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Evaluation of Single Nucleotide Polymorphism and Genetic Diversity at the Myostatin gene Locus in Indigenous and Locally Adapted Exotic Turkey breeds in Nigeria

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Abstract

This research was carried out to characterize single nucleotide polymorphism at the myostatin gene locus in indigenous and Nigerian locally adapted exotic turkeys. A total of 220-day-old poults comprising 120 local and 100 locally adapted exotic turkeys were sourced from reputable hatchery and were managed for 20 weeks under intensive management system. Blood samples were collected from 70 turkeys each form the two breeds via the brachial vein into Ethylene Diamine Tetra Acetic Acid (EDTA) bottles for DNA extraction and amplification of target regions using commercially available kits when the birds were 8 weeks. Genomic regions containing exon 1 and 2 of *MSTN* gene including their surrounding introns were sequenced and analyzed using BioEdit, Codon Code Aligner, DnaSP and MEGA software. Codon-based test was also performed to estimate the ratio of non-synonymous substitutions (dN) to synonymous substitutions (dS). Results from our study showed that both local and exotic turkey breeds had one non-synonymous single nucleotide polymorphism (SNP) (248 G>A) in exon 1 while SNP variant 333 G>A was detected only in exon 2 of exotic turkey. In intron 1, a total of 3 SNPs in local turkey and 2 SNPs in exotic turkey were detected. Intron 2 also revealed 4 and 3 SNPs in local and exotic turkeys respectively. Genetic diversity indices showed that local turkey had higher haplotype diversity at intron 1 (75 %) and intron 2 (84 %) while haplotype diversity of 20 % was estimated at exon 2 in exotic turkey. Our Codon-based test of selection showed dN/dS ratio of <1 (purifying selection) at G248A SNP loci, suggested a possible role of this non-synonymous SNP variant on growth performance. We recommend a population-based study to investigate the effect of this non-synonymous SNP G248A on growth and morpho-structural traits for breed improvement and conservation of our locally adapted turkey populations.

Keywords: Turkey, *MSTN* gene, polymorphism, Genetic diversity
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Introduction

One major poultry bird widely reared today primarily for its meat is turkey, as a result of their high market value, uniqueness of their meat's

taste, increased meat production, resistant nature to harsh climate and their foraging ability (FAO, 2018; Ilori et al., 2017). Turkey is scientifically called *Meleagris gallopavo* belonging to the family of birds called *Meleagrididae* which is native to the North American continent. Turkey domestication probably started by the Indians of Pre-Columbian Mexico. The birds were first taken to Spain in 1519 where they extended throughout Europe, before reaching England in 1541 (Ogundipe and Dafwang, 1980). In Nigeria, there are two main breeds of turkey which are the locally adapted and exotic turkeys. They possess varied characteristics which serve as phenotypic markers used in differentiating them (FAO, 2018). Such phenotypic traits that easily separate the local from the exotic turkeys include body weight, size, and growth rates (FAOSTAT, 2007). The Nigerian local turkeys have the smallest population of about 1.05 million when compared with other poultry species in Nigeria (FAOSTAT, 2010). Local turkey in Nigeria is hardy and better adapted to the harsh condition of the humid tropical condition of the country, their rearing started in the northern part of the country before gaining attraction to other parts of Nigeria (Ilori et al., 2011; Jayeola, 2011). At 20 weeks of age, Nicholas white exotic turkey performed better (4484.74± 52.07 g) than the local turkey with a body weight of 2869.68±46.08 g (Ilori et al., 2010). Adeoye et al. (2017) in a study of 24 weeks also reported that exotic turkey (Nicholas white turkey) had a higher body weight of 8,370 ± 0.24 g when compared to local turkey with an average weight of 3,290 ± 0.06 g at the same age. Although, local turkey weighs lesser than the exotic counterpart at maturity, local turkeys have better feed efficiency than the exotic variety (Ilori et al., 2010; 2017). Local turkey has varied colour ranging from black, lavender to white colour while exotic turkeys are phenotypically known to possess just white colors, except in rare cases as in the Auburn turkey which possesses a light reddish-brown plumage color (Emmah, 1997; Ilori et al., 2010). Local turkey has not been selected for any known trait despite their rich genetic potentials which could be exploited for breed improvement, on the other, their exotic counterpart has undergone several generations of intense selection for improved growth performance (Ilori et al., 2010). With the increasing world's population and its demand for animal protein which exceeds supply (Okewu and Iheanacho, 2015), it is important to increase the availability of animal protein, first by the evaluation of the effect of genetic polymorphism in genes involved in growth performance in poultry, for example myostatin (*MSTN*), chicken growth hormone, *IGF1*, *IGFBP2* and *TGFß3* genes (Saxena et al., 2005; Anh, 2015; Fijabi et al., 2020; Hosnedlova, 2020) and secondly through selection and crossbreeding of those individuals with superior allele with our locally adapted ones. *MSTN* is as growth differentiation factor 8 which plays a vital role in myogenesis. It also acts in part by inhibiting the activity of muscle satellite cells (Carnac et al., 2007). Myostatin polymorphism were observed in Belgian blue cattle with a "double muscling" effect as a result of an 11 bp deletion in the protein which resulted into muscular hypertrophy (Clop et al., 2006). Other effects have been observed in mice, race horse, whippets, and sheep amongst many (Schuelke et al., 2004). Due to increasing population and corresponding demand for animal protein, the need for increase production of poultry is more than necessary. To do this, there is need to evaluate the genetic potentials of local and exotic turkey breeds bred in Nigeria for breed improvement programs. This study therefore attempts to characterize the genetic diversity at *MSTN* gene locus inherent in both local and exotic turkey breeds raised in Nigeria.

Materials and Methods

Experimental Location and Birds

This study was carried out at the Turkey Breeding Unit, Directorate of University Farms (DUFARMS), Federal University of Agriculture, Abeokuta, Nigeria (Latitude $7^0$10'N and Longitude $3^0$2'E). The experimental site was earlier described in Ilori et al. (2010, 2017). A total of 220-day-old poults comprising 120 local turkeys and 100 exotic (hybrid converter) turkey purchased from a reliable hatchery at Ibadan were used for this study. The birds were brooded and raised under deep litter system management and properly tagged for easy identification. Commercial turkey starter diet containing 28% CP and grower diet containing 20% CP were fed to the turkey accordingly. Cool clean water was also provided without restriction. Biosecurity measures were properly engaged during the rearing period to prevent outbreak of diseases. Plate 1 shows the

photograph of both local and exotic turkey breeds at 8th week of age.

Plate 1: Photograph of Exotic hybrid converter (A) and Nigeria local Turkey (B) at 8th week of age

Blood Sample Collection and DNA extraction

About 2 ml of blood was collected via the brachial vein from 70 birds each from a total of 117 local and 96 exotic turkey bird remaining after 8 weeks of rearing into EDTA bottles and were immediately transported to the laboratory for DNA extraction. 200 µl of blood from each blood sample was used for DNA extraction using Zymo kit as described by the manufacturer (Zymo research quick gDNATM miniprep kit-LGC, Biosearch Technologies, USA). The gDNA purity and concentration were determined using Nanodrop spectrometer (ND1000; NanoDrop Technologies, USA). Gel electrophoretic method was used to determine the integrity of the extracted DNA by running 1µl of each eluted DNA sample on 1% agarose gel at 120V for 20 minutes

Primer Design and Amplification of MSTN gene

Pairs of primers set for exons 1 and 2 with the surrounding introns 1 and 2 of Myostatin gene using the *Meleagris gallopavo* gene sequence (accession number: NC_015017.2) were designed at Stab Vida genetics Laboratory in Monte da Caparica, Portugal using Primer Blast of National Centre for Biotechnology Information (NCBI) database, United States of America. PCR was carried out in a final reaction volume of 25 µl. Each reaction volume contained 2 µl of DNA template of 10 -20ng, 10 µl of master mix, 2 µl each of forward and reverse primers and 9 µl of nuclease free water. The amplification procedures were performed as follows: initial denaturation for 1 minute at 96 ºC, 40 cycles of final denaturation at 95 ºC for 30 seconds, annealing was set at 53 ºC for 30 seconds, extension temperature was set at 70 ºC for 1 minute and 40 seconds and final extension at 70 ºC for 5 minutes. PCR products were visualized on 1.5 % gel electrophoresis after staining with 2 µl/ml Gel Red Stain (Phenix Research Products, Candler, NC, USA) and were visualized under UV transilluminator. Purification of PCR products were performed with Magnetic Beads Carboxylate (MCLab, USA) while the DNA sequencing using BigDye Terminator version 3.1 using the instrument 3730 XL

Table 1: Primer sequences used for amplification of fragments of the myostatin gene

Primer name	Sequence (5′–3′)	Region	Annealing temperature
MYTm -F	ATGCAAAAGCTAGCAGTCTATG	Exon 1 and part of intron 1	58^{o}C
MYTE1-R	ACTCCGTAGGCATTGTGATAAT		
MYTE2-F	CTGATTTTCTTGTACAAATGGAG	Exon 2 and part of intron 2	58^{o}C
MYTE2-R	CAATCCATCTTCACCCGGTC		

Determination of Single Nucleotide Polymorphism, genetic diversity and Codon-Based Selection Test at Myostatin locus of turkeys in Nigeria

All sequences for each breed were edited using BioEdit (Hall, 1999) to remove junks from the sequences while sequence alignment was done using ClustalX 2.1 multiple sequence alignment program in MEGA 6.06 (Tamura et al., 2013) software. Polymorphisms (SNPs) within the DNA sequences of *MSTN* were identified using BioEdit software and confirmed using DnaSP v10.01(Librado and Rozas, 2009). Aligned sequences were translated into corresponding amino acid sequences using Open Reading Finder of NCBI. MEGA 6.06 program (Tamura et al., 2013) was used for Codon based test of selection according to Nei and Gojobori (1986) to determine the codon that was under selection and relative rates of non-synonymous (dN) and synonymous substitutions (dS) were corrected using Jukes and Cantor (1969) corrections for multiple hits. Z test was used to compare the relative abundance of synonymous and non-synonymous substitutions and used to infer the type of selective pressure acting on the *MSTN* gene (Nei and Kumar, 2000).

Results and Discussion

Detection of Single Nucleotide Polymorphism at MSTN gene locus in local and exotic turkeys.

The result of the sequence analysis from our study showed presence of SNPs within the first two exons and introns 1 and 2 in both turkeys' myostatin gene (Figure 1). Two transitional SNPs were detected within exon 1 (248G>A: local and exotic) and exon 2 (333G>A: Exotic). Similarly at intron 1, 2 SNPs (exotic turkey) and 3 SNPs (local turkey) were detected (Table 2). At exon 2, one SNP at base position 333G>A was detected in exotic turkey while 4 SNPs were detected in intron 2 (Table 2), indicating presence of variability at these regions. The polymorphism presents in exon 1 is non-synonymous and may cause a change in the type of protein formed which may probably influence growth and skeletal muscle development in the animal. The occurrence of 100% transitional mutation in exon 1 and 2 supports the study on selective basis hypothesis of transition and transversion substitution which states that transition rates are much higher than expected by chance, relative to those of transversion inferring their biological significance by favoring natural selection and overall relative fitness more than transversion (Jiang and Zhao, 2006; Pauly et al., 2017; Lyons and Lauring, 2017). Our results indicated that the exotic turkey which is a commercial broiler turkey is more polymorphic in the exonic region compared to their local counterpart although no variation or loss of function mutation in *MSTN* gene has been reported to influence growth performance in turkey. Several authors have reported different polymorphisms in the gene with some of them associated with growth performance in broiler chickens (Bhattacharya and Chatterjee, 2013; Dushyanth et al., 2016; Zhang et al., 2019). Hu et al. (2013) has also reported the occurrence of Single nucleotide polymorphisms in the upstream regulatory region that alter the expression of myostatin gene in

chicken. Identification of other polymorphisms in the intronic region especially in the local turkey that have not been previously described although still segregating indicates high genetic diversity within the region and any or some of this polymorphism could be in linkage disequilibrium with causative mutation with growth performance in turkey. The absence of SNPs within exon 2 coding region of myostatin gene in local turkeys may suggest conservation of the region. According to Bejarano et al. (2004) conserved regions are underlaying determinants to certain gene functions, as they offer important functions for the organism via proteins which are coded for by conserved sequences.

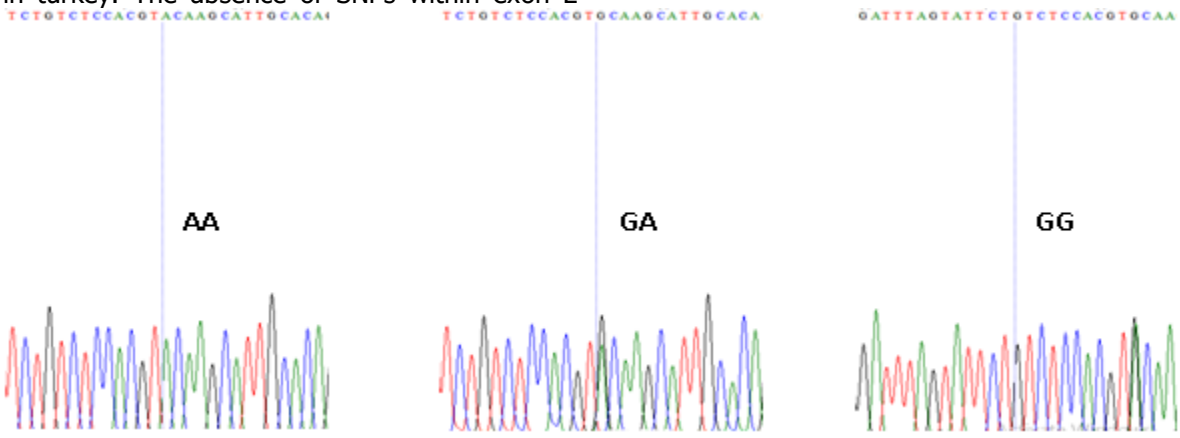

Figure 1: Chromatogram showing polymorphism 248G>A at exon 1 of MSTN gene of Nigerian local and hybrid converter turkey.

Amino Acid Change and Genetic diversity of MSTN gene in Nigeria locally adapted and exotic turkeys.

The resultant amino acid changes detected at *MSTN* gene of the Nigerian local and exotic turkey are presented in Table 3. The SNP (248G>A) detected in exon 1 is non-synonymous in both exotic and local turkey breeds and this resulted into amino acid changes from Alanine to Threonine in exotic turkey and Cysteine to Tyrosine in local turkey which may possibly influence growth and skeletal muscle development in the population (Dushyanth et al., 2016). The occurrence of non-synonymous SNPs in the two breeds resulting into amino acid changes occasioned by base changes or base substitutions within the coding region of exon 1 at *MSTN* gene is suggestive of biological significance of this mutation in the gene probably for growth performance or other important biological function in the populations.

Similarly, at exon 2, we reported a synonymous substitution at SNP 333G>A locus in exotic turkey with no amino acid change. According to Chu and Wei (2019), non-synonymous mutations lead to amino acids changes and it changes protein structure and functions while synonymous mutations could not change the composition of the peptide chain and protein function. However, it is important in the translation process, which could alter the programmed translational velocity and then influence the encoded protein folding and function.

Allelic frequency shows the percentage or proportion of gene copies in a given population (Silver, 2001; Stephenson, 2016). The frequency of an allele ranges from 0 to 1. According to Aquadro (2001) where the frequency of an allele is 1, the population is said to be fixed for that particular allele. In this study, the major allele (G) frequency (MAF) at SNP 248G>A within exon 1 in exotic turkey is 0.75 while MAF for the same allele in local turkey is 0.11. Allele G at exon 2 (333G>A) of exotic turkey has a frequency of 0.11 (Table 2). The evidence of high allelic frequency tending towards fixation suggests the presence of inbreeding in exotic turkey. The occurrence of this polymorphism occurring in a population that have been largely selected for improved productive and reproductive performance may suggest the involvement of this allele in these performance traits but this will need to be ascertained through association studies of these polymorphism with the traits.

The effect of other polymorphisms with low to moderately high frequencies in this study will need to be examined through association studies to detect their influence on production performance if any. The lack of fixed allele or the presence of other alleles in our locally adapted turkey could support its evolution under natural selection and could be a potential genetic resource for future breeding programme. Low heterozygosity reported across all exons and introns at *MSTN* loci in this study could be as result of inbreeding or little genetic variability especially in the exotic turkey which could affect fitness in natural settings (Walling et al., 2011). The polymorphic information content (PIC) value is usually used in measuring the informativeness of a genetic marker for its effectiveness in diversity, population and association studies (Guo and Elson, 1999). In this study, the PIC value for SNP 248G>A in the exon 1 region of exotic turkey was 0.30 which is below the value reported by Hildebrand et al., (1992). From Table 2, the low PIC reported in this study suggest the low level of informativeness of *MSTN* marker and its low ability to detect polymorphism/genetic diversity in the populations of local and exotic turkey and as such, low informativeness of MSTN marker loci in this study may not be suitable for detection of genetic diversity (Botstein et al. (1980) in both local and exotic turkey breeds. According to Botstein et al. (1980), the PIC value is equal or greater than 0.5 suggests high informativeness of genetic markers.

The diversity of exons 1, 2 and intron 1, 2 of *MSTN* gene in Nigerian local and hybrid converter turkeys are shown in Tables 4 and 5. Our results on haplotype diversity at intron 1 showed local turkey with 75 % haplotype diversity at intron 1 while 42 % (Intron 1) was reported in exotic turkey (Table 4). Also, we detected evidence of haplotype diversity within the coding region of intron 2 at *MSTN* gene where local turkey had 84 % while exotic turkey had 22 %. Low haplotype diversity at exon 1 (36 %) was obtained for local turkey, 39 % was reported in exotic turkey and no evidence was reported at exon 2 of local turkey (Table 4). The above genetic information corroborated our earlier report of low genetic diversity within the individual population of turkey breeds examined. The higher genetic diversity of the local turkey in the intronic region may suggest an abundant genetic diversity at this region which may be likely be useful for its natural selection. Polymorphism frequency and nucleotide diversity have been reported to be affected by selection, mutation rates, mating system, effective population size, demography, gene flow between populations, introgression from hybridization and historical effects, for example population bottlenecks on these factors (Frankham et al., 2002).

Selection Pressure at MSTN locus in Local and Exotic Turkey breeds.

For both turkey types, the synonymous substitutions per site was lesser than the non-synonymous substitutions per site. Sites with significantly lower values than 1 is indicative of negative or purifying selection (Yang, 2005) and negative selection is the most common form of natural selection (Bustamante, 2005). Our Condon-based test of selection showed that dN/dS ratio is <1 (negative/purifying selection) at G248A SNP loci, which implies that the base change at this position with amino acid changes may have positive effect on growth performance. From our study, the ratios of non-synonymous to synonymous substitution (dN/dS) in the individual population of Local and Exotic turkey at *MSTN* gene below 1 suggested evidence of negative/ purifying selection, thus indicating selective removal of alleles that are deleterious and purging of deleterious gene or alleles from the population (Grossen, 2020).

Table 2: Genetic Characteristics of SNP Variants Detected within Exon 1 to Intron 2 at *MSTN* Gene in Exotic and Local Turkeys Bred in Nigeria

Breed	Region	SNP	Major allele freq.	Reference allele	Types	Heterozygosity	PIC
Exotic	Exon 1	248G>A	0.75	G	Parsimony/ transition	0.38	0.30
		38C>T	0.75	C		0.38	0.30

	Intron 1	39T>C	0.75	T	Parsimony/ transition Parsimony/ transition	0.38	0.30
	Exon 1	248G>A	0.11	G	Singleton/transition	0.20	0.18
Local	Intron 1	38C>T	0.78	C	Parsimony/	0.34	0.28
		39T>C	0.89	T	transition	0.20	0.18
		50T>C	0.56	T	Parsimony/ transition Parsimony/ transition	0.49	0.37
Exotic	Exon 2	333G>A	0.11	G	Singleton/transition	0.20	0.18
	Intron 2	13T>A	0.89	T	Singleton/transversion	0.20	0.18
		29G>A	0.89	G	Singleton/transition	0.20	0.18
		46C>T	0.89	C	Singleton/transversion	0.20	0.18
Local	Intron 2	13T>A	0.60	T	Parsimony/	0.48	0.36
		29G>A	0.70	G	transversion	0.42	0.33
		36C>T	0.70	C	Parsimony/	0.42	0.33
		46T>G	0.60	T	transition Parsimony/ transition Parsimony/ transversion	0.48	0.36

SNPs was named according to their exact position in exon and intron; C = Cytosine; G = Guanine; T = Thymine;

Table 3: Amino Acid Changes at Exon 1 and 2 of *MSTN* Gene in Exotic and Local Turkeys

Region	Breed	SNP Variants	Nature of SNP variant	Changes in Amino acid	Types of Mutation
Exon 1	Exotic	248G>A	Transition	Alanine>Threonine	Non-Synonymous
	Local	248G>A	Transition	Cysteine>Tyrosine	Non-Synonymous
Exon 2	Exotic	333G>A	Transition	Glutamic acid-Glutamic acid	Synonymous

Table 4: Genetic Diversity of exon 1 and intron 1 at *MSTN* gene locus in Nigerian local and hybrid converter turkeys

	Turkey breed			
Diversity Indices	NLT exon 1	HCT exon 1	NLT intron 1	HCT intron 1
Nucleotide sites	373	373	76	76

Number of sequences	10	9	9	8
Polymorphic site	1	1	3	2
Singleton variable site	0	0	1	0
Parsimony informative site	1	1	2	2
Number of Haplotype	2	2	4	2
Haplotype diversity	0.356	0.389	0.750	0.429
Nucleotide diversity	0.00095	0.00104	0.01535	0.01128
Average number of nucleotide differences(k)	0.356	0.389	1.167	0.857

Legend:
NLT: Nigeria local turkey
HCT: Hybrid Converter Turkey

Table 5: Genetic Diversity at exon 2 and intron 2 of *MSTN* gene in Nigerian local and hybrid converter turkeys

Diversity Indices	Turkey breed			
	NLT exon 2	HCT exon 2	NLT intron 2	HCT intron 2
Nucleotide sites	374	374	104	104
Sequence used	10	9	10	9
Polymorphic site	0	1	4	3
Singleton variable site	0	1	0	3
Parsimony informative site	0	0	4	0
Number of Haplotype	1	2	5	2
Haplotype diversity	0.00	0.200	0.844	0.222
Nucleotide diversity	0.00000	0.00053	0.01923	0.00641
Average number of nucleotide differences(k)	0.000	0.200	2.000	0.667

Legend:
NLT: Nigeria local turkey
HCT: Hybrid Converter turkey

Table 6: Synonymous and Non-synonymous Substitutions at *MSTN* loci in Local and Exotic Turkeys

Genotype	Codon	d_N (±SE)	d_S (±SE)	d_N/d_S	Z-statistic	P value
Local turkey	248	0.457± 0.003	0.00± 0.014	0.457	1.045	0.149
Exotic turkey	248	0.483± 0.011	0.00± 0.003	0.483	1.414	0.080

Conclusion and recommendation

The population of local and exotic turkeys showed low level of heterozygosity at the *MSTN* locus and as such may be homogenous in nature**.** Also, the PIC informativeness of *MSTN* marker is low in both local and exotic turkey breeds suggesting that the marker may not be suitable for linkage and genetic diversity studies in these two populations. Based on the biological information provided in this study, we recommend a population-based study to interrogate the effect of non-synonymous SNP G248A variant reported in this study on growth and morpho-structural

traits for breed improvement and conservation of Nigerian indigenous and locally adapted exotic turkey populations.

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