml sterile syringe. The blood samples (each sample on an area of card) were transferred immediately into Flinder Technology Associate (FTA).

DNA extraction from FTA card

One hundred and twenty samples of chicken's blood (20 samples per genotype) were used for DNA extraction. Five 1 mm discs were punched out from the 120 FTA cards using a 1 mm Harris p-punch on a cutting mat. The discs were placed in the 1.5 ml Eppendorf tube and 200 µl (for each disc) FTA purification reagent was added into the tubes and the tubes were placed in a shaker for it to rotate for 30 minutes, the spent solution was tipped off. The wash process was repeated with 200 µl of distilled water. The wash process was repeated with 200 µl of distilled water for 10 minutes without shaking and the spent solution was tipped off. Fifty microliters (50 µl) of distilled water were added to each tube and were heated in a water bath at 90 °C for 15 minutes after the DNA was ready for use.

Polymerase Chain Reaction and Amplification

Each Polymerase Chain Reaction (PCR) mixture consisted of 12.5 µl mastermix (2x JENA Ruby hot start pol), 1 µl (10 pmol) of the pair of forward and reverse primer of *IGF-2* gene, 1 µl DNA template and 9.5 µl sterile nuclease-free water to make a total reaction of 25 µl. PCR amplification was carried out in an Applied Biosystem 2720 Thermocycler. The primer (Forward: 5' CCAGTGGGACGAAATAACAGGAGGA 3' and Reverse: 5' TTCCTGGGGCCGGTCGCGCTTCA 3', Amill et al., (2003)) was synthesized by Macrogen, South Korea. The mixture was subjected to an initial denaturation at 94 °C for 2 minutes followed by 34 cycles of denaturation at 94 °C at 60 s, annealing at 67 °C for 2 minutes and extension at 72 °C for 3 minutes; and a final extension at 72 °C for 8 minutes. The PCR products were visualized on a 2 % agarose gel containing ethidium bromide in 0.5x Tris- borate buffer (pH 8.0).

DNA digestion by Pst 1 enzyme

Genotyping of the SNP was done using restriction fragment length polymorphism (RFLP) alongside the restriction enzyme. Each reaction mixture contains 10 µl PCR products, 1.5 µl 10 x NEBuffer, 0.1 µl pst 1 restriction enzyme and 3.4 µl of nuclease-free water to make a total reaction volume of 15 µl. The mixtures were incubated at 37 °C for 15 minutes and the enzyme was inactivated at 80 °C for 20 minutes. The enzyme digestion products were visualized on 2 % agarose gel containing ethidium bromide in 0.5 x Tris- borate buffer (pH 8.0) with a 100 bp molecular marker (Jena Biosciences).

Statistical analysis

The parameters of growth curves and indices were estimated by using a non-linear function - Brody, Gompertz, Logistic and von Bertalanffy in the NLIN of SAS.

The models were given as follows:

$$\text{Gompertz: } y_t = Ae^{-b \exp(-kt)} + \varepsilon_t$$

$$\text{Logistic: } y_t = A/(1 + e^{-kt}) + \varepsilon_t$$

$$\text{Brody: } y_t = A(1 - be^{-kt}) + \varepsilon_t$$

$$\text{Von Bertalanffy: } y_t = A(1 - be^{-kt})^3 + \varepsilon_t$$

Where:

- y_t represented the weight of the animal at a given age (t);
- parameter A was the asymptotic weight, if $t \rightarrow \infty$;
- when the adult weight of the animal was not reached, this reflected in an estimate of the weight of the last weighing;
- b was a constant without biological interpretation, but it was important to model the sigmoidal format of the growth curve from birth ($t=0$) up to the adult age of the animal ($t \rightarrow \infty$);
- K was the maturity index, which expressed the ratio of the maximum growth rate in relation to the adult size, where lower k values indicated delayed maturity and higher k values indicated accelerated maturity;
- M was the parameter that shaped the curve; e was the natural base logarithm; the L parameter had no biological meaning, but together with K constituted b , which had the function of modelling the sigmoidal curve; and
- ε represented the residue error associated with each weighing.

The model below was used to associate chicken genotypes, sex and *IGF-2* gene with body weight and growth rate indices:

$$Y_{ijkl} = \mu + C_i + S_j + G_k + \varepsilon_l$$

Where:

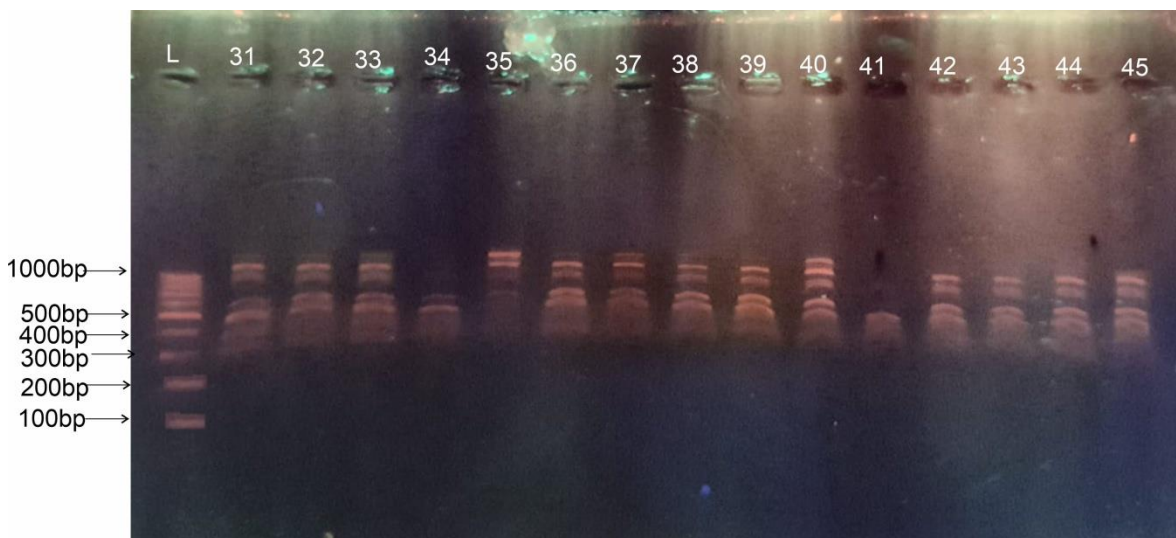
- Y_{ijkl} is the trait of interest,
- μ is the populations mean,
- C_i is the fixed effect of i^{th} chicken genotypes,
- S_j is the fixed effect of j^{th} sex,
- G_k is the effect of k^{th} IGF-2 gene variants and
- ϵ_l is the random residual errors. The heritability for the traits was estimated using Proc mixed in SAS. $h^2 = \sigma_a^2 / \sigma_p^2$.

The phenotypic variance σ_p^2 for each trait was calculated as the sum of the additive genetic variance σ_a^2 and the residual variance σ_e^2 .

RESULTS AND DISCUSSIONS

PCR and RFLP analysis

The digestion of the PCR products of *IGF-2* produced one band size (500 bp) for the AA allele and two band sizes (32.05 %) for the AB allele (500 bp, 1000 bp) while BB allele is represented by single band (1000 bp) that represents which makes up 24.35 percent of the population (Figure 1). Abbasi and Kazemi (2011) reported digestion of amplified product of *IGF-2* reveals the existence of polymorphic fragments with the length of 500 bp 1000 bp, which is line with this study. Several SNPs have been reported in *IGF-2* across several chicken breeds. Wang et al. (2005) identified the polymorphism of *IGF-2* gene using PCR-SSCP and the resultant PCR products derived three types of bands from the exon 2 displayed different band patterns in Silky chickens which is similar to the results of this study.



L= 100bp ladder

Figure 1. *IGF-2* genotypes in Purebred Nigerian indigenous chicken and their crosses with Marhsal chickens using PCR- RFLP

Effect of sex, genotype and *IGF-2* on growth rate indices and body weight of NIC

The analysis of the data presented in Table 1 showed that there was a significant ($P < 0.01$) effect of chicken genotypes on AGR, RGR and BW. There is no significant effect of sex on body weight, AGR and RGR. There is no significant effect of *IGF-2* gene on body weight, AGR and RGR. The mean values of AGR, RGR and BW are presented in Table 2. Although statistical effect of sex did not significantly differ, the mean value observed in males was higher in AGR (1115 ± 103.51) and BW (1533.24 ± 93.93 g) than mean value in female (AGR, 1112.94 ± 72.01) (BW, 1527.95 ± 61.26 g) while the RGR of the female (3.53 ± 0.16) was higher than the male (3.48 ± 0.21). Santos et al. (2005); Rizzi et al. (2013); Eleroğlu et al. (2014) reported that male chickens showed higher values than females in terms of BW and this result was found to be in agreement with the findings. Teleken et al. (2017) reported that parameters in females were lower than in males using the Logistic and Von Bertalanffy models proving our finding

in the present study. Statistically effect of *IGF-2* did not significantly differ; however, the AGR and BW of genotypic allele AB were higher than values obtained for AGR and BW in AA and BB. The AA allele had the highest RGR value than AB and BB. Growth curves parameters are used in selection indices and selecting appropriate growth models in poultry species. The mature weight (A) offered the best opportunity to make comparisons. The estimated value of A, b, k was observed to be highest in FxM genotype closely followed NxM and NKxM while the least A, b, k was observed in NK. The largest A is generally associated with the small estimate of k but this was not observed in this study. This could be a result of gene and environmental influence on the slope of the weight-curve. This implies that the genotypes crossed with Marshal had the best rate of maturing and greater maturing weight. This result was found to be in agreement with the Adenaike et al. (2017).

Table 1. Analysis of variance showing the effects of sex, genotype and *IGF-2* on growth rate indices and body weight at 20 weeks

PARAMETERS	DF	BW	AGR	RGR
Sex	1	19989.059	31831.295	0.2843
Genotype	5	781323.775**	990921.797**	2.5426**
<i>IGF-2</i>	2	34432.053	61764.200	0.9261

Note:**P<0.01, DF: Degree of Freedom, BW: Body Weight, AGR: Absolute Growth Rate, RGR: Relative Growth Rate

Table 2. Effect of sex, genotype and *IGF-2* on growth rate indices and body weight on purebred Nigerian indigenous chicken and their crosses with Marshal chicken

PARAMETER	SUBCLASS	AGR	RGR	BW
Sex	Male	1115.16±103.51	3.48±0.21	1533.24±93.93
	Female	1112.94±72.01	3.53±0.16	1527.95±61.26
<i>IGF-2</i>	AA	1112.25±160.31	4.02±0.42	1537.46±136.80
	AB	1139.12±88.09	3.43±0.18	1556.12±79.95
	BB	1082.97±104.26	3.35±0.19	1494.35±91.58
Genotype	NN	976.23±89.25 ^b	3.41±0.24	1407.69±75.37 ^b
	NxM	1524.23±137.20 ^a	4.18 ±0.27	1894.92±122.81 ^a
	FxM	1445.19±177.24 ^a	4.06±0.39	1840.88±177.86 ^a
	FF	787.52±112.98 ^b	2.74±0.21	1246.50±97.44 ^b
	NK	921.23±136.27 ^b	3.12±0.22	1356.00±115.41 ^b
	NKxM	1168.75±247.89 ^{ab}	3.34±0.24	1557.17±222.56 ^{ab}

Note: ^{ab}Means within the same row with different superscripts differ significantly (P<0.01)

BW: body weight, AGR: absolute growth rate, RGR: relative growth rate

Heritability of growth rate indices and body weight of purebred NIC with crosses with Marshal Chickens

Heritability estimates of AGR, RGR and BW are shown in Table 3. AGR value is low (0.06) while RGR (0.52) and BW (0.77) have high heritability values which implies high genetic variability. Aslam et al. (2011) analyzed the growth data of two commercial turkey parent flocks by using the Logistic function and reported that the heritability estimated for the parameter was 0.30, while the other parameters had low levels (0.05 - 0.11). Mignon-Grasteau et al. (1999) stated that parameters showed medium-high values of heritability which is mandatory to estimate phenotypic-genetic correlations. The moderate heritability estimate is consistent with the findings of Iraqi et al. (2002) in that it shows that the genotype of the hens had adequate genetic diversity and that a faster response to mass selection was anticipated.

Table 4. Heritability of growth rate indices and body weight of purebred Nigerian indigenous chicken with crosses with Marshal chicken

Parameter	Heritability
Absolute growth rate (AGR)	0.06±.011
Relative growth rate (RGR)	0.52±.015
Body weight (BW)	0.77±.024

CONCLUSIONS

It is concluded from this study that the detection of DNA polymorphism through RFLP-PCR shows that *IGF-2* gene is polymorphic and there is only an association between growth curve parameters and chicken genotypes. The genotype crosses with Marshal had the best rate of maturing and greater maturing weight. The Nigerian indigenous chicken and their crosses with Marshal had high heritability value.

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Conflicts of Interest

The authors declare that they do not have any conflict of interest.

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