POLYMORPHISMS OF PROLACTIN GENE ON PLUMAGE COLOUR, GROWTH AND BODY WEIGHT OF NIGERIAN INDIGENOUS CHICKENS

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ABSTRACT
The study aimed at determining the qualitative traits and genotypic and allelic frequencies of prolactin gene polymorphism in Normal (NF) and Frizzle feathered (FF) chickens. Blood was collected and released on a Fast Technology Analysis (FTA) paper gotten from Federal University of Agriculture, Abeokuta. Three plumage colours were observed through visual appraisal which are multi-coloured, white and black. Frequency and percentage of each colour were obtained. The frequency and percentage of each colour in the NF chicken were multi-coloured (48 and 92.31%), white (3 and 5.77%) and black (1 and 5.77%), respectively. No appearance of white colour was seen in FF chicken; however, the frequency and percentage of black were 1 and 1.25% while multi-coloured were 7 and 87.5%, respectively. Three genotypes were observed including II, ID and DD which consequently had two alleles I and D during gel electrophoresis using agarose gel after specific primers have been amplified using PCR machine. The results of the present study showed the presence of polymorphism for the prolactin (PRL) gene which shows two alleles and genotype; I, D and ID respectively in FF chicken and two alleles (I and D) and three genotypes (II, DD and ID) in NF chicken. Conclusively, prolactin gene can be used as a marker in the genetic improvement of Nigerian indigenous chickens due to the presence of polymorphism.
Keywords: Polymorphisms, Prolactin, Gene, Growth, Indigenous

INTRODUCTION
The prolactin (PRL) gene plays a critical role in regulating several physiological and reproductive functions in birds, including egg production, parental behavior, and growth (Angelier, 2024). In Nigerian indigenous chickens, polymorphisms in the prolactin gene have been identified as genetic markers with potential impacts on growth traits and body weight (Zhu and Li, 2024). The Nigerian indigenous chickens are known for their genetic diversity, adaptability to harsh environments, and economic importance to rural households (Olaniyan *et al*., 2024). Exploring prolactin gene polymorphisms offers insights into improving productivity and sustainability in the Nigerian indigenous chicken populations (Goli *et al*., 2024). Prolactin gene polymorphisms are variations in the DNA sequence that can influence the gene's function or expression (Kubba *et al*., 2024). In chickens, such polymorphisms are often linked to traits like body weight at specific ages, feed efficiency, and reproductive performance. The utility of single nucleotide polymorphisms (SNPs) within the PRL gene as markers for selective breeding are being reached (Hoda *et al*., 2024). Specific PRL SNPs have been associated with body weight differences, with notable variation between heavy and light ecotypes of Nigerian indigenous chickens (Hoda *et al*., 2024). The identification and genotyping of these SNPs involve molecular techniques such as PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) and DNA sequencing (Fakhr, Baioumy and Mohamed, 2024; Kaur *et al*., 2024). The role of prolactin gene polymorphisms in growth and body weight emphasizes the potential for genetic improvement programs targeting Nigerian indigenous chickens (Bulent *et al*., 2024). Therefore, the research was carried out to evaluate the polymorphism of prolactin gene on growth and body weight of some selected Nigerian indigenous chickens.

MATERIALS AND METHODS
Experimental Site
This research work was carried out at the Poultry Unit of Teaching and Research farm, Ladoke Akintola University of Technology, Ogbomoso, Oyo State Nigeria. Ogbomoso is located in the derived savannah zone of Nigeria on the longitude 4°15′ East and latitude 8°15′ North East of the Greenwich Meridian (Google Map, 2024). The laboratory experiment was carried out at the Federal University of Agriculture, Abeokuta, Ogun State, Nigeria.

Experimental Birds and Management
Total of eight four (84) birds belonging to two genotypes were used for the experiment. The two genotypes included; Normal (42) and Frizzle feathered chickens (42) which are of different age, sex and body size.

Data collection
Data were collected on the following growth parameters: body weight, body length, breast circumference, shank length plumage colour, thigh length, beak length, comb length and comb height.

Growth parameters
Body weight: It was measured with the use of sensitive scale in grams.
Body length: It was measured as the distance from the wing joint to the vent with the use of tape rule (cm).

Breast circumference: It was measured as the circumference of the breast around the deepest region of the breast with the use of tape rule (cm).
Shank length: It was measured as the length of the tarso-metatarsus from the hock joint to the metatarsal pad with the use of tape rule (cm).
Thigh length: It was measured as the length between the mid region of the thigh hip bone and that of the knee (genu) with the use of tape rule (cm).
Plumage colour: Physical colour appraisal was used.
Beak length: It was measured as the distance between the base of the beak to the tip of the beak.
Beak height: It was measured as the vertical distance from the base of the beak to the top of the beak.
Comb length: It was measured as the horizontal length of the part of the head that comb covered.
Comb height: It was measured as the distance from the base of the comb on the chicken to the highest part of the comb.

Blood Collection and Storage
Blood samples were collected from each chicken using a syringe and needle for individual to avoid contamination from wing vein. Five (5ml) of blood was collected and transferred into a FTA paper from the ACUTIG Laboratory, FUNAAB, Abeokuta Nigeria.

Experimental Design and Statistical Analysis
Data collected was completely randomized and subjected to two-way Analysis of variance using procedure of SAS (2003) and significant mean was separated with Duncan Multiple Range Tests. The following model was adopted.

$Y_{ijk} = \mu + G_i + e_{ijk}$

Where:

Y_{ij} = individual observation
μ = overall mean
G_i = fixed effect of i^{th} genotype ($i = 1, 2$)
e_{ijk} = experimental errors which is evenly distributed

RESULTS AND DISCUSSION
Table 1 revealed the occurrence of plumage colour in Normal and Frizzle feathered of Nigerian indigenous chickens. There are three different plumage colours observed in the population of both chickens. The Normal feather chickens had higher multicoloured frequency (92.31%) while Frizzle feathered chickens had (87.5%). Normal feather chicken had (5.77%) white colour, whereas no white colour was observed in the Frizzle feathered chickens. Frizzle feathered chicken had (12.5%) black colour while Normal feathered chicken had (1.92%) black colour.

Table 1: Qualitative Trait (Plumage colour) Frequency Occurrence in Normal and Frizzle Feathered Nigerian Indigenous Chickens

Trait	Breed	Characteristics	Frequency	Percentage (%)
Plumage Colour	Normal Feather Chickens	Multicoloured	48	92.31
		White	3	5.77
		Black	1	1.92
		Total	**52**	**100**
	Frizzle Feather Chickens	Multicoloured	7	87.5
		White	0	0
		Black	1	12.5
		Total	**8**	**100**

The genotypic frequency and percentage of prolactin gene polymorphism in Normal and Frizzle feathered chickens is revealed in Table 2. Three genotype was observed in Normal indigenous chicken (II, DD, ID) whereas one genotype was observed in Frizzle feathered chickens. The gene with higher frequency and percentage is ID gene, which is the dominant gene for phenotypic expression of Normal indigenous chicken with the frequency of 43 and 84.31% whereas II and DD genotype has the same frequency and consistently have the similar statistical values of frequency 4 and 7.8% respectively. The Frizzle feathered chickens has ID genotype has the dominant gene with frequency of 8 and 100% which is responsible for phenotypic expression in Frizzle feathered chickens. Frizzle feathered chickens had higher percentage of ID genotype (100%) compared to Normal feathered chickens (84.31%).

Table 3 indicated the allelic frequency of prolactin gene polymorphism in Normal and Frizzle feathered chickens. Two alleles (I, D) were deduced from three genotypes observed (II, ID, DD) in both chickens. In the Normal and Frizzle feathered chickens,

both I and D allele have similar statistical values for frequency at 0.36 and 0.33 respectively. The result showed that the Normal feathered chickens had higher frequency compared to Frizzled feathered chicken.

Table 2: Genotypic Frequency and Percentage of Prolactin Gene Polymorphism in Normal and Frizzle Feathered Nigerian Indigenous Chickens

Breed	Genetic Diversity	Frequency	Percentage (%)
Normal Feather Chickens	ID	43	84.31
	DD	4	7.84
	II	4	7.84
	Total	51	99.99
Frizzle Feather Chickens	ID	8	100
	DD	-	-
	II	-	-
	Total	8	100

Table 3: Allelic Frequency of Prolactin Gene Polymorphism in Normal and Frizzle Feathered Nigerian Indigenous Chickens

Breeds	Allele	Frequency
Normal Feather Chicken	I	0.36
	D	0.36
	Total	**0.72**
Frizzle Feather Chicken	I	0.33
	D	0.33
	Total	**0.66**

Table 4 indicated the mean value of body weight and linear body measurements for Normal Feather, Frizzle Feather and Naked Neck Chickens. There are significance differences between the two genotypes and the prolactin gene in relationship to body weight and linear body measurements including body length (BDL), beak length (BKL), chest circumference (CC), beak height (BKH), shank length (SHKL), comb length (CL), comb height (CH) and thigh length (THL). The Frizzle feathered chickens had higher body weight (1739 g) while the Normal feather and Naked Neck chickens had similar statistical values of 1229 g and 1256 g for body weight respectively. There were no significant differences in the BL, CC, BKL, SL, CL, CL, CH and TL of the three breeds of chickens studied.

Table 4: Mean Body Weight and Linear Body Measurements for Normal Feather, Frizzle Feather and Naked Neck Chickens

Parameters	Nf	Ff	Nn
BW (g)	1229±396.08220^{b}	1739±827.15052^{a}	1256±354.81496^{b}
BL (cm)	18.48654±2.21439	19.62500±3.96187	19.50741±2.73115
CC (cm)	26.81346±4.037547	27.37500±4.36504	26.5000±2.15906
BKL (cm)	3.10385±0.34865	3.37500±0.44320	3.09630±0.54029
SL (cm)	7.85769±1.21516	8.18750±1.22292	7.78519±1.5376
CL (cm)	4.03269±2.77501	6.56250±3.21200	5.15926±3.20568
CH (cm)	1.78452±1.95312	3.08750±1.191344	2.66296±1.82886
TL (cm)	14.67115±2.55606	15.18750±2.63137	15.20741±3.68760

DISCUSSION

The growth and body weight traits of Nigerian indigenous chickens are influenced by both genetic and environmental factors, with prolactin (PRL) gene playing a crucial regulatory role. Polymorphisms in the PRL gene, particularly single nucleotide polymorphisms (SNPs), are associated with key phenotypic traits such as body weight, growth rate, and reproductive efficiency. The prolactin gene is responsible for encoding prolactin, a hormone involved in growth regulation, metabolism, and reproductive functions. In Nigerian indigenous chickens, PRL gene polymorphisms are linked to variations in hormonal pathways that influence body weight and growth rate (Hoda *et al*., 2024). Specific SNPs within regulatory and coding regions of the PRL gene can alter gene expression or protein function, impacting growth traits. Prolactin polymorphisms have been associated with differences in body weight at critical growth phases, such as at sexual maturity or at 16 weeks of age (Wang *et al*., 2024). The result in this study is similar to the findings of (Kulibaba *et al*., 2024) who work on molecular diversity of Ukrainian native chicken breeds and research of (Gao *et al*., 2023; Huang *et al*., 2023). Nigerian indigenous chickens are divided into ecotypes, such as heavy and light varieties, which display genetic and phenotypic diversity due to adaptations to local environments. Prolactin gene SNPs are more prevalent

in heavy ecotypes, correlating with higher body weight, while light ecotypes may have distinct SNP profiles linked to reproductive traits (Gao *et al*., 2023).

CONCLUSION

Further research is needed to validate the identified SNPs across larger and more diverse chicken populations, as well as to understand their interaction with other genes involved in growth and metabolism. Additionally, integrating these findings with genomic selection strategies could accelerate genetic gains in local chicken populations.

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