Expression of Ghrelin Gene in Chicken Using Semi-Quantitative Digital Analysis

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Summary

The study investigated Ghrelin gene (GHRL) expression patterns in improved Nigerian indigenous Normal feather and naked neck FUNAAB Alpha chicken. Data on body weight and weight gain were collected weekly for 24 weeks. Beginning from 12 weeks of age, two males and two females were slaughtered per genotype via cervical dislocation fortnightly until the birds reached the point of lay at 24 weeks. Tissue samples from the liver, ovary, and testes were removed aseptically, weighed, and RNA stored for analysis of gene expression. The total RNA of each tissue sample was extracted using the Quick RNA™ MicroPrep kit. Reverse transcription was also carried out using SensiFAST™ cDNA Synthesis Kit. A semi-quantitative analysis of reverse transcription was carried out by densitometric scanning. The collected data were analyzed through two-way analysis of variance (ANOVA) using the general linear SAS model. Genotype and sex were observed to have significant effect ($P<0.05$) on body weight. Normal feather chickens and male chickens had a higher body weight. Semi-quantitative analysis of GHRL mRNA showed significant expression levels in the liver, ovary, and testes samples of both naked and normal feather chickens. Male and female naked neck chickens had significantly higher expression levels of the ghrelin gene at puberty. Normal feather chickens had significantly higher ghrelin mRNA expression levels throughout all weeks in the ovaries and testes. Our results suggest that chicken ghrelin expression may be related to tissue, age and chicken genotype.

Key words

dhrelin gene, growth, expression, indigenous chicken, polymerase chain reaction-electrophoresis

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Introduction

Ghrelin (GHRL) plays an important role in the regulation of energy metabolism due to its involvement in the hypothalamic melanocortin pathway (Jeong et al., 2014). Ghrelin stimulates food intake according to its orexigenic effect by activation of NPY/AgRP neurons (Chen et al., 2004). Ghrelin is usually a product of gastric tissue but its expression has been reported in a large array of tissues and cell types including the small intestine, pancreas, lymphocytes, placenta, kidney, lung, pituitary, brain, liver, and the gonads (Barreiro and Tena-Sempere, 2002; Gualillo et al., 2003; Van der Lely et al., 2004). One of the functional features of ghrelin that has attracted more attention is its ability to stimulate food intake and promote body weight gain. As an orexigenic signal, expression of ghrelin has been shown to increase after food deprivation and its plasma levels are (in most cases) negatively correlated with the body mass index. On this basis, ghrelin has been proposed as a unique circulating signal for energy insufficiency (the only known circulating orexigen), which may play a major role in the short- and long-term control of body weight (Cummings, 2006; Zigman and Elmquist, 2003). It is assumed that ghrelin plays an important role in feed efficiency because it is used to maintain energy balance (Wortley et al., 2004). Furthermore, the potential association between polymorphisms within the GHRL locus and economic traits have been investigated (e.g., feed intake, feed-gain ratio, body weight, the efficiency of growth) in different species of chicken (Jin et al., 2014). Ghrelin participates in the control of major areas of reproductive function (Arvat et al., 2001). It stimulates lactotroph and corticotroph functions, influences the pituitary–gonadal axis, inhibits pro-inflammatory cytokine expression, controls gastric motility and acid secretion and influences pancreatic exocrine and endocrine function (van der Lely et al., 2004; Otto et al., 2005). Ghrelin participates in the control of reproductive function both in male and females while sex differences may exist in the physiological extent of this control. Ghrelin has been found not only in the stomach and other parts of the gut but also in all the tissues (adrenal gland, atrium, breast, buccal mucosa, oesophagus, fallopian tube, fat tissue, gall bladder, human lymphocytes, ileum, kidney, left colon, liver, lung, lymph node, muscle, myocardium, ovary, pancreas, pituitary, placenta, prostate, right colon, skin, spleen, testes, thyroid and vein; (Gnanapavan et al., 2002).

The improved Nigerian indigenous chickens (FUNAAB Alpha) are bred as a dual-purpose chicken. It is newly registered and has been made commercially available for both meat and egg production in the rural and peri-urban areas of the country (Ilori et al., 2017; Yakubu et al., 2019). The traditional Nigerian indigenous chicken from which they were selected and improved for over many generations are found in large numbers distributed across different agroecological categories under a traditional family-based scavenging management system (Sonaiya and Olori, 1990; Ilori et al., 2017). Most of the birds are kept in small flocks in a scavenging system and the feed resources for the birds are household refuse, homestead pickings, crop residues, herbage, seeds, green grasses, earthworms, insects, and a small amount of supplemented feeds offered by the flock owner. They are well adapted to the adverse climatic conditions of the varied tropical environmental and ecological conditions, stresses, and diseases, and characterized by low management inputs. They contain a highly conserved genetic system with high levels of heterozygosity and are highly important farm animals, kept for good sources of animal protein, income and socio-cultural roles (Ebozoje and Ikeobi, 1995; Wimmerset al., 2000; Ajayi, 2010; Ilori et al., 2017). The Nigerian indigenous chickens are slow-growing and possess major genes that assist in early adaptation to the environment so that these genes cause a reduction in tropical heat stress (Peters, 2005). Genetic improvement of indigenous breed of livestock is very valuable because of high adaptability to harsh environmental conditions of climate and disease compared to exotic breeds (Ajayi and Agaviezor, 2016). Growth trait is a very important economic trait in chickens production and is controlled by complex genes. In poultry, growth rate and mature body weight are highly related to circulatory growth hormones level (Anthony et al., 1990), an example of which is the GHRL gene.

The semi-quantitative measurement of PCR amplicons using digital analysis of PCR-electrophoresis gels with ImageJ software is a semi-quantitation method of nucleic acids and immunoblot assays that allow laboratories with limited resources to empirically assess the relative amount of amplified PCR amplicons (Abramoff et al., 2004; Antiabong et al., 2016). It is a process that uses the agarose gel image analysis method for the semi-quantitation of PCR amplicons unlike the quantitative polymerase chain reaction (qPCR) also known as Real-Time PCR which uses different fluorescent reporters technologies in analyzing the relative amplicons of nucleic acid (Van Guilder, 2008). The semi-quantitative digital image endpoint analysis of PCR products is an easily accessible technology in laboratories in developing countries, cost-effective, and requires a less skilled manpower.

One of the most important applications of DNA arrays so far is the monitoring of gene expression (mRNA abundance). The collection of genes expressed or transcribed from genomic DNA sometimes referred to as the expression profile or the transcriptome, is a major determinant of cellular phenotype and function. The transcription of genomic DNA to produce mRNA is the first step in the process of protein synthesis, and differences in gene expression are responsible for both morphological and phenotypic differences as well as indicative of cellular responses to environmental stimuli and perturbations. In terms of understanding the function of genes, knowing when, where and to what extent a gene is expressed is central to understanding the activity and biological roles of its encoded protein. Also, changes in the multi-gene patterns of expression can provide clues about regulatory mechanisms and broader cellular functions and biochemical pathways (Lockhart and Winzeler, 2000).

This study established the temporal changes in expression levels of the ghrelin gene in different tissues of the liver, ovary, and testes during development in male and female improved Nigerian indigenous chickens of naked neck and normal feather chickens as a candidate gene for growth performance using the semi-quantitative digital analysis of PCR gel electrophoresis.

Materials and Methods

Experimental Location and Birds

This experiment was conducted at the Programme for Emerging Agricultural Research Leaders (PEARL) Unit of the Poultry Breeding Unit located at Teaching and Research Farms, Directorate of University Farms (DUFARMS) of the College of Animal Science and Livestock Production (COLANIM), Federal University of Agriculture, Abeokuta, Ogun State Nigeria. The experimental site was earlier described by Ilori et al. (2018). A total of 80 FUNAAB Alpha chicks comprising 16 males and 21 females naked neck, and 19 males and 24 females normal feather chickens were obtained from the FUNAAB hatchery. The birds were brooded from day-old to four weeks of age and reared on a deep litter floor in an open-sided poultry house from day old to six weeks of age and then transferred to the cage until they started laying at 24 weeks. They were fed *ad libitum* with a standard diet at the different phases. Cool and clean water was also provided without restriction. The experiment was conducted in April through September 2018 which is the period of rainfall in Southwest Nigeria. The study was conducted following the code of ethics for animal experimentation with prior approval from the Animal Care and Use Committee of the Federal University of Agriculture, Abeokuta, Nigeria Animal Ethics Committee.

Data and Tissue Collection

Body weight data were collected weekly and recorded in grams, and then weight gain was also estimated from weekly body weights. Four experimental birds for each genotype comprising of two males and two females were slaughtered fortnightly via cervical dislocation, commencing from 12 weeks of age until the birds started laying and the experiment terminated at 24 weeks (at 24th week the birds had reached 50% lay). The method of cervical dislocation was chosen as it quickly separates the spinal cord from the brain, provides fast and painless death of the bird, and also allows for easy extraction of organs. Tissues (liver, ovary, and testes) were removed aseptically, cleaned off, and weighed. The weight in grams of the organs was divided by the weight of the birds from which the organs were extracted and multiplied by 100% expressed as a percentage of the birds' weight at slaughter and then recorded as the relative weight of the organs. Each organ sample was preserved in a 1.5ml Eppendorf tube containing 500 µl of RNA latter and stored in a -80 °C ultra freezer until the expression analysis.

RNA Extraction and Reverse Transcription (RT)-PCR Assay for GHRL Gene Expression

The total RNA of each tissue sample (0.5 g) of liver, testes, and ovaries were extracted using an animal tissue RNA purification kit according to the manufacturer's protocol (Quick RNA™ MicroPrep ZYMO Research USA). The quantity and integrity of isolated RNA were determined for each sample using NanoDrop 2000 Spectrophotometer (Thermo Scientific Inc, Wilmington, Delaware, USA). Reverse transcription was performed with the SensiFAST™ cDNA Synthesis Kit according to the manufacturer's protocol in a Multigene Optimax thermocycler (Labnet International, Inc., U.S.A).

Amplification of cDNA by Polymerase Chain Reaction

This involves amplification of cDNA by polymerase chain reaction (PCR). RT products were used as a template DNA for the polymerase chain reaction (RT-PCR) of chicken Ghrelin gene fragment of 195 bp (Lu et al., 2008). Each PCR was performed in a 15 µl reaction volume containing: 1.0 µl of template with 10 ng cDNA, 3.0 µl of PCR master mix (FIREPol®Solis Biodyne UK) which contains: *Taq* DNA polymerase, dNTPs, reaction buffer, $MgCl_2$, KCl, blue dye, yellow dye and PCR enhancer/stabilizer; 1.0 µl of 0.4 µM forward primer (5'-CTG AGA GAG CAA CGG AAG T-3'), 1.0 µl of 0.4 µM reverse primer (5'-GCA AAT AAA GAG TGA GGG G-3'), and 9.0 µl nuclease-free water. Amplification for Ghrelin primer followed Lu et al. (2008) using annealing temperatures of 47.7 °C for 30 secs. PCR reactions were carried out in a Multigene Optimax thermocycler (Labnet International, Inc., U.S.A). Electrophores of the different PCR amplicons for the different tissue samples was carried out in 1% agarose using 5 µl of amplicon and 100 bp molecular marker (NorgenBiotek Corporation, Canada) and using EtBr (Boi-rad, Australia) dye in tris-acetate ethylenediaminetetraacetic acid (TAE) buffer. Agarose gel electrophoresis was carried out at 120 Volt for 60 min. Fresh TAE buffer was prepared for each electrophoresis to prevent any additive effect of residual EtBr on the PCR band density. The band size and DNA concentration of each PCR amplicon was determined by comparison with the corresponding band in the molecular weight ladder that has been predetermined by the manufacturer. Images of the bands in the gel were captured under ultraviolet (UV) light and documented using a Digital Doc gel documentation system (Bio-rad; Australia) with constant UV exposure time with adjustable exposure time whil eall the experimental conditions and parameters used were kept constant in the study.

Digital Analysis of PCR Bands on eElectrophoresis Gel

Semi-quantitative evaluation of RT signals was carried out by densitometric scanning as described (Antiabong et al., 2016) using image J Gel semi quantification software. A semi-quantitative method for conventional PCR amplicons uses digital image analysis of electrophoretic gel for quantitation of the relative amount of the amplified PCR amplicon. Digital analysis of agarose gel image method for the semi-quantitation of PCR amplicons (Abramoff et al., 2004) has been successfully applied in quantitative immunoblot assays and nucleic acid quantitation (Berchtold et al., 2007; Rai et al., 2010; Antiabong et al., 2016). Since ImageJ software measures the individual band densities by relative comparison of all the band characteristics in a single gel, samples were run on the same gel to ensure accuracy. The trends observed in different gel analyses was then compared. The size, thickness, density, picture format of the agarose gel, reagents, and other conditions was also kept constant. These were ensured to normalize the PCR reactions.

Statistical Analysis

The collected data were analyzed by two-way analysis of variance (ANOVA) using the General Linear Model (GLM) procedures of the SAS Institute (SAS 2001). The means comparison was performed using Duncan's Multiple Range Test at 5%

probability level. Preliminary analysis of the interaction between genotype and sex was not significant and therefore excluded from the analysis.

Model is: $Y_{ijk} = \mu + G_i + Sj + E_{ijk}$

where:

Y_{ijk} = Body weight and weight gains

μ = population mean

G_i = effect ofith genotype

S_j = effect of jth sex

E_{ijk} = residual error

Results

The performance of Nigerian indigenous chicken in terms of body weight and weight gain are presented in Table 1. The result of this study showed a significant effect ($P < 0.05$) of genotype on body weight from week 0 to week 20, except at week 24 where genotype was observed to have no significant effect ($P > 0.05$) on body weight. The mean body weight ranged from 43.91 ± 0.96 g and 48.74 ± 1.59 g at week 0 in the naked neck and normal feather respectively to 2228.0 ± 73.32 g and 2378.5 ± 197.16 g at twenty-four weeks. Genotype also had a significant effect ($P < 0.05$) on weight gain from week 4 to week 16 except at weeks 20 and 24 where there was no significant difference ($P > 0.05$). A steady increase in body weight was observed in the two genotypes with an increase in age of the birds, and the normal feather genotype have a higher body weight and weight gain than the naked neck genotype in all ages.

The effect of sex on body weight and their weight gain of Nigerian Indigenous chicken are presented in Table 2. Sex had a significant effect ($P < 0.05$) on body weight and weekly weight gain of both sexes from week 8 to week 20, however a significant difference was not observed at weeks 0, 4, and 24 in body weight and weight gain of both sexes. The male birds had higher body weight and weight gain than the female birds at all weeks. The mean body weight ranged from 47.95 ± 1.85 g and 45.54 ± 1.08 g at week 0 in male and female chickens to 2391.40 ± 200.62 g and 2101.00 ± 100.01 g at twenty-four weeks respectively.

Table 3 shows the effect of the genotype on the liver weight of Nigerian indigenous chicken at different weeks. The effect of genotype was not significant effect ($P < 0.05$) on liver weight except at week 12 where the naked neck genotype had a higher liver weight than the normal feather genotype. There was no significant effect ($P < 0.05$) of sex on liver weight in all weeks, except at week 18, where males had significantly higher liver weight than the females (Table 4).

Table 5 shows the effect of genotype on testes weight of Nigerian indigenous chicken at different weeks. Genotype had no significant effect ($P < 0.05$) on testes weight from week 12 to week 18. However, a significant effect ($P < 0.05$) of genotype on testes weight was observed from week 20 to week 24 with a higher significant weight in the naked neck genotype.

Table 1. Least squares means of body weight and weight gain as affected by genotype in Nigerian indigenous chickens

Age (weeks)	Genotype	No of observation	Body weight	Weekly weight gain
0	Naked Neck	80	43.91 ± 0.96^{b}	-
	Normal Feather		48.74 ± 1.59^{a}	-
4	Naked Neck	80	272.96 ± 9.34^{b}	52.68 ± 3.64^{b}
	Normal Feather		364.56 ± 15.41^{a}	69.96 ± 5.55^{a}
8	Naked Neck	80	591.27 ± 22.76^{b}	104.14 ± 7.77^{b}
	Normal Feather		840.85 ± 38.66^{a}	143.41 ± 10.04^{a}
12	Naked Neck	72	883.29 ± 36.94^{b}	59.41 ± 6.00^{b}
	Normal Feather		1298.63 ± 66.67^{a}	93.26 ± 9.45^{a}
16	Naked Neck	64	1151.15 ± 46.67^{b}	60.15 ± 7.16^{b}
	Normal Feather		1652.40 ± 99.59^{a}	111.60 ± 10.42^{a}
20	Naked Neck	56	1524.13 ± 89.24^{b}	95.13 ± 16.18
	Normal Feather		2273.17 ± 181.20^{a}	106.00 ± 21.42
24	Naked Neck	48	2228.0 ± 73.32	71.5 ± 11.84
	Normal Feather		2378.5 ± 197.16	86.5 ± 9.52

Note: ^{ab} - means with different superscripts on the same row for the same week are different significantly ($P < 0.05$) according to Duncan's Multiple Range Test

Table 2. Least squares means of body weight and weight gain as affected by sex in Nigerian indigenous chicken

Age (weeks)	Genotype	No of observation	Body weight	Weekly weight gain
0	Naked Neck	80	47.95 ± 1.85	-
	Normal Feather		45.54 ± 1.08	-
4	Naked Neck	80	341.00 ± 12.89	63.09 ± 4.12
	Normal Feather		310.25 ± 15.49	60.79 ± 1.86
8	Naked Neck	80	795.19 ± 40.30^{a}	146.67 ± 11.75^{a}
	Normal Feather		679.00 ± 34.41^{b}	110.11 ± 7.43^{b}
12	Naked Neck	72	1275.87 ± 80.67^{a}	106.73 ± 10.68^{a}
	Normal Feather		978.67 ± 46.17^{b}	56.24 ± 4.97^{b}
16	Naked Neck	64	1784.55 ± 119.65^{a}	123.64 ± 13.29^{a}
	Normal Feather		1191.53 ± 45.86^{b}	64.47 ± 5.68^{b}
20	Naked Neck	56	2370.40 ± 124.79^{a}	149.80 ± 22.34^{a}
	Normal Feather		1541.67 ± 77.31^{b}	72.60 ± 11.49^{b}
24	Naked Neck	48	2391.40 ± 200.62	74.67 ± 9.61
	Normal Feather		2101.00 ± 100.01	92.00 ± 8.01

Note: ^{ab} - means with different superscripts on the same row for the same week are different significantly ($P < 0.05$) according to Duncan's Multiple Range Test

Table 3. Least squares means of liver weight as affected by genotype in Nigerian indigenous chickens

Age (weeks)	Number of observation	Liver weight (g)	
		Normal feather	Naked neck
12	8	1.84 ± 0.08^{b}	2.77 ± 0.05^{a}
14	8	2.25 ± 0.19	2.23 ± 0.12
16	8	2.46 ± 0.43	1.91 ± 0.23
18	8	2.11 ± 0.29	2.37 ± 0.16
20	8	1.83 ± 0.50	2.05 ± 0.29
22	8	2.87 ± 0.25	2.31 ± 0.11
24	8	1.62 ± 0.43	1.69 ± 0.22

Note: ^{ab} - means with different superscripts on the same row for the same week are different significantly ($P < 0.05$) according to Duncan's Multiple Range Test

Table 4. Least squares means of liver weight as affected by sex in Nigerian indigenous chickens

Age (weeks)	Number of observation	Liver weight (g)	
		Male	Female
12	8	2.23 ± 0.27	2.24 ± 0.30
14	8	2.103 ± 0.14	2.43 ± 0.14
16	8	2.34 ± 0.49	2.07 ± 0.08
18	8	2.25 ± 0.06^{a}	1.39 ± 0.08^{b}
20	8	1.26 ± 0.30	2.08 ± 0.31
22	8	2.49 ± 0.23	2.68 ± 0.25
24	8	1.32 ± 0.14	1.97 ± 0.07

Note: ^{ab} - means with different superscripts on the same row for the same week are different significantly ($P < 0.05$) according to Duncan's Multiple Range Test

Table 5. Least squares means of testes weight as affected by genotype at different weeks

Age (weeks)	Number of observation	Testes weight (g)	
		Naked neck	Normal feather
12	8	0.91 ± 0.02	0.73 ± 0.04
14	8	1.22 ± 0.32	0.94 ± 0.34
16	8	1.51 ±0 .43	1.00 ± 0.23
18	8	1.64 ± 0.29	1.35 ± 0.26
20	8	2.86 ± 0.68^{a}	1.96 ± 0.61^{b}
22	8	3.00 ± 0.55^{a}	2.04 ± 0.82^{b}
24	8	3.80 ± 0.98^{a}	2.85 ± 0.35^{b}

Note: ^{ab} - means with different superscripts on the same row for the same week are different significantly ($P < 0.05$) according to Duncan's Multiple Range Test

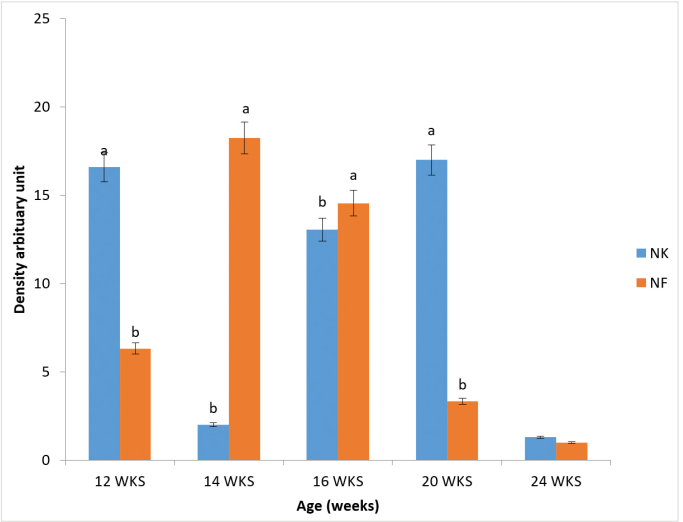
The effect of the genotype on the ovary weight of Nigerian indigenous chicken at different weeks is shown in Table 6. Genotype had no significant effect on ovary weight from week 12 to 18. However, a significantly higher ($P < 0.05$) ovary weight in the naked neck genotype was observed from week 20 to week 24.

Table 6. Least squares means of ovary weight as affected by genotype at different weeks

Age (weeks)	Number of observation	Ovary weight (g)	
		Naked neck	Normal feather
12	8	1.30 ± 0.68	1.15 ± 0.04
14	8	1.41 ± 0.52	1.29 ± 0.14
16	8	1.88 ± 0 .55	1.35 ± 0.27
18	8	2.00 ± 0.29	1.38 ± 0.26
20	8	2.98 ± 0.44^{a}	1.56 ± 0.76^{b}
22	8	3.55 ± 0.55^{a}	2.04 ± 0.82^{b}
24	8	3.99 ± 0.78^{a}	2.22 ± 0.55^{b}

Note: ^{ab} - means with different superscripts on the same row for the same week are different significantly ($P < 0.05$) according to Duncan's Multiple Range Test

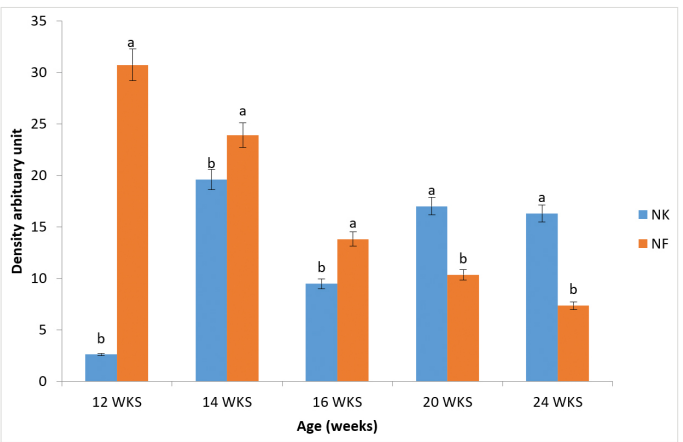
Fig. 1 shows the semi-quantitative analysis of ghrelin mRNA in the liver samples of the naked neck and normal feather male chickens. Significant differences ($P < 0.05$) were observed in the expression levels of ghrelin mRNA in the liver of the two genotypes except at week 24. The naked neck males had a higher expression level of ghrelin mRNA at weeks 12 and 20 while the normal feather males also had a higher level of GHRL mRNA at weeks 14 and 16.



Note: ab - bars with different superscripts for the same week are different significantly ($P < 0.05$) according to Duncan's Multiple Range Test; NK = Naked neck chicken; NF = Normal feather chicken

Figure 1. Expression level of GHRL mRNA in liver samples of the naked neck males and normal feather males chickens at different weeks

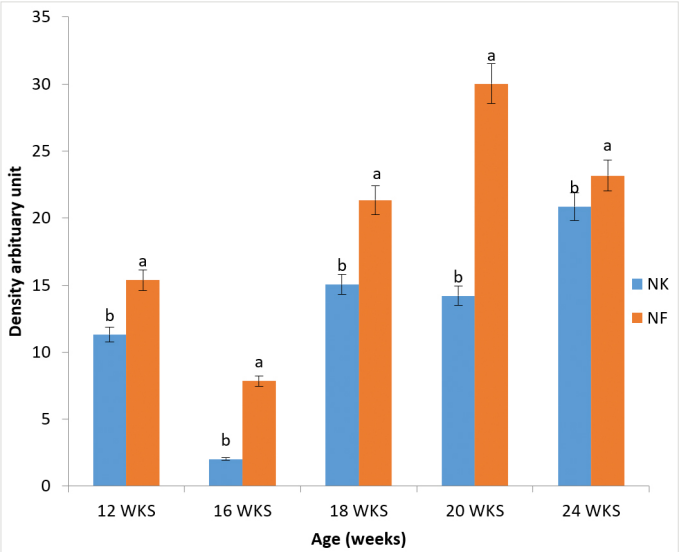
Semi-quantitative analysis of ghrelin mRNA in the liver samples of the naked neck and normal feather female chickens is shown in Fig. 2. Significant ($P < 0.05$) ghrelin mRNA expression levels were observed across all weeks considered, with a significantly higher expression level at weeks 12, 14, and 16 in the normal feather chicken while the naked neck was observed to have a higher expression level at weeks 20 and 24.



Note: ab - bars with different superscripts for the same week are different significantly ($P < 0.05$) according to Duncan's Multiple Range Test ; NK = Naked neck chicken; NF = Normal feather chicken

Figure 2. Expression level of GHRL mRNA in liver samples of the naked neck and normal feather female chickens at different weeks

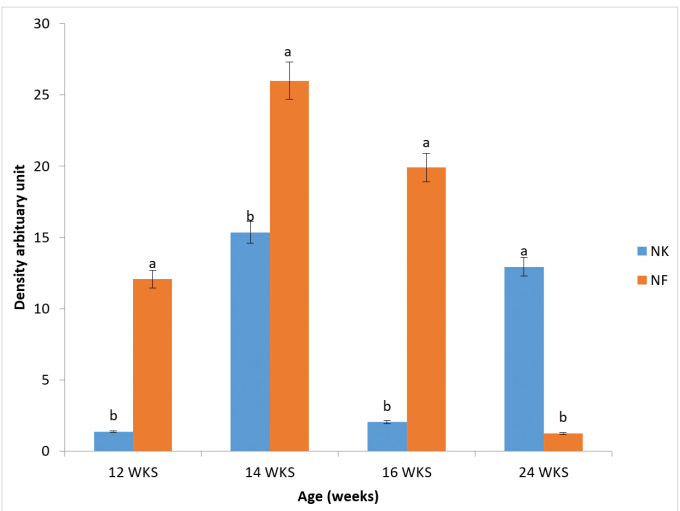
Fig. 3 shows the semi-quantitative analysis of ghrelin mRNA in the ovary samples of the naked neck and normal feather female chickens. Higher significant ($P < 0.05$) expression level of ghrelin mRNA was observed in the ovary of the normal feather chicken than the naked neck chickens across all the weeks.



Note: ab - Bars with different superscripts for the same week are different significantly ($P < 0.05$) according to Duncan's Multiple Range Test ; NK = Naked neck chicken; NF = Normal feather chicken

Figure 3. Expression level of GHRL mRNA in ovary samples of the two chicken genotypes at different weeks

Semi-quantitative analysis of ghrelin mRNA in the testes samples of the naked neck female and normal feather male chicken is shown in Fig. 4. There were significant differences ($P < 0.05$) in levels of ghrelin mRNA expression in the testes of the two genotypes across all weeks. The normal feather males had higher expression levels of ghrelin mRNA at all weeks except at week 24.



Note: ab - bars with different superscripts for the same week are different significantly ($P < 0.05$) according to Duncan's Multiple Range Test ; NK = Naked neck chicken; NF = Normal feather chicken

Figure 4. Expression level of GHRL mRNA in testes samples of the two chicken genotypes

Discussion

The results from this study showed a higher body weight and weight gain in the normal feather genotype than the naked neck genotype. This is not in agreement with the results of Patra et al. (2002) and Adeleke et al. (2011) that reported a higher body weight in the naked neck chickens. According to their study, the higher body weight exhibited by the naked neck could be attributed to the possession of feather distribution genes (*Naked neck gene*) that had been reported to reduce feather mass by 20–40%. The reduction in feather mass improves heat dissipation through the naked area (Singh et al., 2001). In a similar study by Merat (1986), several mechanisms appear to be responsible for higher meat production of chickens with reduced plumage and stated that the more rapid dissipation of heat resulted in less appetite depression and consequently better growth, particularly at high ambient temperature. This research was conducted during April to September and in this period, which isthe most abundant rainfall for the year and ambient temperature is quite low compared to other seasons of the year. Therefore, there is a high likelihood for the normal feather chicken to take advantage of this season of the year for more increased body weight than the naked neck chickens. In a similar study by Yalcin et al. (1997), naked neck chickens were observed to exhibit a highly significant advantage over the normal feather chicken in the hottest climate and they also reported that means were similar in both genotypes in the cool temperate climate.

The male chickens had a higher body weight than their female females for both genotypes (Table 2). This is consistent with previous reports (Garcia et al., 1992; Ikeobi et al., 1995; Ilori et al., 2010) that sexual dimorphism was in favour of males in the performance of strains of birds studied. Also, the comparative advantage of males over females in most of the morphological traits examined in ducks (Yakubu, 2011) and turkey (Ilori et al., 2010) reported that the differences in the size of the birds could be attributed to sex differential hormonal effects on growth.

The significantly higher liver, ovary, and testes weight in the naked neck genotypes agree with the findings of Gunn (2008) who reported that naked neck birds had a higher weight of organs than the normal feather birds. Therefore it can be suggested that despite the lower body weight of the naked neck chicken as observed in this study, they performed better in terms of weight of reproductive organs and liver. This is also in agreement with the study of Oke and Ihemeson (2010) where the testes weight was significantly higher in the naked neck chickens than in the normal feather chickens.

The expression of the ghrelin gene in all the tissues sampled in this study suggests that ghrelin plays a role in all the tissues examined in this study. All tissues expressed the mRNA for the ghrelin gene suggesting that ghrelin protein was secreted by these tissues and with the presence of the receptors are utilized in these tissues. Ghrelin has been implicated in several metabolic (Saito et al., 2005; Chen et al., 2007) and reproductive functions (Tena-Sempere, 2005; Sirotkinet al., 2006) in avian species and also modulates secretion, food intake, and energy homeostasis (Van der Lely et al., 2004). From the results, ghrelin mRNA expression levels were observed to be significantly higher in normal feather chickens than in the naked neck chickens. The

significant expression level of ghrelin mRNA in the testes and ovaries of the normal feather chickens explains that this gene also plays a role in the male and female reproductive tract. The higher level of expression of ghrelin mRNA at maturity in naked neck chicken might not be unconnected with the consistently lower body weight and weekly weight gain compared to the normal feather chicken. One of the functional features of ghrelin that has attracted more attention is its ability to stimulate food intake and to promote body weight gain. As orexigenic signal, expression of ghrelin has been shown to increase after food deprivation, and its plasma levels are in most cases negatively correlated with the body mass index. On this basis, ghrelin has been proposed as a unique circulating signal for energy insufficiency (the only known circulating orexigen), which may play a major role in the short- and long-term control of body weight (Cummings, 2006; Zigman and Elmquist, 2003). The role of ghrelin in the ovary of the chicken has not been clearly defined but at the ovarian level, ghrelin has been implicated in the regulation of gonadotrophin-induced steroidogenesis and ovulation as several studies have reported ghrelin to promote granulosa cell proliferation, inhibit apoptosis, stimulate the release of all the steroid hormones in porcine species (Sirotkinet al., 2010) and also to regulate the ovarian cells secretory activities in chicken (Sirotkin and Grossmann, 2007). The higher expression of Ghrelin in the liver at puberty in the naked neck chicken may explain the low growth performance achieved in the chicken during this period as its plasma levels have been reported to be negatively correlated with the body mass index, proposed as a unique circulating signal for energy insufficiency and orexigenicity which may play a major role in the short- and long-term control of body weight (Cummings, 2006; Zigman and Elmquist, 2003). The results from this study confirm the result from a similar study by Montague et al. (1997) that the expression of these two genes is genotype-dependent.

Conclusion

From the result obtained from this study, it can be concluded that genotype and sex had a significant effect on the body weight of Nigerian indigenous chickens. The Naked neck chickens had higher mRNA expression in the liver at puberty, which may explain their low growth performance in that stage. This study further established the expression of ghrelin mRNA in the liver, ovary, and testes of Nigerian indigenous chicken suggesting and confirming that ghrelin protein is secreted by these tissues and with the presence of the receptors is utilized in these tissues. The method used in the study, although not performed at the exponential phase of the PCR amplification reaction as it is semi-qunatitative optimization with ImageJ quantitation of PCR amplicons, allows the determination of the trend in expression of the target gene with useful information for candidate gene selection in genetic improvement programme.

CRediT authorship contribution statement

Ayotunde Olutumininu Adebambo: Conceived the project and supervised the work. **Babatunde Moses Ilori, Mohammed Okanlawon Onagbesan:** Supervised the work. **Omotomiwa Abiodun Osikomaiya:** Investigation, performed most of the experiments. **Omotomiwa Abiodun Osikomaiya, Babatunde Moses Ilori:** analysed the data and drafted the manuscript. **Babajide Samuel Obimakinde, Samuel Olutunde Durosaro:** Performed some of the experiments, analysed some of the data. **Babatunde Moses Ilori:** edited the manuscript.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest that could bias the integrity or objectivity of this work. There are no financial, personal, or professional relationships that could be perceived as influencing the research presented in this article. No external entity had any involvement in the study design, data collection, analysis, interpretation, or manuscript preparation.

References

Abramoff M. D., Magelhaes P. J., Ram S. J. (2004). Image Processing with Image. J Biophotonics Int 11 (7): 36–42.

Adeleke M. A., Peters S. O., Ozoje M. O., Ikeobi C. O. N., Bamgbose A. M., Adebambo O. A. (2011). Growth Performance of Nigerian Local Chicken in Crosses Involving an Exotic Broiler Breeder. Trop Anim Health Prod 43: 643-650 doi: 10.1007/s 11250-010-9747-3

Ajayi F. O. (2010). Nigerian Indigenous Chicken: A Valuable Genetic Resource for Meat and Egg Production. Asian J Poult Sci 4 (4): 164-172. doi: 10.3923/ajpsaj.2010.164.172

Ajayi F. O., Agaviezor B. O. (2016). Fertility and Hatchability Performance of Pure and Crossbred Indigenous Chicken Strains in the High Rainforest Zone of Nigeria. Int J Livest Prod 7 (12): 141- 144. doi: 10.5897/IJLP2016.0308

Anthony N. B, Vasilatos – Younken R., Emmerson D. A., Nestor K. E., Bacon W. L. (1990). Pattern of Growth and Plasma Growth Hormones Secretion in Turkey Selected for Increased Egg Production. Poult Sci 69 (12): 2057-2063. doi: 10.3382/ps.0692057

Antiabong J. F., Ngoepe M. G., Abechi A. S. (2016). Semi-Quantitative Digital Analysis of pPlymerase Chain Reaction-Electrophoresis Gel: Potential Applications in Low-Income Veterinary Laboratories. Vet World 9 (9): 935–939.

Arvat E., Maccario M., di Vito L., Broglio F., Benso A., Gottero C., Papotti M., Muccioli G., Dieguez C., Casanueva F. F., Deghenghi R., Camanni F., Ghigo E. (2001). Endocrine Activities of Ghrelin, a Natural Growth Hormone Secretagogue (GHS), in Humans: Comparison and Interactions with Hexarelin, a Nonnatural Peptidyl GHS, and GHreleasing Hormone. J Clin Endocrinol Metab 86: 1169–1174. doi: 10.1210/jcem.86.3.7314

Barreiro M. L., Tena-Sempere M. (2004). Ghrelin and Reproduction: A Novel Signal Linking Energy Status and Fertility. Mol Cell Endocrinol 226: 1-9. doi: 10.1016/j.mce.2004.07.015

Barreiro M. L., Tena-Sempere M. (2002). Ghrelin and Reproduction: A Novel Signal Linking Energy Status and Fertility. Mol Cell Endocrinol 226: 1-9. doi: 10.1016/j.mce.2004.07.015

Berchtold C. M., Wu Z. H., Huang T. T., Miyamoto S. (2007). Calcium-Dependent Regulation of NEMO Nuclear Export in Response to Genotoxic Stimuli. Mol Cell Biol 2007: 497–509. doi: 10.1128/MCB.01772-06

Chen H. Y., Trumbauer M. E., Chen A. S., Weingarth D. T., Adams J. R., Frazier E. G., Shen Z., Marsh D. J., Feighner S. D., Guan X. M., Ye Z., Nargund R. P., Smith R. G., Van der Ploeg L. H. T., Howard A. D., MacNeil D. J., Qian S. (2004). Orexigenic Action of Peripheral Ghrelin Is Mediated by Neuropeptide Y and Agouti-Related Protein. Endocrinol 145: 2607–2612. doi: 10.1210/en.2003-1596

Chen L. L., Jiang Q. Y., Zhu X. T., Shu G., Bin Y. F., Wang X. Q., Gao P., Zhang Y. L. (2007). Ghrelin Ligand Receptor mRNA Expression in Hypothalamus, Proventriculus and Liver of Chicken (*Gallus gallus domesticus*): Studies on Ontogeny and Feeding Conditions. Comp Biochem Physiol Part A: Mol Integr Physiol 147 (4): 893-902. doi: 10.1016/j.cbpa.2007.02.017.

Cummings D. E. (2006). Ghrelin and the Short- and Long Term Regulation of Appetite and Body Weight. Physio Behav 89 (1): 71-84. doi: 10.1016/j.physbeh.2006.05.022

Ebozoje M.O., Ikeobi C. O. N. (1995). Productive Performance and Occurrence of Major Genes in the Nigerian Local Chicken. Niger J Genet 10: 67–77.

Garcia E. A., Mendes P. K., Curi P. K., da Silva A. B. P, Gonzalez E. (1992). Effect of Line and Diet on Growth and Carcass Yield of Broilers. Veterinária e Zootecnia 4: 7-15.

Gnanapavan S., Kola B., Bustin S. A., Morris D. G., McGee P., Fairclough P., Bhattacharya S., Carpenter R., Grossman A. B., Korbonits M. (2002). The Tissue Distribution of the mRNA of Ghrelin and Subtypes of Its Receptor (GHS-R) in Humans. J Clin Endocrinol Metab 87: 2988. doi: 10.1210/jcem.87.6.8739

Gualillo O., Lago F., Gomez-Reino J., Casanueva F. F., Dieguez C. (2003). Ghrelin, a Widespread Hormone: Insights into Molecular and Cellular Regulation of Its Expression and Mechanism of Action. FEBS Letters. 552:105-109. doi: 10.1016/S0014-5793(03)00965-7

Gunn H. (2008). Effect of Frizzling and Naked Neck Gene on Growth, Haematology, Carcass Traits and Organ Weights of the Nigerian Local Chicken. PhD Thesis, Department of Animal Breeding and Genetics, University of Agriculture Abeokuta.

Ikeobi C. O. N., Peters S. O., Ebozoje M. O. (1995). Sexual Dimorphism in Two Strains of Broiler Chicken. Niger J Genet. 10: 16-22.

Ilori B. M., Oguntade D. O., Akano K., Durosaro S. O., Ozoje M. O. (2018). Reproductive Performance, Feed Intake and Efficiency of Indigenous and Crossbred Turkeys. J Agric Sci Environ18 (1): 2315 – 7453.

Ilori B. M., Oyeniyi T., Dada Q. A., Ayankeye T. R., Hamzat F., Durosaro S. O., Wheto M., Adebambo A. O., Adebambo O. A. (2017). Effect of Crossbreeding and Selection for Meat on Nigerian Indigenous Chickens. Bull Anim Health Prod Afr 65: 277-287.

Ilori B. M., Peters S. O., Ikeobi C. O. N., Bamgbose A. M., Isidahomen C. E., Ozoje M. O. (2010). Comparative Assessment of Growth in Pure and Crossbred Turkeys in a Humid Tropical Environment. Int J Poult Sci 9 (4): 368-375.

Jeong J. K., Kim J. G., Lee B. J. (2014). Cellular and Molecular Life Sciences Participation of the Central Melanocortin System in Metabolic Regulation and Energy Homeostasis. Cell Mol Life Sci 71: 3799–3809. doi: 10.1007/s00018-014-1650-z

Jin S., Chen S., Li H., Lu Y., Xu G., Yang N. (2014). Associations of Polymorphisms in GHRL, GHSR, and IGF1R Genes with Feed Efficiency in Chickens. Mol Biol Rep 41: 3973–3979. doi: 10.1007/s11033-014-3265-8

Lockhart D. J., Winzeler E. A. (2000). Genomics, Gene Expression and DNA Array Genomics, Gene Expression, and DNA Arrays. Nature 405: 827-836. doi: 10.1038/35015701

Lu F. Z., Wang X. X., Pan Q. X., Huang R. H., Liu H. L. (2008). Expression of Genes Involved in the Somatotropic, Thyrotropic, and Corticotropic Axes During Development of Langshan and Arbor Acres Chickens. Poult Sci 87 (10): 2087-2097. doi: 10.3382/ps.2007-00493

Merat P. (1986). Potential Usefulness of the Naked Neck Gene in Poultry Production. World's Poult Sci J 42: 124-142.

Montague C. T., Prins J. B., Sanders L., Digby J. E., O'Rahilly S. (1997). Depot and Sex-Specific Differences in Human Leptin mRNA Expression: Implications for the Control of Regional Fat Distribution. Diabetes 46 (3): 342-347. doi: 10.2337/diab.46.3.342

Oke U. K., Ihemeson C. (2010). Effect of Genotype on the Morphometric Differentiation of the Reproductive Organs and Sperm Reserves in the Nigerian Local Chicken. Livest Res Rural Dev 22 (3): 53.

Otto B., Cuntz U., Fruehauf E., Wawarta R., Folwaczny C., Riepl R. L., Heiman M. L., Lehnert, P., Fichter M., Tschop M. (2001). Weight Gain Decreases Elevated Plasma Ghrelin Concentrations of Patients with Anorexia Nervosa. Eur J Endocrinol 145 (5): 669-673.

Patra B. N., Bais R. K. S., Prasad R. B., Singh B. P. (2002). Performance of Naked Neck versus Normally Feathered Coloured Broilers for Growth, Carcass Traits and Blood Biochemical Parameters in Tropical Climate. Asian-Aust J Anim Sci 15 (12): 1776-1783.

Peters S. O., Ikeobi C. O. N., Ozoje M.O., Adebambo O. A. (2005). Modeling Growth in Seven Chicken Genotypes. Niger J Anim Prod 32 (1): 28–38.

Rai T., Mosoian A., Resh M. D. (2010). Annexin 2 Is not Required for Human Immunodeficiency Virus Type 1 Particle Production but Plays a Cell Type-Dependent Role in Regulating Infectivity. J Virol 2010: 9783–9792. doi: 10.1128/JVI.01584-09

Saito E., Kaiya H., Tachibana T., Tomonaga S., Denbow D., Kangawa K., Furuse M. (2005). Inhibitory Effect of Ghrelin on Food Intake Is Mediated by the Corticotropin-Releasing Factor System in Neonatal Chicks. Regul Pept 125: 201-2008. doi: 10.1016/j.regpep.2004.09.003

SAS (2001) User's guide: Statistics, Version 8.2. Statistical Analysis System Inc., Cary, NC.

Singh C. V., Kumar D., Singh Y. P. (2001). Potential Usefulness of the Plumage Reducing Naked Neck (Na) Gene in Poultry Production at Normal and High Ambient Temperatures. World's Poult Sci J 57: 140-156. doi: https://doi.org/10.1079/WPS20010011

Sirotkin A., Grossmann R. (2007). The Role of Ghrelin and Some Intracellular Mechanisms in Controlling the Secretory Activity of Chicken Ovarian Cells. Comp Biochem Physiol A Mol Integr Physiol 147 (1): 239-246. doi: 10.1016/j.cbpa.2006.12.038

Sirotkin A. V., Makarevich A. V., Grosmann R. (2010). Protein Kinases and Ovarian Functions. J Cell Physiol 226 (1): 37–45. doi:10.1002/jcp.22364

Sirotkin A.V., Grossmann R., Maria-Peon M. T., Roa J., Tena-Sempere M., Klein S. (2006). Novel Expression and Functional Role of Ghrelin in Chicken Ovary. Mol Cell Endocrinol 257–258: 15–25. doi: 10.1016/j.mce.2006.06.004

Sonaiya E. B., Olori V. E. (1990). Village Poultry Production in Southwestern Nigeria. In E.B. Sonaiya, ed. Rural Poultry in Africa: Proc. of an Int. Workshop held in Ile-Ife, Nigeria, November 13–16, 1989, pp. 243–247. Thelia house, Ile-Ife.

Tena-Sempere M. (2005). Ghrelin: Novel Regulator of Gonadal Function. J Endocrinol Invest 25: 26-29.

Van der Lely A. J., Tschop M., Heiman M. L., Ghigo E. (2004). Biological, Physiological, Pathophysiological, and Pharmacological Aspects of Ghrelin. Endocr Rev 25: 426–457. doi: 10.1210/er.2002-0029

Van Guilder H. D., Vrana K. E., Freeman W. M. (2008). Twenty-Five Years of Quantitative PCR for Gene Expression Analysis. Biotechniques 44: 619. doi: 10.2144/000112776

Wimmers K., Ponsuksili S., Hardge T., Valle-Zarate A., Mathur P. K., Horst P. (2000). Genetic Distinctness of African, Asian and South American Local Chickens. Anim Genet 31: 159–165. doi: 10.1046/j.1365-2052.2000.00605.x

Wortley K. E., Anderson K. D., Garcia K., Murray J. D., Malinova L., Liu R., Moncrieffe M., Thabet K., Cox H. J., Yancopoulos G. D., Wiegand S. J., Sleeman M. W. (2004). Genetic Deletion of Ghrelin Does not Decrease Food Intake but Influences Metabolic Fuel Preference. Proc Natl Acad Sci USA 101: 8227–8232. doi: 10.1073/pnas.0402763101

Yakubu A., Bamidele O., Hassan W. A., Ajayi F. O., Ogundu U. E., Alabi O., Sonaiya E. B. Adebambo O. A. (2019). Farmers' Choice of Genotypes and Trait Preferences in Tropically Adapted Chickens in Five Agro-Ecological Zones in Nigeria. Trop Anim Health Prod 52: 95–107. doi: 10.1007/s11250-019-01993-0

Yakubu A. (2011). Discriminant Analysis of Sexual Dimorphism in Morphological Traits of African Muscovy Ducks. Arch Zootec 60 (232): 11115-11123.

Yalcin S., Testik A., Ozkan S., Settar P., Celen F., Cahaner A. (1997). Performance of Naked Neck and Normal Broilers in Hot, Warm, and Temperate Climates. Poult Sci 76: 930–937. doi: 10.1093/ps/76.7.930

Zigman J. M., Elmquist J. K. (2003). Mini Review: from Anorexia to Obesity- the Ying and Yang of Body Weight Control. Endocrinol 144: 3749-3756. DOI: 10.1210/en.2003-0241

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