EFFECT OF MYOSTATIN GENE POLYMORPHISM ON BODY WEIGHT AND CARCASS TRAITS OF WHITE PLUMAGE NIGERIAN TURKEY

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ABSTRACT

The myostatin gene is a candidate gene whose biological function has been known to influence muscle growth and carcass traits in avian class. Our objective was to determine the association between gene polymorphism and growth traits in White Plumage Indigenous Turkey (WPIT). Genomic DNA was extracted from the whole blood of 93 WPIT. At 21 weeks, WPIT were slaughtered for carcass traits. Specific primers for WPIT were used for amplification of a 700 base segment. Alleles A and B corresponding to genotypes AA, AB and BB were detected at the locus tested. Significant (P>0.05) associations were recorded for all the genotypes detected with bodyweights of the population utilized at weeks 5, 13, 15, 17 and 19 but at 21 week, bodyweights of homozygote BB genotypes were higher than those of AA and AB genotypes. No significant associations (P>0.05) with carcass traits like liver, full gizzard weight, empty gizzard weight, spleen, heart weight, neck weight, defeathered weight, eviscerated weight and dressed weight. The products amplified displayed polymorphism and the variation in exon2 of myostatin gene may be a molecular marker for superior growth in the WPIT populations utilized.

Key words: WPIT, PCR-RFLP, Myostatin gene, SNP

Introduction

Domesticated turkey is an integral avian class with great potential for meat and egg production. An inventory survey revealed that six varieties (white, Black, Bronze, Slate, Bourbon Red, and Narragansett) of domesticated turkey based on plumage are given recognition and description by America Poultry Association in the standard of perfection (Ewuola and Olarinre, 2022). However, black, mottled and white plumage indigenous turkeys are found in Nigeria (Ewuola *et al*., 2022). White plumage confers selective advantage and are more demand driven in the poultry market than their counterparts. This is because its pin feathers are less visible when the carcass is dressed (Ewuola and Olarinre, 2022). We hypothesized that myostatin gene may perhaps pose a great influence on lean muscle distribution of turkey carcasses and live weight.

Myostatin is a key gene for growth and function as an inhibitor factor on muscles growth. Several gene products have been recognised in the sequence of this gene that has a significant effect on growth and carcass traits. These gene products are used as proficient marker in livestock species in order to upturn quality and quantity of meat. Karim *et al*., (2000) reported that, mutations in myostatin controlling regions were correlated with abdominal fat weight, abdominal fat percentage, hatch weight, and breast muscle percentage and weight in poultry. In chicken, the locus of myostatin comprised of three exons and two introns. Baron *et al* . 2002 recorded 373 bp, 374 bp and 1567 bp for the exons 1, 2, and 3 correspondingly. The gene products of myostatin locus had strong associations with survivability and growth rates, feed conversion efficiency, breast depth and percentage, eviscerated carcass weight, leg deformity, blood oxygen level, and hen antibody titer to the Gomboro

disease virus in three selected commercial broiler chicken lines as reported by Ye *et al* . 2007. Improvement of the growth traits of indigenous turkeys at this locus is a possible means to obtaining optimum productivity. Hence, this study was conducted to identifying polymorphisms of myostatin gene associated with bodyweight and carcass traits of white plumage Nigerian indigenous turkey.

Materials and Methods

Experimental turkeys, blood samples collection and DNA extraction: The experiment birds consist of ninety three (93) white plumage indigenous turkeys. Blood samples were collected in EDTA-treated tubes from 10 week-old turkey prior to slaughter at age 21week. Genomic DNA was obtained using Quick-gDNA Miniprep extraction kits following manufacturer protocol, and stored at -20 C prior to PCR amplification. The integrity of eluted DNA were checked using nanodrop spectrophotometer.

Generation of Amplicons using PCR: The primer used in this study was designed from myostatin gene sequence as follow:

Forward primer: AACCAATCGTCGGTTTTGACA and reverse primer: GAAAAGCAGCAGGGTTGTTA. The 700bp of DNA fragment was amplified in a Mastercycler Gradient 5331 thermal cycler (Eppendorf®) in a total reaction volume of 25µL containing 50ng genomic DNA, 12.5pmol of forward primer and the same amount of reverse primer, dNTPs, $MgCl_2$, Taq DNA polymerase, reaction buffer, PCR stabilizer and enhancer at optimal concentrations according to the following program: 94°C for 300s; 35 cycles of 94°C for 45sec, annealing of 62°C for 45sec, and 72°C for 50sec; and final extension step at 72°C for 120sec. Amplicons were detected on 1.5% agarose.

PCR-RFLP Assay: The polymorphisms in turkey myostatin were analyzed using PCR Restriction Fragment Length Polymorphism (RFLP), which was performed by mixing 20µl of molecular graded water, 2µl of 10X FastDigest Green Buffer, 20µl of concentrated PCR products and 1µl of FastDigest enzyme and subjected to digestion for 20 minutes at 65°C following the manufacturers' instructions. The restriction digests were separated on 1.5 agarose gel in 1xTBE at a constant current of 100v for 1hour. The gels were stained with ethidium bromide and the fragments were visualized under UV transilluminator. The three genotypes (AA, AB and BB) were revealed.

Collection of Data

At 21 weeks of age, the Liveweight(LW) of the turkeys from which the blood were sampled at age 10 were slaughtered and the weights of the following 16 carcass traits were measured using a sensitive scale: Defeathered weight, eviscerated weight, dressed weight, full gizzard weight, empty gizzard weight, breast weight, thigh weight, drumstick weight, back weight, wing weight, shank weight, liver weight, spleen weight, heart weight, head weight, and neck weight.

Statistical Analysis

Data collected were subjected to one way analysis of variance (ANOVA) in a completely randomized design following generalized linear mixed model (GLM) procedure of SAS (SAS, 2012) statistical software. Significant differences were determined using Duncan Multiple Range Test (DMRT) at 95% confidence interval of the same software.

The statistical model was $Y_{ij} = \mu + G_i + E_{ij}$. Where: Y_{ij} is the observation of the i^{th} population, μ^{ij} is the population mean, G_i is the fixed effect of i^{th} myostatin genotype, and E_{ij} is the random error.

Result and Discussion

Myostatin is autocrine signal protein secreted by muscle and acts as a negative regulator of muscle growth (Stinckens *et al*., 2005; Bellinge *et al*., 2005) . The high polymorphic tendency of the DNA sequence of the myostatin gene, which has been detected in earlier works (Baron *et al*., 2002; Dunner *et al*., 2003) was further obtained in the current findings in the three strains of indigenous turkey in Nigeria. Therefore, the coding region (exon 2) at myostatin locus was examined for genetic markers. In the current study, three genotypes AA, AB and BB were detected with corresponding alleles A and B were identified in the white

plumage of Nigerian turkey utilized as presented in plate 1. The variation at this locus shows genetic polymorphisms. Allele and genotype frequencies of myostatin gene exon 2 in white plumage population as presented in table 1. The results indicate that A allele is codominant allele in the population examined.

Homozygote BB conferred comparative advantage on bodyweights at weeks 5, 13, 15, 17 and 19 at 21 week. Associations of some of the genotypes with bodyweights at this locus in the population studied may be due to the alteration in amino-acid sequence in exon 2 at myostatin locus, although functional analysis will be needed to verify this. This indicates that, because the key role of myostatin gene is regulation of skeletal muscle growth, therefore, alterations in its protein sequence is a possibility which can transform growth function. In this study, the identified associations of the different genotypes with bodyweights at different ages might be connected to their linkage disequilibrium (LD) with other polymorphisms that occur in exon 2 of myostatin or to quantitative trait loci (QTL) beyond the myostatin gene. The results of this findings is in agreement with the work of Gu *et al*., (2004) who reported a polymorphic site in the 5'regulatory region of myostatin, which was associated with hatch weight in F2 chickens from a broiler by Silky cross.

Polymorphic effects of myostatin gene were also examined on carcass traits of turkey as presented in table 2. In the present study which showed no significant associations with carcass traits like liver, full gizzard weight, empty gizzard weight, spleen, heart weight, neck weight, defeathered weight, eviscerated weight and dressed weight. This is an indication that, the aforementioned carcass traits showed no linkage disequilibrum (LD) with any of the polymorphic variants of myostatin gene in the exon 2. Individuals with homozygote BB showed superior association with spleen and heart weights. Therefore, this polymorphism may have overdominant effects on spleen and heart weights. Animals with genotype AB had better carcass composition with breast, wing, drumstick and back muscles in black and spotted strains of the populations utilised and it did not show significant relationship with all carcass traits in spotted strain. Associations of some of the genotypes with two or more carcass traits might be due to the pleiotropic effect of the myostatin gene in the populations utilised. The results of this study corroborates the report of Gu *et al*. (2004) who found homozygous genotypes AA and BB at a locus in the 5'regulatory region to be associated with higher abdominal fat weight and abdominal fat percentage than AB in the F2 chickens from a cross of broiler and Silky chickens.

Conclusion

Variations at the myostatin locus associated with some of the bodyweights and carcass traits obtained in this study are indications that the studied locus is a potential molecular marker for improvement of growth rate of turkey in Nigeria.

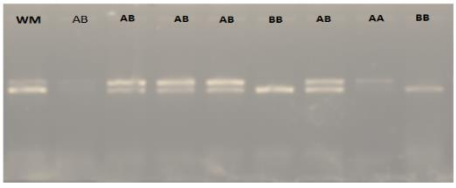

Plate 1: PCR-RFLP fragment of Myostatin gene

Table 2: Effect of Myostatin Gene polymorphism on Carcass Traits (LSM±SE)

Carcass Traits	AA	AB	BB
LIVER	49.75±2.99	45.43±4.55	46.86±4.34
FLGZ	87.63±2.91	85.71±5.76	87.14±4.40
EMGZ	55.00±1.66	53.14±3.88	55.14±2.89
SPLEEN	$4.25±1.03^{ab}$	$2.71±0.89^{ab}$	$1.71±0.18^{b}$
HEART	$9.00±0.66^{ab}$	$11.14±1.30^{a}$	$7.71±1.02^{b}$
HEAD	90.75±25.00	70.86±5.65	56.14±2.31
NECK	121.50±5.14	135.00±10.25	117±11.58
SHANK	$66.88±3.69^{bc}$	$75.43±6.92^{abc}$	$59.14±3.24^{c}$
BRST	$257.31±17.03^{bcd}$	$252.43±29.74^{cd}$	$318.57±35.44^{abc}$
WING	$194.69±10.41^{ab}$	$192.86±18.08^{ab}$	$199.86±15.82^{ab}$
THGH	$189.31±10.54^{ab}$	$181.71±17.6^{ab}$	$196.14±15.66^{ab}$

DRST	233.75±11.82[a]	232.29±15.6[a]	252.00±7.87[a]
BACK	308.75±14.17[a]	298.00±22.81[a]	334.71±16.25[a]
DFWT	1.68±0.07	1.66±0.15	1.59±0.09
EVWT	1.47±0.05	1.39±0.11	1.34±0.09
DRWT	1.16±0.05	1.11±0.10	1.07±0.08

[abcd:] Means with different superscripts along the same row are significantly ($p<0.05$) different
FLGZ: Full gizzard, EMGZ: Empty gizzard, BRST: Breast, THGH: Thigh, DRST: Drumstick, DFWT: Defeathered weight, EVWT: Eviscerated weight, DRWT: Dressed weight

References

Baron E.E., Wenceslau A.A., Alvares L.E., Nones K., Ruy D.C., Schmidt G.S., Zanella E.L., Coutinho L.L., Ledur M.C. (2002). High level of polymorphism in the myostatin chicken gene, in: *Proceedings of the 7th World Congress on Genetic Application to Livestock Production*., 19–23 August CD Rom, Communication 04-35, Montpellier, France.

Bellinge, R.H.S., Liberles, D.A., Iaschi, S.P.A. and O'brien, P.A. (2005) Myostatin and Its Implications on Animal Breeding. *Animal Genetics*, 36, 1-6. http://dx.doi.org/10.1111/j.1365-2052.2004.01229.x

Dunner S.M., Miranda E., Amigues Y., Cañón J., Georges M., Hanset R., Williams J., Ménissier F., (2003). Haplotype diversity of the myostatin gene among beef cattle breeds, *Genetic Selection Evolution*. 35 103–118.

Ewuola M. K, Akinyemi M. O. Osaiyuwu O. H., Fatai R. B., Tiamiyu A. K., Oso Y. A.A and Salako A. E. (2022). Single Nucleotide Polymorphisms of Insulin-like Growth Factor 2 (IGF-2) Associated with Bodyweights of Nigerian Locally Adapted Turkey. Proceedings of *47th Annual Conference of the Nigerian Society for Animal Production (NSAP)* March, 2022*;* **Pp. 60-63**

Ewuola, O. E. and Olarinre, I. O. (2022). Differentiation in poult plumage colour of f1 progeny from crosses between white and black indigenous turkeys. *Translational Animal Science*, Volume 7, Issue1, January 2023, txac168, https://doi.org/10.1093/tas/txac168

Gu Z., Zhu D., Li N., Li H., Deng X., Wu C. (2004). The single nucleotide polymorphisms of the chicken myostatin gene are associated with skeletal muscle and adipose growth, *Science in China Ser. C Life Sciences* 47 (26–31).

Karim L., Coppieters W., Grobet L., Valentini A. and Georges M. (2000). Convenient genotyping of six myostatin mutations causing double-muscling in cattle using a multiplex oligonucleotide ligation assay. *Anim. Genet.* 31, 396-399.

Statistical Analysis System Institute Inc. (2012). User's Guide: Statistics. *SAS programme, Cary,NC: SAS Institute Inc*.

Stinckens, A., Bijttebier, J., Luyten, T., Van Den Maagdenberg, K., Harmegnies, N., De Smet, S., Georges, M. and Buys, N. (2005) Detection of Polymorphisms in the Myostatin Gene in Belgian Pietrain Pigs. *Communications in Agricultural and Applied Biological Sciences*, 70, 37-41.

Ye X., Brown S.R., Nones K., Coutinho L.L., Dekkers J.C. and Lamont S.J. (2007). Associations of myostatin gene polymorphisms with performance and mortality traits in broiler chickens. *Genet. Sel. Evol.* 39, 73-8