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POLYMORPHISM AND ASSOCIATION OF PROLACTIN RECEPTOR GENE WITH THE REPRODUCTIVE TRAITS OF THE NIGERIAN INDIGENOUS CHICKEN ECOTYPES

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Abstract

Prolactin receptor gene can influence the reproductive traits of chickens, such as broodiness and egg production. The aim of the study was to assess polymorphism and association of prolactin receptor genes with the reproductive traits of the Nigerian indigenous chicken ecotypes. A total of 100 chickens drawn from four different groups/populations of chickens were provided for the study. The chickens which comprised of laying hens only were tagged from Hen 1 to Hen 100. For further identification, hen 1 to hen 35 were tagged as population 1, and were drawn from Generation (G) 7, Heavy ecotype selected (35 hens). Hen 36 to hen 72 were tagged as population 2, and were drawn from G 8, Heavy ecotype selected (37 hens). Hen 73 to hen 90 were tagged as population 3, and were drawn from Light ecotype unselected (18 hens), and hen 91 to hen 100 were tagged as population 4, and were drawn from Heavy ecotype unselected (10 hens). Generations G7 and G8 are groups of the Nigerian heavy local chicken ecotype that have undergone seven and eight generations of selection using selection index. Two (2) blood samples were randomly collected from each population with the aid of sterile syringes and needles onto a Whatman FTA card and allowed to air dry for DNA extraction. The DNA was extracted using the GenoAid extraction protocol. PCR was conducted and the product was then viewed on 2% agarose gel. The sequence results showed that a total of 206 base pairs (bp) screened for prolactin receptor PRLR5a gene did not have any polymorphism across the samples studied. However, on prolactin receptor PRLR6 gene, 195 base pairs were screened and variation occurred at polymorphic site 47bp with nucleotide base “A” transcribed to “G”, and the possible genotypes were “AG” and “GG”. The phylogenetic tree analysis clustered the samples into two branches and showed that individuals with “GG” genotype performed better than those birds with AG genotype in the reproductive traits considered. It was therefore, concluded that prolactin receptor PRLR6 gene was

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polymorphic at site 47 bp and positively associated with the reproductive traits: body weight at first egg, body weight at 16th week of egg production, total egg number and average egg weight. The mutated genotype (GG) showed superior performance over the normal genotype (AG) of the Nigerian indigenous chicken ecotypes.

Keywords: Polymorphism, Prolactin Receptor, Association, Reproductive Traits, Heavy and Light Ecotype Chickens.

INTRODUCTION

Prolactin (PRL) is secreted by the anterior pituitary gland and it is a polypeptide hormone which has diverse biological actions in vertebrates (Jin and Fan, 2019; Al-Chalabi *et al*., 2021). Prolactin is known for its capacity to stimulate the mammary gland development, lactation, egg production and numerous other actions necessary to maintain homeostasis. Prolactin's chemical structure resembles that of growth hormone and placental lactogen hormone. This family of hormones shares a common ancestral gene (Li *et al*., 2019). The prolactin in chickens can modulate maternal behaviours such as effect on egg production (Gumulka *et al*., 2021). Along with growth hormones and placental lactogens, PRL is a hormone developed as a result of the duplication of an ancestral gene (Sinha, 1995; Chen *et al*., 2011). More than 300 biological processes, including immunological responses, behavior, development, and metabolism, are influenced by PRL (Al-Chalabi *et al*., 2021). By attaching to its membrane-bound cell surface receptor, PRL can cause different biological effects as prolactin receptor, (PRLR). In other words, prolactin binds to the prolactin receptor to cause a number of physiological and biochemical reactions to exercise functions. All vertebrates possess these binding sites or receptors (Al-Chalabi *et al*., 2021).

Prolactin receptors (PRLRs) has the capacity to activate Janus kinase 2 and signal transducers, and activators of transcription which distinguishes them from other members of the cytokine class-1 receptor superfamily, a wider family that currently comprises more than 20 members (Fleenor *et al*., 2006). According to Wells and De Vos (1996), the prolactin receptor (PRLR) protein is a type of distinct membrane protein that belongs to the cytokine receptor superfamily. It has an intracellular domain, a trans-membrane domain, and a membrane domain. According to published research, female mice with the PRL and PRLR gene knockout displayed some unusual symptoms, including irregular estrus, decreased ovulation rate, false pregnancy, altered maternal behavior, infertility, and loss of luteal function (Hai *et al*., 2015). Thus, the absence of PRLR in female mice resulted in reduced ovulation and fertilization and multiple reproductive abnormalities. Kelly *et al*. (2001) also, reported that PRLR is important for oocyte maturation. Previous studies on hens revealed that the PRLR gene was a promising candidate gene for broodiness. Increased prolactin secretion was what caused the onset of broody behavior in chickens (Cui *et al*., 2006). Reduced egg production, frequent nest occupancy, reduced feed and water intake, regressive ovary, aggressive or defensive behavior, distinctive clucking, and elevated body temperature were all indicators of broodiness in chickens (Jiang *et al*., 2005). It controls vital physiological processes like fish osmoregulation and

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avian nesting behavior, as well as impacts on mammalian reproduction that are widely documented (Elkins *et al*., 2000). Due to the fact that a key gene hypothesized to play a role in broodiness susceptibility is thought to reside on the chicken Z chromosome and manifest as a sex-linked trait, Dunn *et al*. (1998) mapped the chicken PRLR gene as a candidate gene for the control of broodiness. PRLR, a crucial growth and differentiation regulating gene, may be a candidate gene for reproductive characteristics (Van Rens *et al*., 2003) and plays an important role in the PRL signal transduction cascade. Thus, the aim of the study was to assess polymorphism and association of prolactin receptor genes with the reproductive traits of the Nigerian indigenous chicken ecotypes.

MATERIALS AND METHODS

Experimental animal and Blood sample collection

A total of 100 chickens drawn from four different groups/populations of chickens were provided for the study. The chickens which comprised of laying hens only were tagged from Hen 1 to Hen 100. For further identification, hen 1 to hen 35 were tagged as population 1, and were drawn from Generation (G) 7, Heavy ecotype selected (35 hens). Hen 36 to hen 72 were tagged as population 2, and were drawn from G 8, Heavy ecotype selected (37 hens). Hen 73 to hen 90 were tagged as population 3, and were drawn from Light ecotype unselected (18 hens), and hen 91 to hen 100 were tagged as population 4, and were drawn from Heavy ecotype unselected (10 hens). Two (2) blood samples were randomly collected from each population with the aid of sterile syringes and needles onto a Whatman FTA card and allowed to air dry for DNA extraction.

DNA extraction and purification

The DNA extraction and purification was carried out using the GenoAid extraction protocol. The DNA concentration and purity check was done on Nanodrop Spectrophotometer.

PCR Amplification

The process was carried out to amplify the chicken receptor genes. Reaction set up contained 4µl of master mix, 0.6µl of forward primer, 0.6µl of reverse primer, 12.8ml of water and 2µl of template DNA. The set up formed a total of 20µl, to reduce the number of pipetting and pipetting errors, a reaction cocktail was prepared, which was the aliquot in 18µl into each tube for a sample. The 20ul reaction set-up was prepared for each sample and then loaded in the thermal cycler. The product was then viewed on 2% agarose gel. Thermocycling conditions were as follows: initial denaturation 95^{o}C for 5 minutes, denaturation 95^{o}C for 30 seconds, annealing 59.0 (PRLR5a primers) and 57.0^{o}C (PRLR6 primers) for 30 seconds, extension 72^{o}C for 40 seconds, and final extension 72^{o}C for 5 minutes.

The primer sequences and annealing temperatures of Prolactin Receptor genes are presented in Table 1.

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Table 1: Primer Sequences and Annealing Temperatures of Prolactin Receptor (PRLR) Genes

Primer name	Primer sequences (5' – 3')	Product size (bp)	Tm (°C)	Exon
PRLR5a-F PRLR5a-R	TTGTCTGCTTTGATTCATTTCC TGCATTTCATTCTTCCCTTTTT	250	59.0	Exon 5a
PRLR6-F PRLR6-R	GCCAGATCCTCCTGTGAATG TGAGGGGACATGACTAACAAA	236	57.0	Exon 6

Sources (Cui *et al.* 2011; Jiang *et al*. 2005) PRPR = Prolactin receptor gene

DNA Sequencing Protocol

Primer extension sequencing was performed by GENEWIZ, Inc (South Plainfield, NJ) using Applied Biosystems BigDye version 3.1. The reactions were then run on Applied Biosystem's 3730xl DNA Analyzer. The sequencing was conducted at the African Biosciences DNA Laboratory United State of America. Samples with identification numbers 4, 22, 42, 62, 75, 90, 95 and 100 were randomly selected for DNA sequencing. The evolutionary history was inferred by using the Maximum Likelihood method based on the Jukes-Cantor model (Jukes and Cantor, 1969).

Identification of the Single Nucleotide Polymorphisms (SNPs)

The F & R primer sequences of prolactin receptor PRLR5a and PRLR6 genes were edited. Bioedit software was used for the editing and cleaning of the sequences, while, the reference sequences with Genebank accession number JN650613 was used to identify the single nucleotide polymorphisms (SNPs). After the single nucleotide polymorphisms were identified, they were associated with the phenotypic data, using SAS 9.4 version (SAS, 2014).

Reproductive traits under consideration

The reproductive traits associated with the prolactin receptor genes were body weight at first egg (BWFE), body weight at 16^{th} week of egg production (BWT16), total egg number (TEN) and average egg weight (AEW).

RESULTS AND DISCUSSION

The PCR Amplification of Genes of the Chicken Samples of the Nigerian Heavy local chicken ecotype and Light ecotype

The primers PRLR5a (F & R) and PRLR6 (F & R) were successfully amplified and sequenced 206 bp and 195 bp, respectively, in the samples analyzed Fig. 1 and 2. The clarity of the bands on the gel images indicated that the DNA of the chickens stored in the FTA cards were perfectly preserved and successfully extracted. From the gel image of primer PRLR5a (Fig 1), samples 6 and 8 failed which resulted in the absence of visible bands. However, this was different from gel image of primer PRLR6 (Fig. 2) which had all the samples successfully amplified. The failed samples were subsequently re-amplified and successful amplification was achieved. The result from this study was in agreement

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with Ikeh *et al*. (2020) who had previously reported successful extraction of chicken DNA from the FTA card. Similarly, Ikpeme *et al*. (2021) in another study preserved samples of chicken DNA in FTA card and obtained excellent results in genetic relationship among three Nigerian chicken genotypes based on Cythochrome b of mitochondrial DNA. The successful amplification during PCR as well resulted to the ease of sequencing the DNA products.

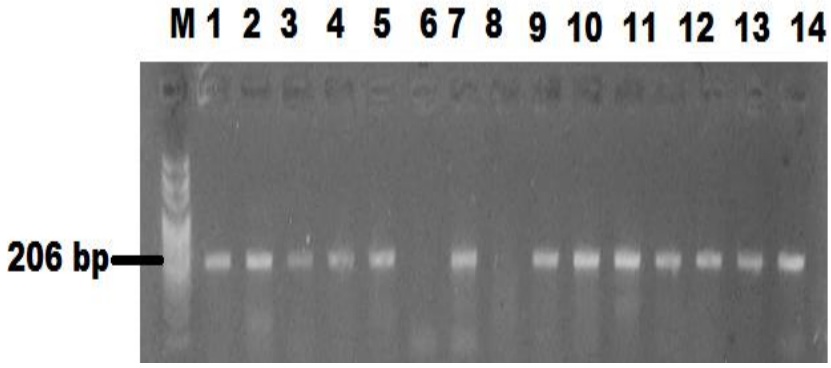

Fig 1: PCR Amplification of Prolactin Receptor PRLR5a gene Primer

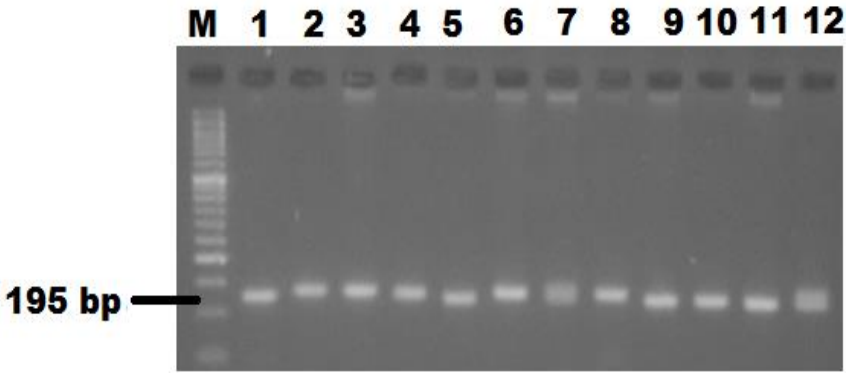

Fig 2: PCR Amplification Prolactin Receptor PRLR6 gene Primer

Sequence Alignment and Polymorphic site on Prolactin receptor PRLR5a gene

The primers of prolactin receptor PRLR5a gene were successfully amplified and sequenced 206 bp of the prolactin receptor gene in all the samples under consideration Fig. 3. The sequences of the prolactin receptor PRLR5a were successful and showed 100% similarities with the first 45 sequences of *Gallus gallus* mRNA prolactin receptor gene from the GeneBank. Among the four populations of the *Gallus gallus* analyzed in this study, there were no difference in the base sequences of the prolactin receptor PRLR5a in all the samples analyzed across the populations under study. The phylogenetic tree analysis did not also, show differences in the samples analyzed (Fig. 4). The molecular phylogenetic relationship of the samples and some *Gallus gallus* (AY547323, AY237376 and AC190411) from the GeneBank which were rooted with

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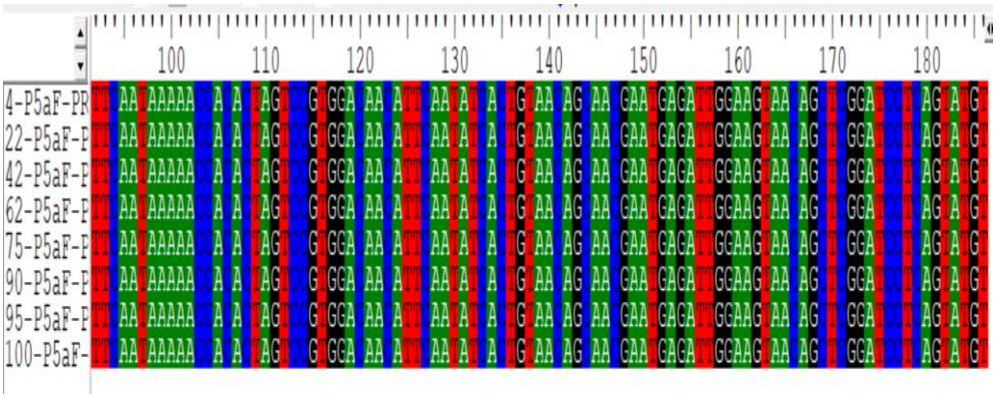
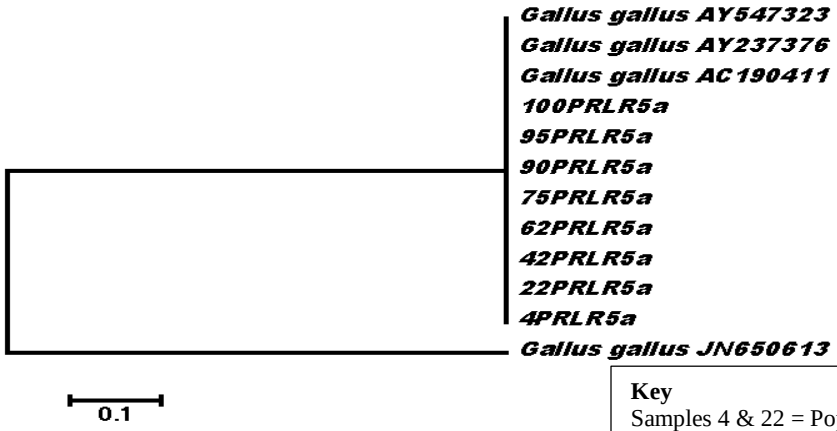
phylogeny of *Gallus gallus* (JN650613) clustered together. This implied that the chickens sampled in this study have similar ancestral descent or origin with the *Gallus gallus* samples obtained from the GeneBank (Wang *et al*., 2020).



No polymorphism dictated in prolactin receptor PRLR5a gene

Key
Samples 4 & 22 = Pop. 1 = G7 Heavy Ecotype Selected
Samples 42 & 62 = Pop. 2 = G8 Heavy Ecotype Selected
Samples 75 & 90 = Pop. 3 = Light Ecotype Unselected
Samples 95 & 100 = Pop. 4 = Heavy Ecotype Unselected

Fig 3: Sequence result of Prolactin receptor PRLR5a gene of the Nigerian local chickens using Bioedit software version® 7.0



Key
Samples 4 & 22 = Pop. 1 = G7 Heavy Ecotype Selected
Samples 42 & 62 = Pop. 2 = G8 Heavy Ecotype Selected
Samples 75 & 90 = Pop. 3 = Light Ecotype Unselected
Samples 95 & 100 = Pop. 4 = Heavy Ecotype Unselected

Fig 4: Phylogenetic tree of Prolactin receptor PRLR5a gene on the Nigerian local chickens

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Sequence Alignment and Polymorphic site on Prolactin receptor PRLR6 gene

The results of the sequence alignment and polymorphic site on Prolactin receptor PRLR6 gene are presented in **(**Fig. 5). The result showed that the primer sequence of prolactin receptor PRLR6 gene was successfully amplified and sequenced 195 base pairs (bp) in all the samples and populations.

The sequences of PRLR6 gene showed 100% similarities with *Gallus gallus* breed Chinese Yellow Wai Chow prolactin receptor gene and *Gallus gallus* breed Huxu on chromosome Z; and 99.32% similarity to the remaining 43 sequences of *Gallus gallus* prolactin receptor gene in the GeneBank (Zhang *et al*., 2012; Liang *et al*., 2019).

Among the samples analyzed, 4 and 95 corresponded with this observation, whereas, samples 22, 42, 62, 75, 90 and 100 showed 100% similarity with 45 sequences of prolactin receptor gene of *Gallus gallus* in the GeneBank (Liang *et al*., 2019).

Variation occurred at 47 bp of the of prolactin receptor PRLR6 with samples 4 and 95 having the base “G” while the rest of the samples had the base “A” at the same location (the polymorphic site) (Fig. 5). These base differences gave rise to the genotypes AG and GG among the *Gallus gallus* populations.

The phylogenetic tree analysis of prolactin receptor PRLR6 gene (Fig. 6) splited the tree into two branches with samples 4 and 95 at one branch, while the remaining samples were clustered at the other branch, in line with the variation observed at the base sequence 47 bp of the samples analyzed.

The phylogenetic tree was rooted with *Gallus gallus* AY547323 from the GeneBank (Fig. 6). The result from the phylogenetic tree was in agreement with the report of Ikpeme *et al*. (2021) who reported that Nigerian indigenous chickens were clustered into two branches from phylogenetic tree analysis.

Similarly, this result corroborates with the report of Adamu *et al*. (2016) who opined that phylogenetic analysis provides important guideline for making conservative decisions among indigenous chickens of Nigeria.

The aligned sequences identified the single nucleotide polymorphism (SNPs) in the samples according to Gamaniel and Gwaza (2017) and Salisu *et al*. (2018) who independently reported that single nucleotide polymorphism is the DNA variation as a result of a change in the nucleotide at single location in the genome.

The result of this study corroborates the findings of Uberu *et al*. (2021) who characterized the Nigerian indigenous chicken ecotypes with prolactin gene and reported that the homozygous TT genotypes showed better egg weight and egg number performances than the heterozygous TC individuals.

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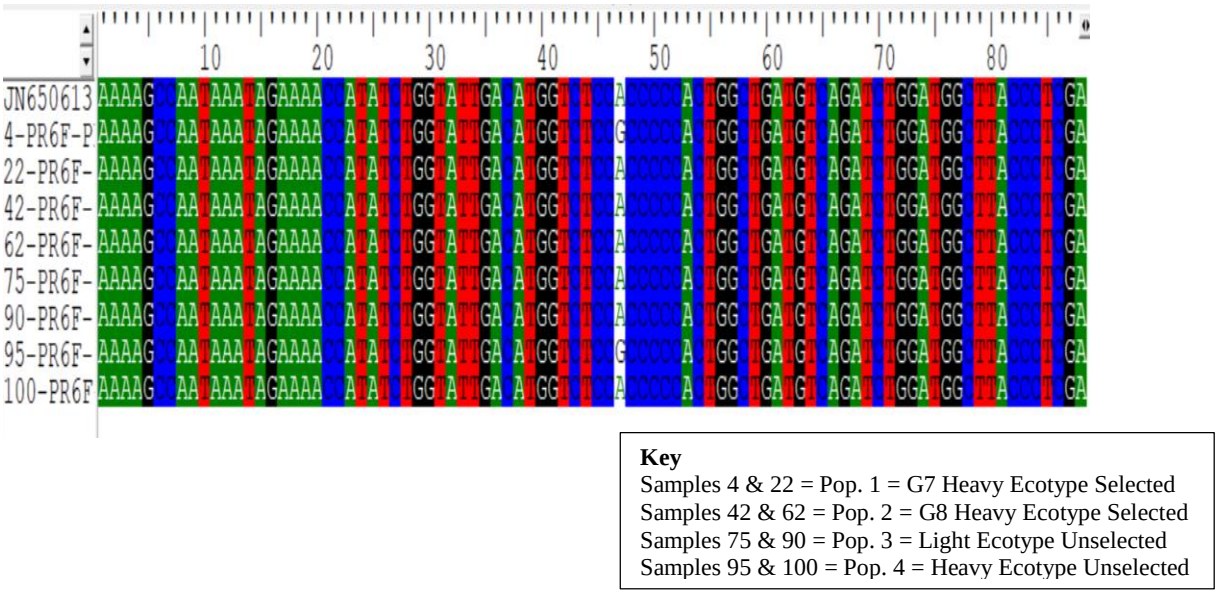

Plate 5: Sequence result of Prolactin receptor PRLR6 gene of the Nigerian local chickens using Bioedit software version® 7.0

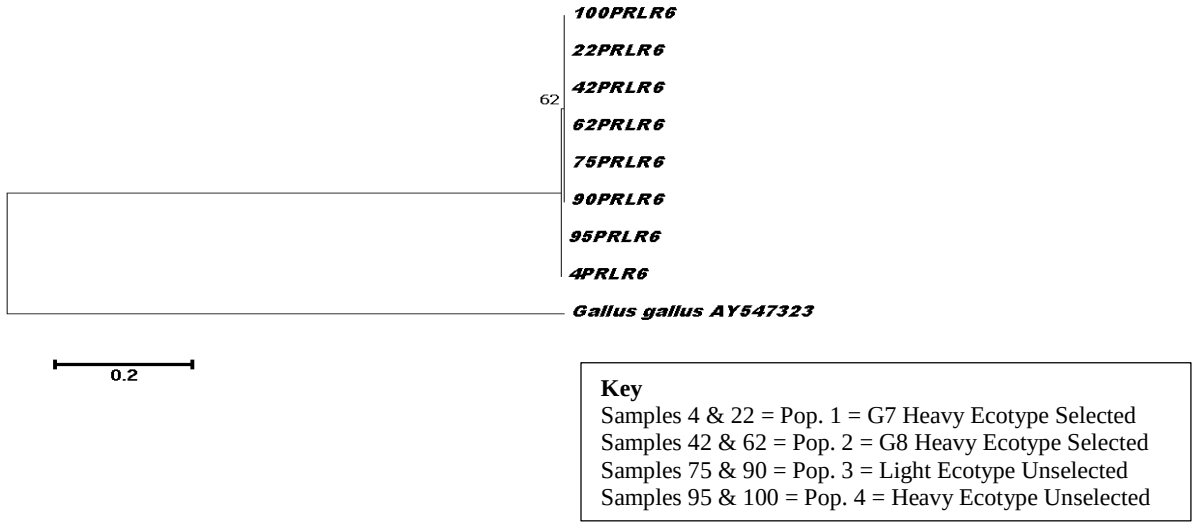

Plate 6: Phylogenetic tree of Prolactin receptor PRLR6 gene on the Nigerian local chickens

Association of prolactin receptor PRLR6 gene with reproductive performance

The information on the polymorphic site, possible genotype and number of base pairs screened are presented in Table 1. From the table, polymorphism occurred at position 47 bp. A total of 195 base pairs were screened, and only one SNP was identified, where nucleotide base “A” changed to “G”. (Fig. 5) The possible genotypes were AA, AG and

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GG. The result of the association of prolactin receptor PRLR6 gene with reproductive traits of the four populations of the Nigerian indigenous chickens is presented in Table 3. The result indicated that in exon 6 of prolactin receptor gene using JN650613 as reference sequence, at the polymorphic site of 47 bp, single nucleotide polymorphism (position 47 A>G) was identified and, and nucleotide base "A" transcribed to "G". The transcription of the nucleotide bases from "A" to "G" resulted to two genotypes AG and GG. The AG genotype was the normal, while, the GG genotype was the mutated genotype. The result showed that mean body weight at first egg, body weight at 112 days of egg production, total egg number and average egg weight were not significantly ($p>0.0$) influenced by the genotypes.

The results of association of prolactin receptor PRLR6 gene to the reproductive traits of the four populations of the Nigerian indigenous chickens showed no significant ($P>0.05$) differences among the genotypes and across the reproductive traits under study. Despite the fact that there were no significant difference among the two genotypes across the reproductive traits under the study, the results indicated numerical increase in the value of the mutated genotype (GG) above the normal genotype (AG). The showed that body weight at first egg (BWFE) increased from 1196.43 to 1371.36g, body weight at 16th week of egg production (BWT16) increased from 1373.85 to 1499.07g, total egg number from 76.11 to 85.56 and average egg weight from 46.71 to 49.05g. This result agreed with Rashidi *et al*. (2012) who reported that CC, CT and TT genotypes were found in chicken species and the chickens performed differently, as a result of the different genotypes they carry. This result was also, in agreement with the report of Silva *et al*. (2013) and Kleyn *et al*. (2021) who reported positive association of genotypes with phenotypic traits in chicken species. It as well, agreed with the report of Adamu *et al*. (2016); Uberu *et al*. (2021) who independently reported that there were association between genotypes and reproductive traits of the Nigerian indigenous chickens. The result revealed that individuals with GG genotype performed better than individuals with AG genotypes in the reproductive traits of the Nigerian indigenous chickens.

Table 2: Polymorphic site, possible genotype and number of base pairs screened of prolactin receptor PRLR6 SNP in the Nigerian indigenous chickens

Sample ID	Polymorphic site on DNA	Possible Genotype	Total bp screened	Population
4 & 22	47 (G & A)	GG, AG, AA	195	Pop. 1
42 & 62	47 (A & A)	AA, AG	195	Pop. 2
75 & 90	47 (A & A)	AA, AG	195	Pop. 3
95 & 100	47 (G & A)	GG, AG, AA	195	Pop. 4

Samples 4 & 22 = Pop. 1 = G7 Heavy Ecotype Selected, Samples 42 & 62 = Pop. 2 = G8 Heavy Ecotype Selected, Samples 75 & 90 = Pop. 3 = Light Ecotype Unselected, Samples 95 & 100 = Pop. 4 = Heavy Ecotype Unselected (wild type)

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Table 3: Association of prolactin receptor PRLR6 gene to the reproductive traits of the four populations of the Nigerian indigenous chickens

Parameters	Genotypes		P-Value
	AG	GG	
BWFE	1196.43±76.82	1371.36±126.13	0.29^{NS}
BWT16	1373.85±100.16	1499.07±96.75	0.44^{NS}
TEN	76.11±5.67	87.56±12.24	0.37^{NS}
AEW	46.71±3.00	49.05±2.48	0.69^{NS}

BWFE = Body weight at first egg, BWT at 16 WKEP = Body weight at 16th week of egg production, TEN = Total egg number, AEW = Average egg weight

CONCLUSION

It was concluded that prolactin receptor PRLR8 gene was polymorphic at 47 bp where base “G” transcribed to base “A” which gave rise to genotypes AG and GG. The gene also positively associated with the reproductive traits of the Nigerian indigenous chicken. The mutant genotype (GG) showed numerical increase over the normal genotype (AG) as body weight at first egg (BWFE) increased from 1196.43 to 1371.36g, body weight at 16th week of egg production (BWT16) increased from 1373.85 to 1499.07g, total egg number from 76.11 to 85.56 and average egg weight from 46.71 to 49.05g. It was recommended that prolactin receptor PRLR8 gene can be inserted in the chickens through genetic engineering for improvement on the reproductive traits.

Acknowledgement

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Conflict of Interest

The authors hereby declare that there is no conflict of interest.

Author’s contribution

UFU: Design, methodology and statistical analysis, OMO: Original drafting of manuscript, UNP: Supervision and writing of manuscript, UVC: Review, editing and experimentation, NC: Assisted in data collection, OCE: Supervisory Assistant, Initial design of the experiment and review editing, OAL: Assisted in data collection, review and analysis.

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