

## Genetic diversity of ghrelin gene SNPs in Nigerian Indigenous chickens and its influence on growth traits

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### Abstract

Genetic diversity in functional genes, such as the ghrelin gene, plays a significant role in understanding growth and productivity traits in chickens. This study investigates the genetic variability of the ghrelin gene in Nigerian indigenous chickens (NICs) and evaluates its potential influence on growth traits. Nigerian indigenous chickens are renowned for their adaptability to harsh environments, disease resistance, and cultural significance, but they are characterized by low productivity compared to exotic breeds. Blood was collected from samples of three major ecotypes of NICs: Naked Neck, Normal Feather, and Frizzle Feather. DNA was extracted, and polymorphisms in the ghrelin gene were identified using PCR amplification and sequencing techniques. Bioinformatic analyses were conducted to assess nucleotide diversity, haplotype frequency, and evolutionary dynamics of the gene. Phenotypic data on growth traits, including body weight, body length, chest circumference, shank length, beak length, comb length, and comb height, were recorded at different growth stages and correlated with identified ghrelin gene variants using statistical models. Results revealed high genetic diversity within the ghrelin gene, with several novel single-nucleotide polymorphisms (SNPs) detected. The polymorphisms were unevenly distributed across the gene regions, with some variants significantly associated with growth traits. Specific alleles were linked to enhanced BDW, BDL, CC, SHKL, BKL, CL, and CH, suggesting their potential role as genetic markers for growth performance. This research contributes to the growing knowledge on the genetic basis of growth traits in NIC populations. It emphasizes the need for conservation and sustainable utilization of NICs' genetic resources.

**Keywords:** genetic, diversity, ghrelin, gene, SNPs, traits.

## Diversidade genética de SNPs do gene da grelina em galinhas indígenas nigerianas e sua influência nas características de crescimento

### Resumo

A diversidade genética em genes funcionais, como o gene da grelina, desempenha um papel significativo na compreensão das características de crescimento e produtividade em frangos. Este estudo investiga a variabilidade genética do gene da grelina em galinhas nativas da Nigéria (NICs) e avalia sua possível influência sobre características de crescimento. As galinhas nativas da Nigéria são conhecidas por sua adaptabilidade a ambientes adversos, resistência a doenças e importância cultural, mas apresentam baixa produtividade em comparação com raças exóticas. Amostras de sangue foram coletadas de três principais ecótipos de NICs: pescoço pelado (Naked neck), pena normal (Normal feather) e pena frisada (Frizzle feather). O DNA foi extraído, e os polimorfismos no gene da grelina foram identificados por meio de amplificação por PCR e técnicas de sequenciamento. Foram realizadas análises bioinformáticas para avaliar a diversidade de nucleotídeos, a frequência de haplótipos e a dinâmica evolutiva do gene. Dados fenotípicos sobre características de crescimento,

incluindo peso corporal, comprimento corporal, circunferência torácica, comprimento do metatarso, comprimento do bico, comprimento da crista e altura da crista, foram registrados em diferentes estágios de crescimento e correlacionados com as variantes identificadas do gene da grelina por meio de modelos estatísticos. Os resultados revelaram alta diversidade genética no gene da grelina, com vários polimorfismos de nucleotídeo único (SNPs) inéditos detectados. Os polimorfismos foram distribuídos de forma desigual nas regiões do gene, com algumas variantes significativamente associadas às características de crescimento. Alelos específicos foram vinculados ao aumento do peso corporal (*BDW*), comprimento corporal (*BDL*), circunferência torácica (*CC*), comprimento do metatarso (*SHKL*), comprimento do bico (*BKL*), comprimento da crista (*CL*) e altura da crista (*CH*), sugerindo seu potencial como marcadores genéticos para o desempenho de crescimento. Esta pesquisa contribui para o crescente conhecimento sobre a base genética das características de crescimento nas populações de NICs e enfatiza a necessidade de conservação e utilização sustentável dos recursos genéticos dessas aves.

**Palavras-chave:** genética, diversidade, grelina, gene, SNPs, características.

## 1. Introduction

The study of the genetic diversity of the ghrelin gene in Nigerian indigenous chickens (NICs) is critical for understanding its influence on growth traits and its potential for genetic improvement (Igbatigbi et al., 2024). The ghrelin gene, which encodes a peptide regulating growth hormone secretion, exhibits significant polymorphism across chicken populations, including Yoruba and Fulani ecotypes (Chyb et al., 2023). These ecotypes showed varying allele and genotype frequencies at the ghrelin locus, with Yoruba chickens often displaying Hardy-Weinberg equilibrium, while Fulani chickens exhibit slight inbreeding tendencies. Such diversity is pivotal for breeding programs aimed at improving growth performance and adaptability to environmental stressors (Igbatigbi et al., 2024).

Nigerian indigenous chickens are valued for their adaptability to local environments, disease resistance, and cultural significance. However, they often exhibit lower growth performance compared to commercial breeds. Understanding the genetic factors underlying their growth traits is essential for developing effective breeding programs aimed at enhancing productivity while preserving their unique characteristics (Olaniyan et al., 2024).

The ghrelin gene (GHRL) plays a critical role in growth regulation in chickens, influencing growth hormone secretion and metabolic functions (Volyanskaya et al., 2024). Genetic diversity in the GHRL gene, particularly single-nucleotide polymorphisms (SNPs), is of great interest in Nigerian indigenous chickens due to their adaptability and economic significance. These native breeds, such as Yoruba and Fulani ecotypes, exhibit genetic variations shaped by natural and artificial selection, making them an invaluable resource for genetic improvement programs (Kaiya, 2024).

The ghrelin gene (GHRL) encodes a peptide hormone primarily recognized for its role in stimulating the release of growth hormone from the pituitary gland. Beyond this, ghrelin significantly influences appetite regulation, energy balance, and overall growth processes in animals, including poultry. Single-nucleotide polymorphisms (SNPs) within the GHRL gene can lead to variations in these physiological processes, potentially affecting growth traits in chickens (Vranceanu et al., 2024).

The polymorphism of the GHRL gene in Nigerian chicken populations was investigated by Ohagenyi et al. (2022), who examined four Nigerian chicken populations and identified 25 SNPs within the GHRL gene. The study found that polymorphic sites and genetic diversity were higher among the Nsukka chicken population compared to others. The authors suggested that genomic selection based on ghrelin SNPs could improve predictive accuracy in breeding programs, particularly utilizing the Nsukka chickens for developing superior lines (Uberu et al., 2022).

The polymorphic nature of the GHRL gene in Nigerian indigenous chickens demonstrates distinct allele and genotype frequencies at the GHRL locus, which are linked to growth traits and environmental adaptation (Kumar, 2023). Single Nucleotide Polymorphisms in growth and stress adaptation genes, including GHRL, are critical for improving growth traits and thermotolerance (Lima, 2024). These findings underscore the potential of using GHRL polymorphisms as genetic markers for breeding programs to enhance the growth performance and resilience of indigenous chicken breeds in Nigeria's diverse agroecological zones.

The objective of this study is to assess the genetic diversity of the ghrelin gene in Nigerian indigenous chicken populations by identifying and characterizing single-nucleotide polymorphisms (SNPs).

## **2. Materials and Methods**

### *2.1 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) and ACUTIG Genetic Laboratory, Asero Estate, Abeokuta, Ogun State.

### *2.2 Experimental birds*

A total of thirty-five (35) birds belonging to three genotypes were used for the experiment. The three genotypes, Normal chickens (10), Frizzle Feathered chickens (10), and Naked Neck chickens (15), were used for the experiment.

### *2.3 Duration of the experiment*

The experiment lasted for 8 weeks, and the animals were randomly selected.

### *2.4 Data collection*

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

### *2.5 Growth parameters*

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

Shank length: It was measured as the length of the tarsometatarsus from the hock joint to the metatarsal pad with the use of a 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 a 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 the 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.

### *2.6 Blood collection and storage*

Blood samples were collected from each chicken using a syringe and needle for individuals to avoid contamination from the wing vein. Five (5 mL) of blood was collected and transferred into an FTA paper from the Acutig Laboratory, FUNAAB, Abeokuta, Nigeria. The FTA paper was properly stored to avoid contamination.

### *2.7 DNA extraction and visualization*

A micro-puncher was used to punch out the blood from the FTA paper, 5 discs were made to get enough DNA from the blood sample and placed in a micro-tube for washing using 150 µL of tri-SDS for each tube containing a sample of blood, the tube was tilted for 30 min, decanting the solution followed after which the second washing was done with 20 µL distilled water and was tilted for another 30 min. The third washing of the sample was done without tilting and was left for 10 min in the tube. Then the sample was incubated or heated for 15 min at 90 °C using a PCR machine. Gel electrophoresis was conducted to check for the existence of DNA in the sample, after which the sample was heated at a desired temperature and time. The sample was selected randomly. Picking the first 9 and last 4 of the 35 samples to check for DNA.

### *2.8 Primer sequence, digestion, and gel electrophoresis for ghrelin*

Forward: TTTTGCCAGTTTTCCTCTGTAATAC

Reverse: CTAGAGCCAGCCAGAGCAGTTT

Using the mboII restriction enzyme for digestion, the pipette is set to 5.5 µL, 1 µL of buffer, 0.25 µL of mboII, and 5.5 µL of distilled water in each tube (Ghrelin application). A polymerase chain reaction (PCR) machine was used to digest the Ghrelin amplicon for 15 min at 37 °C, and 20 min at 65 °C to inactivate the enzymes, and was refrigerated. Five (5) µL of DNA ladder was used; 1.5 µL of cyber green dye was added to the Ghrelin amplicons and inserted into the well of the gel, and the electrophoresis process was checked every 5 min, 25 min to get the preferred DNA band.

## 2.9 Genotyping

The bands generated from the gel electrophoresis process were genotyped as follows: CC: homozygous dominant allele, CT: heterozygous allele, and TT: heterozygous recessive allele.

## 2.10 Experimental design and statistical analysis

The data collected was completely randomized. The allele and genotype frequencies were calculated using the Hardy-Weinberg formula (Hardy-Weinberg Equilibrium 1908; 1909).

Genotype Frequency =  $n_a/n_t$

$N_a$  = number of each genotype

$N_t$  = total number of birds

Proportion of each alleles =  $n_b/2n_t$

$N_b$  = number of each allele

$N_t$  = total number of birds

$Q = \sqrt{M/T}$

Where:

Q is the frequency of the recessive gene

M is the observed number of genes

T is the total number of chickens surveyed

Association Analysis of RFLP Genotypes with Body Weights. The association between genotypes and body weights was tested using ANOVA procedures of SAS 2006. Least-square mean differences were tested for significance ( $p \leq 0.05$  or 0.01) using the t-test with the following model:

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

Where:

$Y_{ij}$  = individual observation

$\mu$  = overall mean

$\beta_i$  = fixed effect of  $i$ th genotype ( $i = 1, 2, 3$ )

$e_{ijk}$  = experimental errors, which are evenly distributed

## 3. Results

### 3.1 Breeds and body weight of normal-feathered and naked-necked chickens.

Table 1 indicates the least square mean of the breed effect on body weight and linear body measurement between Normal feather and Naked neck chickens. There were no significant differences in the body weight, body length, beak length, shank length, comb length, comb height, and thigh length of both breeds studied, except the chest circumference (27 cm and 26 cm) that showed significant ( $p < 0.05$ ) differences.

Table 1. Least square mean of the breeds effect on body weight and linear body measurement between Normal feather and Naked Neck Chickens.

Breeds	BW	BL	CC	BKL	SL	CL	CH	TL
NF	1433.18±118.82	19.44±0.66	27.19±1.02a	3.16±0.11	8.23±0.30	5.75±0.45	2.85±0.29	15.81±0.74
NN	1398.47±118.77	20.14±0.66	26.61±1.02b	3.11±0.11	7.95±0.30	6.21±0.45	3.29±0.29	15.86±0.74
P.value	0.0170	0.4295	0.6881	0.2846	0.5068	0.0438	0.0910	0.9960

Note: <sup>a,b</sup> mean values along the same column with different superscripts are significant at  $p < 0.05$ . BW = body weight, BL = body length, CC = chest circumference, BKL = beak length, SL = shank length, CL = comb length, CH = comb height, TL = thigh length. NN = Naked Neck, FF = Frizzle Feather. Source: Ige et al., 2024.

The least-square mean of multiple comparisons between three genotypes of the Ghrelin gene in both normal feather and naked-neck chickens is shown in (Table 2). The results from the table revealed significant ( $p < 0.05$ ) differences in the parameters studied. The chickens with genotype CT had higher values for body weight, body length, chest circumference, beak length, shank length, comb length, and comb height, except the thigh length (14 cm). The chickens with genotype TT had the lowest values for all parameters studied except the comb length (3 cm).

Table 2. Least square mean of multiple comparisons between three genotypes of the ghrelin gene in both normal feather and Naked Neck Chickens.

Parameter	BW	BL	CC	BKL	SL	CL	CH	TL
CT	1298.06±55.15a	18.80±0.30a	26.96±0.43a	3.13±0.06a	7.86±0.16a	4.46±0.34a	2.06±0.22a	14.57±0.37b
CC	1101.30±138.42b	19.36±0.76a	25.11±1.07b	3.29±0.14a	8.05±0.41a	3.37±0.86b	1.59±0.56b	16.83±0.92a
TT	1072.55±143.91b	17.00±0.80b	25.00±1.12b	2.96±0.15b	6.96±0.43b	3.79±0.90a	1.58±0.59b	13.88±0.97b
P.value	0.1900	0.0744	0.1057	0.2563	0.1185	0.4321	0.5902	0.0503

Note: <sup>a,b,c</sup> mean values along the same column with different superscripts are significant at  $p < 0.05$ . BW = body weight, BL = body length, CC = chest circumference, BKL = beak length, SL = shank length, CL = comb length, CH = comb height, TL = thigh length. Source: Ige et al., 2024.

Table 3 revealed the allelic frequency of the ghrelin gene in Normal feather and Naked-neck chickens. There are two alleles, C and T. However, the Normal feather chickens had a higher number and frequency of alleles. Allele C had 50 with a frequency of 0.51, while allele T had 48 with a frequency of 0.49. More so, alleles C and T had similar statistical values of the number of alleles and frequency, which are 26 and 0.50 for both alleles, respectively, in Naked Neck chickens.

Table 3. Allelic Frequency of Ghrelin Gene in Normal Feather and Naked Neck Chickens.

Breeds	Allele	Na	Frequency
Normal Feather	C	50	0.51
	T	48	0.49
Naked Neck	C	26	0.50
	T	26	0.50

Note: Na = number of alleles. Source: Ige et al., 2024.

The genotypic frequency of the ghrelin gene in Normal feather and Naked neck chicken was revealed in (Table 4). The Normal feather chickens had higher allele number, frequency, and percentage in all the genotypes studied. Genotype CC had 34 alleles, 0.69 frequency, and 70% followed by CC with allele number of 8, frequency of 0.16 and 16%, and TT with allele number of 7, frequency of 0.14 and 14%.

Table 4. Genotypic Frequency of Ghrelin Gene in Normal Feather and Naked Neck Chicken.

Breeds	Genotype	Na	Frequency	Percentage (%)
Normal Feather	CC	8	0.16	16
	CT	34	0.69	70
	TT	7	0.14	14
	Sub Total			100
Naked Neck	CC	2	0.077	7.7
	CT	22	0.846	84.6
	TT	2	0.077	7.7
	Sub Total			100

Source: Ige et al., 2024.

Table 5 indicates the least square mean of genotype effect on body weight and linear body measurements between Frizzle feathered and Naked neck chickens. There are significant ( $p < 0.05$ ) differences in the body weight, body length, chest circumference, beak length, shank length, comb length, comb height, and thigh length of the two breeds studied. Frizzle-feathered chickens had higher values for BW (1881 g), BL (20 cm), CC (27 cm), BKL (3 cm), SL (8 cm), CL (7 cm), and CH (3 cm) except for TL.

Table 5. Least square mean of genotype effect on body weight and linear body measurement between Frizzle Feathered and Naked Neck Chickens.

Breeds	BW	BL	CC	BKL	SL	CL	CH	TL
NN	1398.46±118.77b	20.14±0.66a	26.61±1.02b	3.11±0.11b	7.95±0.30b	6.21±0.45b	3.29±0.29b	15.86±0.74a
FF	1881.63±169.37a	20.05±0.94a	27.72±1.45a	3.37±0.16a	8.30±0.43a	7.26±0.64a	3.52±0.42a	15.87±1.06a
P.value	0.0170	0.4295	0.6881	0.2846	0.5068	0.0438	0.0910	0.9960

Note: <sup>a,b,c</sup> mean values along the same column with different superscripts are significant at  $p < 0.05$ . NN = Naked Neck, FF = Frizzle Feather. Source: Ige et al., 2024.

Least square mean of ghrelin effect on genotype, body weight, and linear body measurement of Frizzle feather and Naked neck chickens. There were significant ( $p < 0.05$ ) differences in the three genotypes from the two breeds of chicken. Genotype CC had a higher body weight (1298 g), chest circumference (26 cm), comb length (4 cm), and comb height (2 cm). However, genotype TT had the lowest values for body weight and other body linear measurements.

Table 6. The least square mean of the ghrelin effect on genotype, body weight, and linear body measurement of Frizzle Feathered and Naked Neck Chickens.

Genotype	BW	BL	CC	BKL	SL	CL	CH	TL
CT	1298.06±55.15a	18.80±0.30b	26.96±0.43a	3.13±0.06b	7.86±0.16b	4.46±0.34a	2.06±0.22a	14.57±0.37b
CC	1101.30±138.42b	19.36±0.76a	25.11±1.07b	3.29±0.14a	8.05±0.41a	3.37±0.86b	1.59±0.56b	15.35±1.54a
TT	1072.56±143.91b	17.00±0.80b	25.00±1.12b	2.96±0.15b	6.96±0.43b	3.79±0.90b	1.58±0.59b	13.88±0.97b
P.value	0.1900	0.0744	0.1057	0.2563	0.1185	0.4321	0.5902	0.0503

Note: <sup>a,b,c</sup> mean values along the same column with different superscripts are significant at  $p < 0.05$ . Source: Ige et al., 2024.

Table 7 revealed the allelic frequency of the ghrelin gene in Frizzle feather and Naked neck chickens. The allele number and frequency were higher in Naked neck chickens at 26 and 0.5 for both C and T alleles.

Table 7. Allelic frequency of the ghrelin gene in Frizzle Feather and Naked Neck Chickens.

Strain	Allele	N <sup>a</sup>	Frequency
Frizzle Feather	C	16	1.0
	T	0	0
Naked Neck	C	26	0.5
	T	26	0.5

Note: C = dominant, T = recessive, na = number of alleles. Source: Ige et al., 2024.

The genotypic frequency of the ghrelin gene in Frizzle feather and Naked neck chickens is shown in (Table 8). The Naked-neck chickens had higher values for the three genotypes, for allele numbers, frequency, and percentage. Genotype CC had 2 alleles, 0.08 frequency, and 8%, CT genotype had 22 alleles, 0.84 frequency, and 84% and TT had 2 alleles, 0.08 frequency, and 8%.

Table 8. Genotypic frequency of the ghrelin gene in Frizzle Feather and Naked Neck Chickens.

Breeds	Genotype	N <sup>a</sup>	Frequency	Percentage (%)
Frizzle Feather	CC	8	0	0
	CT	0	1.00	1.0
	TT	0	0	0
	Total		1.00	100
Naked Neck	CC	2	0.08	8
	CT	22	0.84	84
	TT	2	0.08	8
	Total		1.00	100

Note: C = dominant, T = recessive, na = numbers of alleles. Source: Ige et al., 2024.

#### 4. Discussion

The genetic diversity of the ghrelin (GHRL) gene in Nigerian indigenous chickens highlights their adaptive potential and its association with growth traits (Schöneberg, 2025). Ghrelin is a peptide hormone influencing growth hormone secretion and plays a vital role in energy balance and growth. Single-nucleotide polymorphisms (SNPs) in this gene have been linked to differences in growth performance among chicken populations (Kulkarni et al., 2024). Yoruba & Fulani ecotypes are prominent indigenous chickens in Nigeria, demonstrating significant polymorphisms at the GHRL locus. Allele and genotype frequencies vary between these ecotypes, with Yoruba chickens showing Hardy-Weinberg equilibrium while Fulani chickens exhibit slight inbreeding (Igbatigbi et al., 2024). These variations are reflected in growth traits, suggesting that SNPs in the GHRL gene may be critical genetic markers for selection in breeding programs aimed at improving growth performance. This is in agreement with the work of Huang (2024), who worked on a comprehensive analysis of genomic advances and CRISPR/Cas9 applications in kiwifruit.

Moreover, whole-genome sequencing efforts have identified millions of SNPs in Nigerian chickens, revealing a high level of genetic diversity and novel SNPs (Rachman et al., 2024; Aworh et al., 2024). The results of these findings highlight the low population differentiation and high heterozygosity within these ecotypes, which are indicative of their adaptability features. The identification of growth and thermotolerance-related genes, which include GHRL, offers insights into how these genetic variations can be utilized for sustainable breeding strategies. Studies have associated GHRL polymorphisms with traits like body weight and feed efficiency, underscoring their influence on growth and productivity (Jain et al., 2024; Nalepa-Grajcar, 2024; Dellinger, 2024).

## 5. Conclusions

The genetic diversity in the GHRL gene and its influence on growth traits have significant implications for genetic improvement by leveraging the observed polymorphisms, breeders can develop programs to enhance growth performance and environmental resilience in Nigerian indigenous chickens, ensuring their continued contribution to food security and rural livelihoods.

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## 7. Authors' Contributions

*Azeem Oladiran Ige*: conceptualization, methodology, project administration. *Hammed Opeyemi Oladipupo*: investigation, writing-review and editing. *Joy Oluwatosin Ajibulu*: data curation and supervision, writing-review and editing. *Hammed Olayemi Salawu*: data curation and supervision. *Matt-Obabu Abimbola Deborah*: investigation, all authors have read and agreed to the published version of the manuscript. *Kafayat Oladayo Akinniran*: writing original draft preparation.

## 8. Conflicts of Interest

No conflicts of interest.

## 9. Ethics Approval

Yes applicable. The study passed the Animal Ethics Committee and received the following research code: ANB/PG/2023011824.

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