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Association of Growth Hormone Secretagogue Receptor (GHSR) Gene Polymorphisms with Performance Traits in Four Chicken Genotypes

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A B S T R A C T

Poultry farming is crucial to global food security, with particular significance in developing nations due to its affordability, efficiency, and contribution to rural livelihoods. This study evaluated morphological traits and genetic polymorphisms in the growth hormone secretagogue receptor (GHSR) gene across four chicken genotypes: Normal Feather (NF), Frizzle Feather (FF), Naked Neck (NN), and Exotic (EX). A total of 100 day-old chicks were reared under controlled conditions, and growth performance traits, including body weight, height, body length, and shank length, were measured. Genomic DNA was extracted from blood samples, and the GHSR gene was amplified by Polymerase Chain Reaction (PCR). The resulting PCR products were sequenced and analyzed to identify genetic polymorphisms. The results showed that the EX genotype significantly outperformed the indigenous genotypes (NF, FF, and NN) in all growth parameters. The highest level of genetic variation was observed in the EX chickens, followed by FF, NF, and NN genotypes. The EX genotype also exhibited the highest haplotype diversity (0.946 ± 0.006), whereas the highest nucleotide diversity was recorded in NF chickens (0.131 ± 0.0001). Phylogenetic analysis revealed two distinct clusters, with all indigenous genotypes grouped within the same major cluster, while the EX genotype formed a separate cluster. The lowest genetic distance was observed between the FF and NN chickens. Single-nucleotide polymorphisms (SNPs) in the GHSR gene were significantly associated with body weight, body length, and shank length, particularly in the EX genotype ($p<0.05$). These findings suggest that SNP variation within the GHSR gene may serve as a useful basis for developing molecular markers to enhance growth performance, while also emphasizing the importance of maintaining genetic diversity in indigenous chicken populations.

Keywords: *Chicken Genotypes, Growth Performance, GHSR Gene, Nucleotide Polymorphism, Poultry Breeding.*

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1 Introduction

Poultry production plays a vital role in global food security and human nutrition, providing an affordable and high-quality source of animal protein through meat and eggs. As the global population continues to increase, demand for poultry products has risen steadily due to their relatively low cost, reduced environmental footprint, and wide cultural acceptability (Bist et al., 2024; Vlaicu et al., 2024). Meeting this growing demand requires sustained improvements in poultry productivity, which has become a central objective of modern breeding and research programs. One effective strategy for enhancing productivity is the exploitation of genetic variation, particularly in genes that regulate key physiological processes such as growth, metabolism, and immune function.

Among candidate genes associated with growth regulation, the growth hormone secretagogue receptor *(GHSR)* gene has attracted increasing attention due to its role in neuroendocrine control of growth, energy balance, and feed intake (Cruz & Smith, 2007). The *GHSR* gene encodes the receptor for ghrelin, a hormone that stimulates growth hormone (GH) secretion and influences feeding behavior, energy metabolism, and body weight regulation (Cruz & Smith, 2007; Li et al., 2013). Functional polymorphisms in the *GHSR* gene have been shown to influence growth performance and related traits (Khaerunnisa et al., 2017), making it a promising candidate gene for marker-assisted selection in poultry breeding programmes. Improved understanding of *GHSR* gene variation may therefore facilitate the identification of molecular markers associated with economically important traits.

Recent advances in genomics and transcriptomics have further improved knowledge of the genetic mechanisms underlying growth regulation in poultry. Using transcriptomic analysis of the anterior pituitary gland in broiler chickens selected for divergent body weights, Ellestad et al. (2019) demonstrated the importance of the hypothalamic–pituitary axis in regulating growth-related genes (Ellestad et al., 2019). Differences in somatotropic axis gene expression between high- and low-growth lines highlight the complexity of genetic networks governing growth traits. In addition, polymorphisms in the chicken growth hormone (*cGH*) gene have been associated with key production traits, including weight gain, feed conversion efficiency, and carcass composition (Moniem et al., 2023). Furthermore, several quantitative trait loci (QTLs) related to growth, metabolism, and feed efficiency have been identified, reinforcing the role of genetic architecture in shaping performance traits (Rasheed et al., 2020). Given the complex and polygenic nature of growth traits, further investigation of multiple genetic loci, including *GHSR*, is necessary to improve understanding of growth variability in chicken populations.

In Nigeria, chickens are the most widely distributed livestock species, with a population exceeding 166 million (Food Agriculture Organization of the United Nations, 2012). Indigenous Nigerian chickens are highly valued for their adaptability to local environmental conditions, disease tolerance, and contributions to rural livelihoods (Ushie et al., 2025). These native populations exhibit substantial phenotypic and genetic diversity, serving as an important genetic resource for future breeding programs (Yin et al., 2014). However, the expansion of intensive poultry production systems and increasing reliance on high-yield commercial breeds have contributed to the erosion of indigenous genetic diversity. Although commercial breeds offer rapid growth and high productivity, they often show reduced resilience to environmental stressors and diseases, raising concerns regarding long-term sustainability and animal welfare (Adebowale et al., 2024; Efienokwu & Ekerette, 2024).

Therefore, identifying functional genetic variation in key regulatory genes such as *GHSR* is essential for the development of sustainable poultry production systems. Investigating *GHSR* gene polymorphisms across different chicken genotypes can provide insight into the genetic basis of growth traits and support the identification of molecular markers for breeding applications. Thus, the focus of this study was to evaluate genetic polymorphism in the *GHSR* gene among four chicken genotypes and to assess the association between these variations and growth-related phenotypic traits.

2 Material and Methods

2.1 Study location and management of experimental birds

A total of 100 day-old chicks were used in this study, comprising 25 birds each from the Normal Feather (NF), Naked Neck (NN), Frizzle Feather (FF), and Exotic (EX) genotypes. The NF, NN, and FF genotypes were classified as Nigerian indigenous chicken breeds (Ebozoje & Ikeobi, 1995; Ibe, 1993; Peters et al., 2002), whereas the EX genotype was classified as an exotic commercial breed. All birds were raised under an intensive management system at

the Animal House of the Department of Genetics and Biotechnology, University of Calabar, following standard husbandry procedures described by Ushie et al. (2025) (Ushie et al., 2025). Before the arrival of the chicks, the facility was thoroughly fumigated and disinfected using an organophosphate insecticide and Dettol. Relative humidity was maintained between 55 and 65%, while temperature was adjusted based on the age of the birds. During the first week, the ambient temperature was maintained at 32–35 °C and gradually reduced by 2–3 °C each week until a final temperature range of 20–23 °C was achieved. Following a two-week acclimatization period, birds were classified into four groups based on genotype. Within each genotype, individuals were randomly distributed using a completely randomized design implemented in GraphPad Prism (version 10.0.0) to avoid allocation bias. Birds were fed Chikun Super Starter feed containing 23% crude protein and 3000 kcal/kg metabolizable energy during the first six weeks, followed by Chikun Finisher feed for an additional two weeks containing 18% crude protein and 3200 kcal/kg metabolizable energy. Clean drinking water was provided ad libitum throughout the experimental period.

2.2 Measurement of performance traits

Morphometric and growth traits were measured following the protocol described by Ekerette et al. (2025a). Traits measured included Height (H), Body Length (BL), Head Length (HL), Shank Length (SHL), Shank Circumference (SHC), Wing Length (WL), Toe Length (TL), Neck Length (NL), Back Length (BKL), Toe-to-Back Length (TBL), and Body Weight (BW). All measurements were taken in the morning before feeding to minimize variation.

2.3 Genomic DNA extraction

Genomic DNA extraction was carried out using the Quick-DNA Miniprep Plus Kit, following the manufacturer's instructions and the protocol described by Ekerette et al. (2025b) (Ekerette, Etukudo, et al., 2025). Briefly, four volumes of genomic lysis buffer were added to each blood sample and vortexed for 4–5 s. Samples were incubated at room temperature for 5–10 min to facilitate cell lysis, then centrifuged to remove debris. The supernatant was transferred to a Zymo-Spin column placed in a collection tube, then centrifuged at 10000×g for 1 min. The flow-through was discarded, and the column was transferred to a new collection tube. A volume of 200 µL DNA pre-wash buffer was added, then the mixture was centrifuged for 1 min, followed by the addition of 500 µL g-DNA wash buffer and another 1 min of centrifugation. DNA was eluted into a clean microcentrifuge tube using 50 µL DNA elution buffer. After a 30-second incubation at room temperature, the elution step was repeated to maximize DNA yield. Extracted DNA was stored at −20 °C until PCR amplification.

2.4 PCR amplification and GHSR gene sequencing

The *GHSR* gene region was amplified using PCR. Amplification was performed using the forward primer 5′-ACGTTGGATGAGGAAGAGGAAGAACATCGG-3′ and reverse primer 5′-ACGTTGGATGGCCATACCTAGCATCTTCAC-3′, as described by Jin et al. (2014) (Jin et al., 2014). PCR reactions were carried out in a final volume of 25 µL containing 2 µL genomic DNA, 1 µL of 50 mM $MgCl_2$, 1.5 µL of 2 mM dNTPs, 1.5 µL of 10× PCR buffer, 0.4 µL each of forward and reverse primers, 1 µL of STABVIDA proprietary Taq DNA polymerase, and 17.2 µL of nuclease-free water. The annealing temperature was validated through preliminary amplification trials to confirm specificity and reproducibility. An annealing temperature of 54 °C was selected as it produced clear and specific amplification, consistent with the protocol described by Jin et al. (2014) (Jin et al., 2014). PCR conditions consisted of an initial denaturation at 95 °C for 5 min, followed by 25 cycles of denaturation at 94 °C for 40 s, annealing at 54 °C for 45 s, extension at 72 °C for 1 min, and a final extension at 72 °C for 7 min. PCR products were purified using the ExoFast protocol before sequencing. Purified PCR products were sequenced using an ABI 3730xl DNA Analyzer. Sequencing reactions were prepared in a 20 µL volume containing approximately 20 ng of purified PCR product, 8 µL BigDye Terminator Reaction Mix, 8 µL deionized water, and 2 µL primer. Thermal cycling conditions included 25 cycles of 96 °C for 10 s, 60 °C for 5 s, and 60 °C for 4 min. Sequencing was performed using the forward primer.

2.5 Statistical analysis of performance traits

Performance trait data were log-transformed to improve normality and reduce bias, following Ekerette et al. (2025a) (Ekerette, Ushie, et al., 2025). The transformed data were analyzed using one-way analysis of variance (ANOVA) in a completely randomized design, with genotype included as the main effect. Statistical analyses were performed using the General Linear Model (GLM) procedure in SAS software (Inc, 2008). Mean differences among genotypes

were separated using the least significant difference (LSD) test at a 0.05 probability level.

2.6 Genetic and sequence analysis of the GHSR gene

DNA sequences were visualized and edited using BioEdit software version 7.2.5 (Hall, 1999) and aligned using MEGA software version 7.0 (Kumar et al., 2016). Polymorphism analysis of the *GHSR* gene was conducted using DnaSP version 5.1 (Librado & Rozas, 2009). Phylogenetic relationships and genetic distances among genotypes were estimated using MEGA 7.0. Associations between *GHSR* SNPs and performance traits were evaluated using multiple correlation analysis. For the association analysis, SNPs were coded as categorical independent variables, and performance traits were treated as dependent variables for each genotype.

3 Results

3.1 Growth performance of four chicken genotypes

Growth performance traits of the four chicken genotypes are presented in Table 1. Height and body length were significantly higher in the EX genotype compared with the three indigenous genotypes (NF, FF, and NN), which did not differ significantly from one another ($p>0.05$). A similar pattern was observed for head length, with the EX chickens recording significantly higher values ($p<0.05$). Shank length was significantly higher in the EX genotype, followed by the NN chickens ($p<0.05$). Shank circumference did not differ among the indigenous genotypes but was significantly lower than that of the EX chickens. Wing length was highest in the EX genotype, followed by the NF and NN genotypes, whereas FF chickens recorded the lowest values ($p<0.05$). Toe length was also significantly higher in the EX genotype. Neck length was statistically similar among the EX, NF, and NN genotypes. Back length was similar between EX and NN chickens, but higher than the NF and FF genotypes. Toe-to-back length was significantly higher in the EX genotype. Body weight was highest in the EX chickens, while the three indigenous genotypes showed statistically similar values ($p>0.05$).

Table 1. Growth performance and morphometric traits of four chicken genotypes

	H	BL	HL	SHL	SHC	WL	TL	NL	BKL	TBL	BW (kg)
NF	28.8^{ab} ± 0.62	33.40^{b} ± 0.50	9.55 ± 0.19^{b}	6.70^{b} ± 0.16	4.33 ± 0.13^{b}	24.24 ± 0.39^{b}	9.33 ± 0.27^{ab}	9.25 ± 0.16^{a}	23.83 ± 0.37^{b}	20.98 ± 0.58^{b}	0.87 ± 0.07^{b}
FF	27.44^{bc} ± 0.59	32.97^{b} ± 0.72	9.41 ± 0.20^{b}	6.82^{b} ± 0.18	4.01 ± 0.16^{b}	22.88 ± 2.05^{c}	9.18 ± 0.29^{bc}	8.91 ± 0.14^{b}	23.56 ± 0.56^{b}	20.09 ± 0.45^{b}	0.83 ± 0.06^{b}
NN	29.17^{ab} ± 1.35	32.83^{b} ± 2.34	8.92 ± 0.54^{b}	7.33^{ab} ± 0.33	4.00 ± 0.73^{b}	24.42 ± 2.22^{b}	10.00 ± 0.32^{ab}	9.83 ± 0.28^{a}	25.58 ± 0.47^{a}	19.42 ± 0.73^{b}	0.93 ± 0.05^{b}
EX	29.71^{a} ± 0.53	35.47^{a} ± 0.50	10.16^{a} ± 0.17	7.58^{a} ± 0.19	5.39 ± 0.11^{a}	25.61 ± 0.35^{a}	10.03 ± 0.22^{a}	9.38 ± 0.18^{a}	25.26 ± 0.47^{a}	22.58 ± 0.35^{a}	1.63 ± 0.002^{a}

Values are presented as log-transformed means ± standard error (SE). Mean values with different superscripts within the same column differ significantly *(P < 0.05)*. H (Height); BL (Body length); HL (Head length); SHL (Shank length); SHC (Shank circumference); WL (Wing length); TL (Toe length); NL (Neck length); BKL (Back length); TBL (Toe to back length); BW (Body weight)

3.2 Genetic polymorphism of the GHSR gene

Genetic polymorphism parameters of the *GHSR* gene across the four chicken genotypes are summarized in Table 2. The highest number of polymorphic sites was observed in the NN genotype, followed by the NF and EX genotypes, while the FF genotype recorded the lowest number (450, 449, 262, and 189, respectively). The highest number of haplotypes was observed in the EX genotype, followed by the FF genotype, whereas the NF and NN genotypes each recorded two haplotypes. Haplotype diversity was highest in the EX genotype (0.946±0.006), followed by the FF genotype (0.644±0.023). Nucleotide diversity was highest in the NF genotype (0.130±0.0001), which was identical to that observed in the NN genotype. Sequence conservation was highest in the FF genotype (88.7%), followed by the EX genotype (76.2%).

Table 2. Genetic diversity and polymorphism of the GHSR gene in four chicken genotypes

Polymorphism indices	NF	FF	NN	EX
No of sequences	25	25	25	25
Number of sites	677	662	671	671
Monomorphic sites	228	473	221	409
Polymorphic sites	449	189	450	262
Singleton variable sites	449	78	450	83
Parsimony information sites	0	111	0	179
Number of haplotypes	2	4	2	8
Haplotype (gene) diversity (Hd)	0.200 ± 0.024	0.644 ± 0.023	0.200 ± 0.024	0.946 ± 0.006
Nucleotide diversity (Nu)	0.131 ± 0.0001	0.043 ± 0.005	0.130 ± 0.011	0.0724 ± 0.007
Average number of nucleotide difference	88.40	28.178	87.600	47.393
Sequence conservation	0.421 (42.1%)	0.887 (88.7%)	0.350 (51.1%)	0.762 (76.2%)
Minimum number of recombination	0	12	0	31

3.2 Genetic distance among chicken genotypes

Genetic distances among the four chicken genotypes are presented in Table 3. The highest genetic distance (0.109) was observed between the NF and EX genotypes, followed by a distance of 0.078 between the NN and EX genotypes. The lowest genetic distance (0.021) was recorded between the FF and NN genotypes.

Table 3. Pairwise genetic distances among four chicken genotypes inferred from the GHSR gene

	NF	FF	NN	EX
NF	0.000			
FF	0.069	0.000		
NN	0.068	0.021	0.000	
EX	0.109	0.068	0.078	0.000

3.3 SNP variation in the GHSR gene

The distribution of SNPs identified in the *GHSR* gene across the four chicken genotypes is presented in Tables 4 and 5. In the FF genotype, a total of 189 SNPs were detected, of which 165 (87.30%) were non-synonymous, and 24 (12.70%) were synonymous. Transversion mutations accounted for 130 (68.78%) SNPs, while transition mutations accounted for 59 (31.22%). In the EX genotype, 262 SNPs were identified, comprising 220 (83.97%) non-synonymous and 42 (16.03%) synonymous mutations. Transversions were more frequent (202; 77.10%) than transitions (60; 22.90%). The NN genotype had 450 SNPs, including 443 (98.44%) non-synonymous and 7 (1.56%) synonymous mutations. Transversion mutations accounted for 287 (63.78%), while transitions accounted for 163 (36.22%). In the NF genotype, 449 SNPs were identified, with 447 (99.55%) non-synonymous and 2 (0.45%) synonymous mutations. Transversions (274; 61.02%) were more prevalent than transitions (175; 38.98%).

3.4 Phylogenetic relationships among chicken genotypes

Phylogenetic relationships among the four chicken genotypes are illustrated in Figures 1 and 2. Figure 1 revealed two distinct clusters, with all indigenous genotypes grouped within a single major cluster, whereas the EX genotype formed a separate cluster. In Figure 2, three main clusters were observed. Cluster I comprised two EX genotypes (EX_GRH-9 and EX_GRH-18) with a bootstrap support of 95%. Cluster II consisted of two sub-clusters, with all NN genotypes grouped within one sub-cluster. Cluster III also contained two sub-clusters, with all NF genotypes clustering together. The FF and EX genotypes were observed to intermingle within some sub-clusters, whereas NN and NF genotypes formed more distinct clusters.

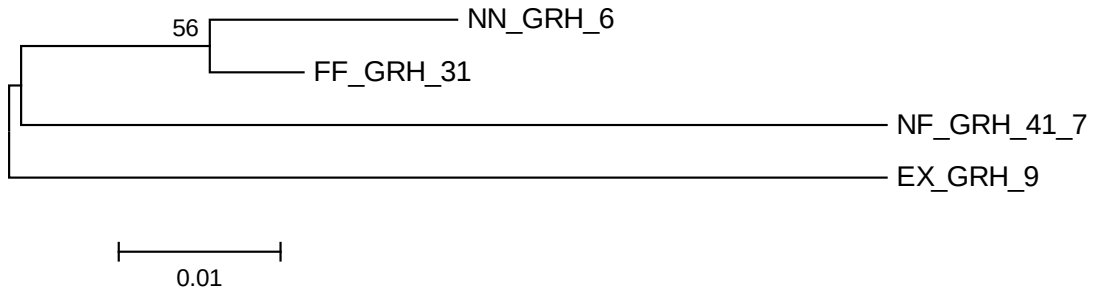

Figure 1. Maximum likelihood phylogenetic tree of four chicken genotypes based on the GHSR gene

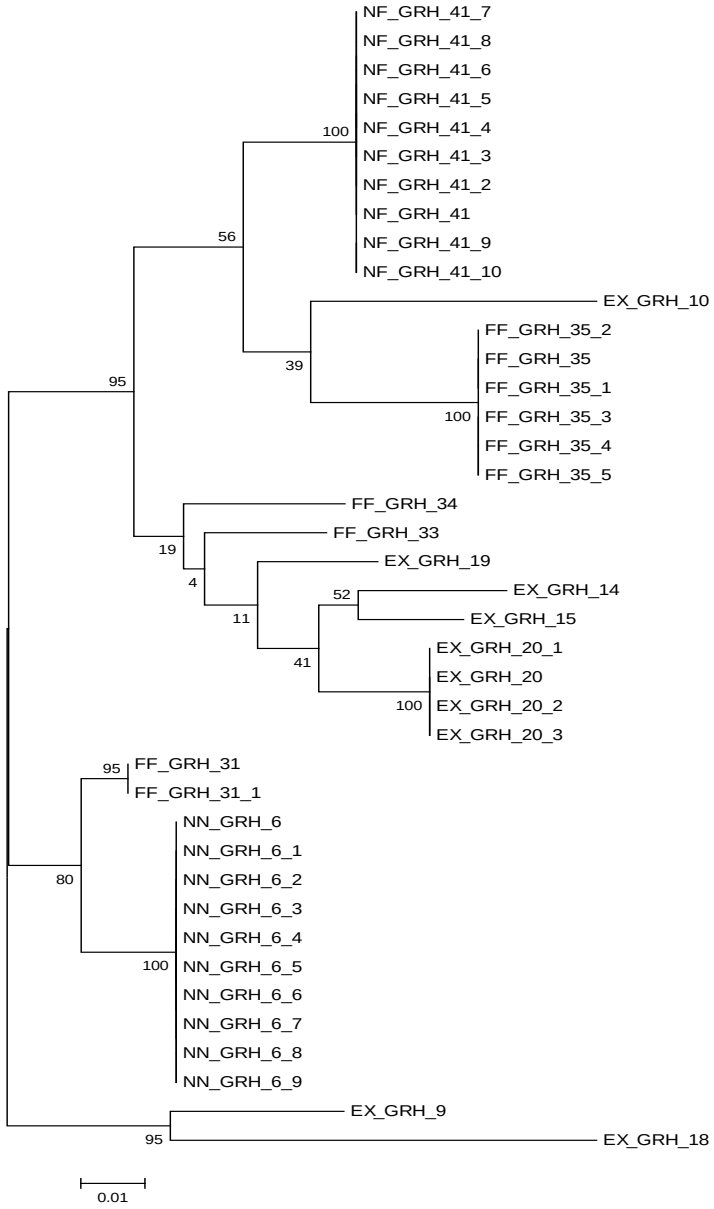

Figure 2. Maximum likelihood phylogenetic tree of chicken genotypes (all individuals) based on the GHSR gene

3.5 Association between SNPs and performance traits

Associations between SNP variation and performance traits are presented in Table 6. No significant association was observed between SNPs and height across the genotypes. SNPs were significantly associated with body length in all genotypes (p<0.05) except the NN genotype (p<0.05). No significant associations were detected between SNPs and head length, shank length, shank circumference, or wing length across the genotypes. However, in the EX genotype, SNPs were significantly associated with shank length and shank circumference. Significant positive associations were observed between SNPs and total length in the FF, NN, and EX genotypes. SNPs were significantly associated with back length in the NF and FF genotypes. In addition, a strong and positive association was observed between SNPs and body weight across all genotypes (p<0.05).

4 Discussion

Chickens play a vital role in food security, particularly in Africa, where indigenous chicken breeds contribute substantially to household nutrition and rural livelihoods (Food Agriculture Organization of the United Nations, 2000). In Nigeria, indigenous chickens are commonly raised under backyard systems and serve as an important source of animal protein (Ajala et al., 2021). These chickens exhibit considerable morphological variation, which has attracted interest due to its potential influence on growth performance and productivity. Morphological traits in chickens are shaped by genetic makeup, environmental conditions, and genotype–environment interactions (Shafiq et al., 2022). In the present study, significant differences were observed in growth performance among the four chicken genotypes evaluated. The EX genotype consistently outperformed the indigenous genotypes across all morphological traits measured. This finding aligns with expectations, given the long-term genetic improvement and selective breeding associated with exotic chicken lines (Afifian et al., 2024; Ahmadzadeh et al., 2025). Despite this, the indigenous genotypes demonstrated substantial phenotypic variation in growth traits, suggesting the presence of valuable genetic resources that could be exploited in breeding programs. The body weights recorded for the indigenous genotypes in this study were lower than the range of 2.1–2.2 kg reported by Balcha et al. (2022) (Balcha et al., 2022) for indigenous chickens in Southwest Ethiopia and lower than the values reported by Hassan et al. (Hassan et al., 2020) for chickens reared across agro-ecological zones in Niger. Comparative studies have reported varying outcomes regarding morphological differentiation among indigenous chicken populations. Adekoya et al. (Adekoya et al., 2013) observed a high level of morphological discrimination among Nigerian indigenous chicken types and recommended the use of molecular tools to validate phenotypic variation. Conversely, Tabassum et al. (Tabassum et al., 2014) reported no significant morphological differences among indigenous chicken genotypes in Bangladesh. Differences between the present findings and previous reports may be attributed to variations in environmental conditions, management practices, feed quality, and genetic background. Environmental factors such as temperature and humidity, as well as genotype–environment interactions, are known to influence growth performance in chickens (Abebe et al., 2024; Shafiq et al., 2022). The observed phenotypic diversity highlights the importance of indigenous chicken populations as reservoirs of adaptive traits that can support sustainable genetic improvement.

Genetic variation within and among livestock populations is fundamental to long-term genetic progress and adaptability. Loss of genetic diversity through inbreeding or uncontrolled mating can negatively affect productivity and resilience (Mehrbani, 2020; Tongsiri et al., 2019). In this study, genetic diversity was assessed using haplotype and nucleotide diversity indices derived from the *GHSR* gene. Haplotype diversity ranged from 0.200 to 0.964, while nucleotide diversity ranged from 0.043 to 0.130, indicating substantial genetic variability among the genotypes. These values are notably higher than those reported by Ikpeme et al. (2021) (Ikpeme et al., 2021), who analyzed the mitochondrial cytochrome b gene. Such differences are not unexpected given the distinct biological characteristics of nuclear and mitochondrial genomes. Mitochondrial DNA is maternally inherited, haploid, and does not undergo recombination, resulting in a smaller effective population size and mutation dynamics that differ from those of nuclear DNA (Ye et al., 2022). In contrast, the nuclear *GHSR* gene is biparentally inherited and subject to recombination, which can increase allelic diversity (Robbins et al., 2023). Furthermore, as a functional nuclear gene involved in growth regulation, *GHSR* may experience selective pressures distinct from those acting on mitochondrial genes primarily associated with cellular respiration. These differences in inheritance patterns, mutation processes, and functional constraints likely contribute to the observed variation in the diversity estimates. The predominance of

non-synonymous mutations observed in this study suggests functional variation within the *GHSR* gene, which may contribute to phenotypic differences among the genotypes. However, this relatively high proportion of non-synonymous mutations should be interpreted cautiously, as it reflects variation within the specific coding fragment of the *GHSR* gene analyzed rather than genome-wide averages. The limited number of total polymorphic sites identified in this region may inflate proportional estimates of non-synonymous substitutions. Furthermore, as *GHSR* is a functional gene involved in growth regulation, it may be subject to selective pressures that favor amino acid-altering mutations. Similar patterns of genetic variability have been reported in chicken populations using candidate gene approaches (Choudhuri, 2014; Ekerette et al., 2026; Ikpeme et al., 2018). The higher haplotype diversity observed in the EX and FF genotypes suggests a broader genetic base, which may be advantageous for selection and breeding programs. Genetic distance analysis further revealed that the EX and NF genotypes were the most genetically divergent, while the FF and NN genotypes were closely related. These findings are consistent with earlier studies that reported genetic similarity between FF and NN chickens and greater divergence between indigenous and exotic genotypes (Ikpeme et al., 2021; Ushie et al., 2025). The relatively high genetic distance between EX and NF chickens observed in this study exceeded values reported for other chicken comparisons, such as dwarf and broiler chickens (Machete et al., 2021). Phylogenetic clustering patterns corroborated these genetic distance estimates, further confirming the close evolutionary relationship between FF and NN genotypes and the distinctiveness of the EX genotype.

Molecular markers have been widely applied to accelerate genetic gains in economically important traits, including growth and production performance in poultry (Anh et al., 2015; Sharma et al., 2023). Understanding the functional roles of candidate genes underlying these traits is therefore essential for the development of effective breeding programs. There were significant associations detected between SNPs in the *GHSR* gene and several growth-related traits, including body length, shank length, shank circumference, toe length, back length, and body weight in some of the chicken genotypes. These results are consistent with previous findings reporting significant associations between SNPs in growth-related genes and performance traits in chickens. Anh et al. (2015) observed significant associations between polymorphisms in the chicken growth hormone (cGH) gene and body weight in broilers (Anh et al., 2015; Sharma et al., 2023), while Li et al. (2006) reported associations between ghrelin gene polymorphisms and growth traits in Chinese chicken breeds (Li et al., 2006). Similarly, SNPs in the cGH gene have been linked to body weight, egg production, and disease resistance in different chicken populations (Ghelghachi et al., 2013; Ilhan & Aygun, 2025; Okafor et al., 2019; Su et al., 2014). While significant associations were identified between *GHSR* SNPs and several growth-related traits, correction for multiple testing was not applied in the association analysis in our study. Given the number of SNPs and traits evaluated, this may increase the likelihood of Type I errors. Therefore, these associations should be interpreted with caution and validated in larger, independent populations to enhance practical application in breeding programs. There was a higher frequency of transversion mutations compared to transition mutations, a pattern that agrees with the findings of Li et al. (2006) (Li et al., 2006) but contrasts with the higher transition rates reported by Wheto et al. (2022) (Wheto et al., 2022). Such differences in mutation patterns across studies and genotypes highlight the complexity of the genetic architecture underlying growth traits and may reflect population-specific evolutionary pressures. Generally, the SNPs identified in the *GHSR* gene appear to contribute to phenotypic variation in growth performance across the chicken genotypes, affirming the potential of this gene as a molecular marker for the genetic improvement of indigenous chicken populations.

5 Conclusion

This study provides insight into the morphological and genetic variation among indigenous and exotic chicken genotypes, integrating phenotypic performance data with polymorphism analysis of the GHSR gene. The identification of significant associations between specific GHSR SNPs and key growth-related traits highlights the potential functional relevance of this gene in growth regulation. From a practical perspective, the significantly associated SNPs represent promising candidate markers for incorporation into marker-assisted selection strategies for improving growth performance while conserving indigenous genetic resources. The observed genetic diversity within the indigenous chicken populations further demonstrates their value for sustainable breeding and long-term adaptability. Further studies involving larger chicken populations would be valuable to confirm the consistency of the identified associations and enhance their applicability in breeding

programs. In addition, complementary functional investigations could provide deeper insight into the biological mechanisms underlying the observed SNP–trait relationships.

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Conflict of Interest

We declare that no conflict of interest.

Author Contributions

E.E.E. and E.V.I. conceived and designed the research and methodology. J.N.E. conducted the experiment, performed the literature search, and drafted the original manuscript. E.E.E. and E.V.I. supervised the research. O.M.E., L.E.O., N.A.N., S.E.A., and P.C.C. assisted with the literature review and editing of the original draft. E.E.E. performed the statistical analyses and data interpretation. J.N.E., E.E.E. and E.V.I. edited and finalized the manuscript. All authors read and approved the final version of the manuscript before submission for publication.

Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Ethical Considerations

The ethical handling of experimental animals was approved by the Research Ethics and Linkage Committee of the Faculty of Biological Sciences, University of Calabar (approval number: FBS/RELC/2023/001). All procedures involving animals were conducted in accordance with the ARRIVE guidelines for animal experimentation.

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