Biotechnology in Animal Husbandry 42(1), 47-59, 2026
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 575.113'636.588
https://doi.org/10.2298/BAH2601047O

GENETIC DIVERSITY OF PROLACTIN GENE IN NIGERIAN FULANI AND YORUBA CHICKEN ECOTYPES

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Original scientific paper

Abstract: Understanding genetic diversity of functional genes is a prerequisite to improving selection and preserve the indigenous animal breeds, known for adaptive traits and ability to utilize low quality feed. This study evaluated variation in the prolactin (PRL) gene, an essential regulator of reproductive characteristics, in two prominent Nigerian indigenous chicken ecotypes: Fulani and Yoruba. Blood samples were collected from 100 local chicken across six geographical locations in Oyo State, Nigeria. Genomic DNA was isolated for PCR amplification of a 24-bp insertion/deletion in the PRL gene. Allele and genotype frequencies, heterozygosity, fixation indices, and Hardy-Weinberg equilibrium (HWE) were assessed using conventional population genetics methodologies. Two alleles, insertion and deletion (A and B, respectively) and three genotypes (AA, AB, BB) were characterized using bands size on the gel electrophoresis. Although allele frequencies were comparable in both ecotypes, Fulani hens demonstrated more heterozygosity and agreed with Hardy-Weinberg equilibrium, indicating genetic stability. Yoruba hens exhibited higher inbreeding and divergence from HWE ($p<0.05$). Genetic distance and differentiation values demonstrated significant similarity between the ecotypes, which is likely resulting from unrestrained mating. These findings show the need for organized breeding programs to preserve genetic integrity and utilize indigenous genetic resources.

Key words: mating, gene, electrophoresis, genetic diversity

Introduction

The sustainability and effective management of indigenous animal as a genetic resource depend on a clear understanding of genetic variability among the locally adapted livestock breeds (Vanvanhossou et al., 2021). In many developing

countries, Nigeria included, indiscriminate mating and inadequate genetic management have led to an increase inbreeding rate and the erosion of essential genetic resources (Martyniuk, 2021). Endris et al. (2023) showed that the loss of local breeds may occur through breed replacement or dilution through crossbreeding. These concerns are very important in poultry farming, where local chicken plays a significant role in rural food security, economic growth, and cultural legacy. Indigenous chickens are valued for their adaptability, disease resistance, low management needs, and socio-economic significance (Singh et al., 2023).

Crossbreeding indigenous hens with an exotic rooster weakens the thermotolerance and disease resistance in the resulting offspring (Kpomasse et al., 2023). The Fulani and Yoruba ecotypes in Nigeria are among the most prevalent and economically important local chicken populations (Sanusi and Oseni, 2020). Typically reared in vast scavenging systems by smallholder farmers, particularly women, these ecotypes are essential for supporting rural livelihoods. Notwithstanding their potential, these indigenous avian species are inadequately used in formal breeding initiatives, and their genetic diversity remains incompletely delineated. Molecular markers serve as efficient instruments for assessing genetic diversity and identifying potential genes associated with production and reproductive characteristics (Kumar et al., 2024). One of such genes is prolactin (PRL), which encodes a hormone integral to essential reproductive functions, including broodiness, oviposition, follicular maturation, and parental care (Hu et al., 2017; Mo et al., 2022).

Polymorphisms in the PRL gene, especially within regulatory and intronic regions, have been linked to age at first lay, clutch size, and incubation behaviour in chicken breeds (Chakraborty and Saha, 2021). Whereas the influence of PRL polymorphisms on trait expression is extensively documented in selectively bred populations (El-Magd et al., 2021), there is a significant paucity of data for PRL gene diversity in African indigenous chickens. Nigerian Fulani and Yoruba chickens, due to their unique evolutionary history influenced by natural selection and conventional breeding methods (Rachman et al., 2024), may possess discrete genetic variants of the PRL gene pertinent to productivity and adaptability. However, given the absence of fundamental genetic data, attempts to formulate focused breeding techniques remain speculative. This study examines the genetic diversity of the PRL gene in Nigerian Fulani and Yoruba chicken ecotypes at a 24-base pair insertion/deletion (Indel) marker to resolve this gap. The study seeks to provide useful data for conservation planning and the formulation of marker-assisted selection strategies specific to indigenous chicken development.

Materials and Methods

Ethical approval was obtained from the animal research ethics committee of the University of Ibadan, Nigeria and the experiment was conducted according to the approved protocol and standard set by the committee.

Sample collection

Blood samples were randomly collected via jugular venepuncture from 100 birds, equally sourced from each ecotype across six distinct locations in Oyo State, Nigeria (Figure 1). The blood samples were stained on FTA cards and maintained at room temperature until further analysis (Murital et al., 2015).

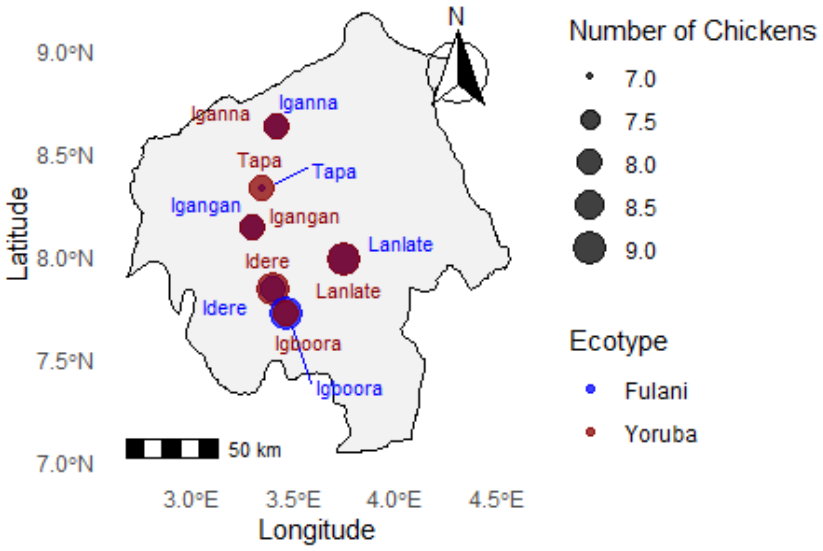

Figure 1. Spatial distribution of the sampled animals in Oyo State, Nigeria

DNA Isolation and quantification

Five pouches from the blood-stained FTA card were taken and transferred into a 200 µl PCR tube. One hundred and fifty microliters of Tris 0.1% SDS buffer was introduced to the samples in the PCR tube and agitated for thirty minutes to disrupt the cell membrane. The supernatant was thereafter discarded with minimum

disturbance to the residue at the base of the PCR tube. Distilled water (150µl) was used to rinse the residue through agitation. The supernatant was subsequently extracted with a micropipette. Subsequently, an additional 150µl of distilled water was introduced to the residue and incubated for 10 minutes, after which the supernatant was discarded. A volume of 50µl of distilled water was added to the residual and incubated at 90ºC in a thermal-cycler (Bio-system veritiTM Gene Amp (R)) for an additional 15 minutes. The extracted DNA was evaluated for quality and quantity using 1% agarose gel electrophoresis and a spectrophotometer. The DNA was preserved at -20ºC for subsequent examination. This was later amplified and genotyped using insertion and deletion alleles— allele A is with the insertion and allele B with the deletion.

Polymerase chain reaction procedure

The amplification of PRL-24-bp Indel (np 358) region using primers (Forward PRL primer -5′-TTT AAT ATT GGT GGG TGA AGA GAC A-3′ and Reverse PRL primer-5′ -ATG CCA CTG ATC CTC GAA AAC TC-3′) described by Cui et al. (2006), in a polymerase chain reaction (PCR) using a thermal-cycler (ABI9700). The PCR master mix (Thermo-scientific) contained 6.25 U of FastStart™ Taq DNA Polymerase (5 U/µl; Roche Diagnostics), about 10-20 ng of DNA template (0.5µl), 5 pmol of each of the forward and reverse primers (0.25µl) and 4µl of Nuclease free water to form 6.25µl solution in each PCR tubes and positioned in the interchangeable blocks of 16 wells of 0.2 ml. with an initial incubation and enzyme activation condition at 94°C for 5 min; followed by 35 cycles of 30 sec at 94°C, 30 sec at 54°C, and 30 sec at 72°C and a final extension at 72°C for 5 min.

Allele determination

For the characterization of the region of interest, the PCR product was mixed with 2 µl of SYBR Green dye and then transferred directly onto a 3% agarose gel for electrophoresis, alongside a standard DNA ladder (100 bp). A UV transilluminator was used to visualize the bands associated with PCR products. The dimensions of the PCR products were assessed based on band migration relative to the molecular weight of the ladder, as described by Kumar et al. (2021), and were used for the characterization of the alleles at the PRL-24-bp Indel (np 358) region.

Statistical analysis

Frequencies of alleles and genotypes, heterozygosity, genetic distance, inbreeding coefficient within subpopulation (F_{IS}), inbreeding coefficient within the entire population (F_{IT}), fixation index (F_{ST}), gene flow and their fit to Hardy-Weinberg equilibrium were estimated using POPGENE (Version. 1.31) software (Yeh et al., 1997). Genetic distance and identity were enumerated according to Nei (1978). Level of significance ($p<0.05$) was evaluated using the 5,000 permutations

of the studied population (Che et al., 2014). Distance-based clustering (average-linkage) was conducted on a normalized genotype-distance matrix to show sample linkages (Borgsmüller et al., 2024). Analysis of molecular variance (AMOVA) was computed to measure the variance components between subpopulations and within subpopulations (Excoffier et al., 1992).

Results and Discussion

PRL gene polymorphism and genotype frequencies

This study provides one of the first comparisons of PRL gene polymorphism in Nigerian indigenous chicken ecotypes. Genotyping of the 24-bp insertion/deletion (Indel) in a locus on the PRL gene showed the presence of two alleles: insertion (A) and deletion (B), resulting in three genotypes: AA (insertion/insertion), AB (insertion/deletion), and BB (deletion/deletion) (Figure 2). A similar report was made by Eichie et al. (2016) where 2 alleles and 3 genotypes were found in Nigerian quail strains. Previously, 2 alleles and 3 genotypes were reported in Nigerian quail strains (Eichie et al., 2016). Also, insertion/deletion polymorphisms were recorded in indigenous and commercial chickens by Cui et al. (2006). Meanwhile Allele frequencies in entire population were similar for the two recorded alleles (A = 0.50 and B = 0.50; Table 1). However, stratification of the allele frequency by ecotype showed that the Fulani chickens had 0.51 and 0.49 for alleles A and B, respectively while Yoruba chickens had frequencies of 0.49 of 0.51 of A and B, respectively. While the overall genotype frequencies were 0.32; 0.36 and 0.32 for genotypes AA, AB and BB, respectively. The ecotype-specific analysis showed that Fulani chickens had AA = 0.31, AB = 0.40, BB = 0.29 and Yoruba chickens had AA = 0.33, AB = 0.33, BB = 0.35. The allelic frequencies observed in the Fulani and Yoruba chickens show a balanced genetic makeup, which reflects a conserved polymorphic structure of the PRL gene in Nigerian ecotypes. The moderate genotype diversity, especially the relatively high observed heterozygote in Fulani chickens, supports the existence of functional genetic variation, which is important for adaptive traits such as reproduction and survival.

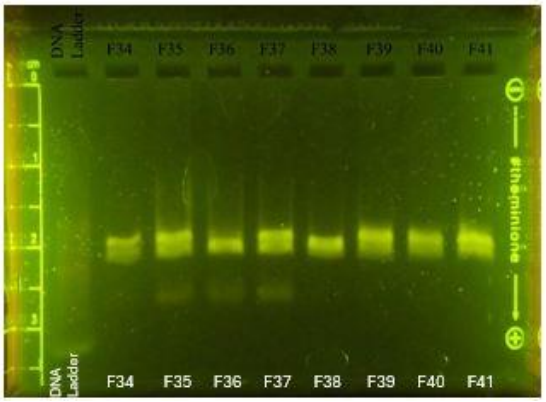

Figure 2. Gel electrophoresis showing the PRL fragment bands (alleles) from Fulani and Yoruba chicken ecotypes

Table 1. Allele and genotypic frequencies of PRL gene in Fulani and Yoruba chicken ecotypes

Breed	Allele Frequency		Genotype Frequency		
	A	B	AA	AB	BB
Fulani	0.51	0.49	0.31	0.40	0.29
Yoruba	0.49	0.51	0.33	0.33	0.35
Overall	0.50	0.50	0.32	0.37	0.32

A-insertion allele; B-deletion allele; AA-insertion-insertion; AB-insertion-deletion; BB-deletion-deletion.

Hardy-Weinberg equilibrium and heterozygosity

The Fulani ecotype exhibited higher observed heterozygosity (H_o) and conformed to Hardy-Weinberg equilibrium (HWE), indicating a stable genetic structure (Table 2). In contrast, the Yoruba ecotype showed a lower H_o and a deviation from HWE, indicating a heterozygote deficit. This was corroborated by higher F_{IS} estimated for the Yoruba chickens (Table 3), suggesting potential inbreeding or non-random mating patterns in that ecotype. The higher heterozygosity and compliance with Hardy-Weinberg equilibrium in Fulani chickens may be attributed to random mating patterns and reduced artificial selection pressure, thereby maintaining a genetically stable population (Luo et al., 2025; Calò et al., 2021). On the other hand, the deficit of heterozygotes and deviation from HWE in the Yoruba chickens suggests the influence of inbreeding or localized non-random mating. This aligns with reports by Davila et al. (2009), who observed reduced heterozygosity in Spanish hens due to selective breeding

practices focused on morphological traits. Higher heterozygosity observed in the Fulani ecotype, together with its conformity to HWE, may also be attributed to the traditional nomadic production system under which these chickens are managed. Under such free-range, village-based systems, uncontrolled mating and frequent inter-flock interactions enhance gene flow and promote random mating, thereby maintaining genetic diversity. This is particularly relevant for the prolactin gene, a key regulator of broodiness and reproductive behaviour in chickens (Jiang et al., 2025). However, selective breeding of highly valued chickens by Yoruba ecotype farmers imposes selection pressure that may explain the observed deviation from Hardy–Weinberg equilibrium.

Table 2. Heterozygosity and Chi-square (X^2) for Hardy-Weinberg Equilibrium (HWE) tests at PRL gene in Fulani and Yoruba chicken ecotypes

Breed	Ne	*Ho*	*He*	F_{IS}	X^2	P value	HWE
Fulani	2.00	0.40	0.51	0.21	2.29	0.13	
Yoruba	2.00	0.33	0.51	0.35	5.58	0.02	*
Overall	2.00	0.37	0.51	0.28	7.15	0.01	*

Table 3. Summary of genetic distance, genetic identity, fixation indices (F_{IT}, F_{IS} and F_{ST}) and gene (Nm) flow for overall populations

Locus	Genetic distance	Genetic diversity	F_{IS}	F_{IT}	F_{ST}	NM*
PRL	0.0010	0.9990	0.28	0.28	0.0005	499.75

*****NM = gene flow estimates from $F_{ST} = 0.25 (1- F_{ST}) / F_{ST}$

Phylogenetic analysis using the neighbour-joining method (Figure 3) confirmed the considerable genetic similarity between the two ecotypes. The clustering pattern demonstrated the lack of divergence at the PRL gene region considered in this study between the Fulani and Yoruba chicken ecotypes. The branch lengths correspond to genetic distance, and clustering did not reveal clear ecotype separation (Figure 3). This corresponds with low estimated Gst (Figure 4), and a permutation test (5,000 replicates) confirmed that the observed value was not statistically significant ((p>0.05); Figure 4). The result from analysis of variance (AMOVA), shows that most variation (~98-99%) was within populations, while only a small proportion (~1-2%) was attributable to differences between Fulani and Yoruba chickens (Table 4). The phylogenetic clustering further illustrates the genetic similarity between individuals from the two ecotypes, reinforcing the notion of limited divergence at the PRL locus. This may reflect a recent common ancestry or ongoing genetic exchange, which maintains allelic continuity across ecotypes. Such a pattern suggests that PRL gene variation is preserved across Nigeria's indigenous chickens, a valuable insight for marker-assisted selection and

breeding strategies aimed at improving productivity and stress adaptability. The findings indicate moderate PRL gene diversity within each ecotype; however minimal genetic divergence exists between them. This shows the need for the establishment of localized and ecotype-specific breeding programs that can promote preserving essential adaptive features. The PRL gene offers information on genetic diversity associated with reproduction, however it constitutes merely a little portion of the genome. For better understanding of the genetic composition and adaptive feature of indigenous Nigerian chickens, further research should employ high-throughput genotyping methods (e.g., SNP arrays, Genome-Wide-Association Study). These should be integrated with phenotypic performance data to uncover functional markers linked to productivity, disease resistance/resilience, and environmental adaptability.

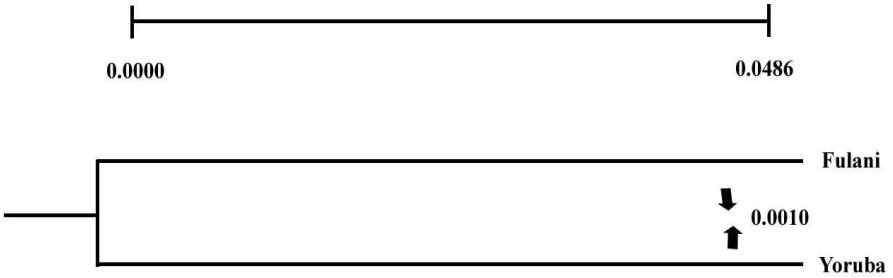

Figure 3. Phylogenetic relationship between Fulani and Yoruba chicken ecotypes based on genotype distances.

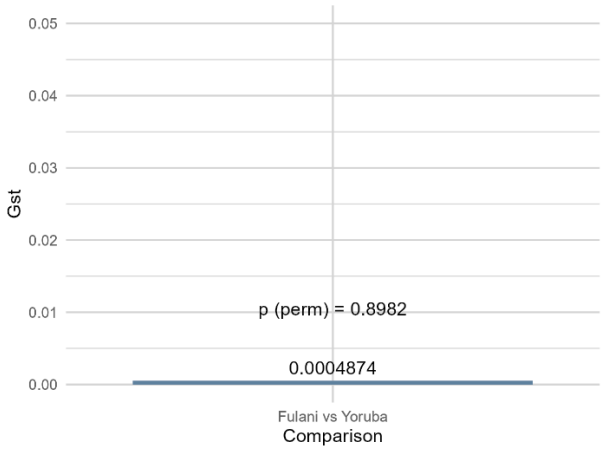

Figure 4. Genetic differentiation index (Gst) between populations

Table 4. Variance partitioning from analysis of molecular variance (AMOVA)

Source of Variation	Df	Sum of Squares (SS)	Variance Components (σ^2)	Percentage Variation (%)
Among populations	1	~0.02	~0.0005	~1–2%
Within populations	89	~44.00	~0.49	~98–99%
Total	90	~44.02	~0.4905	100%

Conclusion

Genetic diversity at PRL-24-bp Indel (np 358) region of the prolactin (PRL) gene in the Fulani and Yoruba indigenous chicken ecotypes of Nigeria were analysed. The characterization of two alleles (A and B) and three genotypes (AA, AB, and BB), along with approximately equal allele and genotype frequencies, signifies modest genetic variation in both ecotypes. The Fulani ecotype showed higher heterozygosity and in agreement with Hardy-Weinberg equilibrium (HWE), indicating a more genetically stable population, whereas the Yoruba ecotype showed higher inbreeding level and deviations from HWE. However, the genetic differentiation between the two ecotypes was negligible. As shown by low F_{ST} and genetic distance metrics. The findings highlight the importance of developing ecotype-specific breeding strategies to promote genetic diversity, thereby providing a valuable genetic resource for resilience against disease outbreaks, climate change, and feed shortages in the future.

Genetska raznolikost gena za prolaktin kod nigerijskih ekotipova pilića Fulani i Joruba

Osamede Henri Osaijuvu, Olubunmi Elizabeth Malomo, Ismaila Muritala

Rezime

Razumevanje genetske raznolikosti funkcionalnih gena je preduslov za poboljšanje selekcije i očuvanje autohtonih rasa životinja, poznatih po adaptivnim osobinama i sposobnosti korišćenja hrane niskog kvaliteta. Ova studija je procenila varijacije u genu za prolaktin (PRL), esencijalnom regulatoru reproduktivnih karakteristika, kod dva istaknuta nigerijska autohtona ekotipa pilića: Fulani i Joruba. Uzorci krvi su prikupljeni od 100 lokalnih pilića na šest geografskih lokacija u državi Ojo, Nigerija. Genomska DNK je izolovana za PCR amplifikaciju insercije/delecije od 24 bp u PRL genu. Frekvencije alela i genotipa, heterozigotnost, indeksi fiksacije i

Hardi-Vajnbergova ravnoteža (HWE) procenjeni su korišćenjem konvencionalnih metodologija populacione genetike. Dva alela, insercija i delecija (A i B, respektivno) i tri genotipa (AA, AB, BB) okarakterisani su korišćenjem veličine fragmenata na gel elektroforezi. Iako su frekvencije alela bile uporedive u oba ekotipa, kokoške rase Fulani pokazale su veću heterozigotnost i bile su u Hardi-Vajnbergovoj ravnoteži, što ukazuje na genetsku stabilnost. Kokoške rase Joruba pokazale su veći stepen inbridinga i divergencije od HWE ($p<0,05$). Genetska udaljenost i vrednosti diferencijacije pokazale su značajnu sličnost između ekotipova, što je verovatno rezultat neograničenog parenja. Ovi nalazi ukazuju na potrebu za organizovanim programima uzgoja kako bi se očuvao genetski integritet i iskoristili autohtoni genetski resursi.

Ključne reči: parenje, gen, elektroforeza, genetska raznolikost

Author Contributions: O.H.O. conceived and designed the analysis; E.M.O. collected the data; I.M. contributed data or analysis tools; E.M.O. performed the analysis; E.M.O. and I.M. wrote the paper.

Acknowledgments and funding
The authors acknowledge Department of Animal Science, University of Ibadan, Oyo State, Nigeria for providing the farm and laboratory facilities for this research. This research was funded by parents of the postgraduate student.

Conflicts of interest
The authors declare no conflict of interest.

Statement of the AI tool use
During the preparation of this paper, the authors used ChatGPT to edit the English grammar and spelling. After using this tool, the authors reviewed and edited the content as necessary and take full responsibility for the content of the published article.

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Received 10 March 2026; accepted for publication 26 May 2026