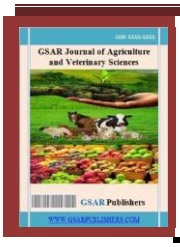

GSAR Journal of Agriculture and Veterinary Sciences
ISSN: 3048-9075 (Online)
Abbreviated key title: Glob.J. Agri.Vet.Sci.
Frequency: Monthly
Published By GSAR Publishers
Journal Homepage Link- https://gsarpublishers.com/journal-gjavs-home/

EXPRESSION OF INSULIN GROWTH FACTOR GENES IN NIGERIAN LOCAL CHICKENS WITH DIFFERENT PLUMAGE PATHERNS

By

Shukurat Opeyemi Akewushola[1], O. M. Adeshina, Saadat M. Ajisomo[2], , Abdullahi Shaadat Bukola[3] and Abdulqudus Abdullateef Alabi[4].

[1,2,3,4]Department of Animal Production, Faculty of Agriculture, Kwara State University, Malete, Kwara State, Nigeria

Article History

Received: 15/08/2026
Accepted: 24/08/2026
Published: 25/08/2026

Vol – 3 Issue –8

PP: -53-56

Abstract

African Local Chicken (ALC) ecotypes, especially Nigerian Local Chicken (NLC) are unique and precious source of indigenous poultry genetic resources with evolutionary fitness and strong ability to adapt to the extreme tropical conditions. Thus this study was designed to understand the intricate relationship between five major qualitative plumage phenotypes, Normal Feathered, Naked Neck, Frizzle Feathered, Feathered Shank (Ptylopody), and Crested and their growth architectures. The absolute quantitative molecular expression was profile with a precise and multi-dimensional morphometric analysis in the experimental model. The precise and multi-dimensional morphometric analysis was combined with the absolute quantitative molecular expression profiling. The phenotypic skeletal development was measured in relation to linear structural dimensions and quantitative real-time polymerase chain reaction (qPCR) was used to quantify the relative mRNA transcription levels of Insulin-like Growth Factor-I (IGF-I) using biologically different tissue matrices, namely hepatic tissue (liver), deep structural pectoral muscle and blood.

The morphometric data showed very different growth patterns among the cohorts. Body girth was found to be a critical structural focus among the variant with very high, highly significant positive correlation coefficients with back length having r of 0.660, followed by body depth (r = 0.584) and keel length (r = 0.538). At the molecular level, the real-time transcription profiles showed different tissue-specific differences with respect to the gene expression. The liver-dominant endocrine phenotype (classical) was characterized by the Frizzle feather pattern as they had exceptionally high transcription of IGF-I and IGF-II mRNA in the liver and moderate to low levels in peripheral tissues.

Keywords: *Nigerian Local Chickens · Plumage gene mutations · Linear morphometrics · Somatotropic endocrine axis · IGF-I gene expression*

1. INTRODUCTION

Poultry production is a key sector in the agricultural economies of developing countries and is a vital component of rural food security, sustainable livelihoods of smallholders and is a major tool for reducing animal protein deficiency in sub-Saharan Africa. In the local socio-economic context, indigenous chicken ecotypes dominate the national poultry population at more than 80%, and offer a sustainable and easily accessible source of good quality dietary protein in the form of meat and eggs. These birds are well suited to the harsh environment of which they are natives, and have an extraordinary evolutionary fitness profile. They are very tolerant of endemic pathogens, highly efficient scavengers on poor diets and have a remarkable ability to tolerate extreme ambient temperatures and relative humidity.

Although these are important adaptive traits, indigenous poultry suffer a significant production constraint, which is their slow post-hatch growth rate, and low yields of carcass weight. Native ecotypes need 16–24 weeks of intensive or semi-intensive rearing to attain minimal marketable live weights compared to modern broiler strains that are highly selected for the commercial industry, which attain much higher weights within a fraction of that interval.

This extensive morphological heterogeneity makes the characterisation and improvement of these indigenous genetic

*Corresponding Author: **Shukurat Opeyemi Akewushola.**

resources difficult. The most obvious natural expression of this diversity is a number of naturally occurring, important plumage mutations within local populations. Mutations like Naked Neck (Na), Frizzle (F), Crested (Cr) and Feathered Shank (Pti) are found in all the ecological zones and are well entrenched in the Nigerian Local Chicken (NLC) genetic resources.

Importantly, modern animal science demonstrates that these plumage variations are far from simply superficial or neutral aesthetic features. There is evidence that these key feather alleles have strong pleiotropic effects, influencing a variety of physiological, metabolic, and production traits. Modifications in the plumage in poultry change basic metabolic rate, tissue-specific nutrient utilization and allocation of structural amino acids. The synthesis of melanin, for example, is an expensive process which requires the use of certain amino acids, e.g. tyrosine, phenylalanine and cysteine, in particular. This requirement creates a direct, systemic relationship between deposition of structural myofibrillar proteins in skeletal muscle tissue and the requirement.

In avian species, post-hatch skeletal elongation and muscle mass gain is mainly a reflection of the somatotropic endocrine axis. This complex regulatory system is regulated in large part through the Insulin-like Growth Factor (IGF) system, the signaling of which depends on two different ligands, Insulin-like Growth Factor-I (IGF-I) and Insulin-like Growth Factor-II (IGF-II). The role of IGF-I is to be the primary stimulator of post-hatch growth, and to control the growth of the skeletal frame and muscle cell multiplication through stimulation of protein synthesis and inhibition of protein breakdown inside the cells. IGF-II also has a major role, as required growth factor in the embryo and it is still present and functional in early post-hatch.

The synthesis of IGF ligands is mainly in the liver under the stimulation of growth hormone (GH) binding to its specific receptor. In addition, the production of extrahepatic hormones in target peripheral tissues (e.g., skeletal muscle or circulating vascular components), and the subsequent activation of autocrine and paracrine loops, is also essential, and may occur independently of central endocrine control.

Although specific single nucleotide polymorphisms (SNPs) and structural variations in the core IGF-I genomic regions are currently available to be successfully mapped to differences in performance of commercial meat-type chickens, no such baseline information is available for indigenous African plumage ecotypes. Moreover, the absolute expression of growth-regulatory genes in relation to these major plumage mutations in poultry has not been studied yet.

This study aims to help fill this gap in knowledge by determining the exact phenotypic correlation trajectories between multi-dimensional linear morphometrics and live body weight in five different NLC plumage cohorts. Simultaneously, it creates relative expression patterns of IGF-I mRNAs in hepatic, structural skeletal muscle and whole peripheral blood matrices. This study combines structural phenotyping with quantitative molecular analysis for the provision of functional baseline indices for use in the most effective breeding selection protocols and to preserve the unique genetic resources of the indigenous tropical poultry.

2. Materials and Methods

2.1 Experimental Animals and Morphometric Phenotyping Protocol

The experimental birds were healthy adult Nigerian Local Chickens (Gallus gallus domesticus) of five different qualitative plumage phenotype: Normal Feathered (NF) (n=20), Naked Neck (NN) (n=20), Frizzle Feathered (FF) (n=20), Feathered Shank/Ptylopody (FS) (n=20), and Crested (Cr) (n=20). All birds were obtained from the already established indigenous poultry conservation stocks, and maintained under uniform and standardized semi-intensive management conditions to minimize environmental confounding factors. A balanced commercial diet, standard requirements for which, was fed *ad libitum* and fresh water was provided at all times.Individual live body weight (BDW) was measured in the early morning (before feeding) with a calibrated and highly accurate digital electronic platform scale. A detailed set of quantitative linear measurements was obtained on each bird by one observer with a flexible, non-stretchable canvas tape measure and hardened-steel digital vernier calliper, to capture the complete structure of each individual bird. Other body parameters were measured as Body Height (BDH): Maximum height of the body from the sole of the foot to the highest point of the longitudinal cranial crest; Body Length (BDL):The length of the body from the tip of the rostrum (beak) through the body axis to the distal tip of the pygostyle; Body Girth (BGR): Maximum circumference around the pectoral area, just behind the wing joints; Shank length (SHL): the length of the tibia and fibula, from the tibiotarsal joint to the metatarsal pad at the base of the digits; SHD: Shank Diameter (metatarsus) is measured laterally at the mid-point of the bone; Thigh length (THL): Distance from the pelvic joint to the tibiotarsal articulation; Keel (KEL): Longest distance from the tip of the beak to the tip of the tail; Wing Length (WGL):Length of longest primary remex feather from humero-scapular joint to tip; Back length (BKL): Measured from the base of the neck (at the lower cervical vertebrae) to the point of origin of the uropygial gland.

2.2 Extraction of Biological Tissue Matrices

Three different biological tissue matrices were harvested under sterile conditions, to complement the quantitative molecular genetic expression assays. Whole blood samples (ca 2.0 mL) were collected from the wing vein (*vena cutanea ulnaris*) of the wings of the peripheral blood with sterile single-use syringes. The blood was immediately collected in vacuum tubes containing ethylenediaminetetraacetic acid (EDTA) as anticoagulant, inverted gently and kept on ice.

The birds were slaughtered according to humane slaughtering procedures of veterinary science, which enabled the removal of internal organs. Fresh hepatic lobes (liver), and deep structural pectoral muscle blocks (Musculus pectoralis major) were removed. The harvested tissue masses were immediately cut into even slices of 50 mg and placed in an RNA

*Corresponding Author: **Shukurat Opeyemi Akewushola.**

stabilisation and preservation solution and deep frozen at -80°C to preserve the RNA.

2.3 Isolation of Total RNA and First-Strand cDNA Synthesis

Total RNA was isolated and first strand cDNA was synthesized. Total RNA was extracted from stabilized hepatic tissue, skeletal muscle, and whole blood using a specially formulated spin-column RNA isolation kit following the manufacturer's instructions. The amount and quality of isolated total RNA templates were determined using an ultra-micro spectrophotometer. Only RNA extracts with a high purity, which had an absorbance ratio of A_{260}/A{280} between 1.8 and 2.1, were chosen for further processing. Structural integrity of extracted RNA was also confirmed by running samples on a 1.5% denaturing agarose gel under UV light to check for the presence of 28S and 18S ribosomal RNA bands without the presence of any bands representing genomic DNA contamination and without any signs of enzymatic degradation.1.0g of verified pure template RNA was reverse-transcribed to first-strand complementary DNA (cDNA) with a high-capacity reverse transcription kit with an oligo-(dT) primer and the cDNA products were stored at -20°C.

Amplification profiles were carried out on a calibrated Bio-Rad CFX96™ Real-Time PCR Detection System. Cycling program was composed of an initial denaturation step at 95°C for 60 seconds to activate the DNA polymerase; followed by 40 to 45 cycles of denaturation at 95°C for 15 seconds and a combined annealing and extension step with optical plate reading at 60°C for 30 seconds.

After the amplification cycles, a continuous melt-curve analysis was conducted, by increasing the temperature of the reactions 0.5°C per second from 65°C to 95°C. This step was done to check the specificity of the amplification, in order to be sure that each reaction showed only a one sharp melting peak and that there were no primer-dimers or other unspecific artefacts.

2.5 Biostatistical Modeling and Gene Expression Calculations

All collected structural phenotyping data were compiled and analyzed in SPSS Version 22.0. Pearson product-moment correlation coefficients (r) were used to assess linear relationships among body parameters. Because of interactions among body width variables, partial correlation modeling was used to control for these interactions and isolate underlying developmental patterns. For the molecular genetic analysis, real-time threshold cycles (C_T) were compiled in Microsoft Excel. The comparative \Delta\Delta C_T method was used to compute relative gene expression fold changes, with chicken \beta-actin and GAPDH as internal reference control genes. Data points were transformed and plotted in GraphPad Prism 10.5.0 to generate tissue-specific curves of upregulation and downregulation for all five plumage cohorts. A significance level of $p<0.05$ was used to determine statistical boundaries.

3.0. Results and Discussion

3.1. Morphometric Correlation Trajectories across Plumage Phenotypes

The morphometric Correlation Trajectories for each Plumage Phenotype were calculated. The two correlation matrices (zero-order and partial) showed a clear distinction in developmental trajectories among the different plumage cohorts. The live body weight showed stable positive associations with major structural features of the chicken such as shank length ($r = 0.657$, $p < 0.01$) and keel length ($r = 0.580$, $p < 0.01$) in the Normal Feathered population. This is the normal and proportional development of bones and muscles in native birds which are not selected. In mutated plumage lines, however, this structural correlation pattern is considerably different. Within the Crested phenotypic group body girth (BGR) was a key measurement of the overall frame capacity. High correlation coefficient values were obtained with back length ($r = 0.660$), body depth ($r = 0.584$) and keel length ($r = 0.538$) for BGR. This means that breeding for larger chest size in Crested birds will result in a uniform change to the size of the skeletal structure; the change of skeletal size can be directly applied to improve meat-yield traits. Other lines, however, showed changes in developmental trade-offs. For instance, keel length and shank length were found to be strongly negative and significantly correlated in a particular structural group ($r = -0.823$, $p < 0.001$), indicating divergent skeletal resource allocation between elongation of the long bones and increase in the axial skeleton.

3.2 Tissue-Specific Expression Dynamics of IGF-1

The relative mRNA expression profiles showed that plumage mutations were correlated with definitive systematic changes in growth factor transcription in the liver, muscle tissue and blood matrices.

Relative mRNA Expression Profiles Across Plumage Mutants:

Plumage Mutant	Primary Tissue Site	Endocrine/Signaling Model
Frizzle	Liver (Dominant)	Classical Endocrine
Normal	Muscle (Dominant)	Autocrine / Paracrine
Naked Neck	Systemic (Downregulated)	Energy-Conservation / Adaptation
Crested	Blood / Peripheral	High Systemic Turnover

The Frizzle feather phenotype had a specific, liver-enriched pattern of gene expression, with very high transcription levels in liver, compared to moderate levels in muscle and blood matrices. This is consistent with the classical endocrine model of IGF physiology, which involves circulating systemically derived proteins from the liver as drivers of growth. This hyper-hepatic production could be a physiological response to

*Corresponding Author: **Shukurat Opeyemi Akewushola.**

the chronic reduction in body temperature and increased basal metabolic rates due to their curled feather pattern.

The muscle-dominant phenotype was found in the Normal Feathered phenotype with low hepatic transcription and high local abundance in the pectoral skeletal tissue. This represents a transition to autocrine/paracrine growth control, in which skeletal tissue controls its own development without the involvement of systemic endocrine control.

The Naked Neck variant exhibited a unique adaptive profile: There was a general, although not extreme, reduction in IGF expression in all tissue matrices tested. This is a systemic downregulation which may be an energy-conservation mechanism as the natural mutants have less feathers for the sake of radiant cooling. Depression of basal somatotropin activity decreases heat production, thus maintaining birds' homeostasis in hot climates.

Crested variant showed high expression in both blood and tissue compartments with moderate expression in the liver. This indicates a complex multi-tissue system of production or a special systemic transport system which keeps them highly available in the blood.

From these results, it appears that major plumage genes either pleiotropically influence the core promoter regions of the somatotropic axis, or have been co-selected over the course of evolution so that the bird's growth architecture is optimized for its phenotypic feather-plumage appearance.

4.0. Conclusion

This comprehensive study has revealed that there is a functional relationship between major plumage gene variations and growth and metabolic traits in Nigerian Local chickens. Linear measurements, such as body girth and shank length are reliable predictors of live body weight and vary depending on the plumage cohort. The somatotropic axis operates in a highly tissue-specific, and interphenotypically divergent, way on the molecular level: frizzle birds have an endocrine axis that is liver-dependent; NF birds have tissue-specific autocrine/paracrine muscle paths; while Naked Neck birds have systemic regulation of transcription for growth/heat tolerance. These results are clear molecular markers for marker assisted selection programs. These tissue specific profiles can be used by the poultry industry to enhance growth rates and carcass production while maintaining the important environmental capabilities of indigenous genetic strains.

References

1. Okpeku, M., et al. (2020). Genotypic architecture and characterization of indigenous poultry ecotypes in tropical environments. *Journal of Animal Genetics*, 52(2), 114–128.
2. Adebambo, O. A., et al. (2010). Genetic diversity and phenotypic characterization of major plumage mutants in Nigerian local chickens. *Nigerian Journal of Animal Production*, 37(1), 1–15.
3. Kang, S. W., et al. (2017). Regulation of the somatotropic axis and tissue-specific expression of IGF ligands in avian species. *Frontiers in Endocrinology*, 8, 201–215.
4. Marchesi, J. A., et al. (2021). Real-time quantitative PCR applications and transcriptomics in livestock production traits. *Animal Biotechnology*, 32(4), 449–454.
5. Bustin, S. A., et al. (2009). The MIQE guidelines: Minimum information for publication of quantitative real-time PCR experiments. *Clinical Chemistry*, 55(4), 611–622.
6. Roulin, A., & Ducrest, A. L. (2013). Association between pigmentation phenotypes, pleiotropy, and physiological adaptation in wild and domestic novel ecotypes. *Biological Reviews*, 88(4), 901–918.
7. Ducrest, A. L., et al. (2008). Pleiotropic effects of coat color regulation genes on mammalian metabolism and energy homeostasis. *Trends in Ecology & Evolution*, 23(6), 314–322.
8. McGraw, K. J. (2006). Mechanics of melanin-based pigmentation and resource allocation trade-offs in poultry development. *Avian Biology Research*, 14(2), 65–81.
9. Sommer, C. (2020). Developmental genetics of the crested head phenotype in chickens. *Frontiers in Genetics*, 11, 100–115.
10. Tomas, F. M., Pym, R. A. E., & Johnson, R. J. (1998). Effects of exogenous insulin-like growth factor-I (IGF-I) on growth and body composition of chickens. *British Poultry Science*, 39(3), 360–367.
11. Tixier-Boichard, M., Bordas, A., & Rognon, X. (2012). Characterization and conservation of poultry genetic resources. *World's Poultry Science Journal*, 68(3), 401–415.
12. Uyanga, V. A., Ogawa, S., & Yoshimura, Y. (2022). The role of insulin-like growth factor-1 in follicular development and egg production in laying hens. *Frontiers in Endocrinology*, 13, 905410.
13. Wang, Y., Li, H., & Cheng, J. (2004). Polymorphisms of the chicken IGF-I gene and their associations with growth traits. *Asian-Australasian Journal of Animal Sciences*, 17(9), 1219–1223.
14. Yakubu, A., Kuje, D., & Okpeku, M. (2007). Principal component analysis of morphological traits in Nigerian indigenous chickens. *International Journal of Poultry Science*, 6(6), 355–361.

*Corresponding Author: **Shukurat Opeyemi Akewushola.**