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Association between Insulin Like Growth Factor-1 (IGF-1) gene polymorphism and carcass traits in improved Nigerian indigenous chickens

Wheto, M.[1], Adenaike, A. S.[1], Sanda, A. J.[1], Ilori, B.M.[1], Akano, K.[2], Sanni, T.1, Olowofeso, O.1, Ikeobi, C. O. N.1 and Adebambo, O. A.[1]

[1]Department of Animal Breeding and Genetics, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. [2]Department of Agricultural Education, Michael Otedola College of Primary Education, Noforija, Epe, Lagos State, Nigeria

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Abstract

The insulin-like growth factor-1 (IGF1) is a key regulator of muscle development and metabolism in birds and other vertebrate. Our objective was to determine the association between IGF1 gene polymorphism and carcass traits in FUNAAB Alpha chicken. Genomic DNA was extracted from the blood of 50 normal feathered birds. At 10 weeks, the birds were slaughtered for carcass traits. Specific primers for chicken IGF1 were used for amplification of a 622 base segment. The amplified gene products were digested with *Hinf1* restriction enzyme and the digested fragments were genotyped. Allele frequencies were 52% and 48% for A and B, respectively. Genotype frequencies were 27%, 50% and 23% for AA, AB and BB genotypes, respectively. All carcass traits values and the IGF1 gene polymorphism observed were subjected to analysis of variance and the mean were separated using Duncan Multiple Range Test. The results showed that the occurrence of the polymorphism did not affect all the carcass traits but AB genotypes had the highest carcass traits values than the AA and BB genotypes. The conclusion of this study demonstrated that IGF-1 gene, to some extent, could be a candidate gene that affects carcass traits in Improved Nigerian indigenous chicken.

Keywords: IGF-1, PCR-RFLP, indigenous chicken

Correspondence: whetom@funaab.edu.ng

Introduction

The major objective of any animal breeder is to select superior animals for breeding purpose (Hossein and Mohsen, 2011). This is because variation exists among animals including poultry. This variation between animals is important to the genes which offspring received from their parents and with the progeny performance they can be selected through chromosomal part (Nie et al., 2002).

Nigeria indigenous chickens are known to be dual-purpose bird that is used both for meat and egg production in the rural and peri-urban area of the country. They are found in large numbers distributed across different agro ecological categories under a traditional family scavenging management system (Sonaiya and Olori, 1990).

The indigenous poultry species represent valuable resources for livestock development because their extensive genetic diversity allows rearing of poultry under varied environmental conditions, providing a range of products and functions (Sonaiya et al., 1999). The Nigeria indigenous chicken has also been said to be small bodied, slow growing, poor feed converters and poor meat animals (Nwosu and Asuquo, 1985).

Nevertheless, these chickens in Nigeria are major source of raw materials from which sustainable protein supply can be developed within the nation, making this as a matter of concentration for researchers as 90% of the 150million chicken in Nigeria are the local variety which contribute 90% and 72% of the egg and meat consumed, respectively (Nwanta et al., 2006).

These findings have led to the conclusion that the indigenous chickens have a great potential for meaningful genetic improvement for growth and therefore contribute to the reduction of protein dearth in the country (Ikeobi et al., 1996).

FUNAAB Alpha strains of chicken was developed at the Poultry Breeding units of the Directorate of the University farm, Federal University of Agriculture, Abeokuta, Ogun state in Nigeria. The selection process for the traits of interest which is the meat and the egg started around 1997 with over 10 generations of selection for improved meat and egg production.

The egg type is a dual purpose which was developed through a rigorous, systematic and selective breeding of the Nigerian indigenous chicken without eroding their tropical adaptive features and disease resistance traits.

The potential variability that exists among the indigenous chickens were utilized to upgrade them. They still maintained the different plumage colours and the three feathering pattern (Normal feathered, frizzled feathered and naked neck) exhibited by the Nigerian Indigenous chicken.

The average chick weight at hatch is between 30 – 35 g, age at first lay ranged between 16 – 18 weeks, average body weight at first lay is between 1200 g – 1728 g, and weight of first egg at lay is between 35 – 40 g. The average egg laid per year ranges between 200 – 250 eggs.

This increasing interest in genetics and animal breeding has led to several researches on how to improve both productive and reproductive traits in chicken. Some of these productive traits include leanness of chicken carcasses, changes in body weight and linear body parameters.

The chicken IGF-I gene maps to 165.95 cM on chromosome 1 and a QTL at 150 cM on chromosome 1 affecting abdominal fat weight has been detected in chicken (Ikeobi et al., 2002). According to Zhou et al. (2005) revealed four exons and three introns. This gene is said to be a candidate for growth, body composition, metabolism, carcass characteristics, growth of adipose tissue and fat deposition in chicken.

Therefore, *IGF-1* gene could be a very useful physiological indicator to assist in screening and selection of animals at an early stage of growth. (Afolayan and Fogerty, 2008). Hence, this study was conducted for identification of the IGF-1 polymorphism and its possible association with carcass traits in improved Nigerian indigenous chicken.

Materials and methods

Experimental birds and study location

The experiment birds consist of fifty (50) Nigerian improved indigenous chickens. The experiment was carried out at the Poultry breeding unit of the Directorate of University Farm (DUFARMS) and the Biotechnology Center Laboratory of the Federal University of Agriculture, Abeokuta (FUNAAB), Ogun state, Nigeria. The University is located within latitude 7°10'N and longitude 3°2'E and lies in the south western part of Nigeria. It has an average temperature of 33.7°C and relative humidity of 80% with rainfall of about 1037 mm.

The vegetation in the University represents an interphase between the tropical rainforest and the derived savannah (Goggle Earth, 2016).

Blood collection

For DNA extraction, blood samples were collected in EDTA-treated tubes from the 10 week-old birds before slaughter. Samples were collected using a syringe from the right jugular vein with a new needle and syringe for each individual to avoid cross contamination.

Genomic DNA Extraction and Quantification

DNA was extracted from the whole blood using Qiagen DNA extraction kit following the manufacturer protocol. The purity and concentration of the extracted DNA were determined using Nano-drop spectrophotometer.

PCR-RFLP assay:

The PCR primers specific for the chicken IGF-1 gene were used (Forward: 5-GACTATACAGAAAGAACCAC-3; Reverse: 5-TATCACTCAAGTGGCTCAAGT-3) (Nagaraja et al., 2000). The DNA amplification by PCR of each bird was performed according to the following conditions: the PCR was performed in a total volume of 20 µl, containing 2 µl of genomic DNA, 2µl of forward and reverse primer (10pmol/µl), 4 µl of 5X Firepol PCR premix and 12 µl of nuclease free water. Cycle parameters were 94ºC for 5 min then 35 cycles of 94°C for 45 sec, 60 ºC for 45 sec and 72ºC for 1 min, with a final extension step for 10 min at 72ºC, the PCR products with length 622 bp were digested at 37ºC for five (5) minutes with 10 U of Hinf1 restriction enzyme.

Restriction digests were electrophoresed at 100 volts for 1h on a 2% agarose gel with ethidium bromide and individual PCR-RFLP fragment sizes in each sample were determined based on a standard DNA molecular weight marker (100bp) by viewing the banding pattern under UV light on the transiluminator. All the three genotypes (AA, AB and BB) were found.

Data collection

At 10 weeks of age, the Live Body Weight (LBW) of the birds from which the blood samples were collected was determined and slaughtered. The weight of the following carcass traits were also determined using a sensitive scale: Bled weight, Plucked weight, Eviscerated weight, Whole gizzard weight, Empty gizzard weight, Liver weight, Wing weight, Leg weight, Thigh weight, Drum stick weight, Neck weight, Breast weight, Back weight and Head weight.

Statistical Analysis

Genotypes of individual birds at different base pair (bp) or marker loci were recorded by direct counting of the bands. The gene frequencies were calculated by counting method as follows:

$$P = \frac{2(AA) + (AB)}{2N}$$

$$q = \frac{2(BB) + (AB)}{2N}$$

Where; P = the gene frequency of allele A, q = the gene frequency of allele B, N = the total number of birds

Obtained results were processed and analyzed using GLM procedure in SAS (SAS. 2010) and means were compared using Duncan's new multiple range test. The statistical model was $Y_{ij} = \mu + G_i + \mathcal{E}_{ij}$

Where: Y_{ij} is the observation of the i^{th} population, μ is the population mean, Gi is the fixed effect of i^{th} IGF1 genotype and $\mathcal{E}_{ij}$ is the random error

Results

The analysis of variance of effects of *IGF-1* gene polymorphism on carcass traits showed that it had no significant (P>0.05) effect on the live body weight before slaughter (1000.63±97.02 g), the bled weight (964.86±93.89 g), the plucked weight (904.87±87.87), the eviscerated weight (722.93±73.75 g), the thigh weight (102.30±11.04), the breast weight (154.70±17.60) and the back weight (145.96±16.12) for genotype AB, although higher mean values were observed in these and every other traits considered but the effects were not significant (Table 1). Genotype AA was the next in values to AB for all the traits except for the plucked weight (641.88±106.69), the leg weight (30.00±4.32), the breast weight (98.55±21.50) and the head weight (27.88±2.76) where the lowest mean values were observed.

Moreover, the lowest mean values were also observed in the genotype BB for all other traits except for the plucked weight (644.57±194.03), the leg weight (30.71±9.07), the breast weight (99.57±39.09) and the head weight (29.14±5.66) which were higher than genotype AA but less than AB, yet the differences were not significant.

Allele and genotype frequencies observed in the analysed samples are given in Table 2. In IGF1 locus, Allelle A was more frequent than B allele with 0.52 and 0.48 respectively. Allele A was identified as a dominant allele in IGF1 locus due to the highest frequency. The frequency of BB homozygous genotype was the lowest among all loci (0.23) whereas AB genotype had the highest frequency of 0.50 as shown in Figure 1.

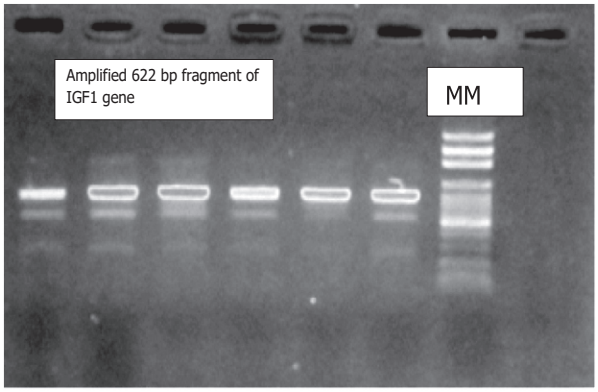

Plate1: IGF-1 gene optimization on agarose gel

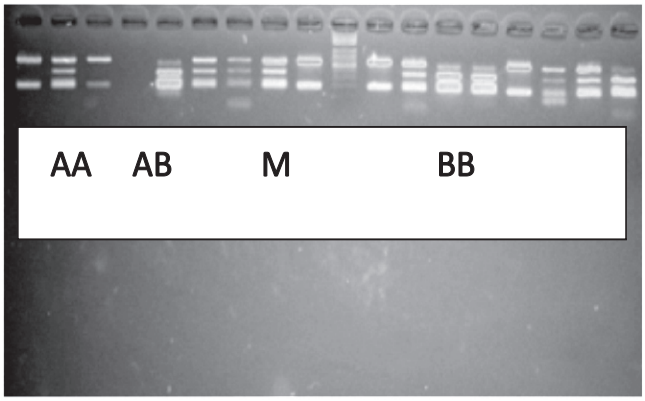

Plate2: IGF-1 gene Polymorphism genotyping

Table 1: Effect of *IGF-1* gene polymorphism on Carcass traits (LSM ± SE)

Carcass traits (Grams)	AA 9	AB 30	BB 7
Body weight	764.78±116.85^{ns}	1000.63±97.02^{ns}	712.00±212.74^{ns}
Bled weight	681.55±114.10^{ns}	964.86±93.89^{ns}	680.85±204.12^{ns}
Plucked weight	641.88±106.69^{ns}	904.87±87.87^{ns}	644.57±194.03^{ns}
Eviscerated weight	493.11±88.85^{ns}	722.93±73.75^{ns}	419.00±162.32^{ns}
Whole gizzard weight	29.66±2.96^{ns}	34.30±2.39^{ns}	29.28±3.98^{ns}
Empty gizzard weight	18.33±1.69^{ns}	21.00±1.55^{ns}	17.57±2.80^{ns}
Liver weight	18.44±2.56^{ns}	25.03±2.29^{ns}	17.42±4.40^{ns}
Wing weight	62.44±9.30^{ns}	89.43±8.32^{ns}	60.71±17.33^{ns}
Leg weight	30.00±4.32^{ns}	42.66±4.15^{ns}	30.71±9.07^{ns}
Thigh weight	69.22±13.65^{ns}	102.30±11.04^{ns}	66.42±24.47^{ns}
Drum stick weight	67.66±12.07^{ns}	94.06±10.00^{ns}	66.28±21.67^{ns}
Neck weight	31.51±4.31^{ns}	44.66±4.37^{ns}	33.28±9.67^{ns}
Breast weight	98.55±21.50^{ns}	154.70±17.60^{ns}	99.57±39.09^{ns}
Back weight	99.00±19.66^{ns}	145.96±16.12^{ns}	93.85±31.24^{ns}
Head weight	27.88±2.76^{ns}	33.73±2.44^{ns}	29.14±5.66^{ns}

Where P> 0.05 show that the value is not significant ns = non significant

Table 2: Allele and Genotype frequencies

Locus	Allele frequencies		Genotype frequencies		
	A	**B**	**AA**	**AB**	**BB**
IGF-1 gene	**0.52**	**0.48**	**0.27**	**0.50**	**0.23**

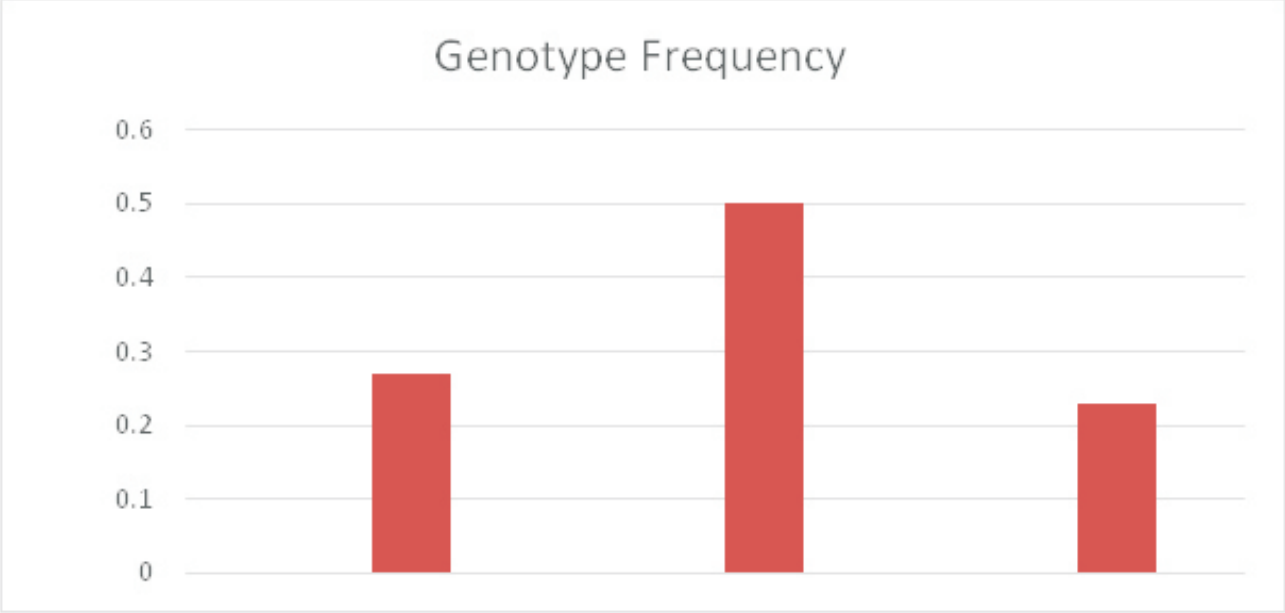

Figure 1: Genotypic frequencies of the IGF1 gene polymorphism

Discussion

The success of poultry enterprise for meat depends on the level of production at a specific time. The success of selection program for improved meat production depends on the identification of the candidate marker and their successful transfer to the flock of interest. In this study, three genotypes of IGF-1 namely: AA, AB, and BB with different fragments using *Hinf1* for PCR-RFLP polymorphisms were detected.. The genotype frequency of AA, AB and BB were 0.27, 0.50, and 0.23, respectively and the allelic frequency of A and B were 0.52 and 0.48, respectively.

In the population of chicken used, the Chi-square for the genotypic frequency was calculated and it was observed that there was no significant difference which shows that the frequency of the genotype does not differ from the expectation of Hardy-Weinberg equilibrium.

Although, polymorphism of *IGF-1* shows that it may be a potential candidate gene associated with growth, body composition and carcass traits of chicken (Zhou et al., 2005). Lei et al. (2007) also reported that the *IGF-1* polymorphism is also significantly related to the breasts and leg muscles of the chickens but this study shows that there was no significant relationship of the *IGF-1* gene mutation with the chicken carcass traits. Although higher mean value were observed in the live body weight, eviscerated weight, breast and back muscles of the chickens with genotype AB but it does not show any significant difference which was also in accordance with the report of Jaromir et al. (2012) that there was no significant difference of the effects of *IGF-1* gene on the carcass traits of the broilers used but that higher values were also observed in the breast muscle weight of the AB genotype chickens.

Since, *IGF-1* gene could be related with different chicken carcass traits, it is therefore, recommended that further research should be carried out on the investigation of *IGF-1* gene and this should be done at specific age as *IGF-1* gene could be associated with the body composition and the carcass traits at a particular stage of growth in chickens because this will in no small way improve the economy of the poultry section of the livestock industry in producing more quality carcass traits with higher values.

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