

Evolution of TLR3 gene in Nigerian indigenous and exotic turkeys

Jeremiah, Taiwo Boluwatife^{1*}, Abdulrahman, Taofeek¹, Olaogun, Aishat Bukolami¹, Ibrahim, Qahharat², Akinyemi, Olusegun Moses¹, Oyetayo, Oluwaseyi Paul³, Ayinde, Omolara Oluwakemi¹ and Babatunde, Moses Ilori¹

¹Department of Animal Breeding and Genetics, Federal University of Agriculture, Abeokuta, Nigeria.

²Department of Microbiology, Crescent University, Abeokuta, Nigeria.

³Department of Animal Science, Faculty of Agriculture, Obafemi Awolowo University, Ife, Nigeria.

*Corresponding author. Email: jeremiahtb.15@student.funaab.edu.ng

Copyright © 2023 Jeremiah et al. This article remains permanently open access under the terms of the [Creative Commons Attribution License 4.0](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Received 9th September 2023; Accepted 28th October 2023

ABSTRACT: This experiment was carried out to determine the evolution of the TLR3 gene in Nigerian indigenous and exotic turkeys. A total of 150 turkeys i.e. 100 exotic and 50 indigenous were used for this study. Birds were allotted treatments and fed formulated grower diets that meet the NRC—Turkey grower nutritional requirements. Genomic DNA was isolated from each blood sample, and the concentration and purity of each DNA sample was determined using a Nanodrop Spectrophotometer. After DNA extraction and quantification, three DNA samples from each genetic group (or breed) were taken for TLR3 gene discovery and polymerase chain reaction (PCR). The source sequence and the retrieved sequences were trimmed and edited while the amino acid sequences of the new turkey TLRs were predicted based on the open reading frames of the expressed nucleotide sequences, and the nomenclatures of turkey TLRs were based on the best hits of the proteins in the database. From the findings of the study, the SNP (172N>N) detected at position 516 was synonymous in both exotic and local turkey breeds, and this resulted in amino acid changes from Alanine to Threonine in exotic turkeys and Cysteine to Tyrosine in local turkeys. Also, in this study, the SNP (7 Q>*) detected in exon 4 was non-synonymous, resulting in amino acid changes from Cytosine to Threonine in the exotic turkey. Positive Tajima's D values were the same for exotic and local turkey breeds. The findings of this study suggest significant instances of purifying selection acting on the gene. Based on the results of this research, the populations of local and exotic turkeys showed a low level of heterozygosity at TLR3 locus and may be homogenous. Also, there was low genetic diversity in the TLR3 gene of both local and exotic turkey breeds. This suggests potential vulnerabilities and challenges for their long-term health and adaptability. Strategies that aim to increase genetic diversity, like cross-breeding should be implemented.

Keywords: Evolution, exotic turkeys, immunology, Nigerian indigenous turkey, TLR3 gene.

INTRODUCTION

Poultry production, especially of domestic turkeys, like Nigerian Indigenous turkey (NIT), occupies a central position in the nation's attainment of animal protein sufficiency (Uberu *et al.*, 2021). This is because the NIT is mainly involved in improving the livelihood of rural people, thereby contributing substantially to the nation's gross domestic product. The NIT are resilient birds that can survive any prevailing local environmental conditions

(Ajayi, 2010). The origin of each strain or ecotype of NIT is the product of mutation, genetic drift, adaptation, and evolution. The different selection pressures imposed on these turkeys include diet, variation in climate, endemic parasites, and diseases. Indigenous animals are both functionally and genetically valuable because they contain genetic materials that may be harnessed for improvement (Ajayi, 2010).

Local turkey has been reported to thrive better under arid conditions, has better heat tolerance, ranges farther, and has better meat quality (Yakubu *et al.*, 2013). Nigeria has a local turkey population of about 1.05 million, the smallest compared to other poultry species (FAOSTAT, 2010). They are nondescript, have multi-coloured plumage, and sometimes appear black or white (Ngu *et al.*, 2014). However, local turkey is one of Nigeria's least studied poultry species, and more effort needs to be directed at characterizing them using evolutionary analysis.

Toll-like receptors (TLR) play a crucial role in host immune response via recognition of pathogen-associated molecular patterns (PAMPs), such as lipopolysaccharides, lipopeptides, flagellins, double-stranded RNA, or CpG DNA motifs (Temperley *et al.*, 2008). So far, at least ten members of the chicken TLR family have been identified, including TLR1 type 1 and type 2, TLR2 type 1 and type 2, TLR3, TLR4, TLR5, TLR7, TLR15, and TLR21 (Temperley *et al.*, 2008). Toll-like receptors (TLRs) play a crucial role in the host's innate immune response via the recognition of pathogen-associated molecular patterns (Jin and Lee, 2008). TLRs recognize PAMPs efficiently and non-self-reactively to initiate pro-inflammatory mediators, culminating in adaptive immune response (West *et al.*, 2006). In addition, TLRs also react to damage-associated molecular patterns (DAMPs) released after cellular damage and, thus, play a crucial role in initiating the innate immune response (Tizard, 2009). Genetic variations found in the genes encoding TLRs have been associated with disease susceptibility and resistance in a variety of animal species (Ogorevc *et al.*, 2009).

Advances in whole genome sequencing and annotation in recent times have led to the identification of TLRs in several vertebrates, including fish, amphibians, birds, and mammals (Medzhitov *et al.*, 1997). However, the present knowledge of the avian TLR family is mainly based on chicken studies and few reports on duck, turkey, and zebra finch.

Chicken TLR repertoire consists of ten genes (TLR1LA, ILB, 2A, 2B, 3, 4, 5, 7, 15, and 21) (Boyd *et al.*, 2007; Temperley *et al.*, 2008). Recently, the whole genome of Turkey has been sequenced and available in the public domain that, has analyzed immune genes including TLRs and antimicrobial peptides (Dalloul *et al.*, 2010). Polymorphisms of TLR may profoundly influence the response of a host to a wide range of pathogens and are associated with resistance and susceptibility to diseases (Misch and Hawn, 2008). The TLR1 gene family cluster, including TLR1, TLR6, and TLR10, is associated with susceptibility to bacterial infections in humans and mice. It was found that variation in the inflammatory responses to bacterial lipopeptides is regulated by a common TLR1 transmembrane domain polymorphism that could affect the innate immune response and clinical susceptibility to a broad spectrum of pathogens. The hypermorphic genetic variation in TLR1 is associated with increased susceptibility to organ dysfunction, death, and gram-positive bacterial infections in sepsis (Wurfel *et al.*, 2008).

In addition, the human TLR1 variant is associated with a decreased incidence of leprosy (Johnson *et al.*, 2007), and the response to N-palmitoyl-Sdipalmitoylglycerol, a synthetic ligand for TLR1 (Misch and Hawn, 2008). Furthermore, human TLR6 polymorphism was associated with lower left ventricular wall thickness and inflammatory response in hypertensive women (Sales *et al.*, 2010).

The main objective of this study was to determine the evolution of the TLR3 gene in Nigerian indigenous and exotic turkeys. In the course of ascertaining polymorphic variations within the TLR3 gene among both indigenous and non-native turkey populations in Nigeria, the objectives encompass the prediction of amino acid types and variations present in the TLR3 gene across these indigenous and non-native turkey groups. Additionally, it was aimed to compute neutrality test statistics for the TLR3 gene sequence in the context of Nigerian and non-native turkey breeds. Finally, this study investigation extends to elucidating the evolutionary dynamics of the TLR3 gene within the turkey species and its comparative evolution vis-à-vis other poultry species. The causative agents for these diseases, such as *Salmonella*, *Escherichia coli*, and *Pasteurella multocida*, are resident opportunistic microorganisms that are difficult to eradicate from flocks. TLR polymorphisms are associated with host susceptibility to diseases. Although it is well known that the susceptibility of Nigerian indigenous turkeys with different genetic backgrounds to pathogenic infection varies, little is known regarding the genetic factors contributing to the susceptibility of turkeys to diseases. Therefore, genetically improving Nigerian indigenous turkeys' resistance to these opportunistic pathogens may be considered an alternative control measure against these bacterial infections. Hence, there was a need to carry out this study to investigate the evolutionary analysis of the Toll-like three receptor gene, which may be periodically used to control disease outbreaks in turkey production.

MATERIALS AND METHODS

Experimental site

The experiment was carried out at the Turkey Unit, Teaching and Research Farms of the College of Animal Science and Livestock Production, Federal University of Agriculture, Abeokuta, Ogun state, Nigeria in accordance with the guidelines of the Animal Care Committee of the University. The farm is located on latitude 7°13'35.48"N, longitude 3°25'39.01"E with an elevation of 415 ft and 700 ft (Google Earth, 2021, Climatesdata.org).

Experimental birds and management

A total of 150 day-old turkey birds comprised of 100 exotic and 50 indigenous turkeys procured from a reputable farm were used for this 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 formulated grower diets that meet the NRC Turkey grower nutritional requirements (NRC, 1999). Biosecurity measures recommended vaccination programs were strictly adhered to, and water was given *ad libitum*.

Data collection

Blood collection

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

DNA extraction

Genomic DNA was isolated from each blood sample using the Zymo Quick-g DNA™ 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 were determined using a Nanodrop Spectrophotometer.

PCR amplification

After DNA extraction and quantification, three DNA samples from the two genetic groups were taken for TLR3 gene discovery and polymerase chain reaction (PCR). The primers for PCR amplification of the target gene Turkey TLR3 (tuTLR3) were designed using the Primer3 and BLAST options at the NCBI database (www.ncbi.nlm.nih.gov). Polymerase chain reactions (PCR) were performed in a 50 µl reaction volume containing ten µl 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 four 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. PCR conditions consist of 1 cycle of 95°C for 4 minutes of initial denaturation, 35 cycles each of 95°C for 30 seconds of denaturation, 62°C for 30 seconds of annealing, 72°C for 1-minute elongation, followed by 72°C for 10 minutes final elongation.

Gel electrophoresis

The amplified product from each sample was separated electrophoretically on 1% gel containing ethidium bromide in 1XTAE buffer at 120V for 1½ h. DNA bands were observed on a UV-transilluminator and photographed by a Gel Cam Polaroid camera.

Sequence analysis

The source sequence and the retrieved sequences were trimmed and edited using the bio edit package before using other bioinformatics packages such as MEGA 10 for the alignment and phylogenetic analysis, DnaSP for the genetic diversity analysis, and RasMol for protein structure prediction.

Detection of amino acid

The amino acid sequences of the new turkey TLRs were 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 blastp. The nomenclatures of turkey TLRs were based on the best hits of the proteins in the database.

RESULTS

The polymorphism and resultant amino acid changes detected at the TLR 3 gene of the Exotic turkey are presented in Table 1. The study detected four polymorphisms in exon 4 of TLR3 of hybrid converter turkey. The resultant amino acids showed that three mutations are non-synonymous, while only one is synonymous. Amino acid substitution at position 7 resulted in a stop codon. At exon 516, it could be observed that a synonymous substitution occurs at the locus in exotic turkey with an amino acid change of 172N>N. However, no change was observed in 19 exons. At exon 953 of the TLR 3 gene of the exotic turkey breed, a non-synonymous single nucleotide polymorphism (318Q>R) was detected, which resulted in the transition of Glutamine and Arginine. Also, at the exonic position 1532, there was a non-synonymous polymorphism (511A>T), which resulted in the transition of Alanine to Threonine.

The result of the amino change is presented in Table 2. At the exonic position 19 of the TLR 3 gene, a non-synonymous (7Q>*) change was observed, resulting in the transition of Cytosine to Thymine. There were two allelic transitions of Cytosine to Thymine at exonic position 19 and 516. There were only two synonymous polymorphisms at exonic position 516 and 1747. At the exonic position 560 of the TLR 3 gene, a non-synonymous (187S>P) change was observed, which resulted in the

Table 1. Resultant amino acid changes detected at TLR 3 gene of the exotic turkey.

S/N	Alleles	Exonic position	Amino acid	Type
1	C>T	19	7 Q>*	Non-synonymous
2	C>T	516	172N>N	Synonymous
3	A>G	953	318Q>R	Non-synonymous
4	G>A	1532	511A>T	Non-synonymous

Table 2. Resultant of the amino acid change in Nigerian local turkey.

S/N	Alleles	Exonic position	Amino acid	type
1	C>T	19	7 Q>*	Non-synonymous
2	C>T	516	172N>N	Synonymous
3	T>C	560	187S>P	Non-synonymous
4	A>G	953	318Q>R	Non-synonymous
5	A>G	1747	582E>E	Synonymous

Table 3. The neutrality test for Nigerian local turkey and exotic turkey.

Breed	Taj. D	Fu & Li's D*	Fu & Li's F*	Fu's FS	Achaz Y
HCT	0.08612	-0.08318	-0.04196	-3.323	0.34249
NLT	0.08612	-0.08318	-0.04603	-3.323	0.34249

Table 4. Adaptive evolution of TLR3 of Nigerian indigenous chicken.

Breed	Ds	Dn	Dn/Ds	Selection type
HCT	1.47	1.54	1.05 NS	Purifying selection
NLT	0.0	1.438	1.438 NS	Purifying selection

Dn, non-synonymous rate; Ds, synonymous rate.

transition of Thymine back to Thymine. There were two transitions of Adenine to Guanine at the exonic position 953 and 1747 (resulting in 582E> and E318Q>R, respectively). The latter resulted in different polymorphisms: synonymous at exon 953 and non-synonymous at exon 1747. The evidence of high allelic frequency tending towards fixation suggests the presence of inbreeding in exotic turkeys. The occurrence of this polymorphism in a population that has been selected mainly for improved productive and reproductive performance may indicate the involvement of this allele in these performance traits but this will need to be ascertained through association studies of these polymorphisms with the traits.

The neutrality test for the two breeds is presented in Table 3. The Tajima' D value was positive and the same for both the exotic and local turkey breeds (0.08612). A negative Fu and Li's D value (-0.08318) was observed for both the local and exotic turkey. There was a negative Fu and Li value observed in the local (-0.04196) and exotic turkey (-0.04603). The Fu's FS value was also negative

(-3.323) for exotic and local turkeys. A positive Achaz Y value (0.34249) was also positive for exotic and local turkey breeds.

The result of the type of selection acting on the gene is shown in Table 4. The result in local and exotic turkeys is insignificant, indicating that purifying selection affects the gene.

DISCUSSION

Most studies conducted in species other than chicken have focused on characterizing particular expressed TLRs (Vinkler *et al.*, 2009). In this study, the SNP (7 Q>*) detected in exon 4 is non-synonymous, and this resulted in amino acid changes from Cytosine to Threonine in the exotic turkey that may influence the immune system. According to Chu and Wei (2019), non-synonymous mutations lead to amino acid changes and changes in protein structure and functions, while synonymous mutations cannot 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 influence the encoded protein folding and function. Overall, it was found that a conservative mode of evolution in avian TLRs, with a predominance of synonymous substitutions but also significant instances of positive selection acting upon a few amino acid sites falling in pathogen-recognition domains. In this regard, the findings bear similarities to a recent and similarly comprehensive survey of TLRs in primates (Wlasiuk and Nachman, 2010). It has been shown that TLRs are associated with innate immune responses in various livestock species (Prakash *et al.*, 2014). Therefore, interest in breeding animals resistant to major infectious diseases using TLR genes has increased tremendously recently (Novak, 2014).

The SNP (172N>N) detected at position 5'6 was synonymous in both exotic and local turkey breeds, and this resulted in amino acid changes from Alanine to Threonine in exotic turkeys and Cysteine to Tyrosine in local turkeys, which may influence growth and skeletal muscle development in the population (Dushyanth *et al.*, 2016). The occurrence of non-synonymous SNPs in the two breeds resulting in amino acid changes occasioned by base changes or base substitutions within the coding region of exon 4 at TLR 3 gene is suggestive of the biological significance of this mutation in the gene probably for immune-related functions or other critical biological processes in the populations. Moreover, the occurrence of synonymous SNPs in exon 4 supports the study on the 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 favouring natural selection and overall relative fitness more than transversion (Jiang and Zhao, 2006; Pauly *et al.*, 2017; Lyons and Luring, 2017). Several authors have reported different gene polymorphisms, with some associated with growth performance in broiler chickens (Bhattacharya and Chatterjee, 2013; Dushyanth *et al.*, 2016; Zhang *et al.*, 2019). Hu *et al.* (2013) have also reported the occurrence of single nucleotide polymorphisms in the upstream regulatory region that alter the expression of the TLR gene in chickens. TLR3 polymorphisms may profoundly influence host responses to a wide range of viruses and may be associated with viral disease resistance or susceptibility (Misch and Hawn, 2008).

The genetic analyses have revealed an excess of synonymous over non-synonymous substitutions during the evolution of the TLR gene in exotic and local turkeys that is compatible with purifying selection. Avian TLR genes are, therefore, mainly evolving under functional constraints, presumably because of the need to preserve a well-established biological function.

Negative Tajima's D values may suggest that positive directional selection may purge deleterious or disadvantageous mutations from turkey populations. These findings

agree with studies conducted in commercial breeds of domestic chickens and wild populations of jungle fowl (Downing *et al.*, 2010). However, this result must be interpreted cautiously because population structure and history can affect Tajima's D values. Therefore, a more detailed consideration of other evolutionary factors in the two species is needed. Recent findings on genetic diversity also show that many of the most frequent non-synonymous mutations are translated into conservative amino acid substitutions. Thus, negative selection (in the form of an excess of silent mutations) and positive directional selection (in the form of low frequencies of deleterious or selectively disadvantageous mutations) reinforces the idea that TLRs may be subjected to functional solid constraints and species-specific patterns of pathogen recognition (Werling *et al.*, 2009). That said, spatially varying selection patterns acting on TLRs have been described in human populations (Barreiro *et al.*, 2009), and variable coevolutionary dynamics between different species have been described in primates (Wlasiuk and Nachman, 2010).

However, despite a global trend to maintain essentially unchanging TLR repertoires in birds, the survey suggests significant instances of purifying selection acting on a few amino acid sites falling in pathogen-recognition domains. These findings would agree with species-specific differences during the recognition of related types of ligands (Werling *et al.*, 2009). Recent studies have emphasized the influence of the number of taxa investigated in the power to detect positive selection with codon-based approaches.

Conclusion

Based on the findings of this study, the following conclusions were deduced:

1. The population of local and exotic turkeys showed a low level of heterozygosity at the TLR3 locus and, as such, may be homogenous.
2. There was low genetic diversity in the TLR3 gene of both the local and exotic turkey breeds. This suggests potential vulnerabilities and challenges for their long-term health and adaptability.

Recommendation

In line with the findings of this study, the authors recommend that crossbreeding should be practised among the local and exotic breeds of turkey. This is commonly practised by breeders, especially when the desired traits would be beneficial to the offspring, such as when its biological fitness is enhanced.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

ACKNOWLEDGEMENT

The authors are grateful to the poultry breeding unit of the Federal University of Agriculture Abeokuta for allowing us to use the site for fieldwork.

REFERENCES

- Ajayi, F. O. (2010). Nigerian indigenous chicken: A valuable genetic resource for meat and egg production. *Asian Journal of Poultry Science*, 4(4), 164-172.
- Barreiro, L. B., Ben-Ali, M., Quach, H., Laval, G., Patin, E., Pickrell, J. K., Bouchier, C., Tichit, M., Neyrolles, O., Gicquel, B., & Quintana-Murci, L. (2009). Evolutionary dynamics of human Toll-like receptors and their different contributions to host defense. *PLoS Genetics*, 5(7), e1000562.
- Bhattacharya, T. K., & Chatterjee, R. N. (2013). Polymorphism of the myostatin gene and its association with growth traits in chicken. *Poultry Science*, 92(4), 910-915.
- Boyd, A., Philbin, V. J., Smith, A. L. (2007). Conserved and distinct aspects of the turkey (*Meleagris gallopavo*) Toll-like receptor (TLR) system: implications for transmission and control of bird-borne zoonoses. *Bio-chemical Society Transactions*, 35(Pt6), 1504-1507.
- Chu, D., & Wei, L. (2019). Nonsynonymous, synonymous and nonsense mutations in human cancer-related genes undergo stronger purifying selections than expectation. *BMC Cancer*, 19, Article number 359.
- Dalloul, R. A., Long, J. A., Zimin, A. V., Aslam, L., Beal, K., Ann Blomberg, L., Bouffard, P., Burt, D. W., Crasta, O., Crooijmans, R. P., & Reed, K. M. (2010). Multi-platform next-generation sequencing of the domestic turkey (*Meleagris gallopavo*): genome assembly and analysis. *PLoS Biology*, 8(9), e1000475.
- Downing, T., Lloyd, A. T., O'Farrelly, C., & Bradley, D. G. (2010). The differential evolutionary dynamics of avian cytokine and TLR gene classes. *The Journal of Immunology*, 184(12), 6993-7000.
- Dushyanth, K., Bhattacharya, T. K., Shukla, R., Chatterjee, R. N., Sitaramamma, T., Paswan, C., & Guru Vishnu, P. (2016). Gene expression and polymorphism of myostatin gene and its association with growth traits in chicken. *Animal Biotechnology*, 27(4), 269-277.
- FAOSTAT (2010). Food and Agriculture Organization of the United Nations. Retrieved from <http://faostat.fao.org/default.aspx>
- Hu, W., Chen, S., Zhang, R., & Lin, Y. (2013). Single nucleotide polymorphisms in the upstream regulatory region alter the expression of myostatin. *In Vitro Cellular and Developmental Biology-Animal*, 49, 417-423.
- Jiang, C., & Zhao, Z. (2006). Mutational spectrum in the recent human genome inferred by single nucleotide polymorphisms. *Genomics*, 88(5), 527-534.
- Jin, M. S., & Lee, J. O. (2008). Structures of the toll-like receptor family and its ligand complexes. *Immunity*, 29(2), 182-191.
- Johnson, C. M., Lyle, E. A., Omueti, K. O., Stepensky, V. A., Yegin, O., Alpsoy, E., ... & Tapping, R. I. (2007). Cutting edge: A common polymorphism impairs cell surface trafficking and functional responses of TLR1 but protects against leprosy. *The Journal of Immunology*, 178(12), 7520-7524.
- Lyons, D. M., & Luring, A. S. (2017). Evidence for the selective basis of transition-to-transversion substitution bias in two RNA viruses. *Molecular Biology and Evolution*, 34(12), 3205-3215.
- Medzhitov, R., Preston-Hurlburt, P., & Janeway Jr, C. A. (1997). A human homologue of the Drosophila Toll protein signals activation of adaptive immunity. *Nature*, 388(6640), 394-397.
- Misch, E. A., & Hawn, T. R. (2008). Toll-like receptor polymorphisms and susceptibility to human disease. *Clinical Science*, 114(5), 347-360.
- Ngu, G., Butswat, I., Mah, G., & Ngantu, H. (2014). Characterization of small-scale backyard turkey (*Meleagris gallopavo*) production system in Bauchi State-Nigeria and its role in poverty alleviation. *Livestock Research for Rural Development* 26(1). <http://www.lrrd.org/lrrd26/1/ngu26019.html>.
- Novak, I. (2014). Evidence-based diagnosis, health care, and rehabilitation for children with cerebral palsy. *Journal of Child Neurology*, 29(8), 1141-1156.
- Ogorevc, J., Kunej, T., Razpet, A., & Dovc, P. (2009). Database of cattle candidate genes and genetic markers for milk production and mastitis. *Animal Genetics*, 40(6), 832-851.
- Pauly, M. D., Procario, M. C., & Luring, A. S. (2017). A novel twelve class fluctuation test reveals higher than expected mutation rates for influenza A viruses. *Elife*, 6, e26437.
- Prakash, K., Radhamani, J., Pandey, A., & Yadav, S. (2014). A preliminary investigation of cultivated and wild species of *Luffa* for oil and protein contents. *Plant Genetic Resources*, 12(1), 103-111.
- Sales, M. L., Schreiber, R., Ferreira-Sae, M. C., Fernandes, M. N., Piveta, C. S., Cipolli, J. A., Cardoso, C. C., Matos-Souza, J. R., Geloneze, B., Franchini, K. G., & Nadruz, W. (2010). Toll-like receptor 6 Ser249Pro polymorphism is associated with lower left ventricular wall thickness and inflammatory response in hypertensive women. *American Journal of Hypertension*, 23(6), 649-654.
- Temperley, N. D., Berlin, S., Paton, I. R., Griffin, D. K., & Burt, D. W. (2008). Evolution of the chicken Toll-like receptor gene family: a story of gene gain and gene loss. *BMC Genomics*, 9, Article number 62.
- Tizard, I. R. (2004). *Veterinary immunology* (3rd edition). Saunders Elsevier: Saint Louis, MO.
- Uberu, N. P., Oleforuh-Okoleh, V. U., Ndofor-Foleng, H. M., Agaviezor, B. O., Ohagenyi, J. I., Udeh, F. U., Ani, A. O., Nwosu, C. C., & Akuru, E. A. (2021). Molecular evolution of prolactin gene single nucleotide polymorphisms in Nigerian chicken ecotypes and their association with light ecotype chickens' egg traits. *International Journal of Veterinary Science* 11(1), 91-97.
- Vinkler, M., Bryjová, A., Albrecht, T., & Bryja, J. (2009). Identification of the first Toll-like receptor gene in passerine birds: TLR4 orthologue in zebra finch (*Taeniopygia guttata*). *Tissue Antigens*, 74(1), 32-41.
- Werling, D., Jann, O. C., Offord, V., Glass, E. J., & Coffey, T. J. (2009). Variation matters: TLR structure and species-specific pathogen recognition. *Trends in Immunology*, 30(3), 124-130.
- West, A.P., Koblansky, A. A., & Ghosh, S. (2006). Recognition and signaling by toll-like receptors. *Annual Review of Cell and Developmental Biology*, 22:409-437.
- Wlasiuk, G., & Nachman, M. W. (2010). Adaptation and constraint at Toll-like receptors in primates. *Molecular Biology and Evolution*, 27(9), 2172-2186.
- Wurfel, M. M., Gordon, A. C., Holden, T. D., Radella, F., Strout, J., Kajikawa, O., Ruzinski, J. T., Rona, G., Black, R. A., Stratton, S., Jarvik, G. P., Hajjar, A. M., Nickerson, D. A., Rieder, M., Sevransky, J., Maloney, J. P., Moss, M., Martin, G., Shanholtz, C., Garcia, J. G., Gao, L., Brower, R., Barnes, K.

- C., Walley, K. R., Russell, J. A., & Martin, T. R. (2008). Toll-like receptor 1 polymorphisms affect innate immune responses and outcomes in sepsis. *American Journal of Respiratory and Critical Care Medicine*, 178(7),710-720.
- Yakubu, A., Abimiku, K., Musa-Azara, I. S., Idahor, K. O., & Akinsola, O. M. (2013). Assessment of flock structure, preference in selection and traits of economic importance of domestic turkey (*Meleagris gallopavo*) genetic resources in Nasarawa state, Nigeria. *Livestock Research for Rural Development*, 25, Article number 18.
- Zhang, X. X., Ran, J. S., Lian, T., Li, Z. Q., Yang, C. W., Jiang, X. S., Du, H. R., Cui, Z. F., & Liu, Y. P. (2019). The single nucleotide polymorphisms of myostatin gene and their associations with growth and carcass traits in Daheng broiler. *Brazilian Journal of Poultry Science*, 21(03), 001-008.