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ASSOCIATION BETWEEN IGF- 1 GENE POLYMORPHISMS AND BODY WEIGHT IN NIGERIA LOCALLY ADAPTED TURKEYS

Oyewola, K. A*, Akinyemi, M. O, Osaiyuwu, O. H, Fijabi, O. E, Shobanke, I. A., Nwokorie, G.I. and Ewuola, K. M.

Animal Breeding and Genetics Unit, Department of Animal Science, University of Ibadan

Corresponding Author: writekhadijat@yahoo.com; +2348060403412

Abstract: The insulin-like growth factor 1 (IGF-1) gene polymorphism plays important roles in development, growth and reproduction. Genetic intervention for the improvement of Nigeria locally adapted turkeys based on growth rate and higher mature weight is important. This study was aimed at investigating the association between IGF-1 gene polymorphism and body weights in Nigeria locally adapted turkeys using PCR-RFLP method. Fifty poults were randomly selected for DNA analysis at 10 weeks. Zymo Miniprep kit was used for genomic DNA extraction from blood samples and 529bp fragment of intron 2 of IGF-1 gene was amplified. The genetic structure of the population was analysed using POPGENE 32 software. Association of the genotypes with body weight was evaluated using the General linear model of SAS 9.2. The enzyme digested products revealed A and B alleles with frequencies of 0.61 and 0.39 respectively. Two genotypes AA (0.38) and AB (0.62) were detected. Chi-square test (0.001830) for Hardy-Weinberg equilibrium showed that the population sampled was not in equilibrium for the gene investigated. Also, significant association was not observed between IGF-1 polymorphs and body weight at 4, 8 and 12 weeks of age in Nigeria locally adapted turkeys.

Keywords: DNA, Hardy-Weinberg equilibrium, Improvement, PCR-RFLP, Polymorphs.

INTRODUCTION

Nigeria is endowed with an impressive array of indigenous and locally adapted livestock, which have significant roles in socio-economic life of the rural and semi urban populace. These livestock are reservoir of untapped valuable rare genes and alleles which are linked to their adaptability and survival. Locally adapted turkeys are functionally valuable because they play vital roles in human nutrition, and contain genetic materials which may have been lost in the improved gene pool. They possess relic traits or genetic variants that are either absent in modern improved stocks or that exist in their rare ancestors which may be of commercial value (Adebambo, 2003). Selection of Nigerian locally adapted turkeys for improved growth and higher mature weight is imperative. Traditional approach of selection which is based on phenotype is time consuming and very difficult to achieve (Zhang *et al*., 2008). Therefore the use of molecular marker which is a powerful tool in animal breeding for genetic improvement to define the genotype and predict the performance of animal very early in life is important.

Polymorphism in growth-related genes like growth hormone (GH), growth hormone receptor(GHR), insulin-like growth factor-1(IGF-1), insulin-like growth factor-2(IGF2) and Myostatin (MSTN) have been closely linked with economic traits in poultry (Musa *et al.,* 2016). Genetic polymorphisms in several animal species can be identified using Polymerase Chain Reaction – Restriction Fragment Length Polymorphism (PCR-RFLP) among other techniques (Sartika, 2007). PCR-RFLP as a technique used to multiply certain DNA fragment to detect whether restriction site difference exists or not in individuals within the same population (Griffiths *et al*., 2003)

Insulin-like growth factor 1 belongs to the family of polypeptide hormones which is a structural homologues of insulin. Circulating IGF-1 is generated by the liver under the control of growth hormone (Akinfenwa *et al.,* 2011). Hegarty *et al*., (2006), reported that Insulin-like growth factor 1 (IGF-1) is a naturally occurring protein responsible for the stimulation of cellular growth, proliferation and differentiation. IGF-1 stimulates systemic body growth and has growth promoting effects on almost every cell in the body especially skeletal muscle, cartilage, bone, liver, kidney, nerves, skin, and lungs (Yilmaz *et al.,* 2011). IGF-1 has a fundamental role in both prenatal and postnatal development and exerts all of its known physiologic effects by binding to

the insulin-like growth factor 1 receptor (Le Roith *et al.,* 2001). IGF-I gene has been reported to influence growth rate, carcass traits and feed efficiency in poultry (Amills *et al.,* 2003). Also studies have established a link between the concentration of the circulating IGF-1 and growth trait in many livestock species and laboratory animals (Hegarty *et al.,* 2006). IGF-1 is a mediator for many biological effects such as; increase in glucose absorption, stimulation of myogenesis, increase in lipids synthesis, and also stimulates progesterone production during DNA, RNA and protein synthesis (Etherton, 2004). In view of these biological functions, IGF-1 can be considered as a candidate gene for predicting growth, egg and meat quality traits in the animal genetics (Andrade *et al*., 2008). This study aimed at evaluating the polymorphism in IGF-1 gene and its association with body weight in Nigeria locally adapted turkeys.

MATERIAL AND METHODS

Experimental location and management of experimental birds

This study was conducted at Poultry section of Duke Farm, Orogun Express Ibadan Nigeria. The experiment was performed with 300 one-day old poult of Nigeria locally adapted turkeys. The birds were tagged at 1-day old and raised on deep litter system with feed and water supplied *ad-libitum*. Body weight data was collected at day old and weekly basis for 12 weeks.

Extraction of DNA: 50 poults were randomly selected and bled from the jugular vein at 10 weeks. 4 ml of blood were collected into heparinized sample bottles and transferred to -20 ℃ freezer. Genomic DNA was isolated using Zymo mini prep kit following manufacturer protocol. DNA was also examined by loading samples on 1.5% agarose gel and visualizing the band under gel documentation system.

PCR-RFLP for IGF-1 gene: Intron 2 region of the IGF-1 gene was amplified to a product of 529 bp using Forward (5′-TGTTCTGCATTTGGCCCATAC-3′) and Reverse: (3′CAGAATGTCAGCTTTTTGTCC-5′) primers according to Nie *et al*., (2005) as follows:
The PCR was performed in a total volume of 25 µL in each PCR tube, containing 10 µL of 2 x PCR master mix, 1 µL each of the forward and reverse primers, 5 µL of genomic DNA and 8 µL of nuclease free water. The PCR tube was put in thermocycler and the PCR condition was set at 94℃ for 5 min for initial denaturing, followed by 35 cycles at 94℃ for 45 s for denaturing, 60℃ for 45 s for annealing, and 72℃ for 60 seconds extension, and a final extension step at 72℃ for 10 min.
Restriction digestion was done using 1 µL of Msp1 enzyme according to the manufacturer's (Thermo Scientific) recommendation and at an incubation temperature of 37℃ for 15 min. The enzyme was subsequently inactivated by heating for 20 min at 80℃. The digested products were electrophoresized on 1.5% agarose gel in 1X TBE and visualized by ethidium bromide staining for 15 min at 100 V. Gels were visualized using a gel documentation system and individual fragment sizes in each sample were determined based on standard DNA molecular weight marker for IGF-1 gene.

Statistical Analysis

POPGENE 32 software package was used to calculate genotypic and allelic frequencies and also to detect the state of population about Hardy-Weinberg equilibrium (HWE). Body weight data obtained was then subjected to analysis of variance following General linear model procedure of Statistical Analysis System (SAS) 2012 and Least Square mean (LSM). The following model was used to investigate effect of IGF-I genotypes on body weight:

$Yij=\mu+Gi+eij$

Where Yij = observed trait (body weight)
µ= the overall mean,
Gi= fixed effect of polymorphic variant,
eij= Random error term

RESULTS AND DISCUSSION

The restriction digest analysis of the PCR products using Msp1 indicated the presence of two restriction patterns as shown in Figure 2 below.

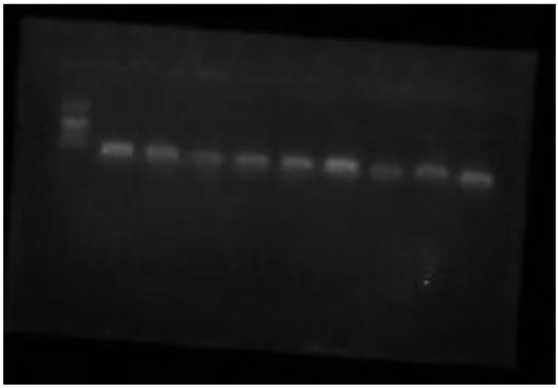

Figure 1: PCR products for IGF-1 gene of Nigeria locally adapted turkeys using 1.5 % agarose gel stained with ethidium bromide and100bp ladder

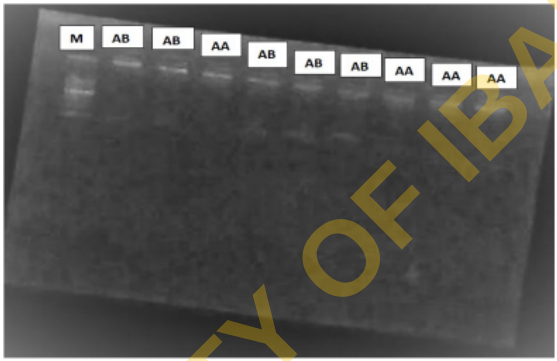

Figure 2: Results of analysis PCR-RFLP for IGF-1 gene in Nigeria locally adapted turkeys by MSP1 restriction enzyme on 1.5% agarose gel and 100bp ladder

M – Molecular weight marker
AA and AB- IGF-1 gene genotypes

Genotypic and Allelic frequencies of the IGF-I gene in Nigeria locally adapted turkeys

Intron 2 of IGF-1 gene in this study was identified to be polymorphic with only two genotypes; AA and AB. The BB homozygous genotype was not identified at all in the Nigeria locally adapted turkey sampled. However, the observed results in this study agreed with that of Babayi *et al.,* (2014) that reported the absence of CC homozygote genotype in west Azerbaijan native poultry. Moe *et al.,* (2009) and Kadlec *et al.,* (2012), also reported 2 polymorphic forms (AA and AC) of the IGF-1 gene with no CC genotype identified in Cobb and Ross broiler chickens. The results of Allelic and Genotypic frequencies are as shown in Table 1. Allele A(0.69) is significantly higher*(0.0018)* than allele B(0.31) in the studied population. Also the frequency of AA genotype was lower (0.38) than the frequency of AB genotype (0.62). In addition, these observed genotype frequencies are in agreement with the results of Abbasi and Kazemi (2011), that reported that allele A(0.51) was predominant over allele B(0.49), and genotype frequency for AA(0.26) was lower than that of AB(0.50) in Mazandaran Native Chicken. Kadlec *et al.,* (2011) reported, in Ross 300 strain frequency of A and C alleles in the UTR of IGF-1 gene were 0.915 and 0.085, respectively, while the genotypic frequencies of AA and AC were 0.83 and 0.17, respectively. Babayi *et al.,* (2014) reported allele A(0.58), and C allele (0.42) with genotype frequencies of AA(51.04), AC(14.58) and CC(34.37) in west Azerbaijan native poultry. The analysis of the turkey IGF-I gene revealed that the population deviated from Hardy Weinberg's equilibrium as evidenced by the significant Chi square values ($P<0.05$), and this is in line with the findings of Wang *et al.,* (2011), and Li *et al.,* (2010), who reported that the population of goats and chickens studied for IGF-1 gene were not in Hardy Weinberg equilibrium. However, Musa *et al.,* (2015), and Abbasi and Kazemi (2011), reported that Nigerian indigenous chicken and Mazandaran Native Chicken population studied were in Hardy-Weinberg equilibrium.

Table 1: Allele and Genotype frequencies at intron 2 locus of IGF-1 gene in Nigeria locally adapted turkeys

Allele frequency	Genotype frequency	X^2	G^2
A(0.69) B(0.31)	AA(0.38) AB(0.62)	0.00183	0.000184

X^2: Chi-square test for Hardy-Weinberg equilibrium
G^2: Likelihood ratio test for Hardy-Weinberg equilibrium.

Effects of IGF-I genotypes on body weight of Nigeria locally adapted turkeys at various ages

The effects of IGF-I genotypes on body weight of Nigeria locally adapted turkeys at ages 4,8 and 12 weeks is presented in Table 2. The results showed that, there were no significant effect ($p>0.05$) of AA and AB genotypes with the body weight at 4, 8 and 12 weeks of age. The results obtained is consistent with that of Nagaraja *et al.,* (2000) who identified that different genotypes had no significant association for 140, 265 and 365 days weight in chickens. Promwatee *et al.,* (2013), reported higher body weights at 4, 8, 12 and 14 weeks of age in the AA genotype than in AB and BB genotype in the Khai Mook Esarn and Soi Pet population of chickens. Amills *et al.,* (2003) identified suggestive associations between IGF1-SNP and average daily gain at 107 days in black Penedesenea chicken. Fang *et al.* (2008), identified a significant correlation between IGF1 polymorphism and egg production in wenchang chickens. Gouda and Essawy, (2010) analyzed the polymorphism of IGF-I gene among Egypt chicken breeds and indicated that their effects on the growth traits of chicken was significant. Wang *et al.,* (2011), reported that a novel of SNP at IGF-1-P1 locus was significantly associated with cashmere production traits in exon 4 of Nanjiang Cashmere goat population in China

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Table 2: Least Square Means (LSM±SD) of IGF-I genotypes on body weight (g) of Nigeria Locally Adapted Turkeys at Different Ages

WEEKS	AA (19)	AB (31)	SEM	P-VALUE
BW4	317.52±48.9	308.71±45.68	0.61	0.52
BW8	593.84±103.47	596.77±97.84	0.20	0.92
BW12	854.00±205.74	852.29±140.86	0.12	0.97

BW4, BW8 and BW12: Body weight at 4, 8 and 12 weeks of age respectively.
SEM: standard error of the means.
AA and AB: observed genotypes.

CONCLUSION

The results obtained in this study indicates that the population analysed deviated from Hardy- Weinberg equilibrium. Allele A in the IGF-1 gene of Nigeria locally adapted turkey is the predominant allele and there was no significant association ($p>0.05$) of IGF-1 genotypes on body weight of the sampled population.

RECOMMENDATION

There is need for further analysis to be performed to validate the association of polymorphic variant (IGF-1 gene) at intron 2 with the body weight of Nigeria locally adapted turkeys using large population sample size from different unrelated farms in other to increase precision rate and accommodate all the assumptions of Hardy–Weinberg principle. Also studies on association of IGF1-SNP (Single Nucleotide Polymorphism) with body weight traits of Nigeria locally adapted turkey should be carried out.

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