Myostatin gene polymorphism and its association with body weight in Nigerian indigenous turkey

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ABSTRACT

Polymorphisms of the myostatin gene have been used as marker–assisted selection in chicken but not in turkey for growth traits. Improvement of Nigeria indigenous turkey will enhance the genetic potentials of local poultry and data on their genetic variation is essential to strategic breeding programs and genetic improvement. Therefore, this study was aimed at investigating the polymorphisms of myostatin gene (*MSTN*) in Nigerian indigenous turkey using polymerase chain reaction-restriction fragment length polymorphism (PCR–RFLP) method with *Msp*I restriction enzyme for digestion. Fifty poults were randomly selected for DNA assaying at 10 weeks of age. Genomic DNA extraction from blood samples was done and 599 bp fragment of myostatin was amplified. Data on the weight of the animals were collected every week. The genetic structure of the sampled population was analyzed by POPGENE 32 software. Association of the genotypes with body weight was evaluated using the general linear model (GLM) of SAS 9.2. Three allelic variants A, B and C with frequencies of 0.79, 0.20 and 0.01 were identified and controlled three genotypes AA, AB and AC with frequencies of 0.58, 0.40 and 0.02, respectively. The probabilities values obtained for the likelihood ratio (G^2; 0.145757) and chi–square test (X^2; 0.342138) for Hardy–Weinberg equilibrium suggest the sampled population were at equilibrium. The observed heterozygosity, Shannon information index, number of effective alleles and Nei's gene diversity indices in the population were 0.4200, 0.5542, 1.5056 and 0.3358 respectively indicating moderate to high diversity among the population. Body weight showed no significant ($P > 0.05$) association with genotype at 4, 8 and 12 weeks of age. Myostatin gene is polymorphic and thus create a room for genetic improvement through selective breeding. However, its polymorphic variants did not significantly ($P > 0.05$) influence body weight in Nigerian indigenous turkey.

Keywords: Association, genotype, myostatin gene, PCR–RFLP, turkey, Nigeria

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INTRODUCTION

Indigenous species of animals constitute a great potential for local production. Poultry, particularly turkey is very important and has been recognized as an important genetic resource among the avian species (Olowofeso, 2005). The description of Nigerian local poultry is based on phenotypic traits (Adebambo *et al*., 1999). However, the association of both phenotypic and genotypic data can form a basis for making correct inferences and can serve as long term plans for improvement programs. The complex relationship between genotypes and economic traits may reflect gene interactions and

indicates that simple models based on additive gene effects may not be adequate for the dissection of the genetic architecture of quantitative traits (Tiago and Nicholas, 2016). The quest for unraveling the genetic mechanism controlling the growth of the muscle, which has a direct link with body weight, has resulted in the development of several factors regulating muscle growth. One of the most important muscle-growth limiting factors is myostatin (MSTN).

Myostatin, a transforming growth factor β (TGF–β) family member is an important regulator of muscle mass. Gene knock–out experiments showed that myostatin null mice exhibited a two to threefold increase in the muscle mass compared to the normal wild type mice (McPherron *et al*., 1997). In addition, it was demonstrated that the loss of myostatin protein due to 11–bp deletion in the third exon or point mutations in coding region of myostatin gene could be a possible genetic cause for the double–muscle mass phenotype found in Belgian Blue and Piedmontese breeds of cattle (Kambadur *et al.,* 1997; McPherron and Lee, 1997; Grobet *et al*., 1998; Szabo *et al*., 1998). Functionally, lack of MSTN results in the excessive growth of skeletal muscle, which delineates the existence of a powerful mechanism to control muscle size in normal individuals (McPherron *et al.,* 1997). These results provide strong evidence that myostatin is a negative regulator of skeletal muscle growth in animals (Thomas *et al.,* 2000; Lee and McPherron, 2001). The MSTN is mainly expressed in skeletal muscles which controls the growth of the muscles by suppressing proliferation and differentiation of myoblast cells in mammals (Rios *et al.,* 2004). Mutations in the myostatin gene were associated with the double–muscling phenotype in cattle (McPherron and Lee, 1997; Grobet *et al*., 1998). The sequence and function of myostatin appear to be highly conserved among vertebrate species, and its role during myogenesis of chickens was expected to be similar to that observed in other mammals (Scheuermann *et al*., 2004). Mutations in myostatin regulatory regions have been shown to be associated with abdominal fat weight, abdominal fat percentage, birth weight, breast muscle percentage and breast muscle weight in chicken (Gu *et al*., 2004). Myostatin gene is highly polymorphic in chicken, composed of 3 exons and 2 introns (Baron *et al*., 2002), and the mode of inheritance is probably monogenic, autosomal and partially recessive with incomplete penetrance (Ménissier, 1982). The attention of poultry farmers in Nigeria has been integrally focused on chicken with minimal attention paid to other poultry species. There are many underrated, but highly promising poultry species such as turkey, quail and guinea fowl. Turkey production has not been fully exploited in developing countries despite its greater potential than the chicken (Shingari and Sapra, 1993; Peters *et al*., 1997; Perez–Lara *et al*., 2013). Turkey thrives better under arid conditions, tolerates heat better, ranges farther and has higher quality meat (Fisinin and Zlochevskaya, 1989; Yakubu *et al*., 2013). A free–range system of rearing is most popular for rearing the local stocks of turkey (Peters *et al*.,1997) in Nigeria. These birds are natural foragers and scavengers and always range farther. Indeed, they thrive best where they can rove and freely feed on seeds, fresh grass, locusts, crickets, grasshoppers, worms, slugs and snails (Singh and Sharma, 2012). Consequently, polymorphisms of the myostatin gene have been used as marker-assisted selection in chicken but not in turkey for growth traits.

Improvement of Nigeria indigenous turkey will enhance the genetic potentials of local poultry and data on genetic variation is essential to strategic breeding programs and genetic improvement. This study was carried out to identify the genetic variation in exon 1 of myostatin gene in Nigeria indigenous turkey and to determine their associations with body weight.

MATERIALS AND METHODS

Body weight of the poults was collected weekly throughout the experiment. Blood samples were randomly collected from 50 poults at 10 weeks of age from the Nigerian indigenous turkey. The poults were sourced from different farms in Oyo state and were intensively managed. A commercial feed with water was supplied to the animals *ad libitum*. About 4 ml of blood were drawn from

each poult by jugular venipuncture. The blood was aseptically collected into EDTA bottles and then stored in –20°C freezer. The DNA extraction was done using the Zymo Quick–gDNA Miniprep kit and the manufacturer's instruction was followed. The integrity and quality of the extracted DNA were viewed using spectrophotometer. The 599 bp in the exon 1 of myostatin gene (Figure 1) was amplified using 300 ng extracted genomic DNA in a final volume of 25 µl containing 10X PCR buffer, 2 m*M* $MgCl_2$, 25 µ*M* dNTP mixture, 1 U *Taq* polymerase and 25 µ*M* of each primer according to as follows:
Forward: 5´–AACCAATCGTCGGTTTTGAC–3´
Reverse: 3´–CGAAAGCAGCAGGGTTGTTA–5´

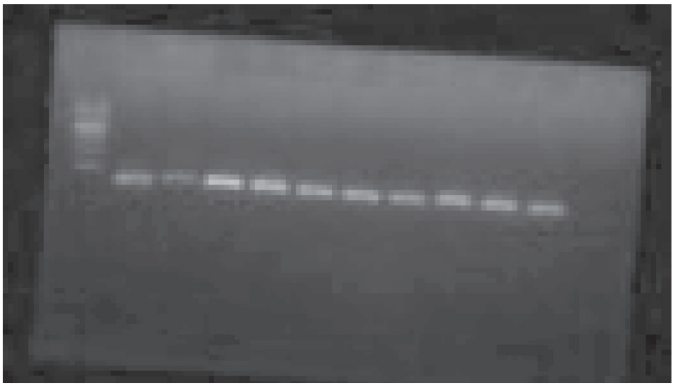

Figure 1 Band scoring in PCR shows the electrophoresis patterns of exon 1 of the myostatin gene of Nigerian indigenous turkey obtained by polymerase chain reaction polymorphism

Reactions were run on a Mastercycler Gradient 5331 thermal cycler (Eppendorf) under the following thermal conditions: initial denaturation at 94°C for 5 min, followed by 35 cycles consisting of denaturation at 94°C for 45 s, annealing at 57°C for 45 s, extension at 72°C for 50 s and a final extension at 72°C for 2 min. The 1.5% agarose gel stained with ethidium bromide was used to view the bands. The 15 µl of amplicons samples were loaded into the well on the gel and were run with 100 volts for 15 min in the electrolysis chamber. The bands were viewed under the gel documentation system. Digestion of amplicons was performed in a 25 µl of the reaction solution at 35°C for 15 min incubation period and at 80°C for 20 min using *Msp*I restriction enzyme. The restriction digest products (Figure 2) were electrophoresed for 20 min at 100V on 2.5% agarose gel. Individual PCR–RFLP fragment sizes in each sample were determined, based on standard DNA molecular weight markers for each gene using Gel Doc system by computer software "Quantity One" of Biorad USA.

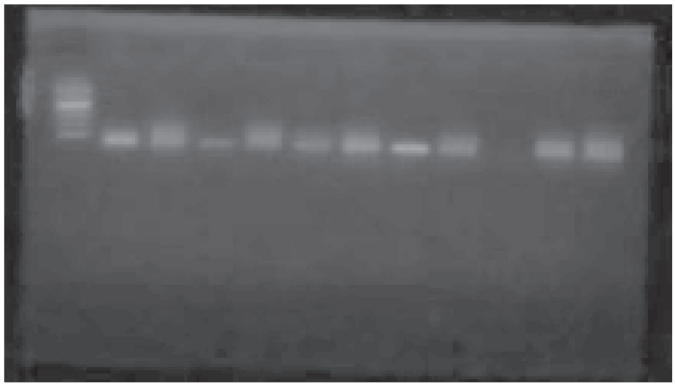

Figure 2 Digested product shows the electrophoresis patterns of exon 1 of myostatin gene of Nigerian indigenous turkey obtained by digestion using *Msp*1. Band 1 = 100 bp ladder, Band 2 = genotype AA, Band 3 = genotype AB

Allele frequency, genotypic frequency and genetic structure of the sampled population for the myostatin locus were analyzed using the POPGENE 32 software for Hardy–Weinberg equilibrium through chi–square analysis. Association between the genotypes and body weight was done using general linear model of SAS 9.2. The statistical model used: $y_{ij} = \mu + G_i + e_{ij}$, where y_{ij} is observed trait (body weight), μ is the overall mean, G_i is fixed effect of polymorphic variant and e_{ij} is random error.

RESULTS AND DISCUSSION

The alleles and genotypic frequencies that were observed are presented in Table 1 for the myostatin gene in the sampled population. It was discovered that the myostatin locus has moderate to high polymorphism as alleles A, B and C and genotypes AA, AB and AC were observed. The observed frequencies for A, B and C alleles were 0.79, 0.20 and 0.01 respectively while the observed genotypic frequencies for the AA, AB and AC were 0.58, 0.40 and 0.02, respectively. These results imply the independence of alleles within genotypes as well as constant allele frequencies across generations. The chi square (X^2) and likelihood ratio test (G^2) for Hardy-Weinberg equilibrium (HWE) fitness probabilities were 0.342138 and 0.145757, respectively. The implication being that the sampled population did not deviate from the principle that underlines Hardy-Weinberg. These assumptions ensure that the breeding population from where sampling was done must be infinitely large, mating must have been random, mutation of the allele was rare, no differential migration and selection (assortative). This probability value implies that the myostatin locus in the sampled population is not significantly different ($P > 0.05$) from Hardy-Weinberg hypothesized population, and thus at equilibrium. Zandi *et al*. (2013) reported a deviation from Hardy-Weinberg in the association of myostatin gene with chicken production traits and attributed the cause to selection while result obtained from the control broiler and IWI layer population (Bhattacharya *et al*., 2010) also deviated from Hardy-Weinberg. However, Khederzadeh *et al*. (2017) reported a conformation to Hardy-Weinberg in an analysis of the third exon of the myostatin gene in gray Shirazi sheep populations.

The allele and genotype with the highest frequency were A and AA, respectively. Exon 1 of the myostatin gene in this study was found to be polymorphic with three genotypes which are in consonance with the findings of Gan *et al.* (2008), Nadjar-Boger and Funkenstein (2011) and Bhattacharya and Chatterjee (2013) in chicken.

The AC genotype obtained in this study deviated from the normal pattern of BB genotypes that usually complement the AA and AB genotypes in several studies in chicken and cattle (Chandan *et al.,* 2014). However, the AC allele was observed at a very low frequency (0.08) in exon 2 of myostatin for IWI broiler line (Bhattacharya *et al*., 2010). The inconsistency and variations in these results may be ascribed to breed differences, population, sampling size, environmental factors, mating strategies, geographical position effect and frequency distribution of genetic variants (Georgieva *et al*., 2015). The frequency of AA was higher (0.58) than the frequencies of AB (0.40) and AC (0.02). In addition, these observed genotype frequencies were in agreement with the result obtained previously by Miranda *et al.* (2000). The result obtained from this study indicated that the A allele is the dominant allele while the C allele is the rarest as the myostatin gene is concerned in the Nigerian indigenous turkey. The polymorphic nature of the studied locus provides us with the information that genetic improvement opportunity is available through selective breeding.

Table 1 Observed allele and genotypic frequencies for myostatin locus in Nigerian indigenous turkey

Allele frequency	Genotypic frequency	X^2	G^2	df
A (0.79)	AA (0.58)	0.342138	0.145757	2
B (0.20)	AB (0.40)			
C (0.01)	AC (0.02)			

Note: X^2 = chi-square test for Hardy-Weinberg equilibrium, G^2 = likelihood ratio test for Hardy-Weinberg equilibrium, df = degree of freedom

The values for the heterozygosity are presented in Table 2 which shows the influence of breeding and selection for myostatin locus in the sampled population. The observed homozygosity (Obs_{Hom}) value of 0.5800 was lower than the expected homozygosity (Exp_{Hom}) value of 0.6608, while the observed heterozygosity (Obs_{Het}) value of 0.4200 was greater than the expected heterozygosity (Exp_{Het}) of 0.3392. Nei's expected heterozygosity (Nei) had the same value with the average heterozygosity (Ave_{Het}) which was 0.3358. The heterozygote frequency (0.42) obtained in this study is greater than those reported earlier by Jaiswal *et al*. (2009) in Kadaknath native chicken (0.32) and Reen *et al.* (2007) in the indigenous chicken of northwestern Himalayan (0.23) which can be attributed to the differences in the population structure. A higher frequency of heterozygote implies a greater tendency of making genetic progress or improvement. This implies that the genetic progress that can be obtained in the Nigerian indigenous turkey can be achieved by selecting poults with genotype AA at a very young age while reducing the cost of production. The observed heterozygosity was higher than the expected heterozygosity indicating moderate variation in the myostatin locus for Nigeria indigenous turkey.

Table 2 Summary of heterozygosity statistics for all loci for Myostatin in Nigerian indigenous turkey

Locus	Obs_{Hom}	Obs_{Het}	Exp_{Hom}	Exp_{Het}	Nei	Ave_{Het}
Myostatin	0.5800	0.4200	0.6608	0.3392	0.3358	0.3358

Note: Obs_{Hom} = observed homozygote, Obs_{Het} = observed heterozygote, Exp_{Hom} = expected homozygote, Exp_{Het} = expected heterozygote, Nei = Nei's expected heterozygosity, Ave_{Het} = average heterozygosity

The number of alleles and the effective number of alleles are presented in Table 3. This table shows the richness and abundance of alleles as it relates to the myostatin gene in Nigerian indigenous turkey. The observed number of alleles (Na) is 3.000. The effective number of alleles (Ne) and Shannon's information index were 50.19% (1.5056) and 18.47% (0.5542) respectively of the observed allele. The oftenest allele was A with an abundance of 0.79 which was greater than B (0.20) and C (0.01) allele, meaning that the most abundant allele was A and the rarest allele was C. Skewness of the population toward allele A and genotype AA may be attributed to random mating that favor this allele owing to the fact that they have the highest frequency in the studied population for several generations, without the introduction of new germplasms from outside (Chandan *et al.,* 2014). This suggests that this population is likely to be at equilibrium. Therefore, it thus implies that the population under consideration is not operating under any force of mutation, assortativeness, genetic drift, founder effect and net migration of any allele.

Table 3 Summary of genetic variation statistics for all loci for Myostatin gene in Nigerian Indigenous turkey

Locus	Na	Ne	I
Myostatin	3.0000	1.5056	0.5542

Note: Na = observed number of alleles, Ne = effective number of alleles, I = Shannon's information index

Table 4 presents genotypes association with the body weight of Nigerian indigenous turkey for myostatin locus. Analysis of variance indicated no significant ($P > 0.05$) association with body weight of the Nigerian indigenous turkey at 4, 8 and 12 weeks of age intervals although numerical differences exist. Genotype AB group had the highest numerical body weight at 4 weeks old with a mean value of 303.80 ± 44.30 g. The mean range of body weight at 4 weeks of age was 234.00 to 303.80 g. At 8 weeks of age, the AA genotype group had the highest body weight (571.28 ± 98.32 g). They had a mean range value of 499.00 to 571.28 g. Consequently, at 12 weeks of age, the AC genotype group had the highest body weight (990.00 ± 0.00 g) and the range of body weight for this week was 786.20 to 850.90 g.

Table 4 Mean ± SD for body weight at 4, 8 and 12 weeks of age of Nigerian indigenous turkey with different myostatin genotypes

Trait/genotype	AA (n = 29)	AB (n = 20)	AC (n = 1)	P–value
BW4 (g)	293.52 ± 35.24	303.80 ± 44.30	234.00 ± 0.00	0.1927
BW8 (g)	571.28 ± 98.32	549.50 ± 91.12	499.00 ± 0.00	0.5956
BW12 (g)	850.90 ± 161.11	786.20 ± 128.65	990.00 ± 0.00	0.1894

Note: BW4, BW8 and BW12 = body weight at 4, 8 and 12 weeks of age respectively, AA, AB and AC = observed genotypes, SD = standard deviation

The genotype groups in this study were not significantly different (P > 0.05) at 4, 8 and 12 weeks of age, however, numerical differences exist. A high numerical value was associated with AB genotype at 4 weeks of age while at 8 weeks of age, higher body weight was associated with AA genotype and at 12 weeks of age, genotypes AA and AC were associated with higher body weight. These results were in agreement with the result obtained by Chandan *et al*. (2014) that reported no significant (P > 0.05) difference between the genotype groups for body weight at 2–6 weeks of age in broiler for the SNP of the minimal promoter region of the myostatin gene. However, Genxi *et al*. (2012) found a significant difference (P < 0.05) between genotype groups at 8–12 weeks in the 5'–upstream region of the myostatin gene in four Bian chicken breeds but no significant difference (P > 0.05) at 10–12 weeks was observed.

CONCLUSION

It could be therefore concluded that exon 1 of the myostatin gene had moderate to a high degree of polymorphism in Nigerian indigenous turkey, and thus have room for genetic improvement through selective breeding. Data obtained from this preliminary study will be a guide for future investigation as well as for conservation strategies of Nigerian indigenous turkey.

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