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SINGLE NUCLEOTIDE POLYMORPHISMS OF INSULIN-LIKE GROWTH FACTOR 2 (IGF-2) GENE ASSOCIATED WITH BODYWEIGHTS OF NIGERIAN LOCALLY ADAPTED TURKEY

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ABSTRACT

The Insulin-Like Growth Factor 2 (IGF-2) gene was studied as a candidate gene for bodyweight of three turkey populations (Black, Spotted and White strains). The primer was designed to detect single nucleotide polymorphisms of exon2 at the IGF-2 locus by polymerase chain reaction-restriction fragment length polymorphism. Co-dominant alleles A and B corresponding to genotypes AA, AB and BB were detected at the locus tested. No significant (P>0.05) associations were recorded for all the genotypes detected with bodyweights of the population utilized at weeks 3, 7, 9, 11 and 21 but bodyweights of homozygote BB genotypes across the three strains were higher than those of AA and AB genotypes. Significant differences (P<0.05) also occurred for male and female turkeys at weeks 15 and 19. No significant (P>0.05) association of different genotypes with bodyweight at other weeks were observed (P <0.05). The products amplified displayed polymorphism and the variation in exon2 of IGF-2 gene may be a molecular marker for superior growth in the turkey populations utilized.

Keywords: Turkey strains, PCR-RFLP, IGF-2 gene, SNP, Genetic association

INTRODUCTION

The traits of production importance in farm animals which are affected by many genes is mainly growth rate. Growth rate and lean meat content are always the primary concern during breeding for their economic value (Zhang *et al*., 2008; Liu *et al*., 2010; Ren *et al*., 2010; Zhang *et al.,* 2019). Growth traits are physiological functions under the control of several genes. Genotyping animals for all the genes encoding a polygenic trait seems impractical and so it appears more realistic to focus on only a few genes having effects that account for a significant part of genetic variation in growth traits (An *et al*., 2011). Insulin-Like Growth Factor 2 (IGF-2) Gene is imperative in regulating the growth of farm animals. IGF-2 gene is responsible for the growth of cell, survival, locomotion and distinction through the IGF1 receptor tyrosine kinase (Wendy and D'Amore, 2008). McMurtry (1998) reported the functional role of IGF2 in controlling rate of growth, body conformation together with fat and oil absorption in avian. Gene products of IGF-2 in mouse through restriction fragment length polymorphism were said to have effect on 21-day weight in cocks and 42-day weight in hens. IGF-2 gene; a product of insulin hormone is a typical growth-influencing gene for identifying polymorphisms that are correlated with the growth rate in farm animals. The Nigerian locally adapted turkey plays a pivotal role in socio-economic wellbeing of rural people and is distributed all over the agro-ecological zones of the country. They are adaptable, less vulnerable to diseases than their indigenous counterparts and somewhat promising amongst indigenous poultry species owing to its fast growth rate and the better carcass yield of toms. In spite all these positive attributes, little or no consideration has been given to the genomic characterization, conservation, diversity and improvement of these valuable genetic resources at DNA level. The three main strains of turkey in Nigeria are black, spotted and white based on

their plumage. Although, recent work has reported phenotypic and biochemical characterization. There is lack of information on genomic variation of Nigerian turkey at IGF-2 locus. The objective of this study was to reveal the polymorphism in Nigerian turkey populations (black, spotted and white strains), and investigate its association with weekly bodyweights in the three populations at IGF-2 locus.

MATERIALS AND METHODS

Resource population, samples collection and preparation: Three different turkey strains [black (55), spotted (102) and white (93)] were used in this study for single nucleotide polymorphism (SNP) detection. Blood samples and weekly (week1-21) bodyweights were collected from the 247 birds consisting of both strains and sexes. Genomic DNA was obtained using Quick-gDNA Miniprep extraction kits following manufacturer protocol, and stored at -20 C.

PCR Amplification: The primer used in this study was designed from IGF-2 gene sequence as follow: GH: Primer forward: AAC ATC CTC CCC AAC CTT TCC and Primer reverse: CCC TGT CAA GGT TAG GCT CAT. The 300bp 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 5min; 35 cycles of 94°C for 45sec, annealing of 57°C for 45sec, and 72°C for 50sec; and final extension step at 72°C for 72sec. Amplicons were detected on 1.5% agarose.

SNP identification and genotyping by PCR-RFLP: The polymorphisms in turkey IGF-2 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. Band scoring was carried out manually.

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 (9) statistical software to test the fixed effects of polymorphic variants, strain and their interactions. Significant differences between polymorphic variants and weekly bodyweights were determined using Duncan Multiple Range Test (DMRT) at 95% confidence interval of the same software.

RESULT AND DISCUSSION

Insulin-like growth factor 2 (IGF2) is an imprinted gene expressed in most tissues affecting muscle content in farm animals and plays a vital role in livestock growth, influencing cell division and differentiation. Therefore, the coding region (exon 2) of IGF-II was examined for genetic markers. At the selected locus, three genotypes (AA, AB and BB) and two co-dominant allele (A and B) were detected across the three strains of Nigerian turkey as presented in plate 1. This is signal of high genetic polymorphisms at the examined locus. Allele and genotype frequencies of IGF-2 gene exon 2 in three turkey populations are presented in Table 1. The results indicate that A allele is preponderant allele across the three populations utilized.

Table 1: Alleles and genotypes frequency for IGF2 gene of three strains of indigenous turkey in Nigeria

Strains	N	Genotype frequency			Allele frequency		HWE		
		AA	AB	BB	A	B	x^2	P<0.05	Sig
Black	55	0.36	0.47	0.16	0.60	0.40	0.01	0.910	*
Spotted	102	0.46	0.36	0.17	0.64	0.35	4.52	0.033	NS
White	93	0.34	0.49	0.16	0.59	0.40	0.831	0.821	NS

Chi-square (x^2)* Significant at P<0.05; ^{ns} not significant (P>0.05) HWE=Hardy Weinberg equilibrum, N=sample size

The frequency of the genotype of the black strain differed significantly (P <0.05) from the expectation of Hardy-Weinberg equilibrium. Frequencies of genotypes and alleles obtained in the present study were dispersed and distributed among the strains. The highest frequency of A allele in spotted population at IGF-2 locus might be vindicated with the discoveries (Tang *et al*., 2010) who indicated that the individual carrying greater frequency of allele A exhibited superior weight gain in comparison with the ones conceded lesser frequency of allele A as is the case of Aseel chicken known for better meat production in comparison with Naked neck and Desi native chickens carrying lesser frequencies and are also low producers.

The results of association analysis between different the polymorphic variants detected and weekly bodyweights of both strains and sexes are given in Table 2 and 3 respectively. In the three strains of

Table 2: Association of exon 3 at locus of IGF2 gene with strain on weekly body weight of Nigerian indigenous turkey

	Spotted			White			Black		
Weeks	AA	AB	BB	AA	AB	BB	AA	AB	BB
3	208.89±6.05	184.86±13.50	224.23±10.86	206.36±5.47	216.35±11.16	208.38±10.84	205.31±6.90	209.80±15.03	196.13±13.92
7	509.71±18.28^{ab}	493.57±27.32^{ab}	586.00±32.73^{a}	518.76±20.42^{ab}	536.29±25.08^{ab}	450.00±34.22^{b}	518.45±17.48^{ab}	482.20±38.9^{ab}	503.88±42.23^{ab}
11	824.18±20.53	829.29±49.95	780.40±72.33	850.33±24.06	835.00±34.26	849.00±35.29	873.24±19.97	878.40±24.66	886.50±40.50
15	1378.55±35.93^{ab}	1391.71±79.98^{ab}	1232.20±78.38^{b}	1416.70±42.72^{ab}	1366.53±63.96^{ab}	1366.63±45.46^{ab}	1500.14±38.20^{a}	1537.40±62.06^{a}	1529.13±80.60^{a}
19	2078.95±37.85^{abcd}	2053.57±86.38^{bcd}	1858.00±171.76^{d}	2122.85±44.59^{abcd}	2062.18±99.78^{abcd}	2025.50±96.52^{cd}	2220.31±62.77^{abc}	2340.00±83.61^{ab}	2366.38±74.50^{a}
21	2347.05±44.49^{a}	1971.43±309.47^{b}	2353.8±116.06^{a}	2441.33±61.82^{a}	2372.35±115.17^{a}	2373.38±112.11^{a}	2575.69±67.55^{a}	2672±101.45^{a}	2684.25±85.61^{a}

$^{abc:}$ Means with different superscripts along the same row are significantly (p<0.05) different

Table 3: Association of Exon 3 at locus of IGF2 gene with sex on weekly body weight of Nigerian indigenous turkey

Weeks	Female			Male		
	AA	AB	BB	AA	AB	BB
3	204.23±5.93	229.13±10.75	212.70±12.30	208.88±4.34	199.43±9.69	202.73±8.58
7	522.35±18.41	520.88±44.23	509.10±37.36	510.48±13.32	515.05±18.2	497.27±32.67
11	827.45±21.24^{ab}	787.13±43.25^{b}	779.70±39.14^{b}	860.10±15.39^{ab}	861.67±27.07^{ab}	908.09±26.41^{a}
15	1388.48±36.82^{ab}	1260.50±60.48^{b}	1305.60±59.00^{ab}	1451.68±28.94^{a}	1456±52.23^{a}	1479.18±61.36^{a}
19	2059.23±43.40^{ab}	1933.13±133.63^{b}	2025.00±107.29^{ab}	2184.57±34.98^{a}	2174.62±71.53^{a}	2197.73±101.36^{a}
21	2327.15±48.24^{ab}	2128.75±169.55^{b}	2421.40±67.67^{a}	2522.68±43.95^{a}	2402.86±131.89^{a}	2546.91±112.51^{a}

$^{abc:}$ Means with different superscripts along the same row are significantly (p<0.05) different

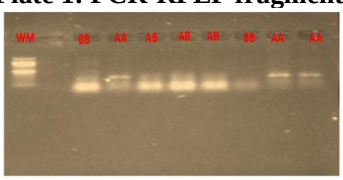
turkey, there were significant associations of genotypes AB and BB with bodyweight at week 1 and week 5 of the spotted strain. Animals with genotype BB for black and spotted strains were significantly associated with bodyweight from week 13 to week 19. No significant associations were recorded for all the genotypes detected with bodyweights of the population utilized at weeks 3, 7, 9, 11 and 21 respectively but bodyweights of homozygote BB genotypes across the three strains were higher than those of AA and AB genotypes respectively. Significant differences also occurred for male and female turkeys at weeks 15 and 19 respectively. No significant (P >0.05) association of different genotypes with bodyweight at other weeks were observed. The result of the present findings revealed the influence of IGF2 on posthatch growth. The alteration in variation in the exons was expedient for estimation of association with advantageous traits. The variation of IGF2 gene exon 3 could be connected to alternative variation in the IGF2 gene resulting in the alteration amino acid sequence. In the present study, no significant associations were recorded for all the IGF2 genotypes AA, AB and BB with bodyweight at weeks 1, 3, 5, 7, 9, 11 and 21 across the three strains studied. This might be due to production of linkage disequilibrium (LD) with another mutation located in the IGF2 locus or another linked gene. However, genotype BB had significantly greater means for growth traits than the genotypes AA and AB in black and spotted strains at weeks 13, 15, 17 and 19 respectively. Though, the chief role of IGF2 gene is to regulate early growth in mammals but the present study showed it has an effect on chick stage of turkey. Individuals with genotype BB showed superior growth rate with black and spotted strains of turkey from week 13 to week 19. This might be attributed to dominant effect of B allele which might be associated with greater growth rate. The results of this study were consistent with the findings of (Nagaraja *et al*., 2000) who studied gene effect on bodyweight, carcass yield and meat quality of locally adapted chicken. However, the results

are in divergence with the findings of (Amills *et al*., 2003), who reported coding region (exon 3) of IGF2 gene. The significant influence may be ascribed to allele A to B substitution; the present outcomes infer that this substitution might lead to alterations in peptide sequence, which could change the action of the hormone. Therefore, variances in the genetic structure among the strains of indigenous turkey used in this study with those studied by (Amills *et al*., 2003) and (Zhang *et al*., 2008) might explain the inconsistent results.

CONCLUSION

Variations at the IGF2 locus associated with some of the bodyweights obtained in this study are indications that the studied locus is a potential molecular marker for improvement of growth rate of turkey in Nigeria and this may help breeders and researchers to develop new characteristics in response to changes in environment and maintain genetic diversity as well as improve productivity.

Plate 1: PCR-RFLP fragment of Insulin-like growth factor-2 gene



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