EFFECT OF IGF1 GENE POLYMORPHISM ON CARCASS TRAITS IN IMPROVED NIGERIAN INDIGENOUS CHICKEN

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Abstract

Molecular genetics selection on individual genes is a promising method to genetically improve economically important traits in chickens. Chicken insulin-like factor 1 gene (IGF1) is a biological and positional candidate gene for growth, body composition, metabolic and skeletal traits, and fat deposition in chickens. In this study, IGF-1 gene polymorphism of improved Nigerian indigenous chickens was investigated using Restriction Fragment Length Polymorphism (RFLP). Blood samples were collected from 46 randomly chosen improved Nigerian indigenous chicken. Genomic DNA was extracted using Qiagen DNA extraction kits and amplified using polymerase chain reaction technique (PCR). The promoter and 5' untranslated region of the fowl IGF-1 gene was amplified to produce a 621 bp fragment. The PCR products were electrophoresed and the amplicon digested with restriction enzyme, HinfI and electrophoresed on 2.5% agarose gel, stained by ethidium bromide and the digested fragments were genotyped. The genotype revealed two alleles A and B. In this population, AA, AB, BB genotypes have been identified with 27%, 50% and 23% frequencies respectively. A and B alleles frequencies were 0.52, 0.48, respectively. The Chi-square (χ^2) test was significant and the population was in Hardy-Weinberg equilibrium ($p<0.05$). The IGF-1 gene polymorphism did not show any significant association with the carcass traits of the chicken used.

Key word: IGF1, Polymorphism, RFLP

Introduction

Insulin-Like Growth Factors (*IGF*) is a family of polypeptide hormones associated with insulin with multiple metabolic and anabolic functions (Hossein and Mohsen, 2011). It was mapped to chromosome 1 and encompasses 50 kb (Klein *et al.* 1996). The IGFs have been shown to regulate body and muscle growth in chickens (Li *et al.* 2009). Several studies have shown that circulating IGF-I affects growth rate in poultry (Scanes *et al.*, 1989; Ballard *et al.*, 1990). Restriction Fragment Length Polymorphism (RFLP) is a technique in which organisms may be differentiated by analysis of patterns derived from cleavage of their DNA. It is also defined by the existence of alternative alleles associated with restriction fragments that differ in size from each other. The objective of this study therefore was to examine the associations between genotypes of the *IGF1* gene and selected carcass traits in improved Nigerian indigenous chicken.

Materials and methods

The experiment was carried out at the poultry breeding unit of the Directorate of University Farm and the Biotechnology Center Laboratory of the Federal University of Agriculture, Abeokuta (FUNAAB), Ogun state, Nigeria. Blood samples were collected randomly from 46 chickens through the wings vein using 2ml syringe and transferred to EDTA bottles to serve as anti-coagulating agent, after which the samples were stored in the laboratory at -20°C. At 8 weeks of age, the birds were slaughtered for carcass analysis. The following carcass traits were measured 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, Head weight.

Genomic DNA was extracted from blood, using Qiagen DNA extraction kits following the manufacturer protocol. Working dilutions of extracted DNA were prepared for each individual at a concentration of 50 ng/µg. Primers 5'-GAC TAT ACA GAA AGA ACC CAC-3' and 5'- TAT CAC TCA AGT GGC TCA AGT-3' were used for polymerase chain reaction (PCR) amplification of IGF-I gene (Nagaraja *et al.*, 2000). The PCR mixture containing 50 ng genomic DNA, 25 pmol of each primer, 25 µM of each dNTP, of unit Taq DNA polymerase and 1×reaction buffer in a 25 µL reaction volume. The reaction mixture was subjected to initial 4 minutes denaturation at 94°C followed by 40 cycles of denaturation at 94°C for 1minute; annealing temperature at 56°C for 45sec;

extension at 72°C for 1minute and final extension at 72°C for 10minutes. The PCR products were digested with *Hinf1* restriction enzyme. 17µl of Nuclease Free Water, 2µl of 10X FastDigest Green Buffer, 10µl of PCR product and 1µl of FastDigest enzyme were subjected to digestion for 20 minutes at 65°C. The restriction digests were separated using 2.0% agarose gel in 1×TBE at a constant current of 100v for 1hour. The gels were stained with ethidium bromide and the fragments were visualized using a UV transilluminator. Genotyping was carried out manually following the scoring procedure of Darabi *et al.* (2010).

The data collected were subjected to one way analysis of variance. Significance between genotypes was assessed by Duncan multiple range test (SAS, 1999).

Allelic frequency and genotypic frequency was calculated using the following formula.

$$A=\frac{2AA+AB}{2N}$$

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

Where

A- Gene frequency of allele A

B- Gene frequency of allele B

N- Total number of birds tested

Results and Discussion,

The effect of *IGF-1* gene polymorphism was not significant ($p>0.05$) on all the carcass traits measured as shown in Table 1. The results observed may be due to the number of bird used for this experiment. though the IGF-1 gene polymorphism on carcass traits were not significant but the findings was in accordance with the findings of Mu'in *et al.* (2010) which stated that the body weight of the chicken with genotype AA is lower than the weight of chicken with the genotype AB. Nagaraja *et al.* (2000) also observed that there were no significant differences between IGF1 gene polymorphism and the weight of chicken aged 140, 265 and 365 days.

Polymorphism of *IGF-1* shows that it is a potential candidate gene associated with growth, body composition and carcass traits in chicken (Zhou *et al.*, 2005). Lei *et al.* (2007) also reported that the *IGF-1* polymorphism is 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 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) who reported 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.

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), and the breast weight (98.55±21.50). Moreover, the lowest mean values were also observed in the genotype BB for all other traits except for the plucked weight and the breast weight which were higher than genotype AA but less than AB.

The three genotypes were found namely: AA, AB, and BB which show fragments at different base pair (bp) using *Hinf1* for PCR-RFLP. 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 as shown in Table 2. The observed frequency was in accordance with the findings of Nagaraja *et al.* (2000). They observed that frequency of genotype AA was lower than the frequency of AB.

The allele and genotypic frequencies as shown in Table 2 shows that the frequency of A allele was higher than that of the B allele with 0.52 and 0.48 for A and B allele respectively. The genotypic frequencies of 27%, 50% and 23% were observed for genotype AA, AB and BB respectively. In the population of birds 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.

A population is said to be in Hardy-Weinberg equilibrium when showed no selection, migration, mutation or genetic drift. This indicates that in the population of the improved Nigerian indigenous chickens in the study area, there were no factors that disrupt genetic equilibrium in the population.

Conclusion

The results of the RFLP analysis showed three fragment after digested with restriction enzyme, *Hinf1* that identified changes in 5′ untranslated region. The *IGF-1* gene polymorphism did not show any significant association with the carcass traits of the chicken even though higher mean values were observed for the live body weight, eviscerated weight, breast and back muscles weight of the chickens with genotype AB

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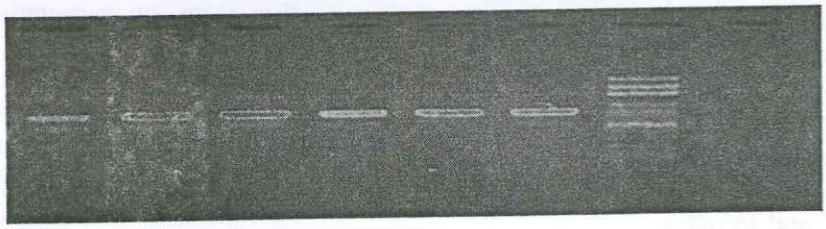

Plate 1: *IGF-1* gene optimization on agarose gel

AA D AB M BB

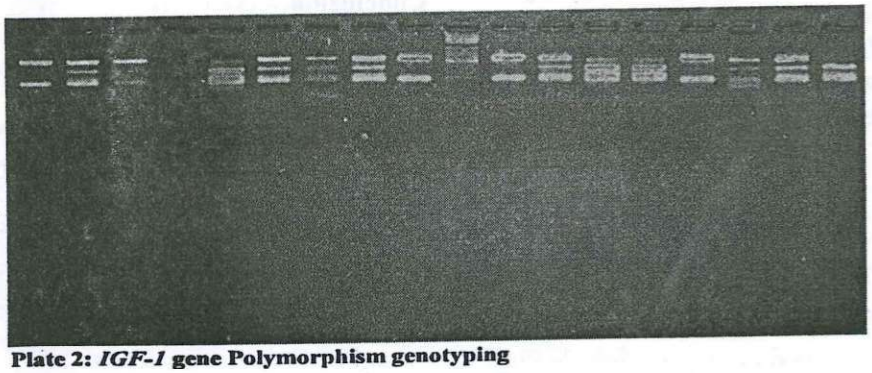

Plate 2: *IGF-1* gene Polymorphism genotyping

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

Carcass traits	AA	AB	BB
	9	30	7
Body weight (g)	764.78±116.85	1000.63±97.02	712.00±212.74
Bled weight (g)	681.55±114.10	964.86±93.89	680.85±204.12
Plucked weight (g)	641.88±106.69	904.87±87.87	644.57±194.03
Eviscerated weight (g)	493.11±88.85	722.93±73.75	419.00±162.32
Thigh weight (g)	69.22±13.65	102.30±11.04	66.42±24.47
Drum stick weight (g)	67.66±12.07	94.06±10.00	66.28±21.67
Breast weight (g)	98.55±21.50	154.70±17.60	99.57±39.09
Back weight (g)	99.00±19.66	145.96±16.12	93.85±31.24

Table 2: The Allele and Genotype frequencies of the IGF1 gene polymorphism

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