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Ghrelin (*GHRL*) Gene Polymorphism and its Association with Growth and Body Size Parameters in Three Nigerian Chicken Breeds

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RESEARCH ARTICLE

Abstract

This study was conducted to identify ghrelin (*GHRL*) gene polymorphism within exons 2 and 3 in three chicken breeds and to determine its association with growth and linear body measurements. Three hundred one-day-old chicks comprising 100 each of three chicken breeds (FUNAAB Alpha, Noiler and Shika Brown) were used for the research and were raised for eighteen weeks. Fifty birds per breed were sampled for blood collection and Genomic DNA was extracted using Zymo miniprep kit. DNA was amplified and PCR products digested with *Eco72I* restriction enzyme. Growth data were analysed using Generalized Linear Model of SAS. Noiler had the highest (P <0.05) growth traits from 10 to 18 weeks among studied breeds. *GHRL* gene polymorphism had no significant (P >0.05) effect on growth traits. However, interaction between *GHRL* gene polymorphism and chicken breeds revealed that Noiler chickens AA and AB had the best (P <0.05) productive performance from 2 to 18 weeks.

Keywords: *Ghrelin* gene; breed; growth; parameters; chickens.

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INTRODUCTION

GHRL is an acylated peptide that stimulates the release of growth hormone from the pituitary. The release of growth hormone from the pituitary might be regulated not only by hypothalamic growth hormone-releasing hormone, but also by ghrelin derived from the stomach. In addition, *GHRL* stimulates appetite by acting on the hypothalamic arcuate nucleus, a region known to control food intake. *GHRL* is orexigenic (appetite-stimulating), it is secreted from the stomach and circulates in the bloodstream under fasting conditions, indicating that it transmits a hunger signal from the periphery to the central nervous system. Taking into account all these activities, ghrelin plays important roles in maintaining growth hormone (GH) release and energy homeostasis in vertebrates (Kojima and Kangawa, 2005). *GHRL* also increased feeding in chickens that are genetically deficient in growth hormone (Nakazato et al., 2001).
Meat of chickens is considered healthier than red meat because of comparably low fat and cholesterol content, also it is preferred for its low price and rare religious restrictions (Jaturasitha et al., 2008). Chickens largely dominate flock composition and make up about 98% (Gueye, 2003) of the total poultry numbers (chickens, ducks and turkeys) including the indigenous breeds kept in Africa.

Genetic improvement of animals has greatly been encouraged as it has proved very efficient in improving the productivity, health status and general management of animals. Growth hormone GH, *GHRL*, and leptin receptor genes have been identified to be significantly related to chicken fatness (Xu et al., 2013). Therefore, the genes of the somatotropic axis not only affected chicken growth and body composition but also are associated with fatness and muscle fibre traits (Date et al., 2000). Wang et al. (2014) reported that identification of *GHRL* gene which is involved in activities such as energy homeostasis, regulation of body weight and modulation of many physiological processes, will help to improve our locally-adapted birds by producing birds of high-quality and fast-growing breeds/strains. This together with the consideration of other financial factors can help to increase the availability of white meat that is low in cholesterol for everyone, thereby playing a major role in alleviating poverty in the rural environment.

MATERIALS AND METHODS

Experimental site

The experiment was carried out at the Poultry Breeding Unit of the Directorate of University Farms (DUFARMS), Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. The site is located on latitude 7°10'N and 3°2'E in Odeda Local Government Area, Ogun State, Nigeria. This lies in the tropical climate with an average rainfall of 1100 mm, a mean temperature of about 34°C and a yearly average relative humidity of 82%. The vegetation represents an inter-phase between the tropical rainforest and the derived savannah (AGROMET FUNAAB, 2015; Google Earth, 2018).

Experimental birds

A total of 300 one-day-old chicks comprising 100 each of three chicken breeds (FUNAAB Alpha, Noiler and Shika Brown) were procured from three reputable hatcheries (Abeokuta in Ogun State, Ibadan and Awe in Oyo State). Each breed was housed in a separate deep litter pen at one-day-old and the birds were wing-tagged for identification purposes. The birds were reared for a period of 18 weeks following standard routine management practices as described by FAO (2012).

Brooding system

The brooding house was prepared prior to the arrival of the chicks and disinfected. Good bedding material (wood shavings) were laid up to 3 cm height. Light was provided to stimulate and keep the chicks active when eating and also to generate heat in the brooding house. Heat was provided through high voltage bulbs and charcoal heated coal pots as alternative sources of heat for the chicks. Black nylon at the open side of the house were provided to maintain the brooding house temperature and humidity at 33-35°C and 82%, respectively. The brooding stage lasted for three weeks.

Management of birds

The management system used was intensive. Commercial feed and water were provided to bird's *ad libitum*. The three breeds were subjected to the same management system from one-day-old to 18 weeks of age. Water containing anti-stress (vitamin supplement and glucose) was given on the first day of arrival to replenish the energy lost during transportation. On the second day through the sixth day, antibiotics were given to help the chicks adapt well to their new environment. The same vaccination and medication programme was used for the three chicken breeds throughout the experimental period.

Biosecurity measures

At the entrance of the poultry house, a foot bath was placed in order to prevent virulent pathogenic microbes and there was restricted entry of visitors in the brooding house to prevent disease outbreak. Also, the litter was changed when necessary, to ensure good hygiene and prevent wet litter which could lead to bacterial build-up within the pens. This was done regularly to ensure proper hygiene and to prevent bad odour.

Feed and feeding

The birds were fed *ad libitum* with a commercial feed containing 23% crude protein and 2840 kcal/kg metabolisable energy (ME) from one-day-old to 5 weeks of age, and also with commercial feed containing 19% crude protein and 2875 kcal/kg ME from 6 to 18 weeks of age. Clean drinking water was also provided *ad libitum* to all the birds.

Data collection

Individual bird weight (100 per breed) was taken on a weekly basis starting from the 1st week till the 18th week of age. Each bird was weighed with a sensitive scale (Camry IS09001 Dial Spring Scale) calibrated to 5 kg to obtain the body weight, and the linear body measurements (body circumference, breast girth, shank length, thigh length and wing length) were measured on weekly basis using a measuring tape as described by Monsi (1992), Adeleke et al. (2011) and Udeh et al. (2011).

To ensure proper record-keeping, the birds were wing-tagged for identification purposes using different colour tags for each breed which was attached to each bird's wing throughout the experimental period.

Growth performance evaluation

- Body weight (g): A sensitive scale was used to determine an individual bird's weight.
- Body circumference (cm): The circumference of the bird's body was measured from the back to the chest region.
- Breast girth (cm): The measurement of the chest circumference around the deepest region (hind breast).
- Shank length (cm): length from the hock joint to the tarsometatarsus of any leg.
- Thigh length (cm): The thigh length was taken at the distance between the hock joint and the pelvic joint.
- Wing length (cm): This was measured from the distance between the tip of the phalanges and the coracoid-humerus joint.

Blood sample collection for DNA analysis

Birds were randomly sampled (Fifty birds per breed) for blood collection at eighteen weeks of age and labelled according to their breed and tag number. One (1) ml of blood was collected from each of the chicken through the brachial venial puncture, aseptically into 5 ml ethylene di-amine-tetra-acetic acid (EDTA) tube using 2 ml sterilised syringe. Blood samples were placed in an icebox and transported to the Biotechnology Laboratory of the Department of Animal Breeding and Genetics, Federal University of Agriculture, Abeokuta, for perseveration against degradation at -20^{o}C before DNA extraction and quantification.

DNA extraction

Genomic DNA was extracted at Biotechnology Laboratory of the Department of Animal Breeding and Genetics, Federal University of Agriculture, Abeokuta from 150 birds comprising 50 each of FUNAAB Alpha, Noiler and Shika Brown using Zymo research quick-gDNA™ miniprep kit (catalogue number: D4068) as prescribed. The manufactural procedure was adhered to strictly.

DNA quantification

The purity and concentration of the extracted DNA were carried out using a Nano-drop spectrophotometer in the Biotechnology Centre, Federal University of Agriculture, Abeokuta. One (1) µl of DNA was placed on the sensor of the Nano-drop spectrophotometer and the sensor was standardised with the DNA elution buffer. Measurements on the Nano-drop spectrophotometer for concentration and optical density (OD) wavelength for purity were noted, and DNA samples having OD value with the range of 1.7-1.9 were used for further analysis.

GHRL gene primers

A pair of primers was used on the basis of chicken *GHRL* gene information which consists of five exons, four introns and a promoter region as reported by Nie et al. (2004). The sequences of the PCR primers used on exon 2 to 3 of the chicken breeds are as shown in Table 1.

Table 1. Sequences of PCR primers for amplification of chicken *GHRL* gene

Fragment name	Primer Sequence (5'-3') (Forward/Reverse)	Primer Location	Length (bp)
F: Forward Primer	CATTTCTAAGCTTTTGCCAGTT	Exon 2-3	774
R: Reverse Primer	GCATTATTCTGACTTTTTACCTG		

Source: Nie et al. (2004).

PCR optimisation and DNA amplification

PCR primers were dissolved with 1X Tris-acetate EDTA (TAE) buffer and vortexed to mix properly; and were later centrifuged briefly. A 1:10 dilution of both forward and reverse primers and nuclease-free water was used for primer optimisation. Frequent freeze-thaw cycles after dissolving primer were avoided and the dissolved stock solution was kept at -20˚C for subsequent use.

PCR reaction was carried out using a total volume of 15 µl which contained 1 µl DNA sample, 2 µl of diluted primers (forward and reverse primers), 3 µl of Master Mix and 9µl of nuclease-free water. The PCR protocol was 94°C for 3 minutes, followed by 35 cycles at 94°C for 30 seconds, annealing at 55°C for 45 seconds, 72°C for 1 minute with a final extension at 72°C for 5 minutes (Nie et al., 2004).

Gel electrophoresis

The agarose gel electrophoresis (1%) and 100 bp DNA ladder were used to investigate and confirm the quality of the PCR products. Amplicons derived were loaded and run on 1 % agarose gel stained with ethidium bromide (0.5µl/ml). A 100 bp ladder was used to determine the fragment sizes. Electrophoresis was performed in a 1xTAE buffer-filled tank and ran at 100 volts for 45 minutes. Detection of the amplified fragments was carried out under an ultraviolet light using an Alphamager^{R} 2200 version 5.5 Gel Documentation Systems.

Analysis of growth data

Growth data was subjected to a factorial experiment and analysed using the Generalized Linear Model of SAS (2002) and the model used is of the form:

$Y_{ijkl} = \mu + B_i + S_j + G_k + (BG)_{ik} + \varepsilon_{ijkl}$ where,

Y_{ijk} = Observation made on traits of interest (body weight, body circumference, breast girth, shank length, thigh length and wing length)

μ = Overall estimate of the population mean.

B_i = Fixed effect of the $_i$th Breed of chickens ($_i$ = FUNAAB Alpha, Noiler, Shika Brown)

S_j = Fixed effect of the $_j$th Sex of chickens ($_j$ = Male, Female)

G_k = Fixed effect of the $_k$th *GHRL* gene genotype ($_k$ = AA, AB, BB)

$(BG)_{ik}$ = Fixed effect of the interaction between breed of the chicken and *GHRL* gene genotype

ε_{ijkl} = Random error associated with each measurement.

Least significant difference (LSD) test was used to separate the means to ascertain if there were significant differences among breeds (Li et al., 2000).

RESULTS AND DISCUSSIONS

Effect of breed and sex on body weight and linear body measurements of chickens at weeks 2, 4 and 6

The least squares mean for body weight as affected by breed and sex of the three chicken breeds (FUNAAB Alpha, Noiler and Shika Brown) at weeks 2, 4 and 6 are presented in Table 2. The results revealed that Noiler chickens performed better (P <0.05) when compared with FUNAAB Alpha and Shika Brown chickens. Both FUNAAB Alpha and Noiler chicken breeds had better early start in life.

Table 2. Effect of breed and sex on body weight and linear body measurements of three chicken breeds at weeks 2, 4 and 6 (LSM±SE)

AGE (Week)	Breed / Sex	BW (g)	BC (cm)	BG (cm)	SL (cm)	TL (cm)	WL (cm)
	FUNAAB Alpha	155.05±2.14^{b}	15.81±0.11^{b}	9.25±0.06^{a}	4.75±0.04^{a}	7.84±0.06	9.68±0.06
	Noiler	161.08±2.40^{a}	16.90±0.11^{a}	8.17±0.06^{b}	4.55±0.04^{b}	7.94±0.06	9.77±0.06
2	Shika Brown	146.72±2.14^{c}	17.07±0.11^{a}	8.10±0.06^{b}	4.54±0.04^{b}	7.86±0.06	9.61±0.06
	Female	154.43±2.34^{b}	16.27±0.13	8.62±0.09	4.62±0.04	7.83±0.07	9.74±0.07
	Male	161.99±2.44^{a}	16.44±0.14	8.80±0.09	4.68±0.04	7.96±0.07	9.74±0.07
	FUNAAB Alpha	364.85±17.23^{a}	22.22±0.32^{a}	10.31±0.12^{ab}	5.88±0.05^{b}	10.59±0.10^{b}	13.26±0.19^{b}
	Noiler	355.20±17.23^{a}	21.43±0.32^{a}	10.64±0.12^{a}	6.31±0.05^{a}	11.26±0.10^{a}	13.85±0.19^{a}
4	Shika Brown	264.95±17.23^{b}	19.87±0.32^{b}	10.15±0.12^{b}	5.74±0.05^{c}	10.64±0.10^{b}	12.86±0.19^{b}
	Female	331.23±20.36^{b}	21.28±0.38^{b}	10.47±0.14	5.95±0.05^{b}	10.77±0.11^{b}	13.13±0.22^{b}
	Male	391.17±21.18^{a}	22.41±0.39^{a}	10.47±0.14	6.25±0.06^{a}	11.09±0.11^{a}	14.02±0.23^{a}
	FUNAAB Alpha	545.10±8.30^{a}	24.51±1.34	11.47±0.17^{c}	7.28±0.08	12.79±0.10^{c}	15.72±0.09^{b}
	Noiler	527.53±8.89^{a}	24.97±1.44	12.50±0.18^{b}	7.45±0.08	13.70±0.11^{b}	16.02±0.10^{a}
6	Shika Brown	444.07±8.30^{b}	26.40±1.34	13.02±0.17^{a}	7.27±0.08	14.14±0.10^{a}	15.12±0.09^{c}
	Female	506.63±8.99^{b}	24.38±0.16^{b}	11.93±0.17	7.11±0.08^{b}	13.09±0.11	15.57±0.09^{b}
	Male	570.89±9.52^{a}	25.12±0.17^{a}	11.97±0.18	7.64±0.09^{a}	13.35±0.11	16.17±0.10^{a}

$^{a, b, c}$ Means on the same column for each parameter with different superscripts are significantly different (P <0.05). BW = Body Weight, BC = Body Circumference, BG = Breast Girth, SL = Shank Length, TL = Thigh Length and WL = Wing Length.

The result of the linear body measurements considered (body circumference BC, breast girth BG, shank length SL, thigh length TL and wing length WL) showed significant (P <0.05) difference(s) in mean values based on breed effect. At week 2, FUNAAB Alpha had a significantly (P <0.05) higher breast girth and shank length followed by

Noiler and Shika Brown chickens while for body circumference, Shika Brown and Noiler had higher least squares means, followed by FUNAAB Alpha. Noiler chicken had highest least squares means (P <0.05) in all the linear body measurements at week 4 while at week 6, Shika Brown performed best (P <0.05) for breast girth and thigh length when compared with the other two breeds. Noiler chicken had a significantly (P <0.05) higher wing length followed by FUNAAB Alpha and Shika Brown while body circumference and shank length were not significantly (P >0.05) different at week 6.

The males were significantly (P <0.05) superior to the female counterparts in terms of body weight at weeks 2, 4 and 6 by a difference of 7.56 g, 59.94 g and 64.26 g, respectively. Also, for the linear body measurements considered the males were superior (P <0.05) to their female counterparts at the aforementioned weeks.

Effect of breed and sex on body weight and linear body measurements of chickens at weeks 8, 10 and 12

The least squares mean obtained at weeks 8, 10 and 12 for body weight and linear body measurements as affected by breed and sex of the three chicken breeds are presented in Table 3. Similar pattern of superiority was observed at weeks 8 and 10 with Noiler and FUNAAB Alpha both having higher and better (P <0.05) body weights than Shika Brown chickens. Noiler and FUNAAB Alpha chickens were significantly (P <0.05) superior to Shika Brown with an average mean of over 200 g at week 8 and over 300 g at week 10. The superiority (P <0.05) observed between Noiler and FUNAAB Alpha at week 12 for body weight showed a difference of 70 g.

Generally, Noiler chicken recorded the highest linear body measurements at week 8 and 12 except for body circumference that was jointly shared by both FUNAAB Alpha and Noiler at week 8, while at week 10 FUNAAB Alpha and Noiler both had better (P <0.05) least squares mean values in all the linear body measurements. FUNAAB Alpha had the best (P <0.05) body circumference, shank length and wing length at weeks 12 followed by Noiler and Shika Brown. For breast girth and thigh length, Noiler chicken performed best (P <0.05) at weeks 12 with BG values that were significantly (P <0.05) superior to the other two breeds. The males were significantly (P <0.05) superior to their female counterparts in body weight and all the linear body measurements considered at the said weeks.

Table 3. Effect of breed and sex on the body weight and linear body measurements of chickens at weeks 8, 10 and 12 (LSM±SE)

AGE (Week)	Breed / Sex	BW (g)	BC (cm)	BG (cm)	SL (cm)	TL (cm)	WL (cm)
	FUNAAB Alpha	840.39 ± 12.71^a	28.83 ± 0.19^a	11.75 ± 0.22^c	8.50 ± 0.08^b	15.18 ± 0.16^b	18.61 ± 0.14^b
	Noiler	871.46 ± 13.60^a	29.32 ± 0.21^a	14.06 ± 0.24^a	8.88 ± 0.08^a	15.99 ± 0.17^a	19.11 ± 0.15^a
8	Shika Brown	649.02 ± 12.71^b	27.72 ± 0.19^b	12.86 ± 0.22^b	8.44 ± 0.08^b	14.09 ± 0.16^c	15.66 ± 0.14^c
	Female	796.73 ± 13.34^b	28.35 ± 0.20^b	12.66 ± 0.26	8.41 ± 0.07^b	15.26 ± 0.12^b	18.40 ± 0.11^b
	Male	920.11 ± 14.13^a	29.86 ± 0.21^a	13.01 ± 0.27	8.98 ± 0.07^a	15.89 ± 0.12^a	19.34 ± 0.12^a
	FUNAAB Alpha	1156.08 ± 17.41^a	32.84 ± 0.19^a	12.67 ± 0.17^c	9.49 ± 0.08^a	16.94 ± 0.12^b	20.35 ± 0.13^a
	Noiler	1179.78 ± 18.64^a	31.43 ± 0.21^b	15.07 ± 0.18^a	8.29 ± 0.09^b	18.13 ± 0.12^a	18.89 ± 0.14^b
10	Shika Brown	843.92 ± 17.41^b	27.94 ± 0.19^c	13.36 ± 0.17^b	6.94 ± 0.09^c	15.23 ± 0.12^c	16.79 ± 0.13^c
	Female	1075.85 ± 18.39^b	31.35 ± 0.21^b	13.45 ± 0.20^b	8.54 ± 0.10^b	17.17 ± 0.13^b	19.13 ± 0.16^b
	Male	1269.56 ± 19.48^a	33.12 ± 0.22^a	14.17 ± 0.22^a	9.36 ± 0.10^a	17.86 ± 0.14^a	20.27 ± 0.17^a
	FUNAAB Alpha	1317.94 ± 20.25^b	35.24 ± 0.25^a	13.29 ± 0.13^b	10.10 ± 0.08^b	18.49 ± 0.14^b	21.34 ± 0.20^a
	Noiler	1389.33 ± 21.68^a	34.80 ± 0.26^a	14.48 ± 0.14^a	10.89 ± 0.08^a	19.48 ± 0.15^a	21.58 ± 0.21^a
12	Shika Brown	1013.72 ± 20.25^c	30.36 ± 0.25^b	13.00 ± 0.13^b	9.42 ± 0.08^c	17.30 ± 0.14^c	17.83 ± 0.20^b
	Female	1236.34 ± 21.12^b	34.11 ± 0.27^b	13.59 ± 0.14^b	9.91 ± 0.08^b	18.34 ± 0.15^b	20.28 ± 0.21^b
	Male	1480.11 ± 22.37^a	36.07 ± 0.28^a	14.13 ± 0.14^a	11.09 ± 0.08^a	19.64 ± 0.16^a	22.77 ± 0.22^a

[a, b, c] Means on the same column for each parameter with different superscripts are significantly different (P <0.05). BW = Body Weight, BC = Body Circumference, BG = Breast Girth, SL = Shank Length, TL = Thigh Length and WL = Wing Length.

Effect of breed and sex on body weight and linear body measurements of chickens at weeks 14, 16 and 18

Table 4 shows the least squares means for body weight and linear body measurements as affected by breed and sex of the three chicken populations at weeks 14, 16 and 18. It was observed that Noiler chicken breed was significantly (P <0.05) superior to FUNAAB Alpha and Shika Brown chickens in that order for both body weight and all linear body measurements considered.

The superiority (P <0.05) observed between Noiler and FUNAAB Alpha for body weight gradually increased to over 180 g at 18 weeks in favour of the Noiler as their age increased, also, FUNAAB Alpha consistently displayed better performance over Shika Brown with an average difference of 600 g at 18 weeks.

The results of the linear body measurements showed significant (P <0.05) difference(s) in mean values based on breed effect. Noiler chicken attained least squares means that were significantly (P <0.05) superior to the other two breeds (FUNAAB Alpha and Shika Brown) for all the traits considered at weeks 14. At week 16 and 18 Noiler chicken was also significantly (P <0.05) superior to FUNAAB Alpha and Shika Brown for all the growth traits except for body circumference at week 16 and breast girth at week 18 where Noiler and FUNAAB Alpha both had better (P <0.05) length when compared with the Shika Brown chickens (Table 4). Similarly, the males were significantly (P <0.05) superior to their female counterparts in all the linear body measurements considered at the aforementioned weeks.

Polymorphisms identified in exons 2 and 3 of *GHRL* gene in the three chicken breeds (FUNAAB Alpha, Noiler and Shika Brown)

The amplified region (exons 2 and 3) of the chicken *GHRL* gene was polymorphic, with two variants (A and B). The three *GHRL* gene genotypes (AA, AB and BB) were identified in Noiler and Shika Brown chicken populations while genotypes AA and AB were observed in FUNAAB Alpha chicken population.

Table 4. Effect of breed and sex on the body weight and linear body measurements of chickens at weeks 14, 16 and 18 (LSM±SE)

AGE (Week)	Breed / Sex	BW (g)	BC (cm)	BG (cm)	SL (cm)	TL (cm)	WL (cm)
14	FUNAAB Alpha	1572.16±22.37^{a}	37.83±0.26^{b}	13.26±0.08^{b}	10.74±0.09^{b}	19.10±0.15^{b}	21.67±0.18^{b}
	Noiler	1611.24±23.95^{a}	42.40±0.28^{a}	13.61±0.09^{a}	11.20±0.09^{a}	20.19±0.16^{a}	23.16±0.19^{a}
	Shika Brown	1051.96±22.37^{b}	34.54±0.26^{c}	12.48±0.09^{c}	9.48±0.09^{c}	17.60±0.15^{c}	20.26±0.18^{c}
	Female	1479.70±24.04^{b}	38.75±0.35^{b}	13.18±0.10^{b}	10.23±0.07^{b}	18.65±0.14^{b}	21.36±0.20^{b}
	Male	1714.56±25.47^{a}	41.32±0.37^{a}	13.70±0.10^{a}	11.77±0.08^{a}	20.67±0.14^{a}	23.49±0.21^{a}
16	FUNAAB Alpha	1729.71±25.65^{b}	39.37±0.23^{a}	14.05±0.09^{b}	10.86±0.10^{b}	19.92±0.15^{b}	22.49±0.17^{b}
	Noiler	1864.61±27.46^{a}	39.02±0.24^{a}	14.99±0.09^{a}	11.67±0.11^{a}	20.91±0.16^{a}	24.07±0.19^{a}
	Shika Brown	1133.14±25.65^{c}	35.93±0.23^{b}	13.41±0.09^{c}	9.93±0.10^{c}	17.97±0.15^{c}	20.75±0.17^{c}
	Female	1640.89±27.04^{b}	37.79±0.21^{b}	14.26±0.10^{b}	10.35±0.08^{b}	18.94±0.12^{b}	21.64±0.14^{b}
	Male	1962.78±28.64^{a}	40.80±0.22^{a}	14.75±0.11^{a}	12.24±0.09^{a}	22.00±0.12^{a}	25.00±0.15^{a}
18	FUNAAB Alpha	1895.25±27.68^{b}	41.75±0.31^{b}	14.93±0.09^{a}	10.94±0.09^{b}	19.93±0.15^{b}	22.68±0.18^{b}
	Noiler	2079.55±29.64^{a}	43.74±0.34^{a}	15.16±0.10^{a}	11.73±0.10^{a}	20.46±0.16^{a}	24.02±0.19^{a}
	Shika Brown	1243.53±27.68^{c}	37.87±0.31^{c}	13.82±0.09^{b}	9.89±0.09^{c}	18.46±0.15^{c}	21.05±0.18^{c}
	Female	1787.03±28.01^{b}	41.25±0.36^{b}	14.55±0.09^{b}	10.44±0.08^{b}	18.86±0.10^{b}	21.72±0.15^{b}
	Male	2199.00±29.67^{a}	44.28±0.38^{a}	15.59±0.10^{a}	12.28±0.08^{a}	21.66±0.11^{a}	25.08±0.15^{a}

$^{a, b, c}$ Means on the same column for each parameter with different superscripts are significantly different (P <0.05)
BW = Body Weight, BC = Body Circumference, BG = Breast Girth, SL = Shank Length, TL = Thigh Length and WL.

Effect of *GHRL* gene polymorphism on growth performance of the three chicken breeds (FUNAAB Alpha, Noiler and Shika Brown) from week 2 to 18

The least square means for body weight as affected by *GHRL* gene polymorphism of the three chicken breeds (FUNAAB Alpha, Noiler and Shika Brown) from week 2 to 18 are presented in Tables 5 and 6. It was observed that *GHRL* gene polymorphisms (AA, AB and BB) had no significant (P >0.05) effect on all the traits studied (body weight, body circumference, breast girth, shank length, thigh length and wing length) from week 2 to 18.

Table 5. Effect of *GHRL* gene polymorphism on growth performance of the three chicken breeds (FUNAAB Alpha, Noiler and Shika Brown) from week 2 to 10 (LSM±SE)

Band Genotype	N	Dependent variable	Age (weeks)				
			2	4	6	8	10
AA	68	BW (g)	153.27±2.12	314.44±6.28	508.98±9.95	801.43±17.20	1069.90±24.40
AB	53		159.55±3.66	320.15±10.83	505.76±17.15	777.58±29.63	1056.67±42.06
BB	29		161.00±9.41	310.00±27.81	502.00±44.07	756.00±56.13	1050.00±68.05
AA	68	BC (cm)	16.46±0.13	21.53±0.47	24.60±0.16	28.69±0.20	30.88±0.29
AB	53		17.03±0.22	21.15±0.82	24.53±0.28	28.31±0.35	30.47±0.50
BB	29		17.10±0.57	20.50±2.11	24.90±0.72	28.40±0.89	30.40±1.29
AA	68	BG (cm)	7.87±0.06	10.29±0.12	12.37±0.20	12.61±0.25	13.86±0.21
AB	53		8.08±0.10	10.68±0.20	12.67±0.34	13.65±0.45	14.27±0.36
BB	29		7.90±0.26	10.90±0.52	11.80±0.87	14.40±1.10	13.20±0.93
AA	68	SL (cm)	4.59±0.04	5.93±0.05	7.43±0.10	8.54±0.07	8.34±0.14
AB	53		4.67±0.06	5.97±0.09	7.21±0.17	8.70±0.13	7.97±0.24
BB	29		4.60±0.16	6.20±0.22	7.40±0.44	9.40±0.33	7.70±0.62
AA	68	TL (cm)	7.87±0.06	10.72±0.12	13.54±0.12	14.99±0.18	16.64±0.18
AB	53		8.08±0.10	10.97±0.20	13.61±0.21	15.12±0.31	16.97±0.31
BB	29		7.90±0.26	11.30±0.52	13.70±0.55	14.60±0.80	17.20±0.79
AA	68	WL (cm)	9.18±0.06	13.18±0.09	15.67±0.10	17.93±0.21	18.79±0.21
AB	53		9.85±0.11	13.20±0.16	15.15±0.17	17.68±0.36	18.30±0.36
BB	29		10.00±0.27	13.40±0.40	15.70±0.43	17.00±0.93	18.40±0.93

Not Significant (P >0.05); BW = Body Weight; BC = Body Circumference; BG = Breast Girth; SL = Shank length; TL = Thigh length and WL = Wing length.

Table 6. Effect of *GHRL* gene polymorphism on growth performance of the three chicken breeds (FUNAAB Alpha, Noiler and Shika Brown) from week 12 to 18 (LSM±SE)

Band Genotype	N	Dependent variable	Age (weeks)			
			12	14	16	18
AA	68	BW (g)	1255.31±27.27	1417.35±33.91	1576.84±42.84	1766.93±49.88
AB	53		1245.76±47.00	1389.70±58.44	1563.64±63.82	1713.03±80.79
BB	29		1220.00±60.73	1364.00±70.13	1604.00±89.65	1760.00±97.55
AA	68	BC (cm)	33.59±0.36	38.36±0.42	38.47±0.28	41.46±0.35
AB	53		33.41±0.61	38.52±0.72	37.77±0.48	41.26±0.60
BB	29		32.30±1.57	39.70±1.86	37.80±1.24	41.80±1.53
AA	68	BG (cm)	13.70±0.14	13.18±0.10	14.28±0.11	14.73±0.11
AB	53		13.64±0.25	13.20±0.17	13.80±0.19	14.26±0.60
BB	29		13.40±0.63	13.10±0.44	14.60±0.49	41.80±1.53
AA	68	SL (cm)	10.21±0.10	10.62±0.12	11.00±0.13	10.97±0.12
AB	53		10.08±0.17	10.38±0.20	10.65±0.23	10.82±0.21
BB	29		10.08±0.45	10.70±0.52	11.00±0.59	11.60±0.54
AA	68	TL (cm)	18.51±0.17	19.04±0.19	19.85±0.20	19.80±0.17
AB	53		18.58±0.29	18.95±0.32	19.33±0.35	19.61±0.29
BB	29		19.10±0.75	18.60±0.83	20.10±0.90	20.50±0.74
AA	68	WL (cm)	20.31±0.28	22.01±0.23	22.74±0.23	22.79±0.23
AB	53		20.12±0.48	21.39±0.39	22.02±0.40	22.43±0.40
BB	29		21.00±1.22	21.30±1.00	22.80±1.02	23.80±1.02

Not Significant (P >0.05); BW = Body Weight; BC = Body Circumference; BG = Breast Girth; SL = Shank length; TL = Thigh length and WL = Wing length.

Interactive effect of *GHRL* gene polymorphism and chicken breed on growth traits (BW, BC and BG) from week 2 to 10

GHRL gene polymorphism and chicken breed interactive effect significantly (P <0.05) affected body weight, body circumference and breast girth at weeks 2, 4, 8 and 10. This is presented in Table 7.

Table 7. Interactive effect of *GHRL* gene polymorphism and chicken breed on growth traits (BW, BC and BG) from week 2 to 10 (LSM±SE)

Band Genotype	Chicken Breed	Dependent variable	Age (weeks) 2	4	6	8	10
AA	FUNAAB Alpha	BW (g)	158.61±3.28^{ab}	339.03±7.98^{a}	551.39±14.73	872.78±22.17^{a}	1193.89±30.18^{a}
AA	Noiler		156.79±3.72^{ab}	340.36±9.04^{a}	535.36±16.70	897.86±25.14^{a}	1191.79±34.22^{a}
AA	Shika Brown		144.71±3.37^{b}	267.06±8.20^{b}	442.35±15.16	646.47±22.81^{c}	838.24±31.05^{c}
AB	FUNAAB Alpha		151.11±6.55^{b}	319.44±15.95^{a}	523.33±29.46	805.56±24.34^{ab}	1102.22±30.35^{ab}
AB	Noiler		175.38±5.45^{a}	375.38±13.27^{a}	538.46±24.51	868.46±36.89^{a}	1196.15±30.21^{a}
AB	Shika Brown		147.73±5.93^{b}	255.45±14.43^{b}	452.73±26.65	647.27±30.11^{c}	854.54±34.59^{c}
BB	Noiler		158.33±11.35^{ab}	360.00±17.63^{a}	536.67±21.03	816.67±36.80^{ab}	1133.33±34.53^{a}
BB	Shika Brown		165.00±13.90^{ab}	235.00±17.84^{b}	450.00±22.49	665.00±34.06^{bc}	925.00±28.02^{ab}
AA	FUNAAB Alpha	BC (cm)	15.89±0.20^{b}	23.33±0.77	24.78±0.27	29.28±0.31	33.14±0.33^{a}
AA	Noiler		16.52±0.22^{ab}	21.30±0.87	24.89±0.30	29.21±0.36	31.57±0.38^{a}
AA	Shika Brown		17.03±0.20^{ab}	19.79±0.79	24.16±0.27	27.65±0.32	27.93±0.34^{b}
AB	FUNAAB Alpha		16.00±0.39^{b}	21.28±1.53	24.17±0.53	28.11±0.62	32.56±0.66^{a}
AB	Noiler		17.65±0.33^{a}	22.08±1.27	25.27±0.44	29.15±0.53	31.04±0.55^{a}
AB	Shika Brown		17.14±0.36^{ab}	19.95±1.39	23.95±0.48	27.50±0.57	28.09±0.60^{b}
BB	Noiler		16.67±0.68^{ab}	21.33±2.66	24.83±0.92	28.67±1.09	31.50±1.15^{a}
BB	Shika Brown		17.75±0.83^{a}	19.25±3.25	25.00±1.13	28.00±1.33	28.75±1.40^{b}
AA	FUNAAB Alpha	BG (cm)	9.17±0.11^{a}	10.15±0.19	11.56±0.31^{ab}	11.88±0.38^{c}	13.00±0.30^{bcd}
AA	Noiler		8.13±0.13^{b}	10.73±0.21	12.61±0.35^{ab}	12.91±0.44^{bc}	14.95±0.35^{ab}
AA	Shika Brown		8.10±0.11^{b}	10.06±0.19	13.03±0.32^{ab}	13.15±0.40^{bc}	13.88±0.31^{abc}
AB	FUNAAB Alpha		9.17±0.22^{a}	10.61±0.37	11.67±0.62^{ab}	11.78±0.77^{c}	12.39±0.61^{cd}
AB	Noiler		8.50±0.19^{ab}	11.15±0.32	12.77±0.52^{ab}	15.42±0.64^{ab}	16.04±0.51^{a}
AB	Shika Brown		8.18±0.20^{b}	10.18±0.34	13.36±0.56^{a}	13.09±0.70^{bc}	13.73±0.55^{bc}
BB	Noiler		7.83±0.39^{b}	11.17±0.66	10.83±1.08^{b}	15.85±1.33^{a}	14.50±1.05^{abc}
BB	Shika Brown		8.50±0.47^{ab}	10.50±0.80	13.25±1.32^{a}	12.25±1.63^{c}	11.25±1.29^{d}

$^{a, b, c, d}$ Means on the same column with different superscripts are significantly different (P <0.05); BW = Body Weight, BC = Body Circumference and BG = Breast Girth.

Among the interactive groups (FUNAAB Alpha chickens AA, AB, Noiler chickens AA, AB, BB and Shika Brown chickens AA, AB, BB) Noiler chicken AB had the best performance in body weight, which was significantly (P <0.05) superior to the other interactive groups while at week 4, the performance of FUNAAB Alpha chicken AA, Noiler chickens AA, AB and BB were significantly (P <0.05) superior to the other interactive groups. Body weight obtained at weeks 8 and 10 showed a similar pattern of superiority among the interactive groups as observed at week 4; with FUNAAB Alpha chicken AA, Noiler chickens AA and AB having better least squares means that were significantly (P <0.05) superior to the other interactive groups.

For the body circumference at week 2, Noiler chicken AB and Shika brown chicken BB both had the best body weight among the interactive groups while at week 10, FUNAAB Alpha chicken AA, Noiler chickens AA and AB had significantly (P <0.05) superior body circumference when compared with the other groups. The result of the breast girth showed that *GHRL* gene polymorphism and chicken breeds significantly (P <0.05) affected breast girth at weeks 2, 6, 8 and 10. At week 2, FUNAAB Alpha chickens AA and AB both had the best breast girth value which is significantly (P <0.05) superior to values observed in the other interactive group. The trend was different at week 6, with Shika Brown chickens AB and BB having the best least squares means which were superior to the means observed in other interactive groups while at weeks 8 and 10, Noiler chickens BB and AB significantly performed best.

Interactive effect of *GHRL* gene polymorphism and chicken breeds on growth traits (SL, TL and WL) from week 2 to 10

GHRL gene polymorphism and chicken breed interactive effect significantly (P <0.05) affected shank length at weeks 4, 8 and 10 while for thigh length and wing length they were significantly (P <0.05) affected at weeks 6, 8 and 10 (Table 8).

Table 8. Interactive effect of *GHRL* gene polymorphism and chicken breed on growth traits (SL, TL and WL) from week 2 to 10 (LSM±SE)

Band Genotype	Chicken Breed	Dependent variable	Age (Weeks)				
			2	4	6	8	10
AA	FUNAAB Alpha	SL	4.77±0.06	5.86±0.07^{bc}	7.38±0.16	8.64±0.12^{ab}	9.68±0.14^{a}
AA	Noiler		4.45±0.07	6.27±0.08^{ab}	7.76±0.19	8.66±0.14^{ab}	8,41±0.16^{bc}
AA	Shika Brown		4.50±0.06	5.74±0.07^{c}	7.22±0.17	8.32±0.12^{b}	6.87±0.14^{d}
AB	FUNAAB Alpha		4.56±0.12	5.61±0.14^{c}	6.94±0.33	8.22±0.24^{b}	9.11±0.28^{ab}
AB	Noiler		4.81±0.10	6.46±0.12^{a}	7.35±0.27	8.96±0.20^{ab}	8.00±0.23^{c}
AB	Shika Brown		4.59±0.10	5.68±0.13^{c}	7.25±0.30	8.77±0.22^{ab}	7.00±0.25^{d}
BB	Noiler		4.50±0.20	6.50±0.24^{a}	7.17±0.57	9.50±0.41^{a}	8.33±0.48^{bc}
BB	Shika Brown		4.75±0.25	5.75±0.30^{c}	7.75±0.69	9.25±0.51^{a}	6.75±0.59^{d}
AA	FUNAAB Alpha	TL	7.97±0.10	10.44±0.19	12.79±0.18^{cd}	15.31±0.26^{ab}	17.11±0.20^{abc}
AA	Noiler		7.84±0.11	11.23±0.21	13.82±0.20^{abcd}	15.77±0.30^{ab}	18.14±0.23^{ab}
AA	Shika Brown		7.79±0.10	10.59±0.19	14.09±0.18^{abc}	14.01±0.27^{b}	14.91±0.20^{d}
AB	FUNAAB Alpha		8.17±0.19	10.55±0.38	12.56±0.36^{d}	14.72±0.53^{ab}	16.94±0.39^{bd}
AB	Noiler		8.27±0.16	11.54±0.31	13.77±0.30^{abcd}	16.31±0.44^{a}	18.42±0.33^{a}
AB	Shika Brown		7.77±0.17	10.64±0.34	14.27±0.32^{ab}	14.05±0.48^{b}	15.27±0.36^{d}
BB	Noiler		8.00±0.33	11.50±0.65	13.00±0.62^{bcd}	16.33±0.91^{a}	17.83±0.69^{ab}
BB	Shika Brown		7.75±0.40	11.00±0.79	14.75±0.76^{a}	12.00±1.11^{c}	16.25±0.84^{cd}
AA	FUNAAB Alpha	WL	9.81±0.10	13.32±0.14	15.88±0.14^{ab}	18.86±0.23^{a}	20.49±0.23^{a}
AA	Noiler		9.64±0.11	13.42±0.16	16.05±0.16^{ab}	19.20±0.27^{a}	19.23±0.27^{ab}
AA	Shika Brown		9.59±0.10	12.84±0.14	15.13±0.15^{bc}	15.91±0.24^{b}	16.63±0.24^{d}
AB	FUNAAB Alpha		9.78±0.20	12.94±0.29	15.44±0.29^{bc}	18.17±0.47^{a}	20.06±0.47^{a}
AB	Noiler		10.15±0.17	13.69±0.24	16.08±0.24^{ab}	19.12±0.39^{a}	18.38±0.39^{bc}
AB	Shika Brown		9.55±0.18	12.82±0.26	15.32±0.26^{bc}	15.59±0.42^{b}	16.77±0.42^{d}
BB	Noiler		10.17±0.35	13.67±0.49	16.50±0.50^{a}	19.00±0.81^{a}	19.17±0.81^{ab}
BB	Shika Brown		9.75±0.43	13.00±0.60	14.50±0.61^{c}	14.00±0.99^{c}	17.25±0.99^{cd}

$^{a, b, c, d}$ Means on the same column with different superscripts are significantly different (P <0.05); SL = Shank Length, TL = Thigh Length and WL = Wing Length.

Noiler chickens AB and BB had the best (P <0.05) performance for shank length at week 4. While at week 8 and 10, Noiler chicken BB and Shika Brown chicken BB both had the best shank length at week 8 and at week 10, FUNAAB Alpha chicken AA had the best shank length. For the thigh length at week 6, Shika Brown chicken BB had the best thigh length among the interactive groups while at week 8, Noiler chickens AB and BB had significantly (P <0.05) superior thigh length when compared with the other interactive groups. Also, at week 10, Noiler chicken AB performed best among the interactive groups with significantly (P <0.05) superior least squares means. Considering wing length, Noiler chicken BB had the best wing length which was significantly (P <0.05) superior to the other interactive groups. The least value was recorded in Shika Brown chicken BB with a least square means of 14.50±0.61 cm at week 6. At week 8, Noiler chickens AA, AB and BB were significantly (P <0.05) superior to the other interactive groups. At week 10, the pattern / trend of superiority shifted to FUNAAB Alpha chickens AA and AB. The lowest wing length value was observed in Shika Brown chicken AA with least squares means of 16.63±0.24 cm.

Interactive effect of Ghrelin gene polymorphism and chicken breed on growth traits from week 12 to 18

GHRL gene polymorphism and chicken breed interactive effect significantly (P <0.05) affected body weight, body circumference and breast girth at weeks 12, 14, 16 and 18 (Table 9). The trend of superiority was similar from week 12 to 18 with FUNAAB Alpha chicken AA, Noiler chicken AA, Noiler chicken AB and Noiler chicken BB having significantly (P <0.05) superior body weight than the other interactive groups.

Table 9. Interactive effect of *GHRL* gene polymorphism and chicken breed on growth traits from week 12 to 18 (LSM±SE)

Band Genotype	Chicken Breed	Dependent variable	Age (Weeks)			
			12	14	16	18
AA	FUNAAB Alpha	BW (g)	1374.16±33.80^{a}	1617.22±34.90^{a}	1748.88±24.16^{a}	1969.44±25.44^{ab}
AA	Noiler		1411.43±38.32^{a}	1612.50±29.57^{a}	1905.36±30.07^{a}	2133.57±21.52^{a}
AA	Shika Brown		1000.88±34.78^{b}	1045.00±25.91^{b}	1124.12±25.44^{b}	1250.59±26.76^{c}
AB	FUNAAB Alpha		1234.44±37.59^{ab}	1491.11±29.80^{a}	1661.11±38.32^{a}	1763.33±30.89^{b}
AB	Noiler		1434.61±36.23^{a}	1601.53±28.08^{a}	1861.54±33.49^{a}	2073.84±35.62^{ab}
AB	Shika Brown		1031.81±31.14^{b}	1056.36±23.14^{b}	1131.82±39.89^{b}	1245.45±32.21^{c}
BB	Noiler		1316.67±27.07^{a}	1550.00±20.90^{a}	1883.33±32.98^{a}	2066.67±37.42^{ab}
BB	Shika Brown		1075.00±23.38^{b}	1085.00±48.07^{b}	1185.00±37.36^{b}	1300.00±32.80^{c}
AA	FUNAAB Alpha	BC (cm)	35.93±0.43^{a}	38.54±0.42^{b}	40.24±0.36^{a}	42.72±0.35^{ab}
AA	Noiler		34.50±0.49^{a}	42.68±0.48^{a}	39.48±0.40^{ab}	44.27±0.39^{a}
AA	Shika Brown		30.35±0.45^{b}	34.60±0.43^{c}	35.77±0.37^{d}	37.84±0.36^{c}
AB	FUNAAB Alpha		34.72±0.87^{a}	37.00±0.84^{bc}	38.56±0.71^{ab}	41.28±0.69^{b}
AB	Noiler		35.42±0.72^{a}	42.85±0.70^{a}	38.73±0.59^{ab}	44.23±0.57^{a}
AB	Shika Brown		29.95±0.78^{b}	34.64±0.76^{c}	36.00±0.64^{cd}	37.73±0.62^{c}
BB	Noiler		32.83±1.50^{ab}	42.33±1.46^{a}	38.33±1.23^{abc}	44.00±1.20^{a}

BB	Shika Brown		31.50±1.84^{a}	36.25±1.79^{bc}	37.00±1.51^{bcd}	38.50±1.47^{c}
AA	FUNAAB Alpha	BG (cm)	13.51±0.22^{ab}	13.58±0.14^{a}	14.28±0.14^{bcd}	15.26±0.14^{ab}
AA	Noiler		14.68±0.24^{a}	13.43±0.16^{ab}	15.29±0.16^{a}	15.25±0.16^{ab}
AA	Shika Brown		13.10±0.22^{ab}	12.54±0.14^{c}	13.46±0.14^{de}	13.74±0.15^{c}
AB	FUNAAB Alpha		13.22±0.43^{ab}	13.44±0.28^{ab}	13.88±0.28^{cde}	14.89±0.28^{b}
AB	Noiler		14.31±0.36^{a}	13.85±0.23^{a}	14.81±0.23^{abc}	15.19±0.23^{ab}
AB	Shika Brown		13.18±0.39^{ab}	12.23±0.25^{c}	13.05±0.25^{e}	13.82±0.26^{c}
BB	Noiler		14.00±0.75^{ab}	13.67±0.49^{a}	15.00±0.48^{ab}	16.00±0.49^{a}
BB	Shika Brown		12.50±0.91^{b}	12.25±0.60^{c}	14.00±0.59^{cde}	14.50±0.60^{b}

^{a,b,c,d,e} Means on the same column with different superscripts are significantly different (P <0.05); BC = Body weight, BC = Body circumference, BG = Breast girth.

For body circumference at week 12, FUNAAB Alpha chicken AA, Noiler chicken AA, FUNAAB Alpha chicken AB and Noiler chicken AB performed better than the other interactive groups while at weeks 14 and 18, Noiler chicken AA, Noiler chicken AB and Noiler chicken BB were significantly (P <0.05) superior to the other interactive groups, but at week 16, FUNAAB Alpha chicken AA was outstanding among the interactive group.

Noiler chicken AA and Noiler chicken AB chickens attained least squares means that were significantly (P <0.05) superior to the other interactive groups at week 12 for breast girth while at week 14, three interactive groups significantly (P <0.05) distinct themselves; they are FUNAAB Alpha chicken AA, Noiler chicken AB and Noiler chicken BB. At weeks 16 and 18, Noiler chicken AA and Noiler chicken BB were significantly (P <0.05) superior to the other interactive groups for breast girth.

Interactive effect of *GHRL* gene polymorphism and chicken breed on growth traits (SL, TL and WL) from week 12 to 18

Table 10 shows the interactive effect of *GHRL* gene polymorphism and chicken breed on growth traits (shank length, thigh length and wing length) from week 12 to 18. Varying levels of superiority was displayed in shank length from week 12 to 18 with Noiler chicken AA, Noiler chicken AB and Noiler chicken BB having the best shank length at week 12. Week 14 featured FUNAAB Alpha chicken AA, Noiler chicken AA, Noiler chicken AB and Noiler chicken BB was highest (P <0.05) among the interactive groups while at weeks 16 and 18, Noiler chicken AA and Noiler chicken BB performed best. The result of thigh length at weeks 12, 14, 16 and 18 revealed that Noiler chicken BB had the highest thigh length which was significantly (P <0.05) superior to the other interactive groups at week 12, while at week 14, 16 and 18 the trend of superiority was the same with the following interactive groups (FUNAAB Alpha chicken AA, Noiler chicken AA, Noiler chicken AB and Noiler chicken BB) being significantly (P <0.05) superior to the others. Wing length at weeks 12 and 18 both had a similar pattern of superiority with Noiler chicken BB having the best wing lengths which were significantly (P <0.05) superior to the other interactive groups for both weeks, while at week 14, Noiler chicken AA performed best. Wing length at week 16 showed a similar pattern of superiority displayed in thigh length from week 12 to 18 with FUNAAB Alpha chicken AA, Noiler chicken AA, Noiler chicken AB and Noiler chicken BB that were significantly (P <0.05) superior to the other interactive groups.

Table 10. Interactive effect of *GHRL* gene polymorphism and chicken breed on growth traits (SL, TL and WL) from week 12 to 18 (LSM±SE)

Band Genotype	Chicken Breed	Dependent variable	Age (Weeks)			
			12	14	16	18
AA	FUNAAB Alpha	SL	10.38±0.13^{ab}	11.13±0.14^{a}	11.26±0.18^{abc}	11.44±0.15^{b}
AA	Noiler		10.98±0.15^{a}	11.30±0.16^{a}	11.95±0.21^{a}	11.68±0.17^{ab}
AA	Shika Brown		9.40±0.14^{c}	9.53±0.14^{b}	9.94±0.19^{d}	9.90±0.15^{c}
AB	FUNAAB Alpha		9.66±0.26^{bc}	10.17±0.28^{b}	10.27±0.36^{bcd}	10.22±0.30^{c}
AB	Noiler		10.81±0.22^{a}	11.38±0.23^{a}	11.46±0.30^{ab}	11.92±0.25^{ab}
AB	Shika Brown		9.55±0.24^{bc}	9.36±0.25^{b}	10.00±0.31^{cd}	10.00±0.27^{c}

BB	Noiler		10.66±0.46^{a}	11.17±0.48^{a}	11.50±0.63^{ab}	12.50±0.51^{a}
BB	Shika Brown		9.50±0.56^{bc}	10.00±0.59^{b}	10.25±0.77^{bcd}	10.25±0.63^{c}
AA	FUNAAB Alpha	TL	18.67±0.22^{bc}	19.49±0.24^{a}	20.58±0.24^{a}	20.57±0.22^{a}
AA	Noiler		19.71±0.25^{ab}	20.27±0.27^{a}	21.23±0.28^{a}	20.36±0.25^{ab}
AA	Shika Brown		17.35±0.23^{cd}	17.54±0.25^{bc}	17.94±0.25^{b}	18.51±0.23^{c}
AB	FUNAAB Alpha		18.06±0.45^{cd}	18.72±0.48^{ab}	18.83±0.49^{b}	19.00±0.44^{c}
AB	Noiler		19.92±0.37^{ab}	20.27±0.40^{a}	20.85±0.41^{a}	20.88±0.37^{a}
AB	Shika Brown		17.40±0.40^{cd}	17.59±0.43^{bc}	17.95±0.44^{b}	18.59±0.40^{c}
BB	Noiler		20.67±0.77^{a}	20.33±0.83^{a}	21.67±0.84^{a}	21.83±0.77^{a}
BB	Shika Brown		16.75±0.95^{d}	16.00±1.02^{c}	17.75±1.04^{b}	18.50±0.94^{c}
AA	FUNAAB Alpha	WL	21.79±0.34^{ab}	22.46±0.31^{abc}	23.40±0.29^{a}	23.52±0.30^{b}
AA	Noiler		21.61±0.38^{ab}	23.55±0.35^{a}	24.38±0.32^{a}	24.04±0.34^{ab}
AA	Shika Brown		17.68±0.35^{c}	20.25±0.32^{cd}	20.69±0.29^{b}	20.97±0.31^{c}
AB	FUNAAB Alpha		20.39±0.68^{b}	20.25±0.51^{bcd}	21.00±0.57^{b}	21.44±0.60^{c}
AB	Noiler		21.62±0.56^{ab}	22.88±0.51^{ab}	23.81±0.48^{a}	24.31±0.49^{ab}
AB	Shika Brown		18.13±0.61^{c}	20.05±0.55^{d}	20.73±0.52^{b}	21.00±0.54^{c}
BB	Noiler		23.00±1.17^{a}	21.33±1.07^{bcd}	24.17±0.99^{a}	25.67±1.04^{a}
BB	Shika Brown		18.00±1.43^{c}	21.25±1.31^{bcd}	20.75±1.21^{b}	21.00±1.27^{c}

$^{a, b, c, d}$ Means on the same column with different superscripts are significantly different ($P < 0.05$); SL = Shank Length, TL = Thigh Length and WL = Wing Length.

Digestion of PCR products

The PCR products were digested with *Eco72I* restriction enzymes. The PCR component included 5 µl of 10X FastDigest Green Buffer, 10 µl of PCR product, 1µl of FastDigest enzyme and 9 µl of nuclease free water were subjected to digestion for 20 minutes at 80^0 C. Genotyping was carried out manually following the scoring procedure of Darabi *et al.* (2010).

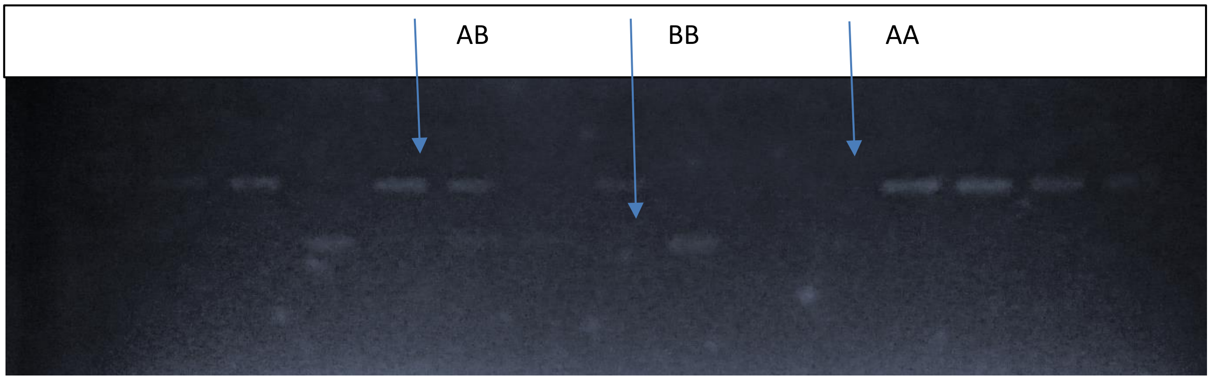

Figure 1. Ghrelin gene digestion with Eco 72I showing the three genotypes (AA, AB and BB).

The three chicken breeds studied (FUNAAB Alpha, Noiler and Shika Brown) have been developed and improved through a progressive programme of breeding and selection for excellent performance and good processing yield to meet multiple market demand around the world. Live weight and body measurements are important parameters in assessing the potential of genetic improvement and development of any livestock breed/strain, which is the reason for the choice of morphometric traits considered in this study. An on station performance evaluation by the African Chicken Genetic Gains Nigeria (ACGG-NG) carried out in Nigeria showed that the Nigerian indigenous chickens attained average body weights of 793 g at 16 weeks in Nigeria station (Tadelle, 2017). The improved indigenous chicken (FUNAAB Alpha) attained an average body weight of 1729 g at 16 weeks in this study. A difference of close to 1 kg (936 g) observed, which means a high level of genetic improvement has been achieved over the years.

The indigenous poultry species represent valuable resources for livestock development because their extensive genetic diversity allows for rearing of poultry under varied environmental conditions, providing a range of products and functions. FUNAAB Alpha is the first improved breed of chicken developed in the Federal University of Agriculture, Abeokuta which is superior to Shika Brown and competes favourably with Noiler in terms of body weight and linear body measurements. Reported matured body weights of Noiler chicken at 20 weeks were between 2 and 2.6 kg (Bamidele et al., 2019) which are within the result of this study with 2.3 kg at 18 weeks. The observed body weight of FUNAAB Alpha is in agreement with the findings of Adebambo (2015) who reported an average of

1200-1800 g at between 18 and 21 weeks of age for the FUNAAB Alpha pullet line. It is also in agreement with the report of Bamidele et al. (2019) who reported body weight that ranged between 1635-2097 g at 20 weeks of age. Shika Brown chicken had an average body weight that is in line with the findings of Bamidele et al. (2019) but is slightly lower when compared with the findings of Dairo (2004) who reported a range of between 1400-1680 g at 18-20 weeks and Abubakar et al. (2011) that reported an average body weights of 1400 g at 18 weeks. The trend of superiority observed in the body weight of the three chicken breeds (FUNAAB Alpha, Noiler and Shika Brown) from week 12 to 18 in this study is similar to the report of Suleiman et al. (2019) who reported an average body weight of 1904.57 g, 1511.83 g and 900.05 g for males at 18 weeks of age in favour of Noiler. Male body weight of the chicken breeds between 90 and 130 days observed in this study showed a similar pattern of superiority to their female counterparts with Noiler being the heaviest breed. It is in line with the finding of Bamidele et al. (2019) who carried out similar research in most of the agro-ecological zones. *GHRL* is a key factor in the hypothalamic melanocortin system which is involved in various bioactivities. In general, as a regulatory component of the complex brain-gut anabolic neuroendocrine network, which controls food intake, energy balance, and body weight, ghrelin may play a fundamental role in coordination of energy needs with the processes involved in growth (Richards et al., 2006). Although, several studies have shown a significant association between the ghrelin gene polymorphism and growth traits, body weight, shank girth at 16 weeks (Li et al., 2006), body weight and body composition traits (Fang et al., 2007), and chicken hatch-weight (Fellaheti et al., 2011), there was no significant effect on all the economic traits of interest investigated in this study.
The result of this study indicated that the amplified region of the *GHRL* gene was polymorphic, with two variants (A and B). The three *GHRL* genotypes (AA, AB and BB) were identified in FUNAAB Alpha, Noiler and Shika Brown chicken populations. The effect of *GHRL* gene polymorphism on body weight and linear body measurements was not significant, which is corroborated by similar report of Hamed et al. (2015) on polymorphism in exon 1 and exon 2 of *GHRL* gene and its association with growth traits in broiler chickens (Cobb and Ross). The author also found no significant association between the patterns and the growth traits. One of the reasons could be that the sample size was not large enough to detect a significant difference in the least squares means of the studied traits and the *GHRL* gene polymorphism. The other reason could be that polymorphisms do not always cause obvious phenotypic variations. Even if these polymorphisms lead to changes in the *GHRL* gene expression and consequently phenotypic variation, it is not easy to explain the effect of these changes because of controversial findings regarding physiological effects of *GHRL*. In another study conducted by Li et al. (2006) on the relationship between *GHRL* gene polymorphism and growth traits in twelve Chinese indigenous chickens and commercial chickens, significant differences were observed among TT, CC, and CT genotypes in association with body weight which revealed that CT had the highest growth rate. The *GHRL* gene polymorphism in exon 3 and its relationship with body growth traits in the duck breed Chaohu were investigated using the PCR-RFLP technique and three band genotypes of AA, AB and BB were observed in the duck population similar to this study. Similarly, significant interaction between *GHRL* gene polymorphism and chicken breed's growth traits observed in this study is in line with what has been reported in other studies. Association of chicken *GHRL* polymorphisms with growth traits has also been reported by Fang et al. (2007). *GHRL* gene is considered to be a good candidate marker for the identification of economically important traits in poultry production such as feed intake, growth or carcass quality (Tyra et al., 2019). Benso et al. (2013) reported that accumulating evidence indicates *GHRL* plays a role in regulating food intake and energy homeostasis and it is a reasonable candidate gene for obesity-related co-morbidities. It is known that population-specific differences in reported associations exist (Ukkola, 2011). *GHRL* plays a significant role in feeding regulation and is the strongest stimulator of growth hormone secretion.

CONCLUSIONS

From the results obtained in this study, it can be concluded that Noiler chicken breed had the best body weight and linear body measurements (body circumference, breast girth, shank length, thigh length and wing length) from week ten to eighteen followed by FUNAAB Alpha and Shika Brown.
Sexual dimorphism favoured male birds in terms of body weight and linear body measurements from week 2 to 18. *GHRL* gene polymorphism had no significant effect on all the traits of interest (body weight, body circumference, breast girth, shank length, thigh length and wing length). Noiler chickens AA and AB had the best productive performance in most of the traits from week 2 to 18 for the interaction between *GHRL* gene polymorphism and chicken breeds.

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Conflicts of Interest

The authors declare that they do not have any conflict of interest.

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