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Prolactin gene promoter polymorphism in Nigerian indigenous hens and tropically adapted layer breeds

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Abstract

Poultry eggs remain a vital source of affordable animal protein in Nigeria; however, rising costs associated with maintaining commercial layer breeds have limited access to this protein source for many households. Indigenous hens, though more cost-effective to rear, lay fewer eggs due to broodiness: a natural behavior regulated by prolactin. Polymorphisms in the promoter region of the prolactin gene (PRL) have been associated with non-broodiness and higher egg production, making them potential targets for genetic selection. This preliminary study was designed to investigate the PRL promoter polymorphisms in Nigerian indigenous normal feather hens, improved Nigeria indigenous chicken– FUNAAB Alpha hen, and two exotic layer breeds–ISA Brown, and ISA Black Layers, aiming to identify genetic markers that could support breeding strategies for improved egg productivity. Blood samples were collected from chickens at the Federal University of Agriculture, Abeokuta (FUNAAB) and a commercial farm in Ijebu-Ode. Genomic DNA was extracted using a Zymo DNA kit, and two PRL promoter regions were amplified using PCR. At the -358 bp region, PCR results revealed both the normal sequence (D allele) and a 24 bp insertion (I allele) across all breeds. The II genotype was absent; only heterozygous (ID) and homozygous normal (DD) genotypes were observed. The distribution of alleles was not significantly associated with chicken breed ($\chi^2 = 2.34$; $p = 0.126$), suggesting that non-broodiness in the adapted layers may be influenced by other genetic or environmental factors. This study highlights the presence of PRL polymorphism in Nigerian chicken breeds but shows no association with non-broodiness. Further genetic studies are recommended to explore additional markers for enhancing productivity in indigenous hens.

Keywords: Nigerian chicken breeds, Egg production, Broodiness, Prolactin, Polymorphism.
Running Title: Prolactin polymorpism in Nigerian chicken breeds.

Polymorphisme Du Promoteur Du Gène De La Prolactine Chez Les Poules Indigènes Nigérianes Et Les Races De Pondeuses Adaptées Aux Régions Tropicales

Résumé

Les œufs de volaille restent une source vitale de protéines animales abordables au Nigeria ; cependant, la hausse des coûts associée à l'entretien des races de poules pondeuses commerciales a limité l'accès à cette source de protéines pour de nombreux ménages. Les poules indigènes, bien que plus économiques à élever, pondent moins d'œufs en raison de la couvaison : un comportement naturel régulé par la prolactine. Des polymorphismes dans la région promotrice du gène de la prolactine (PRL) ont été associés à l'absence de couvaison et à une production d'œufs plus élevée, ce qui en fait des cibles potentielles pour la sélection génétique. Cette étude préliminaire a été conçue pour étudier les polymorphismes du promoteur de la PRL chez les poules indigènes nigérianes à plumage normal, la poule améliorée nigériane indigène – FUNAAB Alpha, et deux races de pondeuses exotiques – ISA Brown et ISA Black Layers, visant à identifier des marqueurs génétiques qui pourraient soutenir les stratégies d'élevage pour une productivité ovipare *améliorée. Des échantillons de sang ont été prélevés sur des poulets à la Federal University of Agriculture, Abeokuta (FUNAAB) et dans une ferme commerciale à Ijebu-Ode. L'ADN génomique a été extrait à l'aide d'un kit Zymo DNA, et deux régions promotrices de la PRL ont été amplifiées par PCR. Au niveau de la région -358 pb, les résultats de la PCR ont révélé à la fois la séquence normale (allèle D) et une insertion de 24 pb (allèle I) dans toutes les races. Le génotype II était absent ; seuls les génotypes hétérozygotes (ID) et homozygotes normaux (DD) ont été observés. La distribution des allèles n'était pas significativement associée à la race de poulet (X^2 = 2,34 ; p = 0,126), suggérant que l'absence de couvaison chez les pondeuses adaptées pourrait être influencée par d'autres facteurs génétiques ou environnementaux. Cette étude met en évidence la présence du polymorphisme de la PRL chez les races de poulets nigérians mais ne montre aucune association avec l'absence de couvaison. D'autres études génétiques sont recommandées pour explorer des marqueurs supplémentaires afin d'améliorer la productivité des poules indigènes.*

Mots-clés : Races de poulets nigérians, Production d'œufs, Couvaison, Prolactine, Polymorphisme.

Introduction

The Nigerian poultry industry faces a range of challenges, particularly regarding the rising cost of maintaining commercial layer chicken breeds. While these breeds—such as the ISA Brown and Black Layer—are renowned for their high egg-laying capacity, they also require specialized feed, housing, and healthcare, resulting in high production costs (Alemneh and Getabalew, 2019; Justina and Ugwu, 2023). These increased expenses often translate into higher egg prices, making them less affordable for the average consumer.

In contrast, indigenous or local chicken breeds in Nigeria are widely recognized for their dual-purpose nature, adaptability to local environments, disease resistance, and low maintenance requirements. These attributes make them economically viable for smallholder and rural farmers (Adebambo *et al.*, 2022). Typically raised in extensive systems, local chickens serve as a critical source of income and animal protein—through both meat and eggs—for the rural poor 55(Mujyambere *et al*, 2022). However, their relatively low egg production remains a major constraint to their commercialization, despite their proven resilience to harsh tropical environments. This resilience reflects a complex interplay between genotype and environment. Additionally, their ability to thrive on forage-based diets and minimal input makes them less dependent on commercial feeds, further lowering production costs (Bello *et al*., 2021).

One of the primary limitations of indigenous breeds is broodiness: a natural maternal behavior where hens stop laying eggs in order to incubate and rear chicks (Manyelo et al., 2020). While this trait is valuable in traditional systems, it significantly reduces annual egg output and limits the potential of local chickens to meet increasing protein demands when compared to commercial layers. Broodiness is hormonally regulated, with prolactin—a hormone produced by the anterior pituitary gland, playing a central role. Elevated prolactin levels are associated with the onset and maintenance of broodiness, during which egg-laying ceases (Dunn, 1998; Sharp, 2009). Prolactin inhibits the hypothalamic-pituitary-gonadal axis, thereby suppressing reproductive hormones required for ovulation and egg-laying (Szukiewicz, 2024). Polymorphisms in the prolactin gene *(PRL)*, particularly in its promoter region may cause a reduction in prolactin expression, which results in non-broodiness, and higher egg production (Jiang *et al*., 2005; Eltayeb *et al*., 2010; Bai *et al*., 2019).

Tropically adapted commercial egg-laying breeds such as the ISA Brown and Black Layer chickens have been selectively bred for high productivity. The ISA Brown, for instance, is known for laying between 300–320 eggs annually under optimal conditions (Jesuyon, 2015; Mesele, 2023), while Black Layer chickens typically produce 250–300 eggs per year (Nelson, 2011). Similarly, the FUNAAB Alpha breed developed by the Federal University of Agriculture, Abeokuta, is a highperforming Nigerian layer adapted to local conditions, capable of producing 280–300 eggs annually with good shell quality and resilience (Adebambo *et al.*, 2022).

Local Nigerian chickens, by contrast, are diverse and typically categorized into three types: normal feathered, frizzle feathered, and naked neck (Bolatito *et al*., 2022). While they are hardy and require minimal input, their egg production is significantly lower—averaging 40–60 eggs per year with average egg weights of 40–45 grams (Ajayi, 2010; Adedeji *et al*., 2021). Despite the documented role of prolactin in broodiness and reproductive performance, limited research has been conducted on the genetic mechanisms influencing these traits in Nigerian chicken breeds (Owolabi, 2023; Uberu *et al.,* 2022). Understanding genetic polymorphisms in the prolactin gene promoter could inform breeding strategies aimed at mitigating broodiness and enhancing egg production in local hens.

This study aims to investigate polymorphisms in the promoter region of the prolactin gene in Nigerian local chickens and three commercial layer breeds - FUNAAB Alpha, ISA Brown, and Black Layers. The goal is to identify genetic markers that could be targeted to reduce broodiness and improve egg productivity in indigenous hens. Specifically, the study will amplify PRL gene regions associated with broodiness and egg-laying, and compare the genetic variation between local and commercial breeds.

Materials and methods

Sample sites and sample collection

Birds were sampled from two farming locations; Federal University of Agriculture, Abeokuta (FUNAAB) Poultry Farm (7.23° N, 3.44° E) and the Ijebu Ode Farm (6° 49' N, 3° 55' E), Ogun State, Nigeria. The university's poultry farm serves as a key research facility for poultry breeding and management. The Ijebu Ode Farm, located in Aiyepe-Odogbolu axis, is a privately owned agricultural enterprise affiliated with FUNAAB, playing a significant role in advancing poultry production practices while also serving as a research extension facility.

Blood samples were collected from a total of 45 chickens, comprising of four different breeds of layer chickens. These included FUNAAB Alpha layers (10) and ISA Brown (10) from the FUNAAB farm, and Black layers (10) and ISA Brown layers (5) from the Ijebu Ode farm (making a total of 15 brown layers) and 10 local chickens sourced from a subsistence farm within FUNAAB. Blood sample was collected using a 5 mL syringe from the brachial vein of the birds. The blood samples were immediately transferred into ethylenediaminetetraacetic acid (EDTA) tubes to prevent coagulation and were placed on ice for temporary storage. To minimize stress, D-anhydrous glucose was administered to the chickens post-sampling. The blood samples were subsequently stored at 4°C in a refrigerator until further processing for DNA extraction.

Genomic DNA Extraction and Quantification

Genomic DNA was extracted from chicken blood samples using the ZYMO DNA extraction kit, following the manufacturer's protocol. 200 µL of blood was lysed with 800 µL of lysis buffer in a 1.5 mL Eppendorf tube, vortexed, and incubated at room temperature for 5–10 minutes. The lysate was transferred to a Zymo spin column in a collection tube and centrifuged at 10,000 g for 1 minute, after which the flow-through was discarded. The spin column was placed in a new collection tube, and 200 µL of DNA prewash buffer was added, followed by centrifugation at 10,000 g for 1 minute. Subsequently, 500 µL of genomic DNA wash buffer was applied, and the column was centrifuged at 10,000 g for 1 minute. The spin column was then transferred to a clean microcentrifuge tube, and 50 µL of DNA elution buffer was added. After incubation at room temperature for 2–5 minutes, the DNA was eluted by centrifugation at 10,000 g for 30 seconds. The extracted genomic DNA was quantified using a NanoDrop spectrophotometer (Thermo Scientific), and its integrity was assessed via agarose gel electrophoresis.

Polymerase Chain Reaction (PCR)

Polymerase chain reaction (PCR) was used to amplify two polymorphic sites in the 5′ flanking region (promoter) of the prolactin (PRL) gene, associated with non-broodiness. Primer pair PRL24 amplified a 24 bp insertion at position -358, while primer pair PRL5 amplified a 439 bp fragment containing the AGCT sequence for AluI restriction enzyme, with a C2402T single nucleotide polymorphism (SNP) at position -2402.

PCR reactions were performed in 25 µL volumes using OneTaq PCR master mix, consisting of 12.5 µL of 2× PCR master mix, 1 µL each of 10 µM forward and reverse primers, 7.5 µL of distilled water, and 3 µL of genomic DNA. Amplification conditions for PRL24 included an initial denaturation at 96°C for 2 minutes, followed by denaturation at 94°C for 15 seconds, annealing at 54°C for 30 seconds, extension at 72°C for 1 minute, and a final extension at 72°C for 5 minutes. For PRL5, the conditions were identical except for an annealing temperature of 62°C. Primers were designed by INQABA Biotech. Reconstitution of primers, PCR and downstream analyses were carried out in the Molecular Biology and Biotechnology laboratory, Chrisland University. The sequences of the primers are shown in Table 1.

Locus	Primer sequences	Annealing temperature	Expected amplicon size
PRL24	F: 5'-GGCTCTCCATGGGTATTAGGA-3'	54℃	130 bps and 154 bps
	R:5'-GCTGGTGAACCAATCTCGGTT-3'		
PRL5	F:5'-CTAAAGGACCTGGAAGAAGGG3'	62℃	439 bps
	R: 5'-AACTTGTCGTAGGTGGGTCTG-3'		

Gel Electrophoresis

Gel electrophoresis was performed using a 2% agarose gel prepared in TBE buffer and stained with ethidium bromide. The molten gel was poured into a casting tray, allowed to solidify, and placed in an electrophoresis tank containing TBE running buffer. DNA samples (5 µL) were mixed with 2 µL of loading dye and loaded into the wells alongside a 100 bp marker (SM0251). Electrophoresis was conducted at 100V for 30 minutes. DNA bands were visualized under a UV transilluminator (SYNEGENE), and images were captured for analysis. Band sizes were determined by comparison with the DNA marker.

Restriction enzyme digest

To determine the single nucleotide polymorphism at position -2402 bp, PRL5 amplicons were digested using the AluI restriction enzyme. The reaction was prepared on ice in a 50 µL volume containing 1 µg of DNA, 5 µL of 10X NEBUFFER (rCutSmart), 1 µL of AluI enzyme, and nuclease-free water. The mixture was gently mixed, briefly centrifuged, and incubated at 37°C for 5–15 minutes, followed by enzyme inactivation at 80°C for 20 minutes. Digested products were analyzed on a 2% agarose gel.

Data Analysis

Genotype frequencies for each allele of the 24 bp insertion were determined using the formulae:

$p = [2(DD)+(ID)] / 2N$

$q = [2(II)+(ID)] / 2N$

where p represents the frequency of the normal (D) allele (130 bp), and q represents the frequency of the insertion (I) allele (154 bp). The distribution of genotypes among brooding and non-brooding chicken breeds was analyzed using Fisher's test while allelic association with chicken breed was analyzed using Pearson's Chi-square.

Abbreviations:
DD: Homozygous dominant genotype for the D allele.
II: Homozygous recessive genotype for the I allele.
ID: Heterozygous genotype.

Ethical consideration and animal handling

The handling of the experimental birds was done following the rules and regulations of the Animal Welfare Committee of the Federal University of Agriculture, Abeokuta, Nigeria with the study approval number FUNAAB/COLANIM/AEWC/2024/09/011

Results

Detection of polymorphism in the PRL Gene Promoter Region

The *PRL* -358 promoter region was amplified to assess genetic polymorphism across different chicken layer breeds. In FUNAAB Alpha layers, eight individuals exhibited the normal 130 bp fragment, while two carried the 24 bp insertion. Among the brown layers, amplification failed in two samples, five birds were homozygous for the normal 130 bp fragment, and eight were heterozygous for the 24 bp insertion. In black layers, four samples failed to amplify, while the remaining six were evenly distributed between homozygous normal and heterozygous genotypes. For local chickens, two individuals were homozygous for the normal allele, while the remaining eight carried both variants in a heterozygous state. The amplification of the PRL24 region are shown in Fig. 1. PCR amplification of the PRL5 region produced the expected 439 bp DNA fragments as shown in Fig. 2.

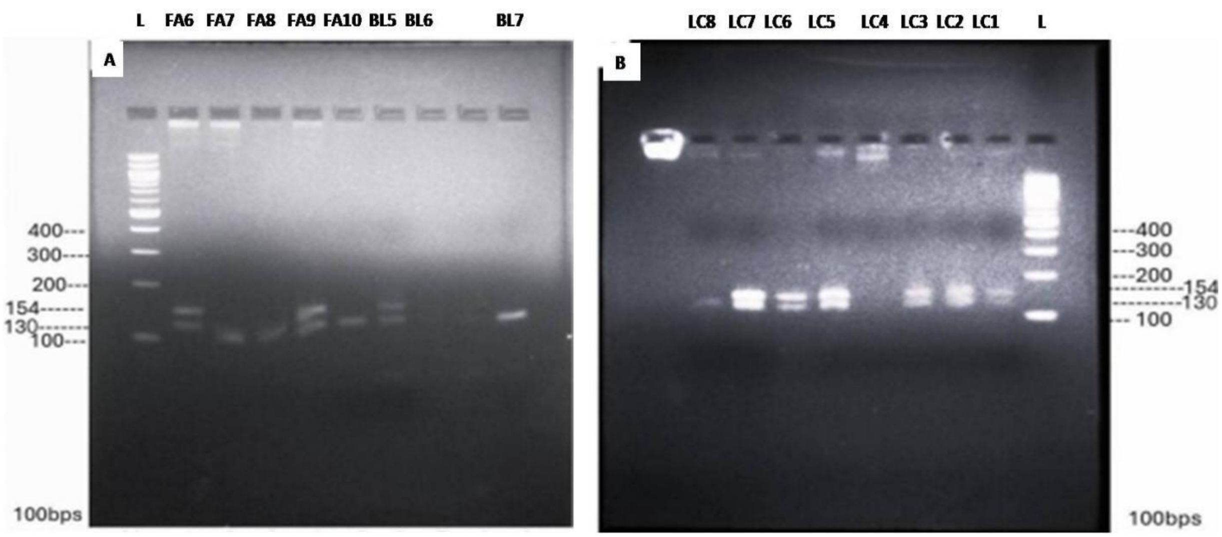

Fig. 1: Genotypes of the 24 bp indel at -358 in the PRL promoter region detected by PCR and agarose gel electrophoresis. Single bands at 130 bp shows homozygous gennotype for the normal allele (DD), double bands at 130 bp and 150 bp shows heterozygote genotype for the normal and insertion allele (ID). FA - FUNAAB Alpha, BL - Black layer, LC - Local hens

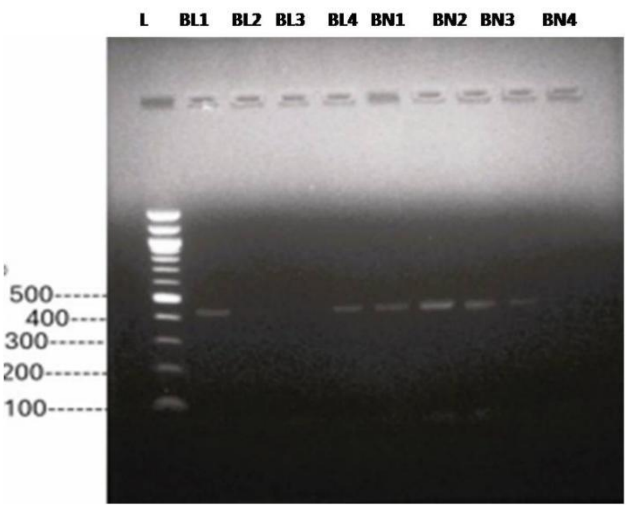

Fig. 2: Bands showing PRL5 amplicons at 439 bp (NB: BL = Black layers; BN = Brown layers)

Allelic and genotypic frequencies of PRL gene promoter polymorphism

The frequency of the D and I alleles in the sampled population was 0.073 and 0.027, respectively. Genotypic analysis revealed that 46% of the individuals were homozygous for the normal allele (DD), while 54% carried the heterozygous genotype (ID). No individuals were observed with the homozygous insertion genotype (II) (Table 2). Representative DNA amplicon bands are shown in Figure 1 (A & B). The 24 bp insertion polymorphism was most prevalent in indigenous local chickens, with an allele frequency of 0.4, while the FUNAAB Alpha breed exhibited the highest frequency (0.9) for the normal sequence. The genotypic and allelic distribution are presented in Table 2. Fisher's exact test ($p = 0.074$) and Pearson's Chi-squared test ($\chi^2 = 2.34$; $p = 0.126$) indicated no significant association between genotype/allele distribution and chicken breed respectively (Table 3).

Table 2: Allele and genotype frequencies of the -358 bp variant in the prolactin gene promoter region

	N	Genotype frequency			Allele Frequency	
		DD	ID	II	D	I
FUNAAB Alpha	10	0.8	0.2	-	0.9	0.1
Brown Layers	13	0.39	0.61	-	0.69	0.31
Black layers	6	0.50	0.50	-	0.75	0.25
Local hens	10	0.20	0.80		0.60	0.40
Total	39	0.46	0.54	-	0.073	0.027

Table 3: Calculation of Fisher's exact test and Pearson's Chi-square for association of genotype and allele frequencies respectively in local hens and the adapted layers

Group	Genotype		Total
	DD	ID	
1	2	8	10
2	16	13	29
Total	18	21	39

Fisher's exact = 0.074 1-sided Fisher's exact = 0.058

Group	Allele	Total

	D	I	
1	12	8	20
2	45	13	58
Total	57	21	78

Pearson $chi^2(1)$ = 2.3378 Pr = 0.126

Group 1 = Local hens showing broodiness
Group 2 = Tropically adapted layers showing non-broodiness

Identification of PRL5 gene polymorphism

No digestion products were observed for the PRL5 amplicons following AluI restriction enzyme treatment, despite multiple optimization attempts.

Discussion

The prolactin (PRL) gene plays a crucial role in regulating reproductive traits such as egg production and maternal behavior in poultry. Genetic variations, particularly in the promoter region of the PRL gene, have been reported to influence its expression and, consequently, reproductive performance in different chicken breeds (Bhattacharya *et al*., 2011; Kilatsih *et al*., 2020; Osman *et al*., 2017). This preliminary study investigated PCR-based polymorphisms in two regions of the PRL gene promoter among Nigerian indigenous chickens and three commercial adapted layer breeds—FUNAAB Alpha, Brown Layers, and Black Layers—with the aim of identifying genetic factors that may be harnessed to improve egg productivity in local hens.

Amplification of the -358 bp promoter region revealed the presence of both the normal (D) and insertion (I) alleles across the studied chicken breeds. The 24 bp insertion allele was most prevalent among indigenous chickens, with an allele frequency of 0.4. In contrast, the FUNAAB Alpha breed exhibited a much lower insertion allele frequency of 0.1, while the normal allele (D) was predominant (0.9). Genotypic distribution showed that heterozygous individuals (ID genotype) were the most common (54%), while homozygous normal genotype (DD) accounted for 46%. No homozygous insertion genotype (II) was observed. Pearson's Chi-square test revealed no statistically significant association between genotypic distribution and the studied chicken breeds.

Interestingly, the findings contradict expectations based on previous studies, which reported an association between the 24 bp insertion and non-broodiness. This insertion is thought to disrupt transcription factor binding, thereby downregulating prolactin expression and inhibiting broody behavior (Jiang *et al*., 2005). In White Leghorns, which have been selected against broodiness, all individuals carried the II genotype, while most Chinese indigenous chickens with strong broodiness exhibited the DD genotype (Cui *et al*., 2006). In native Iranian breeds, the insertion allele was more frequent, with values of 0.59 and 0.76 reported in Mazandaran and Yazd provinces, respectively, and these frequencies were attributed to breed-specific selection pressures (Rashidi *et al*., 2012; Emamgholi-Begli *et al*., 2010).

Our findings suggest that the 24 bp insertion may not be a definitive marker of non-broodiness in tropically adapted commercial layers. The predominance of the D allele in these breeds implies that other genetic mechanisms may contribute to the suppression of broody behavior. Furthermore, the absence of the II genotype in this study precludes evaluation of its functional impact in Nigerian indigenous chickens. It has been suggested that the presence of a single D allele, even in heterozygous form, is sufficient to sustain prolactin expression and enable broodiness (Jiang *et al*., 2005).

The consistent presence of the insertion allele in heterozygous form across all breeds points to a potential heterozygote advantage or reflects the retention of ancestral genetic variation. Studies have shown that certain polymorphisms persist in populations not due to selection, but rather genetic drift or shared ancestry (Granevitze *et al.*, 2007; Muir *et al*., 2008). Given the limited sample size in this preliminary study, the statistical power to detect subtle population-level differences may have been constrained (Van Tassell *et al.,* 2008).

This study also aimed to assess the presence of the C2402T single nucleotide polymorphism (SNP), which has previously been associated with non-broodiness and increased egg production (Cui *et al.,* 2006). While PCR successfully amplified the expected 439 bp product of the PRL5 region, restriction digestion using the AluI enzyme failed to yield any visible fragments. This outcome persisted despite multiple optimization efforts. Such challenges have been reported in earlier studies and are often attributed to mutations in restriction enzyme recognition sites or suboptimal enzyme activity (Jiang *et al*., 2005). Sequence analysis of this region will be necessary to confirm the presence or absence of the C2402T SNP.

Conclusion

This study revealed the presence of polymorphisms in the -358 bp promoter region of the prolactin gene among Nigerian indigenous and tropically adapted commercial layers.. The 24 bp insertion allele was found across all breeds in the heterozygote condition with no significant association with breed type, and the II genotype was absent. These findings suggest that non-broodiness in commercial layers may not be driven solely by the 24 bp insertion, pointing to other underlying genetic mechanisms.While this study contributes valuable preliminary insights into the genetic diversity of Nigerian poultry, future research involving larger sample sizes and broader genetic screening is needed to uncover the complex genetic architecture governing broodiness and egg production. These insights could ultimately inform breeding programs aimed at improving egg productivity in indigenous hens, thereby enhancing food security and reducing poultry production costs in Nigeria.

Conflict of interest statement.

The authors declare no conflict of interest.

References

Adebambo, O. A., Adebambo, A. O., Wheto, M., Kehinde, O., Udoh, J. E., Peters, S., & Adeleke, M. (2022). Development of improved indigenous chickens (FUNAAB Alpha) for smallholder poultry production in Nigeria. In *Agricultural Biotechnology, Biodiversity and Bioresources Conservation and Utilization* (pp. 251-276). CRC Press.

Adedeji, T., Amao, S., & Popoola, D. (2021). Evaluation of Carcass and Sensory characteristics of Nigerian Indigenous Chickens and their Crossbreds raised in Derived Savannah Environment of Nigeria. *PAT, 17*(2), 1-8.

Ajayi, F. (2010). Nigerian indigenous chicken: A valuable genetic resource for meat and egg production. *Asian journal of poultry science*, *4*(4), 164-172.

Alemneh, T., & Getabalew, M. (2019). Exotic chicken production performance, status and challenges in Ethiopia. *International Journal of Veterinary Science and Research*, *5*(2), 039-045.

Bai, D.-P., Hu, Y.-Q., Li, Y.-B., Huang, Z.-B., & Li, A. (2019). Polymorphisms of the prolactin gene and their association with egg production traits in two Chinese domestic ducks. *British poultry science*, *60*(2), 125-129.

Bello, K. O., Adeola, A. A., Akerele, D., Adeyeye, O. A., Irekhore, O. T., & Famakinde, S. A. (2021). Optimisation and Multiplication of Large Fulani Eco-

Type Chicken for Sustainable Production and Genetic Security in Nigeria. *Food Security and Safety: African Perspectives*, 255-269.

Bhattacharya, T., Chatterjee, R., Sharma, R., Niranjan, M., Rajkumar, U., & Reddy, B. (2011). Identification of haplotypes in promoter of prolactin gene and their effect on egg production and quality traits in layer chicken. *Animal biotechnology*, *22*(2), 71-86.

Bolatito, O., Adebambo, A., Ilori, B., Folorunso, O., Ajayi, D., Hasan, T., & Aladele, S. (2022). Utilisation and registration assessment status of chicken genetic resources in Nigeria. *Asian Journal of Research in Animal and Veterinary Sciences*, *9*(3), 28-36.

Cui, J.-X., Du, H.-L., Liang, Y., Deng, X.-M., Li, N., & Zhang, X.-Q. (2006). Association of polymorphisms in the promoter region of chicken prolactin with egg production. *Poultry science*, *85*(1), 26-31.

Dunn, I. (1998). Genetic mapping of the chicken prolactin receptor gene: a candidate gene for the control of broodiness. *British poultry science*, *39*(S1), 23-24.

Emamgholi Begli, H., Zerehdaran, S., Hassani, S., Ali Abbasi, M., & Khan Ahmadi, A. (2010). Polymorphism in prolactin and PEPCK-C genes and its association with economic traits in native fowl of Yazd province. *Iranian journal of biotechnology*, *8*(3), 172-177.

Eltayeb, N. M., Wani, C. E., and Yousif, I. A. (2010). Assessment of Broodiness and its Influence on Production Performance and Plasma Prolactin Level in Native Chicken of the Sudan. *Asian Journal of Poultry Science, 4: 1-6.*

Granevitze, Z., Hillel, J., Chen, G., Cuc, N. T. K., Feldman, M., Eding, H., & Weigend, S. (2007). Genetic diversity within chicken populations from different continents and management histories. *Animal genetics*, *38*(6), 576-583.

Jesuyon, O. (2015). Growth pattern, reproductive performance and seasonal sensitivity of Bovan Nera and Isa Brown parent-stock chickens in Ibadan Nigeria (PhD Thesis, October 2011) https://www.researchgate.net/publication/319669198_

Jiang, R.-S., Xu, G.-Y., Zhang, X.-Q., & Yang, N. (2005). Association of polymorphisms for prolactin and prolactin receptor genes with broody traits in chickens. *Poultry science*, *84*(6), 839-845

Justina, E.-O. I., & Ugwu, S. O. C. (2023). Bottleneck in Creating Layer Breeds of Chickens in Nigeria. In *Poultry Farming-New Perspectives and Applications*. IntechOpen.

Kilatsih, R., Perdamaian, A., Joko, T., Purwanto, S., & Daryono, B. (2020). Effect analysis of Prolactin (PRL) gene polymorphisms on chicken egg productivity (Gallus gallus domesticus) BC1 from crossbreeding between Pelung and Layer chicken. *Iranian Journal of Applied Animal Science*, *10*(4), 717-726.

Manyelo, T. G., Selaledi, L., Hassan, Z. M., & Mabelebele, M. (2020). Local chicken breeds of Africa: their description, uses and conservation methods. *Animals*, *10*(12), 2257.

Mesele, T. L. (2023). Reproduction and production performance of improved chickens, their production constraints, and opportunities under Ethiopian conditions. *Tropical Animal Health and Production*, *55*(4), 245.

Muir, W. M., Wong, G. K. S., Zhang, Y., Wang, J., Groenen, M. A., Crooijmans, R. P., Megens, H.-J., Zhang, H., Okimoto, R., & Vereijken, A. (2008). Genome-wide assessment of worldwide chicken SNP genetic diversity indicates significant absence of rare alleles in commercial breeds. *Proceedings of the National Academy of Sciences*, *105*(45), 17312-17317.

Mujyambere, V., Adomako, K., Olympio, S. O., Ntawubizi, M., Nyinawamwiza, L., Mahoro, J., et al. (2022). Local chickens in East African region: Their production and potential. *Poultry Science*, *101*(1), 101547. https://doi.org/10.1016/j.psj.2021.101547

Nelson, M. G. (2011). *The Complete Guide to Poultry Breeds: Everything You Need to Know Explained Simply*. Atlantic Publishing Company.

Osman M. M., Hemeda, S. A., Hassanin, A. A., & Husseiny, W. A. (2017). Polymorphism of prolactin gene and its association with egg production trait in four commercial chicken lines. *Journal of the Hellenic Veterinary Medical Society*, *68*(3), 391-404.

Owolabi A. O, O. C. O., Atansuyi A. J, Adu O. A, Chineke C. A, & Adebayo I. A. . (2023). Effects of strain and sex on growth performance with prolactin gene expression in different tissues of exotic (cobb 500) and nigerian improved chicken strains (noiler). . https://doi.org/10.5281/ZENODO.10250487

Rashidi, H., Rahimi-Mianji, G., Farhadi, A., & Gholizadeh, M. (2012). Association of prolactin and prolactin receptor gene polymorphisms with economic traits in breeder hens of indigenous chickens of Mazandaran province. *Iranian journal of biotechnology*, *10*(2), 129-135.

Sharp, P. (2009). Broodiness and broody control. In *Biology of breeding poultry* (pp. 181-205). CABI Wallingford UK.

Sharp, P., Macnamee, M., Sterling, R., Lea, R., & Pedersen, H. (1988). Relationships between prolactin, LH and broody behaviour in bantam hens. *Journal of Endocrinology*, *118*(2), 279-286.

Szukiewicz, D. (2024). Current Insights in Prolactin Signaling and Ovulatory Function. *International Journal of Molecular Sciences*, *25*(4), Article 4. https://doi.org/10.3390/ijms25041976

Uberu, N., Oleforuh-Okoleh, V., Ndofor-Foleng, H., Agaviezor, B., Ohagenyi, J., Udeh, F., Ani, A., Nwosu, C., & Akuru, E. (2022). 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

Van Tassell, C. P., Smith, T. P., Matukumalli, L. K., Taylor, J. F., Schnabel, R. D., Lawley, C. T., Haudenschild, C. D., Moore, S. S., Warren, W. C., & Sonstegard, T. S. (2008). SNP discovery and allele frequency estimation by deep sequencing of reduced representation libraries. *Nature methods*, *5*(3), 247-252.

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