

Short communications

**THE RELATIONSHIP BETWEEN BODY WEIGHT AND
GROWTH HORMONE GENE POLYMORPHISM IN
INDIGENOUS TURKEYS OF NIGERIA**

UNDER PEER REVIEW

Abstract

The candidate gene approach is a valuable tool for improving breeding programs, particularly in poultry, by identifying traits for enhanced production performance. This study investigates the growth hormone (GH) gene as a potential determinant of body weight in Nigerian indigenous turkeys. DNA was extracted from 60 twelve-week-old turkeys using blood samples, and a 466bp DNA fragment was amplified via PCR with primers designed from chicken sequences. The PCR product underwent MspI restriction enzyme digestion and was visualized on a 1.5% agarose gel. Genotypes associated with body weight were assessed using a General Linear Model. The GH/MspI locus revealed Two alleles (A and G) and three genotypes (AA, AG, and GG), with allele G being predominant. Genotype AG had the highest frequency (0.55), followed by AA (0.42), and GG had the lowest frequency (0.03). However, the least square means analysis showed that the polymorphism in the GH gene of the studied population was not significantly associated with body weight ($p > 0.05$). Nonetheless, the polymorphic nature of the GH gene in Nigerian turkeys suggests potential for further exploration in quantitative traits.

Key words: Polymorphism; PCR-RFLP; Growth Hormone gene (GH); Nigerian indigenous turkey; body weight.

Introduction

Genotyping animals for every gene that encodes polygenic trait seems unreasonable therefore it is important to focus on genes having effects that is responsible for a significant part of genetic variation observed (*Andrés Legarra and Vitezica, 2015*).

Indigenous or native poultry exhibit considerable functional and genetic significance, owing to their possession of genetic constituents that might have been relinquished within the enhanced gene reservoir. They harbor genetic variations that are either non-existent in contemporary improved breeds or that persist within their scarce progenitors, which sometime hold commercial value (*Sun et al., 2022*). With rising global demand for Turkey's products, its production is both important and profitable in agricultural industry, which makes it crucial in the economy of the country and extensive work is required to improve the economic traits through modern techniques that aid in selection (*Ahmad et al., 2019*).

Growth, carcass quality, meat quality, and reproductive qualities are just a few of the traits that have been linked to genes or markers through advancements in the science of molecular genetics in livestock animals (*Yurnalis et al., 2017*).

The candidate gene approach is effective for genetic advancement in breeding programs, particularly for poultry. Before ~~now~~ characterization for genetic improvement have been done using phenotypic approach but since the advent of molecular method such as candidate gene approach, it has made breeding for desirous trait faster and better in the Nigerian indigenous turkey. Work that has been done includes the use of candidate genes such as the myostatin gene (*Fijabi et al., 2020*) and IGF-1 (*Oyewole et al., 2018*). Investigating relationships between gene polymorphisms and economically significant features in farm animals can be done extremely well using this strategy. (*Khalil et al., 2021*). The GH gene has been identified as a candidate gene in numerous research for traits related to growth and carcass in domestic animals and poultry (*Anh et al., 2015*); (*Akhmad Dakhlan et al., 2022*); (*Wang et al., 2023*).

The growth hormone is a protein with a polypeptide chain consists of 191 amino acid residues with a molecular weight of about 22,000. Among the array of candidate genes, the growth hormone gene (GH) appears promising. This gene encodes the growth hormone known as somatotrophic hormone, which has a wide-ranging influence on cellular function throughout the body which includes enhancing protein, DNA, and RNA synthesis ~~to~~ and also facilitating glycogen biosynthesis (*Singh et al., 2015*). Therefore, the use of candidate gene approach may increase efficiency in identifying the desired traits needed to improve production performance of Nigerian indigenous turkeys.

The purpose of this study is to detect polymorphism in growth hormone gene of the Nigerian indigenous turkeys using PCR-RFLP technique.

Materials and Method

At random, 60 indigenous Nigerian turkeys were chosen for the experiment. Body weight measurements were taken at 4, 8, and 12 weeks, and whole blood samples were obtained at 10 weeks of age through the jugular vein into EDTA tubes, which were immediately placed into an ice box and transported to the laboratory.

DNA was extracted by using Zymo mini prep kit following manufacturers protocol. The quality and concentration of the extracted DNA was assessed using agarose gel electrophoresis and spectrophotometer respectively.

PCR-RFLP ASSAYS

GH gene was amplified using primers based on the sequence of chicken. The PCR primer 5'-ATCCCAGGCAAACATCCTC(forward) and 5'-CCTCGACATCCAGCTCACAT (reverse) for Msp1 site was used (*Thakur et al 2006*). The PCR for the GH gene was prepared in a total volume of 25 µl. The PCR products with length 466 bp were digested at 37-°C with Msp1 restriction enzyme for 15min and then inactivated at 80°C for 20 minutes. We ran the digested product

on electrophoresis using 1.5 % agarose for 30 minutes after which it was viewed under the gel documentation system.

Statistical Analysis

Genotype information was processed in Excel and determined using Pop Gene Version 1.31 software as described by *Yeh et al. (1999)*. Hardy-Weinberg equilibrium test was calculated using chi-square test through Pop Gene Version 1.31 software (*Yeh et al., 1999*). The effect of GH genotypes on body weight was analyzed using the GLM procedure in SAS ver. 9.1 using Least Squares Means (LSM).

Statistical Model

$$Y_{ijk} = \mu + g_i + \epsilon_{ij}$$

Where Y_{ij} = observed trait (body weight)

μ = the overall mean,

G_i = fixed effect of polymorphic variant (genotype),

e_{ij} = residual effects associated with j th observation of i th genotype

Results

Table 1: Genotype and allele frequency of GH gene in Nigerian Indigenous Turkey

	No	FREQUENCY			Allele		X^2 (HWE)
		Genotype AA	AG	GG	A	G	
Turkey	60	0.42	0.55	0.03	0.6917	0.3083	***

HWE = Hardy-Weinberg Equilibrium, *** = $P < 0.05$

Note: X^2 = chi-square test, G^2 = likelihood ratio test and df = degree of freedom

Table 2: Least square means and standard error of mean of body weight (g) of Nigerian indigenous turkey.

Age(weeks)	Body weight (g)		
	AA (n=25)	AG (n=33)	GG (n=2)
4	298.60±8.07	311.09±6.60	269.00±40.00
8	567.92±20.40	569.09±16.38	579.50±51.50
12	837.2±36.49	836.1±30.35	885.5±114.50



Figure 1: Electrophoresis patterns of intron 1 of the growth hormone (GH) gene of Nigerian indigenous turkey obtained by digestion using MspI restriction enzyme.

Discussion

Enhancing economic traits in poultry has become very important because of its relatively short life cycle and large number of progeny (Nassar, 2018). Identifying and utilizing molecular markers provides lots of potential for genetic improvement in selection programme. In local or domestic breeds of animals, genetic diversity allows breeders and researchers to maintain genetic diversity while improving production by developing new traits in response to environmental changes like heat stress and diseases (Olschewsky *et al.*, 2021). Recently the DNA polymorphism of different genes with its effect in chickens and other animals has been studied. In chicken GH gene, several polymorphisms have been discovered at the intron which includes intron1, 3 and 4 (Kulibaba *et al.*, 2017); (Wheto *et al.*, 2023).

In this study, genotype GG was found to have the lowest frequency among the population while AG has the highest frequency, and this agrees with the report of Wu *et al.* (2012) in Cherry Valley duck where it was observed that the TT genotype has the lowest genotypic frequency (0.30) while CT has the highest genotype frequency but contrary to Nie *et al.* (2005) whose reports says that GG genotype has the highest genotype frequency and AA has the lowest genotype frequency in

Leghorn and Taihe silkies chickens. The difference observed may be due to the population structure that was sampled which is different.

The probability of random mating was estimated by chi-square (χ^2) in the Nigerian indigenous population test to examine Hardy Weinberg equilibrium (HWE) which was found to be significant ($p < 0.05$), this shows that the population deviates from Hardy Weinberg equilibrium, which is like the findings of Wu *et al.* (2012) on Cherry Valley duck and Muscovy duck populations. There are several reasons that could be responsible for this deviation which could be the fact that the sampled population had experienced intensive selection, assortative mating, migration within the population and mutation. Deviations of genotype frequencies from the Hardy–Weinberg equilibrium is expected for loci with impact on traits in population under selection (Abramovs *et al.*, 2020); (Fijabi *et al.*, 2020).

For this research, the GH gene was investigated as a candidate gene to evaluate the polymorphisms and its association with body weight in Nigeria indigenous turkey populations. Two alleles was discovered with three genotypes which are A and G allele, and AA, AG and GG genotypes, the same result was reported by Wu *et al.*, (2012) and Anh *et al.*, (2015) in Thai broiler though contrary to Thakur (2006) who discovered three alleles and six genotypes in domestic poultry strains. This can be due to environmental effects which may have contributed to selection for this genotypes in different population. We assessed the association between different genotypes with weight at 4, 8 and 12 weeks. The results showed that GG was associated with superior non-significant ($p > 0.05$) body weight with slight numerical increase over genotype AA and AG at 8 and 12 weeks. This is supported by Wu *et al.* (2012) report on avian growth hormone gene polymorphism effect on growth and carcass characteristics in duck, it was shown that TT genotype has higher body weight than CC and TC genotype at 56days.

In Pitalah Ducks, the dominant allele is G, and the most frequent genotype is AG which is similar to this study on Nigerian indigenous turkey. Three genotypes (AA, AG, and GG) were discovered as shown by Yurnalis *et al.* (2017). It was observed that AG genotype was characterized by higher body weight than the GG genotype which is contradictory to the findings of this research. No significant difference was found in this study between the body weight and the genotypes which is contrary to the findings of Sinpru *et al.* (2021), where a significant difference between body weight and genotype was reported. Polymorphisms of GH gene have been reported in chickens at intron1, 3 and 4. In this-the present study, polymorphism was discovered at msp1 site in the Nigerian indigenous turkey and this is in accordance to Ghelghachi *et al.* (2013) that discovered polymorphisms in intron1 regions of the Arain chicken GH where he discovered three alleles (A₁, A₂ and A₃) and six genotypes. Ghelghachi *et al.* (2013) he reported a significant difference between Growth hormone gene and body weight at 6 weeks, also in In Awassi Lambs where the genotype of GH gene did not have a significant effect on weaning weight, body measurements, or the growth rate of lambs as reported by

| *Qasimi et al.* (2019). However, further research should be conducted on Nigerian indigenous turkey using markers associated with quantitative traits to elaborate more on the relationship of GH gene polymorphism with body weight.

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Conclusion

In summary, it can be concluded that in Nigerian indigenous turkey population, the growth hormone gene exhibit a moderate to high level of polymorphism. This indicates the potential for genetic enhancement through selective breeding initiatives. The result from this preliminary study can serve as a foundational resource for further investigations and inform conservation strategies aimed at preserving the genetic diversity of Nigerian indigenous turkey populations.

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