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Effects of insulin-like growth factor I (IGF-I) polymorphism on bodyweight of Nigerian indigenous chickens

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A total of 101 randomly selected birds from a population of 601 birds (obtained from a 3×3 diallel mating system involving normal, frizzle and naked neck Nigerian indigenous chickens) were used to evaluate the effects of insulin-like growth factor I (IGF-I) polymorphism on bodyweight. Blood samples for DNA analysis were collected at 20 weeks of age. IGF-I genotypes were identified using polymerase chain reaction (PCR)-RFLP method. Data obtained were analysed for frequencies and impact on body weight (BWT). Obtained results showed three polymorphic variants (designated AA, AB and BB) of the IGF-I gene. The overall frequencies for genotypes AA, AB and BB were 55.45, 37.62 and 6.93%, while alleles A and B were 74.26 and 25.74%, respectively. The analysis of the chicken IGF-I gene revealed that the chicken population was in Hardy Weinberg equilibrium, thus, samples were obtained from a large random mating population. IGF-I gene significantly (P<0.05) influenced BWT with the AA genotype having higher BWT than AB and BB at all ages (4-20 weeks) except at hatch. Therefore, the effect of IGF-I gene on BWT indicated that it could be appropriate as a candidate gene in selection for BWT in Nigerian indigenous chickens.

Key words: Insulin-like growth factor I, polymorphism, bodyweight, indigenous chicken, PCR-RFLP.

INTRODUCTION

Nigerian indigenous chickens have been described as small bodied, slow growing, poor feed converters and poor meat animals (Ajayi, 2010). Despite these drawbacks, they play vital role in socio-economic life of the rural and semi urban populace. Therefore, the need to genetically improve the performance of Nigerian

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indigenous chickens through selection and breeding cannot be overemphasized.

Much genetic research is now directed towards determining the relationship between physiological, biochemical and metabolic product/markers on the productive efficiency of farm animals (Isidahomen et al., 2011). Insulin-like growth factor (IGF) consists of a family of polypeptide hormones structurally associated with insulin with multiple metabolic functions (Li et al., 2003). The IGF are important regulators of growth, protein synthesis and cell proliferation and differentiation in a variety of cell types (Scanes et al., 1999).

Since candidate gene approach has become a powerful technique for genetic improvement (Zhu and Zhao, 2007), IGF-I gene has been reported by several authors to influence growth rate, carcass traits and feed efficiency in poultry (Amills et al., 2003; Zhou et al., 2005; Promwatee and Duangjinda, 2010). Although numerous population studies have focused on exotic and pure bred chickens, the information on the specific effects of the IGF-I gene on performance traits of Nigerian indigenous chicken genotypes and their crosses have not been reported or it is scanty. Consequently, there is need to investigate the possibility of using the IGF-I gene as a candidate gene in Nigerian indigenous chicken. The current work therefore, aimed at determining the effect of IGF-I gene polymorphism on body weight of pure and crossbred Nigerian indigenous chicken.

MATERIALS AND METHODS

Experimental location

This study was conducted at the Department of Animal Science Teaching and Research Farm, Faculty of Agriculture, Ahmadu Bello University, Zaria, Nigeria. The farm was located at latitude 11° 09' 06' N and longitude 7° 38' 55' E, at an altitude of 706 m above sea level (Ovimaps, 2012).

Source of experimental birds and management

A total of 601 birds were generated from a 3×3 diallel mating design (involving normal, frizzle and naked neck Nigerian indigenous chickens) as described in an earlier study (Musa et al., 2015). They were fed starter (2652.00 kg/kg DM and 21.00% CP), grower (2400.00 kg/kg DM and 16.08% CP) and breeder (2520.08 kg/kg DM and 18.05% CP) diets at starter, grower and breeder phases, respectively. The birds were tagged at day old and raised on deep litter system. Feed and water were offered *ad libitum*.

Data collection

Data was collected on body weight at day old and on monthly basis until the end of the study (20 weeks). 101 birds were randomly selected and bled from the population of 601 birds. 2 ml of blood was collected from each bird using a 2 ml syringe (needle gauge, 23G x 11/4) into ethylene diamine tetra acetic acid (EDTA) sample bottles. The blood samples were immediately taken for DNA analysis at Centre for Biotechnology Research and Training (CBRT), Ahmadu Bello University, Zaria.

DNA extraction

Genomic DNA was isolated by using Thermo Scientific GeneJET Genomic DNA Purification kit. The protocol used is as described for DNA purification from nucleated blood. A spectrophotometer was used to investigate the quality and quantity of DNA. The purity and concentration of DNA samples was estimated using UV-visible range spectrophotometer. DNA was also examined by loading samples on 0.75% agarose gel and visualizing the band under gel documentation system.

Polymerase chain reaction (PCR)

The IGF-I gene primer was selected from a previous publication (Nie et al., 2005) for use in amplifying the Nigerian indigenous chicken ortholog. According to Nie et al. (2005), the sequences of the candidate gene of the somatotropic axis is from Genbank (http://www.ncbi.nlm.nih.org).The primers had been designed using the GENETOOL program (http://www.biologysoft.com). The primer were synthesised through a commercial service (BioNEER Corp., USA). Information on the primer is given in the Table 1. The PCR was performed in a total volume of 50 µL in each PCR tube, containing 25 µL of 2 x PCR master mix, 1 µL each of the forward and reverse primers, 1 µL of genomic DNA and 22 µL of nuclease free water. The PCR tube was put in thermocycler (FTGENE5D by TECHNE Cambridge) and the PCR condition was set at 94°C for 5 min for initial denaturing, followed by 35 cycles at 94°C for 30 s for denaturing, 52°C for 45 s for annealing, and 72°C for 90 seconds extension, and a final extension step at 72°C for 5 min.

Restriction digest and restricted fragment length polymorphism

Restriction digest was done using 1 µL of Fast Digest enzyme (EcoRI) according to the manufacturer's (Thermo Scientific) recommendation and at an incubation temperature of 37°C for 20 min. The enzyme was subsequently deactivated by heating for 5 min at 80°C. The digested products were loaded on a gel. Then, the electrophoresis tank was set up and connected to electrical source to run for 20 min at 75-100 V. The gel was removed and viewed under UV light to observe the bands. The restriction patterns were visualized by 0.75% agarose gel electrophoresis; gels were stained with GR Green DNA stainer. Gels were visualized and photographed using a gel documentation system (Uvipro Silver by Uvitec).

Statistical analysis

General linear model procedure of Statistical Analysis System program (SAS, 2002) was used to test the effects of the genetic groups and sex. Significant means were separated using Tukey-Krammer HSD (honestly significant difference) multiple comparison test (Tukey, 1953; Kramer, 1956). The following model was used to investigate effect of IGF-I genotypes on body weight:

$$Y_{ijk} = \mu + G_i + S_j + e_{ijk}$$

Where: Y_{ijk} = body weight of bird of the j^{th} sex in the i^{th} IGF-I genotype; μ = overall population mean; G_i = effect of the i^{th} IGF-I genotype; S_j = effect of the j^{th} sex; e_{ijk} = random error.

The gene frequencies were calculated according to Hardy-

Table 1. Details of primer used for amplifying IGF-I gene in Nigerian indigenous chicken.

Primer	Gene	Sequence of oligonucleotide primers (Forward primer 5' – 3'/ Reverse Primer 5' – 3')	Sequence ID[1]	Length (bp)	Annealing temperature for PCR amplification (°C)[2]
309	IGF– I	AGCTGTTCGAATGATGGTGTTTT / GCCCCAGCATTCTCTTTCCTT	AY253744	583	56.4 58.2

Source: Nie et al. (2005); [1]Sequence accession numbers used for primer designing. [2]Annealing temperature as specified by the manufacturer (BioNEER Corporation, USA).

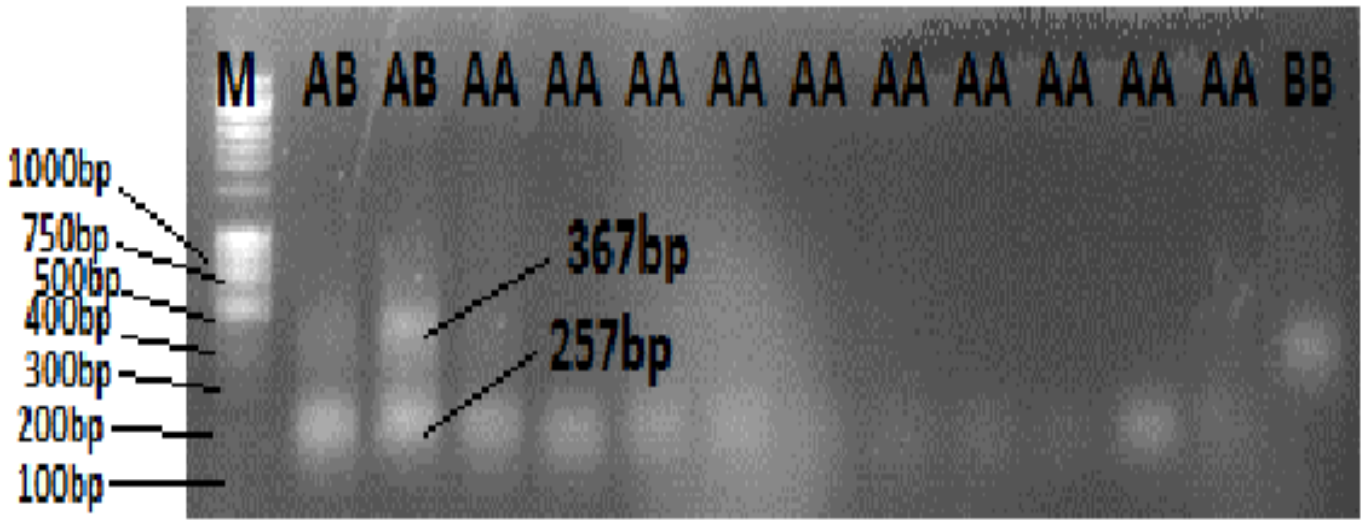

Plate 1. Electrophoregram showing genotyping profiles of IGF-I gene in Nigerian indigenous chickens detected by PCR-RFLP method.

Weinberg's equation 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 and; N = the total number of birds tested.

A Chi-squared test for goodness-of-fit was performed to verify if genotype frequencies agree with Hardy-Weinberg's equilibrium (HWE) expectations with the following formula:

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

Where: O= observed frequency; E= expected frequency.

RESULTS AND DISCUSSION

Genotyping of insulin-like growth factor I (IGF-I)

The IGF-I gene was successfully amplified using genomic DNA samples. The restriction digest analysis of the PCR products using EcoR1 indicated the presence of two restriction patterns. Fragment sizes of 257 and 367bp were observed in the first and second restriction patterns and were assigned AA and BB genotypes, respectively while those with both were assigned AB genotype (Plate 1).

Effects of IGF-I genotypes on body weight at various ages

The effects IGF-I genotypes on body weight at various ages is presented in Table 2. Birds with the AA genotype had significantly ($P<0.05$) higher body weights than birds of AB and BB at all ages except at hatch. The results of higher body weights in AA genotype was consistent with those of Promwatee et al. (2013) who reported higher

Table 2. Effects of IGF-I genotypes on body weight (g) of Nigerian indigenous chickens at various ages.

IGF-I	N (101)	Day old	Week 4	Week 8	Week 12	Week 16	Week 20
AA	56	23.32^{b}±0.57	107.69^{a}±2.28	354.61^{a}±7.46	667.80^{a}±13.47	856.31^{a}±17.82	1206.89^{a}±22.56
AB	38	26.13^{ab}±0.57	93.61^{b}±2.27	314.91^{b}±7.40	597.32^{b}±13.37	765.49^{b}±17.69	1058.44^{b}±22.39
BB	7	26.37^{a}±1.45	93.34^{b}±5.80	285.19^{b}±18.94	583.00^{b}±34.21	743.42^{b}±45.25	1051.36^{b}±57.28
SEM		0.32	1.29	4.21	7.60	10.05	12.72
CV (%)		12.1	13.03	12.99	12.3	12.71	11.39
LOS		*	*	*	*	*	*

SEM= Standard error of means; CV= coefficient of variation; LOS= level of significance; *= (P<0.05); N= number of observation.

Table 3. Genotypic and allelic frequencies of the IGF-I gene in various genetic groups of Nigerian indigenous chickens.

Groups / Sex	N	Allelic frequency (%)		Genotypic frequency (%)			χ^2
		A	B	AA	AB	BB	
Male	49	70.41	29.59	51.02	38.78	10.20	0.24
Female	52	77.88	22.12	59.62	36.54	3.85	0.19
Overall	101	74.26	25.74	55.45	37.62	6.93	0.03

N= number of observations.

body weights at 4, 8, 12 and 14 weeks of age in the AA genotype than in the BB genotype in the Khai Mook Esarn and Soi Pet population of chickens. However, the result of significantly higher body weights in the BB genotype at hatch differed with those of Promwatee et al. (2013), who observed higher body weight in AA genotype in the Soi Nin population. Furthermore, Amills et al. (2003) reported mutation of the IGF-I gene in two chicken strains of the Black Penedesenca breed and significant association of IGF-I SNP1 was found for body weight up to 107 days of age in one of the strains. According to the authors, IGF-I-SNP1 marker was located in the promoter region of the IGF-I gene, so the existence of suggestive associations among IGF-I-SNP1 and growth might be interpreted in the light of differences in the transcriptional rate of both alleles. In fact, the analysis of the promoter sequence revealed that the substitution A→C involved the suppression of one potential chicken homeobox containing gene (CdxA) transcription factor binding site (Amills et al*.,* 2003). Thus, the chicken IGF-I gene is likely a potential marker for use in body weight selection programme.

Genotypic and allelic frequencies of the IGF-I gene in Nigerian indigenous chickens

The genotypic and allelic frequencies varied across sex (Table 3). The observed genotypic frequencies for all the males were 51.02, 38.78 and 10.20% for AA, AB and BB genotypes, while the allelic frequencies were 70.41 and 29.59% for A and B alleles, respectively. For the females, the genotypic frequencies were 59.62, 36.54 and 3.85% for AA, AB and BB genotypes while the allelic frequencies were 77.88 and 22.12% for A and B alleles, respectively. However, the overall frequencies obtained were 55.45, 37.62 and 6.93% for AA, AB and BB genotypes, 74.26 and 25.74% for A and B alleles, respectively. The frequency of allele A was however, higher than allele B in Nigerian indigenous chickens. The analysis of the chicken IGF-I gene revealed that the population was in Hardy Weinberg's equilibrium as evidenced by the insignificant Chi square values. The observed result in this study was not consistent with those of Li et al. (2010) and Shah et al. (2012) who reported that the population of chickens studied were not in Hardy Weinberg equilibrium. However, the observed results in this study agreed with those of Abbasi and Kazemi (2011) who reported 3 polymorphic forms of the gene and population being in Hardy-Weinberg equilibrium which implies that samples were obtained from a large random mating population.

Conclusions

The Nigerian indigenous chickens conformed to Hardy Weinberg's equilibrium based on IGF-I locus, thus, samples were obtained from a large random mating population. The IGF-I gene significantly influenced body weight at all ages with the AA genotype having heavier weight than AB and BB except at hatch. Thus, IGF-I gene may be appropriate as a candidate marker for selection for body weight in Nigerian indigenous chickens. A

breeding plan that incorporates individuals who possess the AA genotype of the IGF-I gene should be considered for possible improvement in body weight of the indigenous breeds of chicken in Nigeria. Further studies on the influence of the IGF-I gene on reproductive performance is recommended.

Conflict of interests

The authors have not declared any conflict of interestS.

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