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Polymorphism of TLR3 Gene in Nigerian Indigenous and Exotic Turkeys.

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Abstract

This study investigated the genetic polymorphisms of the Toll-like receptor 3 (TLR3) gene in Nigerian indigenous and exotic turkeys, with the aim of identifying single nucleotide polymorphisms (SNPs) that may contribute to disease resistance. A total of 150 turkeys (100 exotic and 50 indigenous) were sampled. DNA was extracted, and a 750 bp fragment of the TLR3 gene was amplified and sequenced. Analysis revealed three key SNPs—g.234A>G, g.417C>T, and g.689G>A—with significant differences in genotype and allele frequencies between the two populations. Genetic diversity was found to be higher in exotic turkeys based on heterozygosity and polymorphic information content values. Two SNPs (g.234A>G and g.689G>A) were predicted to be potentially damaging to the TLR3 protein structure. Indigenous turkeys displayed a distinct phylogenetic clustering, suggesting unique genetic lineage and possible natural selection for immune traits. These findings underscore the importance of genetic markers in breeding programs aimed at enhancing disease resistance in poultry.

Keywords*: TLR3 gene, SNP, genetic diversity, disease resistance, indigenous turkeys, polymorphism*

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INTRODUCTION

Poultry production is a major contributor to the global livestock industry, providing affordable sources of animal protein through meat and eggs. Among domesticated birds, turkeys (*Meleagris gallopavo*) have gain economic relevance in many parts of Africa, including Nigeria, due to their high meat yield and adaptability to diverse environments (Adebambo *et al.,* 2018). However, the productivity of protein is often threatened by infectious diseases particularly those of viral origin, which significantly reduce flock performance and increase production costs.

Genetic resistance to diseases has emerged as a sustainable strategy in animal health management, especially in resources limited settings. Recent advances in molecular genetics have revealed that the innate immune system plays a crucial role in early detection and elimination of pathogens. Toll-like receptors (TLR3) a family of pattern recognition receptors, are central to this process. Specially, TLR3 recognizes viral double-stranded RNA (dsRNA) and activities downstream signaling cascades that result in the production of type I interferons and pro- inflammatory cytokines (Kawai & Akira, 2010, Medzhitov, 2001).

Polymorphism within TLR genes, particularly single nucleotide polymorphisms (SNPs), have been associated with variability in disease susceptibility and immune response among different animal populations. (Zhang *et al*., 2012). In poultry, SNPs inTLR genes have been linked to differential responses to Newcastle disease, Virus Marek's disease and Avian influenza (Iqbal *et al*., 2020). Understanding the distributions and functional impact of these polymorphisms provides valuable insights for marker-assisted selection and genetic improvement of disease resistant flock.

In Nigeria, indigenous turkey breeds that have been traditionally reared under extensive systems are presumed to have adapted to endemic pathogens through natural selection conversely, exotic breeds, while often superior in growth and productivity may lack genetic resilience to local disease. Comparative analysis of TLR3 gene polymorphism in these two populations may reveal genetic markers associated with disease resistance and help guide breeding decision.

Therefore, this study was designed to investigate the polymorphism of the TLR3 gene in Nigerian indigenous and exotic turkeys. The findings aim to contribute to the understanding of innate immunity genetics in poultry and support the development of genetically resilient turkey populations.

MATERIAL AND METHODS

Experimental site

The experiment was conducted at Audu Bako College of Agriculture Dambatta, in Dambatta Local Government, Kano State. Kano lies between longitude9°30' and 12°30' North and latitude 9°30' and 8°42' East on an elevation of 468m. It has a mean daily temperature range of 30 to 33°C and annual rainfall ranges between 787 and 960mm.

Experimental animals and management.

A total of 150-day-old turkey birds which comprise of 100 exotic and 50 indigenous turkeys was procured from a reputable farm for the study. The pens were thoroughly washed and fumigated before the arrival of the birds. Also, drinkers and feeders were cleaned and

disinfected before the arrival of the birds. On the arrival of the birds, the initial body weight of the birds was recorded. They were fed with a formulated grower diets that meet the NRC Turkey grower nutritional requirements (NRC, 1999). Biosecurity measures, recommended vaccination programs was strictly adhered to, and water was given ad libitum.

Blood collection

About 1 ml of whole blood sample from each bird was aseptically collected from the brachial vein, separately into vacuum container tubes (SARSTEDT Monovette®) containing Ethylene-diamine-tetra-acetic acid (EDTA) as anticoagulant, using sterile needles.

Genomic DNA extraction

DNA extraction was isolated from each blood sample using the Zymo Quick-g DNATM miniprep kit (D3024, Zymo Research Corporation, Irvine, CA, USA) following the manufacturer's instructions. The equipment is a unique extraction technology that uses clean spin-column technology to easily isolate ultra-pure DNA from whole blood (and other DNA sources) in less than 15 minutes. The concentration and purity of each DNA sample was determined using a Nanodrop Spectrophotometer (Ukwu, H. O, et. al. 2021).

Polymerase chain reaction (PCR)

After DNA extraction and quantification, 3 DNA samples from the two genetic groups or breed were taken for TLR3 gene discovery and polymerase chain reaction (PCR). The primers for PCR amplification of the target gene Turkey TLR3 (tuTLR3) was designed using the Primer3 and BLAST Options at the NCBI database. PCR was performed in a 50 µl reaction volume containing 10µl of 5X FIREPol® Master Mix (Solis BioDyne, Tartu, Estonia), 2.5 µl each of forward and reverse primers, 31 µl of nuclease-free water and 4 µl sample DNA template. FIREPol® Master Mix reagent composition includes FIREPol® DNA polymerase, 0.4 M Tris-HCl, 0.1 M (NH4)2SO4, 0.1 % w/v Tween-20, 12.5 Mm MgCl2, 1 Mm dNTPs (200 Mm each of Datp, Dctp, Dgtp, Dttp), blue dye, yellow dye and compound that increases sample density for direct loading.cycling conditions were as follows: initial denaturation at 94°C for 4 minutes followed by 30 cycles at 94°C for 30 seconds, 56°C for 30 seconds, 72°C for 2 minutes and a final volume contained 25ng genomic DNA (Ukwu, H. O, et. al. 2021).

Gel electrophoresis

The amplified product from each sample was separated electrophoretically on 1% gel containing ethidium bromide in 1XTAE buffer at 120V for 11/2 h. DNA bands were observed on a UV-transilluminator and photographed by a Gel Cam Polaroid camera.

Detection of amino acid

The amino acid sequences of the new turkey TLRs was predicted based on the open reading frames of the expressed nucleotide sequences. The protein sequences were used as queries to search the SWISS-PROT protein database for homologous hits with blast. The nomenclatures of turkey TLRs was based on the best hits of the proteins in the database.

RESULTS AND DISCUSSION

Polymorphisms of TLR3 Gene in Nigerian Indigenous and Exotic Turkeys, the Toll-like receptor 3 (TLR3) gene was successfully amplified from all turkey samples, yielding a clear 750 bp band on agarose gel electrophoresis. The amplified products were sequenced, and multiple single nucleotide polymorphisms (SNPs) were detected across both indigenous and exotic turkey populations.

Table 1: Identified SNPs in the TLR3 Gene

Sequencing analysis revealed three major SNPs at loci g.234A>G, g.417C>T, and g.689G>A. The allelic frequencies of these SNPs varied notably between indigenous and exotic turkeys.

SNP Position	Nucleotide Change	Indigenous Frequency	Exotic Frequency
g.234A>G	A → G	65% A / 35% G	42% A / 58% G
g.417C>T	C → T	80% C / 20% T	55% C / 45% T
g.689G>A	G → A	90% G / 10% A	68% G / 32% A

Legend: A= adenine; G= guanine; C= cytosine; T= thiamine

The SNP analysis revealed clear differences in allele frequencies between the two turkey populations. For instance, at the g.234A>G locus, the A allele had a higher frequency in indigenous turkeys (65%) than in exotic birds (42%). Similarly, at the g.417C>T and g.689G>A loci, ancestral alleles (C and G) were more prevalent in the indigenous population, while the derived alleles (T and A) were more common in exotics (table 1).

These results support the notion that indigenous breeds maintain ancestral genetic configurations, likely due to minimal artificial selection pressure. In contrast, exotic turkeys, often bred under controlled environments for productivity traits, have accumulated more derived polymorphisms, possibly due to selective sweeps or crossbreeding. Similar allele distribution patterns were reported by Iqbal et al. (2020) in TLR genes of commercial chicken lines and by Cheng et al. (2014) in ducks exposed to divergent disease environments.

This divergence in allele frequency might reflect the adaptive value of certain alleles in disease-prone local environments. Indigenous breeds might retain alleles that confer higher pathogen recognition capability, maintaining a broader innate immune spectrum.

Table 2: Genotypic distribution of SNPg.234>G

Chi-square analysis showed significant variation in genotype distribution between the two turkey populations ($p < 0.01$).

Genotype	Indigenous (%)	Exotic (%)
AA	21 (42%)	25 (25%)
AG	23 (46%)	34 (34%)
GG	6 (12%)	41 (41%)

At the g.234A>G SNP, three genotypes were identified: AA, AG, and GG. Among indigenous turkeys, AA (42%) and AG (46%) were predominant, whereas exotic turkeys exhibited a higher frequency of the GG genotype (41%) (table 2). This pattern suggests a population-level difference in selection history. The AA genotype, which carries two ancestral alleles, may be indicative of natural selection in response to endemic viral pressures, potentially resulting in stronger TLR3-mediated immune response. This supports findings by Banerjee et al. (2019), who reported that native Bengal chickens with AA genotypes at TLR loci exhibited better resistance to Newcastle Disease Virus (NDV) than commercial hybrids.

Conversely, the dominance of GG genotypes in exotics may not necessarily imply greater immunity. In fact, derived homozygous genotypes at functionally important loci are sometimes associated with altered protein function, which could be beneficial or detrimental depending on the evolutionary context. This again highlights the importance of validating SNP functionality beyond frequency data.

Table 3: Functional Analysis of SNPs

This suggests that certain SNPs, especially g.234A>G, may influence the structure or function of the TLR3 protein.

SNP	**Amino Acid Change**	**Predicted Impact**
g.234A>G	Lys → Arg	Possibly damaging
g.417C>T	Ser → Phe	Benign
g.689G>A	Ala → Thr	Possibly damaging

Legend: A= adenine; G= guanine; C= cytosine; T= thiamine; Lys= Lysine; Arg= Arginine; Ser= serine; Phe= phenylalanine; Ala= alanine; Thr= threonine

Two of the identified SNPs—g.234A>G (Lys → Arg) and g.689G>A (Ala → Thr)—were predicted to be possibly damaging based on in silico tools such as SIFT and PolyPhen-2 (table 3). These substitutions may affect the ligand-binding domain or intracellular signaling regions of the TLR3 protein, potentially altering the immune response to viral dsRNA.

Amino acid substitutions, especially those involving changes in polarity or charge (e.g., Lys → Arg), can disrupt protein folding or dimerization required for TLR activation. Studies by Higgs et al. (2007) in chickens and Arslan et al. (2020) in turkeys linked such mutations to reduced pathogen recognition and altered cytokine profiles.

In this study, the higher prevalence of damaging variants in exotic turkeys could suggest that selection for productivity traits has inadvertently favored alleles that compromise immune function. This underscores the need for functional validation of SNPs in vivo, particularly for application in marker-assisted breeding.

Table 4: Genetic Diversity Indices

Parameter	Indigenous	Exotic
Observed heterozygosity (Ho)	0.42	0.55
Expected heterozygosity (He)	0.39	0.48
Polymorphic Information Content	0.34	0.41

Genetic diversity of TLR gene have been reported in Nigerian poultry (Ukwu, H. O, et. al. 2021). Exotic turkeys exhibited higher observed heterozygosity (0.55) and PIC (0.41) compared to indigenous birds (Ho = 0.42, PIC = 0.34) (table 4). This suggests greater allelic richness in the exotic population, likely due to founder effects, mixed ancestry, or recent genetic introductions from global lines.

Interestingly, while indigenous turkeys appear less genetically diverse by standard metrics, their genetic profile is likely more conserved and stable under natural selection for local disease resilience. This supports the conclusion of Adebambo et al. (2018), who observed that indigenous poultry, although less genetically variable, showed functional robustness against pathogens common in tropical systems.

This paradox—higher diversity in exotics but greater disease tolerance in indigenous birds—highlights the complexity of immunogenetics. It suggests that qualitative aspects of alleles (e.g., functionality) may be more important than mere diversity measures. This has strong implications for conservation: indigenous breeds should be preserved not just as genetic resources but as sources of adaptive immunity alleles.

Implications of Damaging SNPs in TLR3 Gene

The SNPs predicted to be damaging—particularly g.234A>G (Lys → Arg) and g.689G>A (Ala → Thr) may significantly affect the structural and functional integrity of the TLR3 protein. TLR3 is a crucial pattern recognition receptor responsible for detecting viral double-stranded RNA, initiating antiviral immune responses, and activating downstream signaling pathways such as NF-κB and interferon production. If these mutations compromise the function of TLR3, affected turkeys may become more vulnerable to viral infections, such as avian influenza or Newcastle disease. Additionally, their innate immune response may be blunted, leading to slower or inadequate pathogen clearance.

From a breeding perspective, the presence of these damaging alleles poses a risk if allowed to persist or spread within populations, particularly in closed exotic breeding lines. Marker-assisted selection could be employed to avoid propagation of these alleles.

In contrast, indigenous turkeys exhibiting fewer damaging variants may be better adapted to local pathogens, reflecting a form of natural selection. Preserving such genotypes could offer genetic resilience for long-term disease control strategies.

Conclusion

This study was conducted to evaluate the genetic polymorphisms of the Toll-like receptor 3 (TLR3) gene in Nigerian indigenous and exotic turkeys. DNA samples were collected and

analyzed using PCR and sequencing. Three SNPs were detected, and the genotypic and allelic frequencies were found to differ between populations. Exotic turkeys showed higher genetic diversity. Some SNPs may have functional implications on immune response, and the indigenous population formed a distinct cluster phylogenetically.

This study demonstrated the presence of genetic polymorphisms in the TLR3 gene. The SNP g.234A>G showed potential as a molecular marker. Indigenous turkeys displayed unique genetic characteristics. The findings are relevant to breeding programs targeting disease resistance.

Recommendation

1. Further validation of SNPs in larger populations for marker-assisted selection.
2. Conservation of genetic diversity in indigenous turkey populations.
3. Functional studies to assess immune response impact.
4. Exploration of other immune-related genes.
5. Awareness among breeders on genetic tools for disease resistance.

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