VARIATION IN SELECTEDGROWTH-INFLUENCING GENES AND THEIR ASSOCIATION WITH GROWTH, CARCASS CHARACTERISTICS AND THERMO-TOLERANCE OF INDIGENOUS CHICKENS IN NIGERIA

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CERTIFICATION

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DEDICATION

This thesis is dedicated to:

- Almighty Allaah, the Ever living; the Sustainer and Protector of all that exists; the self-sufficient Master; whom all creatures need
- The upholders of justice, care givers and supporters of the weak, oppressed and less privileged throughout the world.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

PCR: Polymerase Chain Reaction

RFLP: Restriction Fragment Length Polymorphism

MSTN: Myostatin

GH: Growth Hormone

PIT-1: Pituitary Transcription Factor-1

MYOG: Myogenin

HSP70: Heat Shock Protein 70

GHRL: Ghrelin

YEC: Yoruba Ecotype Chicken

FEC: Fulani Ecotype Chicken

FUNAAB: Federal University of Agriculture Abeokuta

FAC: FUNAAB Alpha Chicken

NIC: Nigeria Indigenous Chicken

FAO: Food and Agriculture Organisation of United Nations

NAPRI: National Animal Production Research Institute

NACGRAB: National Center for Genetic Resources and Biotechnology

SNP: Single Nucleotide Polymorphism

SSCP: Single-strand Conformation Polymorphism

Mme: Membrane metalo-endopeptidase

ECORI: *Escherichia coli*; strain R

Hinf1: *Haemophilus Influenza* strain 1

Alu1: *Artrobacter luteus* strain 1

PCV: Packed Cell Volume

RBC: Red Blood Cell

CORT: Corticosterone

AIC: Akaike Information Criterion

BIC: Bayesian Information Criterion

DNA: Deoxyribonucleic Acid

CT: Cloaca Temperature

RR: Respiratory Rate

PR: Pulse Rate

HSI: Heat Stress Index

BLAST: Basic Local Alignment Search Tool

MEGA: Molecular Evolutionary Genetic Analysis

NCBI: National Center for Biotechnology Information

Rsa : *Rhodopseudomonas sphaeroides*

MSE: Mean Square Error

SD: Standard Deviation

ABSTRACT

Slow growth rate and acute heat stress are the main constraints to Indigenous Chicken (IC) production in Nigeria. Characterisation of growth-influencing and heat-tolerance genes are requisites for selective breeding of poultry for improved growth, carcass traits and heat-tolerance. However, information on the variation in growth-influencing and thermo-tolerance genes of IC in Nigeria is inadequate. Therefore, variation in some growth-influencing genes and their association with growth and heat-tolerance traits of IC in Nigeria were investigated.

One-day old chicks(n=358) comprising 118 Yoruba Ecotype Chicken-YEC, 102 Fulani Ecotype Chicken-FEC and 138 FUNAAB Alpha Chicken-FAC were tagged and fed *ad libitum* on commercial diets for 24 weeks. The bodyweight (g) of each chicken was measured weekly. At week 12, blood (5 mL) was sampled from all surviving 96 YEC, 89 FEC and 113 FAC. Genomic DNA was extracted, amplified and electrophoresed using standard procedures. Myostatin, ghrelin, Heat Shock Protein-70 (HSP70), Pituitary Transcription factor-1 (PIT-1) and myogenin genes were genotyped using *Alu1, Hinf1, Mme1, Msp1 and EcoR1*restriction endonucleases, respectively. Association between variants of the genes and weekly bodyweight of each chicken were assessed. At week 23, six chickens per identified HSP70 genotypes (AA, AB and BB) selected from each of YEC, FEC and FAC were exposed to 40±1°C for one hour and blood (5 mL) was sampled and analysed for Packed Cell Volume-PCV and erythrocyte. Cloaca temperature was recorded and Heat Stress Index (HSI) calculated. At week 24, 40 chickens from each of YEC, FEC and FAC were randomly selected and sacrificed. Thigh, breast and dressed weights(g) were measured. Data were analysed using descriptive statistics and ANOVA at $\alpha_{0.05}$.

The bodyweight of FAC (2323.6±258.4) was heavier than YEC (998.2±159.0) and FEC (1156.2±181.3). Co-dominant alleles A and B with corresponding genotypes: AA, AB, and BB were detected in myostatin, ghrelin and HSP70 genes. Genotypes AA and AB were observed in PIT-1 while myogenin was monomorphic. The bodyweight of FEC AB-myostatin genotype (627.7±86.2) was higher than 576.0±51.7 (BB-myostatin) and 582.4±80.8 (AA-myostatin) at week 12. Weekly bodyweight of YEC AA-myostatin genotype was similar to those of AB-myostatin and BB-myostatin. The bodyweight of

FAC AB-myostatin genotype (1257.9±30.3) was significantly higher than 1160.5±24.3 (AA-myostatin) and 1094.4±43.4 (BB-myostatin). The bodyweight of FEC BB-ghrelin genotype (618.4±24.1) was significantly higher than 566.4±17.1 (AB-ghrelin) but similar to 607.3±9.2 (AA-ghrelin) at week 12.The PCV,erythrocyteand cloaca temperatures were similar for YEC, FEC and FAC. Within FAC, HSI of BB-HSP70 (1.35±0.03) was significantly lower than 1.59±0.08 (AA-HSP70) but similar to 1.46±0.02 (AB-HSP70), while within FEC, BB-HSP70 (1.32±0.05) had significantly lower HSI than 1.48±0.13 (AA-HSP70) but similar to 1.40±0.04 (AB-HSP70). In YEC,HSI of AA-HSP70 (1.44±0.06), AB-HSP70 (1.37±0.11) and BB-HSP70 (1.42±0.08) were similar. Dressed weight of FEC AB-myostatin (864.7±54.3) was higher than 792.1±18.9 (AA-myostatin) and 777.7±0.5 (BB-myostatin). Genotypes AA and AB (PIT-1) did not have significant effect on thigh and breast weights of YEC, FEC and FAC.

The growth-influencing genes were polymorphic except myogenin which was monomorphic. Genotype AB-myostatin was associated with growth and carcass traits while BB-HSP70 was associated with thermo-tolerance.

Keywords: Indigenous chickens, Genetic variation, Selective breeding, Heat stress
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CHAPTER ONE

1. INTRODUCTION

Indigenous chicken production is an integral component of the livelihoods of nearly all rural households in Nigeria. Nigerian Indigenous chickens (NIC) constitute 80-97% of the 200 million chickens in Nigeria (FAOSTAT, 2010). They survive under scavenging system withlittleorno supplemental feeds and are adaptive toadverse environmental condition. They also possess abilitytohatch ontheir own with appreciable immunityfrom endemic diseases (Salako and Ige, 2006).Interestingly, its production does not require recurrent expenditure for feed, other inputs and labour since they are often raised on free range with occasional assistance of excess family labour (Bishop, 1991). Indigenous chicken in Nigeria are often classified based on body structure(e.gnakedneck, dwarftypes),plumage colour, feather pattern or geographical region and the location where they are found (Olori, 1992). Nevertheless, recent findings revealed that NIC can be classified based on mature body weights as heavy and light ecotype, also referred to as Fulani and Yoruba ecotype respectively (Ajayi, 2010). The Fulani ecotype are found in the montain regions, dry Savannahs and cattle kraals of Northern Nigeria with few of them among the Fulani descendants in South western states, while, the Yoruba ecotype chickens are found around the Rainforest as well as the Derived-Savannah zones of Nigeria (Ajayi 2010).

In addition to various socio-cultural uses, meat and eggs from indigenous chickens serve as a source of income and nutritional reserves for Nigerian rural households particularly women. They also contribute to future food security through maintaining biodiverse genomes. The highgenetic diversities and possible occurrence of rare alleles makes the indigenous poultryspecies more valuable to animal breeders. Thus, NIC are essential genetic resource for the developmentof high yielding and tropically adapted breeds. Nigeria indigenous chickens are characterized by slow growth rate, small mature weights and low egg production.

Previous attempts towards genetic improvement of Nigeria indigenous chickens have primarily focused on mass selection and crossbreeding(Musa *et al.,* 2016). However, rapid genetic gain is difficult via mass selection (Zhang *et al.,* 2008). Earlier studies towards improving NIC include morphometric and biochemical characterization (Ige *et al.,* 2014), assessment of egg and meat production performance of Nigeria indigenous chickens (Adedeji, *et al.,* 2015), association of Insulin-like Growth factor-1gene polymorphism with growth traits of Nigeria indigenous chickens (Ilori *et al.,* 2016). More recently, the National Animal Production Research Institute Nigeria and Federal University of Agriculture Abeokuta, Nigeria have recorded greater success by developing improved lines named Shika brown and FUNAAB- Alpha through several selections and crossbreeding with exotic chickens (Ilori *et al.,* 2016). However, crossbreeding with exotic stocks may lead to loss of important genotypes or alleles (Cahaner *et al.,* 1993) that are responsible for their excellent tolerance to various forms of stress such as low management input, stressful tropical climate and disease resistance.

To provide basis for further improvement of these important stocks, adoption of advanced molecular techniques such as Restriction fragment length polymorphism (RFLP), Amplified Random Length Polymorphism, Random Amplified Polymorphic DNA, Single-strand Conformation Polymorphism, Single Nucleotide Polymorphism and gene sequencing is essential. Sequencing of farm animal genomes provides an increasing amount of information for genotype-trait association studies, population genetic and structure analyses (Zhao *et al.,* 2004). The RFLP is a highly robust but simple methodology with high discriminatory power and codominantly inherited abilities(Musa *et al.,*2016).The RFLP has been used to detect polymorphism in several genes that influence growth and thermo-tolerance in poultry which include but not limited to Melanocortin 4 receptor-like, transforming growth factor b-3, Growth hormone, Growth hormone receptor, Insulin-like growth factor, Pituitary specific transcription factor-1, Ghrelin andHeat shock protein 70 genes *etc.*(Khoa *et al.,* 2013, Musa *et al.,* 2016). Quantitative traits-associated genetic markers allow selection based on genotype thereby providing a quick tool for early identification of animals with best productive performance and consequently accelerate the rate of genetic improvement. The establishment of these genetic markers requires an understanding of the genetic diversities in candidate genes

that influence productive traits and impacts of their variants on the traits of choice. Earlier studies (Xu *et al.,* 2011, Khoa *et al.,* 2013) had assessed variation in several genes (including: Growth hormone, Pituitary specific transcription factor-1, Growth hormone, Ghrelin,Heat shock protein 70, Myostatin, and Myogenin) and the effect of their variants on growth and carcass traits of chickens.

Pituitary specific transcription factor-1, Growth hormone and Ghrelin genes are members of somatotropic genes that play several complementary roles in muscle growth and development of poultry. Functionally, PIT-1 induces differentiation of hepatic progenitor cells into prolactin-producing cells as well as a transcription factor for growth hormone and transforming growth factor-ß genes that play the most pivotal role in controlling growth in chickens (Renaville *et al.,* 1997). Earlier, significant positive association between genotype AA ofchicken PIT-1gene and the body weight at week 8 have been reported (Nie *et al.,* 2008). Growth hormone (GH) affects growth rate, body compositions, health, milk production and aging by regulating postnatal growth and metabolism in animals while ghrelin regulates growth hormone, adiposity, and appetite in farm animal. Myogenin and myostatin genes induce and regulate the balance between the differentiation and proliferation of muscle precursor cells, repair of skeletal muscle and hypertrophy (Delfini and Duprez, 2004). Zhiliang *et al.,* (2004) observed that homozygote genotypes AA and BB of MSTN had high positive correlation with abdominal fat weight and higher breast muscle weight values in F2 Broilers and Silky chickens.The heat shock proteins (HSPs)gene, encodes proteins that protect cells against heat stress. Of the many expressed HSPs, HSP70 have been extensively studied in chicken (Gu *et al.,* 2012). The polymorphisms of the HSP70gene and its effect on heat stressed chickens have been reported in broiler and chickens that are native to Taiwan (Mazzi *et al.,* 2003).

1.1 Justification for the Study

Indigenous chickens are characterized by small mature weight and low egg production performance (Ige *et al.,* 2014). Nigeria Indigenous chicken production is an integral component of livelihoods of the rural dwellers. They are extensively distributed across all Nigeria agro-ecological zones and contributed substantially to the livelihood of the rural households particularly the women. Nigeria indigenous chickens are hardy; possess good

fertility and hatchability, better flavor of meat and good maternal qualities(Salako and Ige, 2006).In addition to the low productivity constraints of indigenous chickens, it is well documented that high temperatures occasioned by global warming adversely affects poultry production and reproduction in the tropics(Melesse *et al.,* (2011). This necessitates the use of cooling and ventilation systems in improved poultry breeds reared intensively, this does not only increase production costs but also practically difficult to adopt under extensive management that dominate indigenous chicken production systems in sub-Sahara Africa (Cahaner *et al.,* 1993). Genetic selection of tropically adapted chickens for better growth traits with concomitant resistant to heat stress could thus be advantageous. It offers a cost effective and sustainable way of stimulating economic growth and poverty reduction in rural households in sub-Sahara Africa(Benitez, 2002). Knowledge of genetic variation in economic and adaptive traits related genes is a primary step towards designing effective animal genetic improvement program. These among other factors afforded this research a funding opportunity by African Chicken Genetic Gains-Nigeria that was sponsored by the Bill and Melinda Gates foundation.

General Objective

To study the variation in selected growth-influencing genes and their association with production and thermo-tolerant traits of Nigeria indigenous chickens

Specific Objectives

To:

i. estimate the allelic and genotypic frequencies as well as heterozygosity of MSTN, GH, PIT-1, GHRL, MYOG and HSP70 genes in Yoruba, Fulani and FUNAAB-Alpha chickens

ii. determine the effect of the polymorphic variants of MSTN, GH, PIT-1, GHRL, MYOG and HSP70 genes on growth and carcass traits of Yoruba, Fulani and FUNAAB-Alpha chickens

iii. evaluate the adaptive responses of different HSP70genotypes of the of Yoruba, Fulani and FUNAAB-Alpha chickens to acute heat stress

iv. estimate the genetic distance among the Yoruba, Fulani and FUNAAB-Alpha chickens at MSTN, GH, PIT-1, GHRL, MYOG and HSP70 genes regions

v. evaluate and compare the growth patterns of Yoruba, Fulani and FUNAAB-Alpha chickens with the aim of providing information on their growth rate for further genetic improvement

1.2 Hypotheses

1a.H_{O1}: the genotype and allele frequencies of the six selected genes in Yoruba, Fulani and FUNAAB-Alpha chickens in the selected population conform with the Hardy-Weinberg equilibrium principle

1b.H_{A1}: the genotype and allele frequencies of the selected genes in Yoruba, Fulani and FUNAAB-Alpha chickens in the selected population do not conform with the Hardy-Weinberg equilibrium principle

2a.H_{O2}: variants of the six selected genes has significant effect on growth and carcass traits of Yoruba, Fulani and FUNAAB-Alpha chickens

2b.H_{A2}: variants of the six selected genes has no significant effect on growth and carcass traits of Yoruba, Fulani and FUNAAB-Alpha chickens

3a.H_{O3}: polymorphism of HSP70 gene has significant effect on the physiological and hematological responses of Yoruba, Fulani and FUNAAB-Alpha chickens to acute heat stress

3b.H_{A3}: polymorphism of HSP70 gene has no significant effect on the physiological and hematological responses of Yoruba, Fulani and FUNAAB-Alpha chickens to acute heat stress

4a.H_{O4}: The selected population of Yoruba, Fulani and FUNAAB-Alpha chickens are phylogenetically different

4b.H_{A4}: The selected population of Yoruba, Fulani and FUNAAB-Alpha chickens are not phylogenetically different

CHAPTER TWO

2. LITERATURE REVIEW

2.1 Nigeria Indigenous Chickens

Indigenous chickens are the most preferred breed of chicken by smallholder farmers that dominate sub-Sahara Africa. They are often kept on free range with little or no supplemented feed. They are highly adaptive to the poor husbandry practices with excellent natural immunity against common diseases (Atteh, 1990). Nigeria Indigenous chickens (NIC) are characterized with small mature body weight but well-fleshed and compact bodies. The growth rate and egg laying performance of Nigeria Indigenous chickens are comparatively lower than those of the exotic chickens. However, they have good mothering abilities with excellent ability to hatch on their own. Like other chickens that are native to African countries; they possess high tolerance to the harsh environmental condition(Ogundipe, 1990). These birds usually search around the homestead areas for leftover food, crop residues, insects, kitchen waste, grasses and other edible feedstuffs. These feedstuffs which are often discarded as waste are utilized by indigenous birds to produce cheap source of quality animal protein (Adedokun and Sonaiya, 2001, Ajayi, 2010)

2.1.1 Description, classification and distribution of Indigenous chickens in Nigeria

Nigeria Indigenous chickens are characterized with varied plumage colours: white, black, brown, mottled *etc.* (Plate 2.1 and 2.2). The indigenous stocks are often dominated by normal feathered individuals with very small percentage of frizzled feathered and naked necks. Ajayi and Agaviezor, (2009) reported 91.8%, 5.2% and 3.0% for distributions of normal, frizzled and naked neck chickens respectively in Bayelsa State of Nigeria. Nigeria Indigenous chickens are often classified based on the location or geographical region where they are found e.g Yoruba or Fulani ecotype (Olori, 1992). Recent findings revealed that Nigeria Indigenous chickens can be classified based on the

mature bodyweights as heavy and light ecotype also referred to as Fulani (0.9-2.5 kg mature bodyweight) and Yoruba ecotype (0.68-1.5 kg at maturity) respectively (Ajayi 2010). The Fulani chickens are often found in the dry Savannahs, mountain regions and cattle kraals of Northern Nigeria with few of them among the Fulani descendants in south western states, while, Yoruba ecotype are found around Swamp and Rainforest agro-ecological zones of Nigeria. Adedokun and Sonaiya (2001) reported body weight at 20th week as 1300.6g and 1096.0 for Nigeria indigenous chicken cocks and hens respectively. Further, Adedokun andSonaiya(2001) submitted thatNigeria indigenous chickens had157±3.7, 160±3.8 and 165±3.7 mean ageatfirsteggrespectively. Eggproduction under extensive system was about40eggs per year whereas the eggyield was doubledunder improved conditions(Sola-Ojo and Ayorinde 2009). The authors reported 146 eggs per year when the chickens were housed in battery cages. Thus, improved husbandry system increased the laying performance of local chickens. More so, the comparative evaluation of the growth traits of Nigerian local chickens and their crossbred showed that crossbreeding could potentially increase the currently obtainable mature body weight of the Nigerian local chickens(Momoh *et al.,* 2010).

Plate 2.1. The Fulani Ecotype Chickens

Plate 2.2: The Yoruba Ecotype Chickens

2.1.2 Socio-Economic Importance of Nigeria Indigenous Chickens

The rural households of sub-Saharan Africa including Nigeria benefit substantially from native chickens. The indigenous chickens play several critical roles which varied from locality to locality and even from region to region. Nigeria indigenous chickens contribute crucially to livelihood of the rural households particular the women. They serve as a source of high quality animal protein (egg and meat) and incometo smallholder farmers (Sonaiya and Adedokun, 2002). Nigeria indigenous chickenscontributedabout 23,887 metric tons of eggs to the total eggs produced in Nigeria (Atteh, 1990). Additionally, indigenous chickens' production acts as savings for the unforeseen expenses, income diversification, used in cultural events, traditional festivals, sacrifice to deities and play an important role in traditional medicine. Some people believe in some spirituals being or deities which they often sacrifice indigenous chickens to. The commitments of such people are often measure by the quality or value of the animals including indigenous chickens that the person sacrificed (Ogundipe, 1990). This among others contributed to the increase in demand for indigenous chickens particularly those with certain morphological features or characteristics (Ige *et al.,* 2014).

Chickens with special morphological features including but not limited to white or red plumage colours, frizzle feathered are usually commanded higher prices than others of similar age because of their preferences for certain cultural sacrifices which due culminated into higher demand for them (Osaiyuwu *et al.,* 2009). Thus, increase production of indigenous chickens with special or rare traits that fit to cultural and improved socio economic condition should be encouraged. In addition to the taste and suitability to the local cooking methods; most rural households often preferred the meat and eggs from indigenous chickens to those produced by exotic egg producing or meat-type birds (Sonaiya and Adedokun, 2002). Although the meat and egg productivity of indigenous chickens are comparatively lower than their exotic counterparts, however, they play an indispensable roles in the sociocultural life of rural households.

Nearly all rural household keep indigenous chickens under free range systems, i.e without expensive housing, labour or conventional feeds (Momoh *et al.,* 2010). Instead the chickens are often generally kept by women with occasional assistance of their children.

Indigenous chicken production should therefore be encouraged; in order to conserve the biodiversity and maximise the potential of indigenous chicken as a reservoir for the genetic resources for the current improvement and future demand in need.

2.2 Improved Nigeria Chickens

2.2.1 Shika Brown Chicken

The National Animal Production Research Institute (NAPRI) at Ahmadu Bello University, Nigeria, started the poultry breeding project to develop an egg strains parent stock chicken in 1985. The research began with importation of two strains of grandparent chicks from 2 different undisclosed sources. Series of crosses between the two specialized lines of the foundation stock followed by subsequent selections for high body weight at eight weeks were carried out for several generations (Jesuyon, 2011). Inbreeding was minimized by avoiding Full and half sib mating (Kallah, 1999). After the recorded success of the stock in the multi- locational performance testing at all the six Nigerian geo-political zones, the chicken was named Shika Brown after the Shika village, in Kaduna state. In addition, having satisfied all necessary registration conditions; it was registered by National Council for Animal Genetic resource and Biotechnology (NACGRAB) and released in the year 2000. Shika brown recorded low mortality (less than 7%), High persistency of lay with Average egg weight and body weight at end of lay of 60g and 1.8kg respectively (http://www.naerls.gov.ng/extmat/bulletins/Local%20Chicken.pdf).

2.2.2 FUNAAB-Alpha Chicken

The FUNAAB Alpha chicken is a dual purpose breed of chicken that was developed by a team of scientists headed by Prof. Olufunmilayo Adebambo from the Federal University of Agriculture Abeokuta (FUNAAB), Nigeria. The foundation stock (FS) were collected randomly all-over SouthWest, Nigeria. The FS consisted of a total of 1000 chickens with varied colours (Brown, Black, Mottled and White), structure and feather patterns (Normal Fethered, Frizzle Feathered and Naked Necks). They were screened against diseases and broodiness and the successful birds were then multiplied and mated with all possible combinations of mating, back-crossing and criss-crossing, which eventually resulted into the FUNAAB-Alpha chickens (Ilori *et al.,* 2016). FUNAAB Alpha birds have been tested for about 5 months in the year 2017 under rural households and was recently registered by

the National Council for Animal Genetic resource and Biotechnology-NACGRAB(https://unaab.edu.ng/2018/08/).

2.3 Growth in Chicken

Growth is a complex biological process that involves cell replication, tissues differentiation, matrix formation, cell death and many other interrelated mechanisms (Raji *et al.,* 2013). The growth mechanism is controlled by several metabolic hormones such as insulin, glucagon, growth hormone and thyroid hormones. The interactions between hormonal, nutritional and genetic factors also affect growth (Khoa *et al.,* 2013). Genotype is an important factor determining growth because an animal cannot exceed its maximum genetic potential in growth, even when environmental conditions are ideal. The present growth rate of broiler chickens resulted from progressive selective breeding over time (He *et al.,* 2007). The slaughter age of broilers was 12 weeks with an average body weight of 1.3 Kg and about 29% feed efficiency in 1940s. By 1970, this had improved tremendously and the age at slaughter had reduced to 8 weeks with an average body weight of 2.2 Kg and about 50% feed efficiency (Olawumi and Fagbuaro, 2011). Currently, continuous selective breeding of broiler chickens for improve performance has resulted in higher average body weight with increased feed efficiency far above the later values in 40 days. Both body weight and body fat contents have been reported to be moderately heritable and abdominal fat pad contributed 2% or more to total body weight and exhibited strain differences in weight (Mohsen, 2011). Abdominal fat deposition in chicken is a highly variable characteristic and positive genetic relationship between growth rate and fatness in broiler chickens has been reported.

Changes in body weight as well as other parts rely largely on influencesof the genotype or genetic composition of an individual. The growth of pectoralis muscle was greater in the broilers and that of gastrocnemius muscle was greater in the slow-growing(Laseinde, 1991).Also, the growth of the pectoralis and supracoracoideus muscles was more rapid in the fast-growing cross than the White Leghorn chickensroaster (Supakorn and Pralomkarn, 2013). At day old, broiler chickens have higher number of muscle cell, though small-sized than egg-type bird and at 10 weeks, the heavier broiler chickens had superior muscle fibers than the lightlayer chickens (Mohsen, 2011). The

growth rate of muscles varied among species of birds in relation to adult body weight, developmental maturity of the chicks at hatching and postnatal growth increments of skeletal muscles of the legs. The greatest relative growth rate of major tissues is achieved during the first three weeks of the chickens' growth while muscles of chickens grew faster than the leg muscle in the first two weeks after hatching (Laseinde, 1991).

Organs and systems of the body grow and function in accordance with the body size and physiological demand.Some organs characteristically have smaller cells than others and these have a particular high rate of metabolism. For example, liver and kidneys have smaller cells than the heart. Young cells are usually smaller than older mature cells. Further, cell division is always most rapid in the early stages of animal development during which period, growth in all species is due entirely to cell division without significant increase in the cells' size. While, the number of muscle fibres depend on genetics, the size of the fibres depend on nutrition, size of individual animal and how much the muscle is exercised (Sajee, 2013).

In mature non-ruminant mammals, skeletal muscle contributes 50% of total body protein and provides a store that is utilized as a source of amino acids under circumstances of metabolic or nutritional stress. Scatchard(1979) reported that the amount of degradation that occurred in the living muscles was estimated to account for the destruction of up to 70% of the total protein synthesized. The authorsfurther stated that a reduction in the rate of protein breakdown would have a significant effect on increasing the total protein accumulationand muscle growth, provided the synthesis rate remained the same. Thus, proteins of skeletal muscles were subject to continual degradation and synthesis and changes in the relative rates of either or both of these processes must underlie any change in protein deposition.

Adipose tissues comprise a variety of cell types including adipocytes (fat cells) and stromal vascular cells (other cells including endothelia cells of the blood vessels, adipocytes precursor cells, mastcells and macrophages). Adipocytes are the dominant cell type of the tissue in terms of size and lipid metabolism. There are two distinct types of adipose tissues differing in function, colour, vascularity and metabolic activity. They are the white adipose tissue which comprises the major part of the body fat and stores fat and

brown adipose tissue. The later occurs only in specific sites and its colour is due to its specific vascularity and high content of cytochromes (Hui-Liang and Zhong-Xiao, 2006). Fatty acids mobilized from white adipose tissue are transported via plasma to the liver and peripheral tissues for oxidation while brown adipose tissue, the fatty acids are oxidized in situ with immediate release of energy as heat. Such heat is rapidly transferred to the essential organs. Brown adipose tissue is primarily a heat producing tissue while white adipose tissues are known for fat storage (Sajee, 2013).The percentage lipid in the depots is greatly influenced by the stage of development and plane of nutrition, animals on low planes of nutrition having more water and less fat than those on higher planes of nutrition.

Fowler (1976) reported that when plane of nutrition approached maintenance, fat deposition ceased although fat-free tissues continued to grow at a slow rate. Fat-depleted cell retains its ability to deposit fat again once energy balance is regained. Process of cellular growth can occur by hyperplasia or cell division or by hypertrophy or the enlargement of existing cells without a concomitant increase in number. The growth can on the other hand be by a combination of the two processes. Laseinde (1991) indicated that there are two conflicting views on the growth of adipose tissues. One held that hyperplasia occurs only early in life, followed by hypertrophy. Another, opinion held that hyperplasia could be stimulated during adult life. They argued that the problem with adipose tissues is that the cells which give rise to mature fat cells were difficult to identify prior to the formation of fat loculi. More so, adipose tissue cells rise directly from connective tissue and revert into connective tissue when depleted of fat.

In adult meat-producing animals, the major deposit of fat are subcutaneous, intramuscular, perirenal, omental, mesenteric, intramuscular (marbling), pericardial and channel i.e. pelvic area (Leat and Cox, 1980). The bones which are specialised forms of connective tissues perform among others, growth functions in the body, support and protection of body framework, force transmission caused by body weight and muscle contraction, body movement, sense organ and mineral depot, majorly for calcium and phosphorus (Hui-Liang and Zhong-Xiao 2006).

2.4 Growth and Thermo-tolerance Related Genes

2.4.1 Growth Hormone

Growth hormone (GH) is a protein with a single chain of amino-acid residues (approximately190 amino-acids) and 2 disulfide bridges that maintain its tertiary structure. The GH is synthesized by somatotropic cells of the pituitary into the blood stream after stimulation by hypothalamic releasing factors. Growth hormone stimulates triglyceride breakdown in adipocytes and consequently improving fat utilisation. It also modulates nutrient partitioning between adipose tissues and skeletal muscles in mammal, such as pig. Thus, growth hormone reduces fat deposition and stimulates muscle growth. Further, growth hormone is widely involved in the regulation of differentiation and proliferation of follicular cells, as well as oocyte maturation(Supakorn and Pralomkarn, 2013).

In the domestic fowl, the somatotrophs are further separated spatially being situated only in the caudal 43 portion of the anterior pituitary gland. Chicken growth hormone gene consists of 4,101 base pairs with five exons and four introns just like pig, rat, sheep, goat and human (Mohsen, 2011). The large intron size (3.5kb) of Chicken growth hormone gene made it to be comparatively larger than the mammalian genes. The chicken growth hormone gene play critical roles in chicken growth and metabolism and consequently influences chicken performance traits. Therefore, its potential as marker for selection for improved performance of indigenous chickens needs to be fully explored.

2.3.1.1 Effects of Growth Hormone Gene Polymorphism on Economic Traits of Poultry

Polymorphism in growth hormone gene has earlier been reported (Enayati and Rahimi-Mianji, 2009)particularly in the intron region. Khoa *et al.,* (2013) evaluated single nucleotide polymorphisms in GH gene among Noi, Tau Vang and Cobb 500 chicken strains using PCR-RFLP. The CC genotype was not found in Noi breed at GH1 locusbut appreciably higher genotype frequency was observed for TT genotype (Khoa *et al.,* 2013). Similar results were also reported in Iranian native chicken and other meat type chickens (Tanmankaur *et al.* 2008). Conversely, Mohsen, (2011) reported three alleles A, B and C which led to the identification of four genotype patterns (AA, BB, CC and AC) in Panjab Broiler1, Indian Cornish 3 and University Male Line strains of Indian chickens via PCR-

RFLP and DNA sequencing technique.In a related study; Nie *et al*. (2005)identified a new allele D at a high frequency in Taihe Silkies and Beijing Fatty chicken strains.Most of the diversities in GH gene were reported to be single nucleotide polymorphism (SNP) occasioned by nucleotide deletion, insertion or substitution.The C--T mutation was identified by Yan *et al.,* (2003) through SNPs analysis of chicken growth hormone gene. The authors found mutations at the 3rd and 4thintrons. The observed polymorphism was also confirmed via restriction digestion usingMsp I. Yan *et al.,* (2003) further reported significant association between SNP in chicken growth hormone gene and measured carcass traits such as breast muscle weight and abdominal fat weight. However, SNPs of chicken GH gene have been shown to have potential influence on the chickens' performance traits (Huang *et al*. 1993).

The results of the SNPs analysis in the chicken GHgene of four divergent chicken breeds showed significant association between weekly body weight, average daily gain and SNPs in chicken growth hormone gene (Nie *et al*., 2005). Tanmankaur *et al.* (2008) analysed 776 bp amplicon of chicken GH gene and identified three different genotype patterns (AA, AC and CC) were found. More so, a significant influence of those genotypes with body weight of meat type chicken at week five was reported. However, no correlations were observed between chicken GH/*Msp*I patterns and growth traits of White Leghorns chickens (Tanmankaur *et al.* 2008).

2.3.2 Ghrelin Gene

Ghrelin is predominantly expressed in the stomach and hypophysis or pituitary gland. Ghrelin is characterized with unique acyl-modification structure at the third amino acid - serine which is responsible for the most of its biological activities (Kaiya *et al.,* 2002). The GHRL gene varies in structure among fish, poultry and mammals. Chicken ghrelin composed of 26 amino acids compared to mammalian ghrelin which consists of 28 amino acids. The mice, humans, chicken among other animal have been completely sequenced at ghrelin locus (Nie *et al.,*2004).

2.4.2.2 Physiological Roles of Ghrelin Gene

Ghrelin is produced mainly in the gastrointestinal organs. However, very low concentration of ghrelin is also secreted from some peripheral tissues during hunger. The

main physiological functions of ghrelin include: releasing growth hormone, appetite stimulation, increase food intake and control of energy balance, cardiomyocyte cell proliferation via ERK1/2 and PI3K/Akt activation or the cAMP/PKA pathway and contributes to the regulation of glucose metabolism (Rossi *et al.*, 2008).Ghrelin regulates multiple activities, including the release of growth hormone, adiposity, and appetite hence its active involvement in growth regulation have been reported in several animal species including poultry (Mohsen, 2011).

2.4.2.3 Effects of Ghrelin Gene Polymorphism on Economic Traits of Poultry and other Livestock

Significant associations have been reported between variants of cGHRL gene and several economic traits of poultry as well as other farm animals. Nie *et al.,* (2004) reported two SNP variants (C223G SNP and A2355G) in exon 5 of chicken ghrelin gene. In related studies, Richards *et al.,* (2006) and Kgwatalala*et al.,* 2012 identified a total of 19 and 25 SNPs in cGHRL gene in meat birds and three Tswana indigenous chickens respectively. A silent mutation (G→A) was detected at 54th bp in the third exon of duck ghrelin gene and significantly higher body weight valueswas observed in BB genotype than in AA, AB and AC**(**Li *et al*., 2010**)**. More so, a significant association among 8 bp indel in exon 1 of *cGHRL* gene and chicken body weight at different ages and 10 of the measured carcass traits was reported by He *et al.,* (2007). The authors also identified C2100T polymorphism in chicken ghrelin gene. He *et al.,* (2007) further reported significant association between the observed SNPsin GHRL and chicken growth traits.

Ghrelingene, being an appetite stimulating peptide, its polymorphism has been expectedly reported to have associations with the growth traits in cattle. Zhang *et al*. (2008) found a mutations at nucleotides 456 (G > A) and 667(C > T) in exon 1 of the GHRLgene and comparatively, the best performance traits values was observed in the cattle with MM genotype of GHRL. Further findings by Jiajie *et al.,* (2010) reported eleven SNPs in GHRL gene however; the polymorphism had no association with both the growth traits and milk performance traits of in five Chinese cattle breeds.

2.4.3 Pituitary Transcription Factor 1 Gene

Pituitary transcription factor is a tissue-specific transcriptional factor that controls the gene products synthesis via the use of the coded genetic instructions for the growth-related genes such as prolactin and thyrotropin(Renaville *et al.,* 1997). Earlier studies reported 6 and 7 exons in mammalian and poultry Pit-1 genes respectively (Yamada *et al.,* 1993). Mutations or inhibition in the chicken's Pit 1 gene has been attributed to underdevelopment of the endocrine cells that produces growth hormone, prolactin and thyrotropin in response to hormone signals. More so, decrease in growth hormone, prolactin and thyrotropin were often experienced in PIT-1 deficient birds (Cheng and Jefferson, 2008).

2.4.3.2 Physiological Roles of PIT-1

The PIT-1 plays a critical role in the transcriptional regulation, binding and transactivation of promoters of multiple genes in the pituitary (Renaville *et al.,* 1997). Transcriptional activation is achieved via the Serine/Threonine Activation Domain. PIT-1 also involves in proliferation and survival of three pituitary cell types(Nie *et al.,* 2008). The chicken Pit 1 gene also plays crucial roles in endocrine cell differentiation and mediation of cell proliferation.

2.4.3.3 Polymorphism in PIT-1 Gene and Its Association with Growth Traits In Poultry

Single nucleotide polymorphism with varied allele frequencies have been reported in layer and meat type chickens (Jiang *et al.,* 2004). The genotype AA ofchicken PIT-1gene was positively associated with body weight at week 8, though it has no significant effects on their carcass traits (Nie *et al.,* 2008). Conversely, higher values of body weight at 6 weeks, breast muscle weight, and back weight were recorded in chickens with BB genotype at Pit1-Taq1 loci than those of other genotypes. In the same study, chickens with CC genotypes at Pit1-MspI loci had higher carcass weight, drum stick weight and back weight than the AA and BB genotypes (Bastos *et al.,*2006). It is important to note that the influence of genotype obtained via RFLP on a trait of interest is both restriction enzyme and locus specific. This could be associated with the fact that different enzyme has specific and different restriction site. Insertion/deletion in intron 2 of chicken Pit-1 gene

influenced body weight at 8 weeks as well as other measured carcass traits including leg muscle weight, breast muscle weight. Similarly, two mutations in intron 1 of goose Pit-1 gene were positively associated with early body weight of goose (Cheng and Jefferson, 2008).

2.4.3.4 PIT1 gene polymorphismand its Association with Growth Traits in other Livestock

The bovine PIT1gene is located in chromosome 1 and PCR –RFLP analysis of PIT- HinfI in Holstein-Friesian dairy cattle revealed two alleles (A and B) corresponding to three genotypes named BB,AB and AA (Renaville *et al.,* 1997). The detected A allele at PIT-1/Hinf1 had a positive association with milk quality traits particularly the milk protein and yields. In a related study, Moody *et al.,* (1995) reported significant association between allele A of PIT-1 gene and higher milk yield and qualities. The authors observed high milk yield in cattle with genotype AA and ABthan the BBgenotype. Conversely, bovine PIT-1 gene variants had no effect on the growth traits of Angus beef cattle (Zhao *et al.,* 2004). Similar conclusion was drawn when impacts of PIT-1 gene variants on pig growth and carcass traits was assessed (Yu*et al.,* 1995). The authorsalso reported significant association between PIT-1 polymorphisms and pig birth weight and backfat. In addition, Yu*et al.,* (1999) reported significantinfluence of PIT-1 haplotypes on piglets early body weights and growth rate. Similar trend was observed between PIT1/ *MspI* polymorphism and backfat thickness, but not with PIT1/ *RsaI* polymorphism in pig. The AA genotype obtained via PCR-RFLP analysis of *PIT1/ Rsa*I locus had lower values of feed:gain ratio and daily feed intake compared to BB locusin Pietrain gilts. These results were similar but not consistent with the reports of the influence of the A allele of bovine PIT-1 gene in the available literature(Yu*et al.,* 1995).

2.4.4 Myostatin Gene

Myostatin (MSTN) is a multifunctional cytokines; highlyexpressed in the skeletal muscles of vertebrates with diverse effects on cell proliferation, growth and proper functioning (Piek *et al*., 1999). Myostatinis localized between 190,628,670bp and 190,635,699 base pair on chromosome 2. Earlier studies had proven the MSTN abilities to affects muscle

mass and composition and a good example is MSTNknockout mice which possessed 2-3 folds muscle mass than the wild-type mice (McPherron and Lee, 2002).

2.4.4.2 Myostatin Gene Polymorphismin Poultry

Several studies have detected SNPs in myostatin gene and some of the influences of these SNPs have been reported in farm animals. Gu *et al.,* (2004) evaluated the effects of myostatin gene polymorphisms on chicken growth and carcass traits. The authors reported positive association among the chicken MSTN gene, growth, carcass traits and fattiness traits. PCR-RFLP analysis of myostatin gene in chicken showed two alleles (A and B) and two genotypes (AB and BB); however, Saxena *et al.,* (2013) reported a monomorphoic pattern of genotype myostatin in turkey.

In a related study, Zhu *et al.,* (2007) discovered polymorphism at exon 1 of chicken MSTN gene via PCR-RFLP analysis. The SNPs discovered had positive influence on the chickens' growth and carcass traits. Cloning and sequencing of myostatin gene in three broiler chicken lines revealed 5 SNPs with varied alleles frequencies and haplotypes between the lines. In addition, the identified SNPs influenced broilers' resistance to infectious bursal disease and the mortality rate (Ye *et al*., 2007). The authors further observed that association were inconsistent across all the studied broiler lines. The SSCP and sequencing analysis among two broiler lines and a layer chickens revealed 13 haplotypes. The identified haplogroups had a positive influence on body weight at differing ages in two studied broiler lines (PB-1 and CB). However, myostatin haplogroups had no noticeable effects on all the measured growth traits in the layer line. Gu *et al., (*2004) cloned and sequenced 2517 bp of myostatingene fragments in two selectedchicken strains. The authors reportedsix variants of myostatin protein and interestingly; five of the identified myostatin variants were found in the egg laying strains.

Dushyanth *et al.,* (2016) identified 12 haplotypes of myostatin gene in three chicken lines using SSCP and DNA sequencing analysis. The obtained haplogroups had influenceon growth and carcass traits at 6 weeks. Similarly, Zhiliang *et al.,* (2004) detected SNPs in F2 Broilers and Silky chicken crosses using PCR-SSCP. Three genotypes with two homozygotes genotypes were obtained with all the three pairs of primers used. The authors found no statistical difference in the obtained average carcass

and leg muscle weight values among all the chicken myostatin genotypes (AA, BB and AB). However, homozygotes AA and BB had high positive correlation with abdominal fat weight and highest average breast muscle weight values were recorded in birds with genotype EF. Recently, Zhao *et al.,* (2016) identified six SNPs in exon 3 of duck MSTN gene by direct sequencing. Association results showed that Sansui ducks with genotype GG were comparatively bigger compared to other obtained genotypes studied (GA and AA). In addition, the SNP (106G>A) had a positive association with some of the measured carcass traits viz. breast muscle weight, carcass and slaughter weight among others. Complete linkage relationship was observed between SNPs g.5368G>A and g.5389A>C with significant influence on the measured carcass traits (Zhao *et al.,* 2016).

2.4.4.3 Myostatin Gene Polymorphism in Other Livestock

Bovine Myostatin gene just like rabbit; has3 exons and 2 introns (Fontanesi *et al*., 2008). Nineteen SNPs and 20 haplotypes in MSTN gene were detected in European bovine breeds. Several studies have attributed mutation in the exons 2 and 3 of MSTN to phenotypic differences in animals and particularly muscle hypertrophy. However, sequenced results of MSTN genein rabbit showed C-T transition at position 34 and one SNP in intron 2 with no significant association with all the measured performance traits in rabbits (Fontanesi *et al*., 2008). The lack of effect of the only identified SNP in rabbit MSTN gene was adduced to the fact that it was localized to the intron region.In goats, body weight was positively associated with goat myostatin gene genotypes. Recently, Ahad*et al.,* (2017) studied polymorphism in exon 1of goat MSTN gene and evaluated its association with Boer and Bakerwal goats' performance traits. Boer goats with MSTN genotype AA had higher body weight and higher values of wither height at 12, 13 and 14 months of age compared to AC genotype. Similar trend was observed in Bakerwal goats.Goats with genotype AA had superior average values of body weight, chest circumference and body length compared with the AC genotypes at 13 months of age. Ahad *et al.,* (2017) concluded that the identified SNP (368A>C locus) in exon 1 of MSTN gene could be harnessed in selective breeding for improved growth performance in goats.

2.4.4.4 Myogenic Regulatory Factor

The myogenic regulatory factor (MRFs) include: Myf5, MyoD, Myogenin and MRF4. The MRFs are structurally classified into three domains namely: basic helix-loop-helix (bHLH) domain, cysteine/histidine domain and the serine/threonine-rich domain. However, the bHLH domain has been found to be the main contributor to myogenesis initiation. The MRFs mainly play significant roles in the formation of muscular tissue. However, their roles in non-muscle cells differentiation had also been established. The MRFsinduce and regulate the muscle precursor cells differentiation and proliferation (Delfini and Duprez, 2004). They have also been associated with repair of skeletal muscle and hypertrophy.

2.4.5 Myogenin Gene

The myogenin gene is a muscle-specific transcription factor that plays significant roles in the regulation of skeletal muscle growth and repair via its active involvement in myoblast differentiation. Myogenin is situated on chromosome 26 with three exons and two introns (Naka *et al.,* 2013).

2.4.5.2 Effect of MYOG gene on Growth Traits of chickens

The PCR-SSCP analysis revealed three mutations in the myogeningene region in chickens (Wang *et al.,* 2008). The authors further reported that FF genotype of myogenin gene had higher values of body weight than the EE genotype at 6-10 weeks in Bian chickens. However, no significant relationship was detected between the myogenin genotypes and body weight at the other observed weeks. The chickens with genotype AA were heavier than those with AB and BB.Wang *et al.,* (2008) also established a significant influence of the two observed polymorphic loci at myogenin gene of the chicken with carcass traits and muscle development. More so, Yin *et al.,* (2011) studied polymorphisms in MYOGgene using PCR-SSCP and DNA sequencing. The authors discovered significant association ($p<0.05$) between the SNP in MYOG gene (154T>C) and the measured growth and carcass traits of chickens including: live weight, eviscerated weight and breast muscle weight. The authors hypothesised that MYOGgene had strong relationship with the QTL affecting the carcass traits of the studied populations. Similar repeated reports on the association of the polymorphisms in the chicken MYOG gene with growth traits led to the

proposition that MYOG is a potential candidate gene for selection for improved growth performance of the chickens.

2.4.6 Heat Stress

Stress represents the body's response to stimuli that disrupt normal physiological balance. Continuous adverse effects of the environment on animals could be detrimental to animal survivability and productivity (Liang *et al.,* 2016). Domestic animals suffer from different types of stress including nutritional, thermal stress, physical among others. The severity of heat stress on the animals as well as the corresponding physiological, hormonal and behavioural responses vary in intensity due to animal's genetic make-up, heat regulation mechanisms or gender (Salah *et al.,* 1995). Earlier workers have ranked heat stress as one the topmost constraints to animal agriculture particularly at the tropical and sub-tropical region due to its adverse effects on the productive (e.g egg, meat or milk) and reproductive (reduces libido, lower birth weight, reduced semen volume and quality,conception rate or immunity) performance of the livestock (Cahaner *et al.,* 1993, Sakatani *et al.,* 2004, Franco-Jimenez *et al.,* 2007). Thus, understanding the biology of heat stress with a view to develop sustainable solutions to the current livestock production constraints is a crucial step towards improving animal productivity and farmers' welfare particularly in the tropics.

2.4.6.2 Physiological Response to Heat Stress

The degree of discomfort/comfort in the animals can easily be measured through the physiological and haematological responses to the heat stress. Body temperature represents the overall heat gain and loss process of the body, making it a good measure of heat tolerance in animals (Mahmoud, 2000). The rectal temperature is recognized as an ideal indicator for heat stress valuation in animals (Adedeji *et al.,* 2015). Since a rise of less than 1°C in rectal temperature has been reported to negatively reduce most livestock performance, thus,changes in rectal temperature would be a useful measure of the impacts of thermal-stress in poultry (Isidahomen *et al.,* 2012).Franco-Jimenez*et al.,* (2007) reported 41^{o}C as the chickens' rectal temperature value under optimum atmospheric condition. The author then submitted that significant increase or decrease in body temperature beyond the normal range of 41^{o}C will alter the homeostasis and consequently

affects the normal body functioning and productivity of the animals. Genotypes influence the physiological responses of chickens to heat stress including rectal temperature, purse rate *etc*. In addition, the severity of heat stress was significantly higher in naked neck chickens than their normal feathered counterparts. This was attributed to the incomplete covering of their body with feathers (Isidahomen *et al.,* 2012).

Respiratory rate is one of the acceptable indices of thermal density and discomfort estimation in farm animals including poultry (Melesse*et al.,* 2011). Lemerle and Goddard (1986) reported thatimpact of the heat stress can be measured by measuring the changes in respiratory rate occasioned by increase in ambient temperature of the animal above its thermo-neutral range. This often occurs when animals are trying to maintain the thermal equilibrium. The equilibrium of heat is maintained in animals by various mechanisms and there is mainly a loss of heat through the respiratory tract. Defra, (2003) observed significant influence of breed and body size on the respiratory rate of chickens. The authors noted that the heaviest breed had the highest respiratory rate value due to their larger surface area and consequent increased gas exchange. This finding was corroborated by the submission of Robert, (1994) that body size affects the respiratory rate of chickens under heat stress.

Pulse rate is widely considered as the simplest way to determine the physiological condition of an animal particularly under heat-stress. However, there is no consensus in the accessible reports on the relationship between ambient temperature and heartbeat rate. Though, a decline in heartbeat rate was reported with increment in ambient temperature in poultry. Increment in pulse rate builds blood spill out of the center to the surface and because of it more warmth is lost (Marai *et al.,* 2007).

2.4.6.3 Haematological Response to Heat Stress

Rise in ambient temperature alters animal's homeostatic mechanism thereby resulting in impaired erythropoiesis. In heat stressed animal, increased ambient temperature influences oxygen consumption by increasing respiration rate (Nascimento*et al.,* 2012). The rise in oxygen intake decreases the blood formation process which in turn reduces the number of circulating red blood cells thus packed cell volume (PCV) and haemoglobin (Hb) values (Kumar *et al.,* 2011). Other causes of decrease in hematocrit and Hb include but not

limited to attack of free radicals on the red blood cell (RBC) membrane, poor haemoglobin synthesis and consequently lysis of RBC (Archana *et al.,* 2017).

It has been well documented that heat stress evokes significant changes in the Heterophil:Lymphocyte (H/L) ratio in birds (Tamzil *et al.,* 2014). In addition, heat stress is often accompanied with increase corticosterone (CORT) followed by induced increase in the H:L ratio (Tamzil *et al.,* 2014). Similarly Oke *et al*., (2007) reported noticeable increase in in the H/L ratio, increase blood concentration of leukocytes and decrease in the concentrations of monocytes following the administration of CORT without conspicuous changes in PCV values of heat stressed chickens. However, noticeable differences were reported between the PCV values of heat stressed frizzle feathered, naked neck and normal feathered chickens of Nigeria (Isidahomen *et al.,* 2012). The authors ascribed the decreased PCV (24-27%) values to adverse climatic condition. While, the frizzle feathered birds were reported to have the highest PCV values compared to the naked neck and normal feathered chickens.

2.4.6.4 Heat Shock Proteins

Heat Shock Proteins (HSPs) are produced when there is an increase in ambient temperature above the thermo-neutral zone of the animals (Mazzi *et al.,* 2003). They contribute to the cell survival by eliminating the impaired polypeptides within cells. The HSPs also play active roles in the maintenance and prevention of proteins degradation. Increased in HSPs Expression have been reported during hyperthermic stress (Archana*et al.,* 2017). Thus, HSPs aid the protein turn over as well as regeneration of denatured proteins. The predominant and temperature sensitiveHSPs are HSP70 and HSP90. Both of them have protective roles during heat stress in farm animals(Liang *et al.,* 2016). However, HSP70 has been linked with heat stress in animals.Moreover, HSPs plays a key role in protein assembling and disassembling as well as protein translocation(Marai *et al.,* 2007).

2.4.6.5 Effects of HSP70 Gene on Poultry Performance Traits

Responses of chickens to heat have been reported to be influenced by their genetic backgrounds (Franco-Jimenez *et al.,* 2007). For instance, cockerels exposed to acute heat stress (42°C) for 15 min had reduced plasma total free and essential free amino acids.

More so, acute heat stress on broilers causes decrease in feed intake, body weight, blood pressure without significant change in hematocrit and hemoglobin values (Krista, *et al.,* 1979). Liang *et al.,* (2016) identified SNPs in chicken HSP70gene and evaluated their association with growth and egg production performance of acute heat stressed chickens. The HSP70genotypes have also been associated with thermo-tolerance in chickens (Tamzil *et al.,* 2014). The birds with only one *Pst*I HSP70allele were more resistant to heat compared with others that showed two different alleles for *Pst*I HSP70gene (Mahmoud, 2000). In contrast, Mazzi *et al.*, (2003) reported no correlation between the differential expressions of HSP70 protein at the promoter region. The successful linking of HSP70gene variants with heat-tolerance would be useful in selection for heat-tolerant chickens.

2.5 Growth Models

Growth is a complex biological process that involves cell replication, tissues differentiation, matrix formation, cell death and many other interrelated mechanisms (Raji *et al.,* 2013). Growth models are mathematical equations or functions which describe the pattern of growth of animals for body weight or body parts. Appropriate growth functions summarise the information provided by observation of an animal and mathematically express its life time growth course (Aina, 2017). It summarises the information provided on animals into concise interpretable biological parameters that can be used to describe animal growth over time and to estimate the expected weight of animal at a specific age (Salako, 2014). Growth curves are usually considered infinite-dimensional or function-valued traits because they are described by infinite set of instruments (Zhang *et al*., 2008).

Growth curves are important to breeding plans because they shift in response to selection. Growth curve parameters are better indicators of growth patterns and thus likely to be related to mature growth. Thus, livestock researchers often used growth curves to estimate mature body weight and increase in live weight in animals (Salako, 2014). For example in ruminants just like other animals, the final adult weight can be estimated even when animals are slaughtered before maturity (Gbangboche *et al.,* 2008). The models and shape of the curve is however changeable according to species and environmental conditions. Therefore, finding an appropriate model which adequately described the

weight-age relationship of indigenous chickens in Nigeria such as intended in this study is crucial. In poultry breeding, the growth curve parameters and the values calculated from the model can be used as selection criteria (Va´zquez *et al.,* 2012). Growth curves are also used to express the time-dependent non-linear variation of live weight through a mathematical function. Studies on growth curves are generally gathered under three main titles, namely the determination of the most concordant model, model application and quantitative genetic examination of the growth curve (Osei-Amponsah *et al.,* 2014).

2.5.1 Animal Growth Curves

Growth is an increase in bodyweight, girth, height and length that occurs when a healthy animal is given adequate water and foodwith good shelter (Ozdemir and Dellal, 2009). Live weight is the most commonly measured of these features and may be used to plot a growth curve if recorded at regular intervals (Aggrey, 2002). It is however difficult to give a perfect definition of meat animal growth because many of the changes involved are reversible(Ozdemir and Dellal, 2009). For instance, if the body weight of an animal increases by accumulating fat, it could be accepted as the true growth increments. However, these fat depots might readily be lost if the animal is placed on reduced feed.

Generally models can be broadly classified into two; linear and non-linear. However, non-linear growth models often describe growth and development of animals better than the linear models because growth phenomenon is illustrated in sigmoid form as growth of animals is not the same in every part of their lifetime (Gbangboche *et al.,* 2008). These models have 3 or 4 parameters of biological significance. The parameters of non-linear growth curve models are estimated by iterative procedures minimizing the error variance or minimizing the likelihood by assuming that the residuals are independently distributed (Roush *et al.,* 2006). However, growth curves are built up based on repeated live body weight measurements on the same experimental unit. These serial data usually have underlying relationships or correlations among the serial body weight observations. Therefore, heavier animals at birth or hatch usually have a competitive advantage and remain heavier than the other animals of the group in the later age stages. The variation among the animals for live body weight increases as age increases.

Several modes of growth can be distinguished depending on age or stage of growth (Osei-Amponsah *et al.,* 2014). The modes of growth include the exponential, the sigmoid and the bell shaped. The exponential growth is frequently found for both skulls and brain growth and is characterized by a steadily decreasing growth rate and therefore no point of inflection. Body weight and most organs show sigmoid growth, that is, a period of an increasing growth rate followed by a period with a decreasing one. The bell-shaped growth is found for some organs that are characterized by involution (thymus, bursa of fabricius). The size of the organ first increases and after reaching a maximum it decreases (Laseinde, 1991).

Several studies have been carried out to model the growth pattern of Chicken (Aggrey, 2002, Roush *et al*., 2006, Kucuk and Eyduran 2009, Narinc *et al.,* 2010 *etc*), Turkey and Ostriches (Ersoy *et al.,* 2006), Japanese quail (Ozkan and Kocabas,2004, Raji *et al.,* 2014), Sheep and goats (Gbangboche *et al.,* 2008, Raji *et al.,* 2013, Ozdemir and Dellal, 2009) and Cattle (Salako, 2014) by fitting the most common non-linear growth curve functions such as Gompertz, Logistic, Von Bertalanffy, Brody and Richard models to the time-body weight information. Aggrey, (2002) assessed the appropriateness of the spline linear regression, Gompertz, logistic and Richards models in modeling the growth of random bred, unselected chicken population. The nonlinear models produced the best fit to the data compared to the spline regression model. Richards and Gompertz models gave similar prediction for the random bred chicken growth data used. However, the logistic model predicted significantly different growth parameters compared to those of the Gompertz as well as Richards models. Aggrey, (2002) then submitted that growth parameters predicted with different models with fixed inflection points are not practically comparable.

Teleken *et al.,* (2017) found that Gompertz model fitted Athens-Canadian chickens' growth pattern best. Growth curves parameters of three models (Gompertz, Beterlanffy and Logistic) were compared using data collected on 141 broilers from two commercial lines (Lines 1 and 2): through 6 weeks of age. Results for parameter estimates show that the Gompertz had higher than acceptable estimates for parameter A (mature weight), t1 (age at the inflection point), and b (a measure of duration of growth). Biological interpretations of these estimates, therefore, are difficult. Biological

interpretations of estimates from the Bertallanfy, also, are not meaningful. For these reasons, Atil *et al.,* (2007) concluded that the Logistic was preferred among the three models. Estimation of the average growth curve for studied cattle breeds of Nigeria using Mitsherlich, Richard and Gompertz functions revealed that White Fulani breed was consistently heavier than that of N'dama cattle breed from birth through thirty months. However, Gompertz model indicated that N'dama cattle attained maturity at approximately 4 years of age while growth was still continued in the White Fulani cattle. Similar trend was observed in Mitscherlich model. Mitscherlich model overestimatedthe birth weight of N'dama cattle but gave better estimate of its mature body weight. Salako, (2014) further reported that Gompertz model gave perfect estimate of the degree of maturity in bothN'dama and White Fulani breeds having the best parameter estimatescompared to those of Richard and Mitschelich in bothN'dama and White Fulani breeds.

In a small ruminant study, $R^2 = 0.957$ and $R^2 = 0.956$ were found as coefficient of determination for Logistic and Gompertz growth models, respectively in young Angora goats (Ozdemir and Dellal, 2009). In a similar study, Abdul Waheed *et al.,* (2011) reported on predicted live weight, weight at turning point and rate of growth of 120 Beetal goats. The monthly live weight data was fitted to Brody and Gompertz models. The R^2 values were 0.9979 and 0.9976 for Brody and Gompertzmodels, respectively. Another study aimed at determining the best non-linear model for indigenous sheep was conducted at the University of Maiduguri, Nigeria. Weekly body weights (1-20 weeks) obtained from 51 Yankasa crossbred lambs were fitted to Gompertz, Logistic and Monomolecular non-linear models. The goodness of fit statistics; coefficient of determination (R^2), Mean Square error, standard deviation and Akaike Information Criterion (AIC), in addition to model parameters were used for model comparison (Raji *et al.,* 2013).

2.5.2 Ease of Computation of Growth Models

Various forms of growth models have been successfully fitted into the growth of farm animals. However, there are also cases where growth models do not properly describe animal growth or do not converge at all (Roush *et al.,* 2006). Computational difficulty varies with the choice of curves and the data set. Many functions are sensitive to

frequency and regularity of data (Hruby *et al.,* 1996). Fitting weekly data may work smoothly, whilst monthly data may contain sudden disruptions that might significantly decrease the quality of the fit. Mores so, iterative algorithms can be sensitive to choice of starting values and may not even converge (Forni*et al.,* 2008). There is also the risk of obtaining mathematically correct, but physically meaningless, estimates of parameters in growth functions. Intrinsic uncertainty in the data under observation is also a problem (Selvaggi *et al.,* 2015). It is also worthy ofnote that eachgrowth model hasits strengths as well as limitations. And thatcertain growth model can neither be always perfect nor provide the best fit for all possible cases.

Aina, (2017) reported that Richards model failed to converge when FUNAAB Alpha chicken growth data was fitted. However, the author easily succeeded in computing and fitting of Brody, Logistic and Von Bertalanffy models into the same data set. This is not in agreement with the report that showed the superiority of Richards model over other models in fitting chickens' growth data (Osei-Amponsah *et al.,* 2014). Generally,useful growth models are those that could be easily computed,describes data well and contains biologically and physically meaningful parameters that could be used in decision making such as culling of animals with slow growth rate(Raji*et al.,* 2014).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental Birds

The experimental birds comprised three indigenous chicken ecotypes in Nigeria; namely Fulani and Yoruba ecotypes and FUNAAB-Alpha chicken. Fulani ecotype birds are commonly found in the Guinea and Sahel Savannah as well as in the cattle kraals of Northern Nigeria with few of them among the Fulanis in the South Western states, while, Yoruba ecotype are often found around Rainforest and Derived Savannah zones of Nigeria (Ajayi, 2010). Through crossbreeding and intensive selection over many generations; the FUNAAB-Alpha chicken was developed at FUNAAB in the year 2013 (Ilori *et al.,* 2016)and fully registered as a breed in Nigeria in the year 2018.

3.2 Foundation Stock

A total of 196 sexually mature Fulani and Yoruba ecotype chickens (84 hens and 14 cocks per ecotype) constituted the foundation stock for this study.

3.3 Sampling Location and Sampling Method

Mature Fulani and Yoruba chickens were purposefully bought from the Fulani settlements and Yoruba households across the nine purposefully selected local governments in Oyo State. The selected local governments cut across the three senatorial districts in Oyo state. The map of Oyo state showing the sampling locations is presented in Figure 3.1.

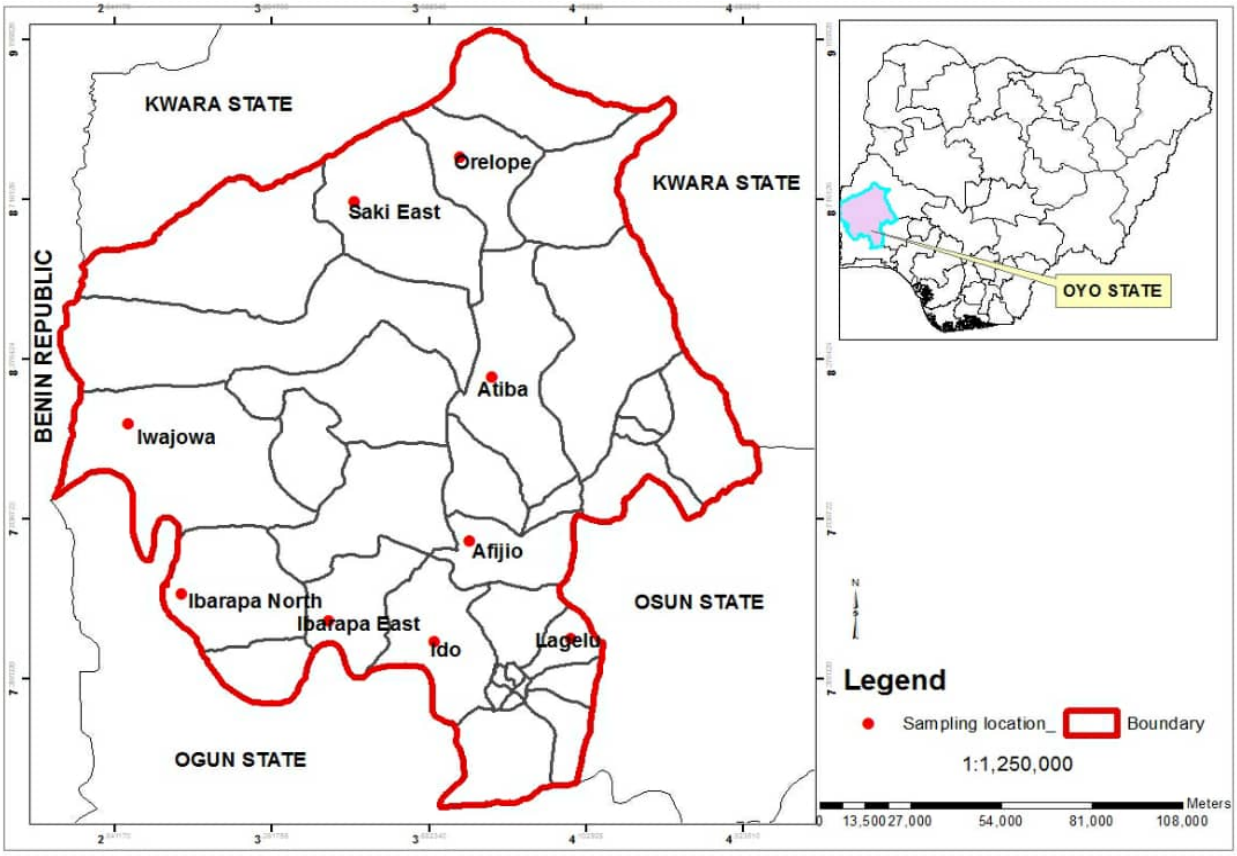

Figure 3. Map of Oyo State showing the sampling locations

3.4 Housing and Management of Foundation Stock

The Foundation Stock were managed under the deep litter system and fed commercial growers and layers' diets at respective stages of the growth. The birds were pen mated in a ratio of 1 cock: 8 hens contrary to the conventional 1 cock: 10 hens in order to get higher fertility. Eggs collected were stored for five days at room temperature (average temperature: 27°C) prior to incubation. Also, two hundred fertile eggs of FUNAAB-Alpha chickens were bought from the Federal University of Agriculture, Abeokuta, Ogun State, Nigeria.

Egg settings and incubation

The procured fertile eggs of FUNAAB-Alpha chickens were set alongside those that were generated from the foundation stocks of Yoruba and Fulani chickens at a reputable hatchery in Ibadan, Oyo state, Nigeria.

3.5 Housing and Management of Experimental Birds

A total of 358 apparently healthy chicks that were hatched from the eggs obtained were used for this study (FUNAAB-Alpha: 138, Yoruba ecotype: 118 and Fulani ecotype: 102). The chicks were tagged at day-old and managed on a deep litter for 24 weeks. The birds were fed commercial diets*ad-libitum* with unrestricted access to fresh clean water. Routine vaccination and medications were administered.

3.6 Data Collection

Body weights of Yoruba, Fulani and FUNAAB-Alpha chickens were individually taken at hatch and weekly for 24 weeks. On each weighing days, measurements were taken early in the morning prior to feeding.At week 24, a total of 120 chickens (20 Cocks and 20 Hens, per ecotype) were randomly selected, fasted overnight and sacrificed. The dressed, thigh, drumstick, breast, empty gizzard, back and wing weights were measured.

3.7 Blood Sampling and DNA Extraction

3.7.1 Blood Sampling

Blood samples (5 mL each) was collected via the jugular vein from a total of 298 birds (Yoruba = 96, Fulani = 89 and FUNAAB-Alpha = 113) using five mL needle and syringe

into sterilized tubes with EDTA as anticoagulant. The contents in the tubes were gently mixed, kept immediately in icebox and transported to the laboratory for DNA extraction.

3.7.2 DNA Extraction

Genomic DNA was extracted with the Zymo® Quick-DNA™ Mini Prep kit by following the manufacturer's protocols:

a. 100 µl of whole blood was pipetted into a micro-centrifuge tube
b. 700 µl of genomic lysis buffer was added to lyse cell walls
c. The mixture was vortex for 5-6 minutes, transferred into a Zymo-spin column and then centrifuged for one minute at 13,000 rpm
d. The spin-column was replaced with another collection tube before addition of 200 µl of DNA pre-wash buffer followed by centrifugation at 13,000 rpm for one minute
e. 500 µl of genomic DNA Wash-buffer and 50 µl DNA elution buffer was added separately and centrifuged for one minute each at 13,000 rpm
f. The DNA was kept in the collection tube following centrifugation and stored at -20 ^{O}C prior to further analysis

3.7.3 DNA Quality Assessment

The quality of the DNA was tested through agarose gel electrophoresis as follows:

a. The edges of the clean gel tray were taped and placed on a flat table
b. 1g agarose powder was dissolved in 100 mls of 1X TBE buffer in a conical flask and microwave for 1 minute
c. The content was allowed to cool for six minutes
d. 10 ml E-Z vision dye was added and gently whirled to ensure even distribution
e. The comb was inserted into the gel tray and cooled agarose containing E-Z vision dye was gently poured into the tray and allowed to solidify
f. The seals and the comb were carefully removed after solidification
g. 10µl of 100 bp DNA ladder was loaded to the first well; 10µl of the DNA were carefully loaded into the subsequent wells and electrophoresis was carried out at 85V for one hour
h. The bands were then viewed on the gel with BIO-RAD gel documentation system (USA)

Table 3.1: Primer Sequences and Restriction Enzymes used for DNA Amplification of the Selected Genes

Gene	Primer sequence(5′–3′)	Length (bp)	Restriction Enzyme	Reference
MSTN	F: ATGCAAAAGCTAGCAGTCTATG	373	*Alu I*	Bhattacharya and Chatterjee, 2013
	R: ACTCCGTAGGCATTGTGATAAT			
GH	F: AAAACCAGGCAGGAAAATCA	195	*Van911*	Su *et al.,* 2014
	R: TACGGAGATGGAAAGGTTGG			
GHRL	F: CATTTCTAAGCTTTTGCCAGTT	431	*TspR I*	Nie*et al.,* 2005
	R: GCATTATTCTGACTTTTTACCTG			
PIT-1	F: GCCTGACCCCTTGCCTTTAT	210	*Hinf I*	Nie *et al.,* 2005
	R: CCAGCTTAATTCTCCGCAGTTT			
MYOG	F: CTCCCCCTCCTCTCTCAGAT	157	*Msp1 & EcoR1*	Wei *et al.,* 2015
	R: CTTTGCGCCAGCTCAGTT			
HSP70	F: AACCGCACCACACCCAGCTATG	360	*MmeI*	Akaboot *et al.,* 2012
	R: CTGGGAGTCGTTGAAGTAAGCG			

3.8 PCR Amplification and RFLP Analysis

Restriction digestion mixture for each reaction:

No	Components	Quantity
1.	Nuclease free water	9 µl
2.	10 x assay buffer	1 µl
3.	Restriction Enzyme	0.5 µl
4.	PCR product	5 µl
	Total	15.5 µl

3.8.1 PCR amplification of MSTN gene

Chicken MSTN gene has 12 exons and 4 introns. In this study, Exon-1 of MSTN gene was amplified with the primer sequences which were previously designed by Bhattacharya and Chatterjee,(2013). Exon 1 was chosen for this study because earlier studies have revealed polymorphism at this locus with possible impacts on growth traits of chickens (Paswan *et al.,* 2013, Bhattacharya and Chatterjee, 2013). The designed primers were synthesized at the Inqaba Biotec West Africa Ltd and used for this study. Details of the primer sequences that were used are presented in Table 3.1.

PCR Reaction Mixture for MSTN gene

The PCR was performed for MSTN gene in a 50-µL total reaction volume. Each PCR tube contained 25 µL one taq quick® load 2X Master mix buffer (M0486S) (Biolabs; New England), 5 µL genomic DNA, 1 µL forward primer, 1 µL reverse primer and 18 µL nuclease free water. Details of the PCR conditions that were used are presented in Table 3.2.

Table 3. 2PCR protocol used for the amplification of MSTN gene

Step	Temperature (^{o}C)	Time (minute)
a. Initial denaturation	94	5
b. Denaturation	94	0.5
c. Annealing	51	1
d. Extension	72	1
Step a – d was repeated for 30 cycles		
Final Extension	72	5

3.8.2 PCR amplification of Growth Hormone gene

The primers sequences that were earlier designed based on chicken GH gene sequence with accession No: AY461843 by Su *et al.,* (2014) were used for this study. The primers were synthesized at Inqaba biotec West Africa Ltd and used for this study. Details of the primer sequences that were used are presented in Table 3.1.

PCR Reaction mixture for GH gene

Of the 8 currently identified exons in chicken GH gene, exon 1 was investigated in this study based on its high polymorphic nature coupled with its potential influence on production traits of poultry (Nie *et al.,* 2005, Tanmankaur *et al.,* 2008). The PCR was performed for GH gene in a 50-µL total reaction volume. Each PCR tube contained 25 µL one taq quick® load 2X Master mix buffer (M0486S) (Biolabs; New England), 5 µL genomic DNA, 1 µL forward primer, 1 µL reverse primer and 18 µL nuclease free water. Details of the optimized PCR conditions are presented in Table 3.3.

Table 3. 3: PCR protocol that was used for the amplification of GH gene

Step	Temperature (^{o}C)	Time (minute)
a. Initial denaturation	94	5
b. Denaturation	94	0.5
c. Annealing	56	1
d. Extension	72	1
Step a – d was repeated for 35 cycles		
Final Extension	72	5

3.8.3 PCR amplification of GHRL gene

Exon-1 of GHRL was chosen for this study, having been established as a polymorphic locus with potential influence on growth and carcass traits of poultry (Mohsen, 2011, Bhattacharya and Chatterjee 2013).Exon-1 of GHRL gene was amplified with the primer sequences which were previously designed based on GHRL gene sequence with accession number AY303688 (Bhattacharya and Chatterjee 2013).The designed primers were synthesized at the Inqaba biotec West Africa Ltd and used for this study. Details of the primer sequences that were used are presented in Table 3.1.

PCR Reaction mixture for GHRL gene

The PCR was performed for GHRL gene in a 50-µL total reaction volume. Each PCR tube contained 25 µL one taq quick® load 2X Master mix buffer (M0486S) (Biolabs; New England), 5 µL genomic DNA, 1 µL forward primer, 1 µL reverse primer and 18 µL nuclease free water. The PCR reaction protocol is presented in Table 3.4.

Table 3.4PCR protocol that was used for the amplification of GHRL gene

Step	Temperature (^{o}C)	Time (minute)
a. Initial denaturation	94	5
b. Denaturation	94	0.5
c. Annealing	51	1
d. Extension	72	1
Step a – d was repeated for 35 cycles		
Final Extension	72	5

3.8.4 PCR amplification of PIT-1 gene

Chickens PIT-1 gene has 14 exons and 4 introns (Bastos *et al.,* 2006). However, variation in only exon 2 and its impacts on growth and carcass traits of Nigeria indigenous chickens were investigated in this study. The primer sequences that were earlier designed based on chicken PIT-1 gene (AF029892) were used in this study (Nie *et al.,* 2005). The selected primer sequences were synthesized at Inqaba Biotec West Africa Ltd. Details of the primer sequences that were used are shown in Table 3.1.

PCR Reaction mixture for PIT-1 gene

The PCR was performed for PIT-1 gene in a 50-µL total reaction volume. Each PCR tube contained 25 µL one taq quick® load 2X Master mix buffer (M0486S) (Biolabs; New England), 5 µL genomic DNA, 1 µL forward primer, 1 µL reverse primer and 18 µL nuclease free water. Details of the optimized PCR reaction protocols are presented in Table 3.5.

Table 3. 5PCR protocol that was used for the amplification of PIT-1 gene

Step	Temperature (^{o}C)	Time (minute)
a. Initial denaturation	94	5
b. Denaturation	94	0.5
c. Annealing	51	1
d. Extension	72	1
Step a – d was repeated for 30 cycles		
Final Extension	72	5

3.8.5 PCR amplification of MYOG gene

Of the 8 reported exons of chicken myogenin gene, exon 2 of MYOG locus was investigated using the primer sequences that were earlier designed based on MyoG gene sequence with GenBank accession number NC_006113.3 (Wang *et al.,* 2008, Wei *et al.,* 2016). The primers sequences were synthesized at Inqaba biotec West Africa ltd (South Africa).Details of the primer sequences that were used are presented in Table 3.1.

PCR Reaction mixture for MYOG gene

The PCR was performed for MYOG gene in a 50-µL total reaction volume. Each PCR tube contained 25 µL one taq quick® load 2X Master mix buffer (M0486S) (Biolabs; New England), 5 µL genomic DNA, 1 µL forward primer, 1 µL reverse primer and 18 µL nuclease free water. Details of the optimized reaction protocol that was used are presented in Table 3.6.

Table 3. 6 PCR protocol used for the amplification of MYOG gene

Step	Temperature (^{o}C)	Time (minute)
a. Initial denaturation	94	5
b. Denaturation	94	0.5
c. Annealing	55	1
d. Extension	72	1
Step a – d was repeated for 35 cycles		
Final Extension	72	5

3.8.6 PCR amplification of HSP70 gene

The chicken HSP70 gene has 28 exons and 6 introns. However, intron 1 with a tracked record of high polymorphism and significant association with thermo-tolerance (Akaboot *et al.,*2012, Liang *et al.,* 2016) were investigated using earlier reported primers by Akaboot *et al.,* (2012). The primer sequences were synthesized at Inqaba Biotec West Africa Ltd (South Africa) and then utilised in this study. Details of the primer sequences that were used are presented in Table 3.1.

PCR Reaction mixture for HSP70 gene

The PCR was performed for HSP70 gene in a 50-µL total reaction volume. Each PCR tube contained 25 µL one taq quick® load 2X Master mix buffer (M0486S) (Biolabs; New England), 5 µL genomic DNA, 1 µL forward primer, 1 µL reverse primer and 18 µL nuclease free water. The optimized PCR reaction protocols are presented in Table 3.7.

Table 3. 7PCR protocol used for the amplification of HSP70 gene

Step	Temperature (^{o}C)	Time (minute)
a. Initial denaturation	94	5
b. Denaturation	94	0.5
c. Annealing	60	1
d. Extension	72	1.5
Step a – d was repeated for 35 cycles		
Final Extension	72	5

3.9 Acute Heat Stress Exposure

At week 23, a total of 54 birds (18 birds per ecotype) whose HSP70 genotype group have earlier been determined were randomly selected from the flock and exposed to an acute heat stress test at 40-41°C for 1.0 hour. Respiration rate (RR), Cloaca temperature (CT) and Pulse rate (PR) were measured at 0 and 1 h after heat stress (Isidahomen *et al.,* 2012). Blood samples were collected at 0 and 1 h after heat stress for determination of Packed Cell volume (PCV), hemoglobin (Hb), red blood cells, lymphocyte, heterophil and heterophil/lymphocyte ratio using standard procedures as described by Ewuola and Egbunike, (2007).

3.9.1 Physiological Parameters Measurement

The following physiological parameters were determined by:

Cloaca temperature: inserting a clean clinical thermometer into the vent for one minute after which the readings were taken**.**

Respiration rate: counting the number of movements of abdominal region or vent of each bird for a minute using a stopwatch.

Pulse rate: placing the stethoscope under the wing vein and counting the number of beats per minute

Heat stress index: The heat stress index (HSI) was derived by using the formula bellow:

$$HSI = \frac{\text{Average respiratory rate value}}{\text{Average pulse value}} \times \frac{\text{Normal pulse rate value}}{\text{Normal respiratory rate}}$$

3.10 Sequence Analysis

DNA Sequencing: A total of 36 quality PCR products (six samples per each of the studied genes) were sequenced at INQABA® Biotech, South Africa. The sequences obtained were subjected to NCBI Basic Local Alignment Search Tool (BLASTn) and similar sequences of earlier reported *Gallus gallus* that areavailable in the GenBank database were retrieved from the (http://ncbi.nih.gov/BLAST/).The multiple sequence alignments, phylogenetic tree and genetic distance were generated using Molecular Evolutionary Genetic Analysis version 7.0 (Kumar *et al.,* 2016).

3.11 Statistical Analysis

Allele and genotype frequencies, test for Hardy-Weinberg equilibrium and Heterozygosity were obtained using POPGENE 1.32 software package (Yeh, 1999). Data on growth, carcass and thermal-tolerance parameters were analysed using the generalized linear model (GLM) of SAS (2010). The following linear model was employed:

$Y_{ij} = \mu + G_i + S_j + (GS)_{ij} + e_{ij}$

Where:

Y_{ij}: is observed phenotypic traits

μ: is the overall mean

G_i: is the fixed effect of $_i$th genotype

$S_{j:}$ is the fixed effect of jth sex

$(GS)_{ij}$ is the interaction effect of ith genotype and jth sex

$e_{ij:}$ is random error associated with each record

3.12 Growth Models

Five commonly used non-linear growth model for poultry (Logistics, Gompertz, Brody, Richard, Von bertalanffy and Mitscherlich) were applied to describe the weight-age relationship of Yoruba, Fulani and FUNAAB-Alpha chickens. Details of the adopted models are presented in Table 3.8.

Table 3. 8Non-linear models used and their parameters

S/N	Name	Expanded Model
1	Mitscherlich	Y = a-b * exp (-k* age)
2	Gompertz	Y = a * exp (-b*exp (-k* age))
3	Logistics	Y = a/(1+b* exp (-k* age))
4	Von bertalanffy	Y = a*(1-b* exp (-k* age))**3
5	Brody	Y = a*(1-b* exp (-k* age)

y = weight of animals in time t;a = asymptotic weight or potential final weight;b = integration parameters;

k = maturity index.

3.12.1 Determination of Goodness of Fit

Models in this study were compared using coefficient of determination (R^2), Akaike's Information Criterion (AIC) and Bayesian Information Criterion (BIC). The coefficient of determination (R^2) is a measure of the proportion of the total variation accounted for by the explanatory variable (age). The best growth model is expected to have the highest value of R^2 but lowest values of AIC and BIC respectively (Roush*et al.,*2006).

$R^2 = 1-(SSE/SST)$

$AIC = n.ln (SSE/n) + k\ lnn$

$BIC = kln(n) – 2ln(x)$

Where:

SSE: Sum of Squared errors, SST: Total sum of Squares, R^2: Coefficient of Determination

n= number of observation, k= maturity rate, n: number of parameters.

3.13 Fitting of selected models to the observed growth Data

All the five selected models were fitted to the individual body weights for each age using the non-linear regression procedure and llevenberg-Marquardt iteration method of SPSS package version 22.

CHAPTER FOUR

4.0 RESULTS

4.1Descriptive Statistics of body weight of Yoruba, Fulani and FUNAAB-Alpha Chicken Ecotypes

At day-old, Yoruba chicken ecotypes had the least initial body weight followed by Fulani then FUNAAB-Alpha chicken. The final body weight at week 24 followed similar trend. More so, body weight of FUNAAB Alpha chickens was about 1000g heavier than those of Yoruba and Fulani chickens from weeks 20 – 24 (Table 4.1). At day-old, the body weights of the cocks and hens appeared similar across the ecotypes. However, the body weight (at week 24) of the cocks was heavier than those of their corresponding hens across the ecotypes. Body weights of FUNAAB-Alpha cocks and hens were consistently higher than those of Yoruba and Fulani cocks and hens throughout the study (Table 4.2).

Table 4. Descriptive Statistics of Body Weight (g) of Yoruba, Fulani and FUNAAB-Alpha Chickens

Breed	N	Week 0	Week 4	Week 8	Week 12	Week 16	Week 20	Week 24
Yoruba	91	24.5±1.1	109.5±14.1	265.9±30.1	474.2±70.5	695.9±119.3	897.8±146.1	998.2±159.0
Fulani	79	26.8±2.7	165.8±20.9	350.8±43.0	591.7±79.8	887.0±126.6	1061.5±164.4	1156.2±181.3
FUNAAB-Alpha	98	34.6±2.1	315.9±56.0	770.0±123.1	1177.0±173.7	1580.7±223.0	2102.6±285.7	2323.6±258.4

N: sample size

Table 4. 2 Descriptive Statistics of Body Weight (g) of Yoruba, Fulani and FUNAAB-Alpha Cocks and Hens

Genotype	N	Week 0	Week 4	Week 8	Week 12	Week 16	Week 20	Week 24
Yoruba								
Cock	37	24.2±1.3	110.6±9.5	261.0±17.4	517.6±42.3	801.3±55.2	1033.6±71.9	1162.1±73.2
Hen	54	24.7±1.0	108.8±16.3	236.1±35.6	447.6±71.3	631.0±100.2	814.2±113.7	897.3±103.3
Fulani								
Cock	33	26.8±2.0	166.2±12.4	357.7±19.6	636.2±42.2	984.1±81.8	1205.2±96.9	1319.9±115.5
Hen	46	26.7±2.4	151.1±22.1	324.9±43.3	555.2±57.2	799.1±69.8	940.5±81.2	1015.9±77.8
FUNAAB-Alpha								
Cock	56	34.9±2.0	326.0±61.7	784.9±114.1	1285.0±123.6	1713.8±152.6	2294.4±158.3	2496.9±183.8
Hen	42	36.6±2.2	302.0±44.3	719.6±121.0	1029.1±125.7	1398.7±169.2	1840.1±165.3	2086.3±161.5

N: sample size

VARIATION IN MSTN GENE AND ITS ASSOCIATION WITH GROWTH AND CARCASS TRAITS OF YORUBA, FULANI AND FUNAAB-ALPHA CHICKENS

4.3.1 Amplification of exon-1 MSTN gene

PCR Amplification of exon-1 MSTN gene produced 373 bp fragments. The gel picture of the PCR product is presented in Plate 4.1. The amplified product of MSTN gene was digested with *Alu1* enzyme. The digested products yielded two alleles (A and B) which led to identification of three genotypes (AA, AB and BB) in Yoruba, Fulani and FUNAAB-Alpha chickens. Sample of the gel image of PCR product of MSTN and MSTN/*Alu1* restriction digested products in the studied chickens is presented in Plate 4.1 and 4.2, respectively.

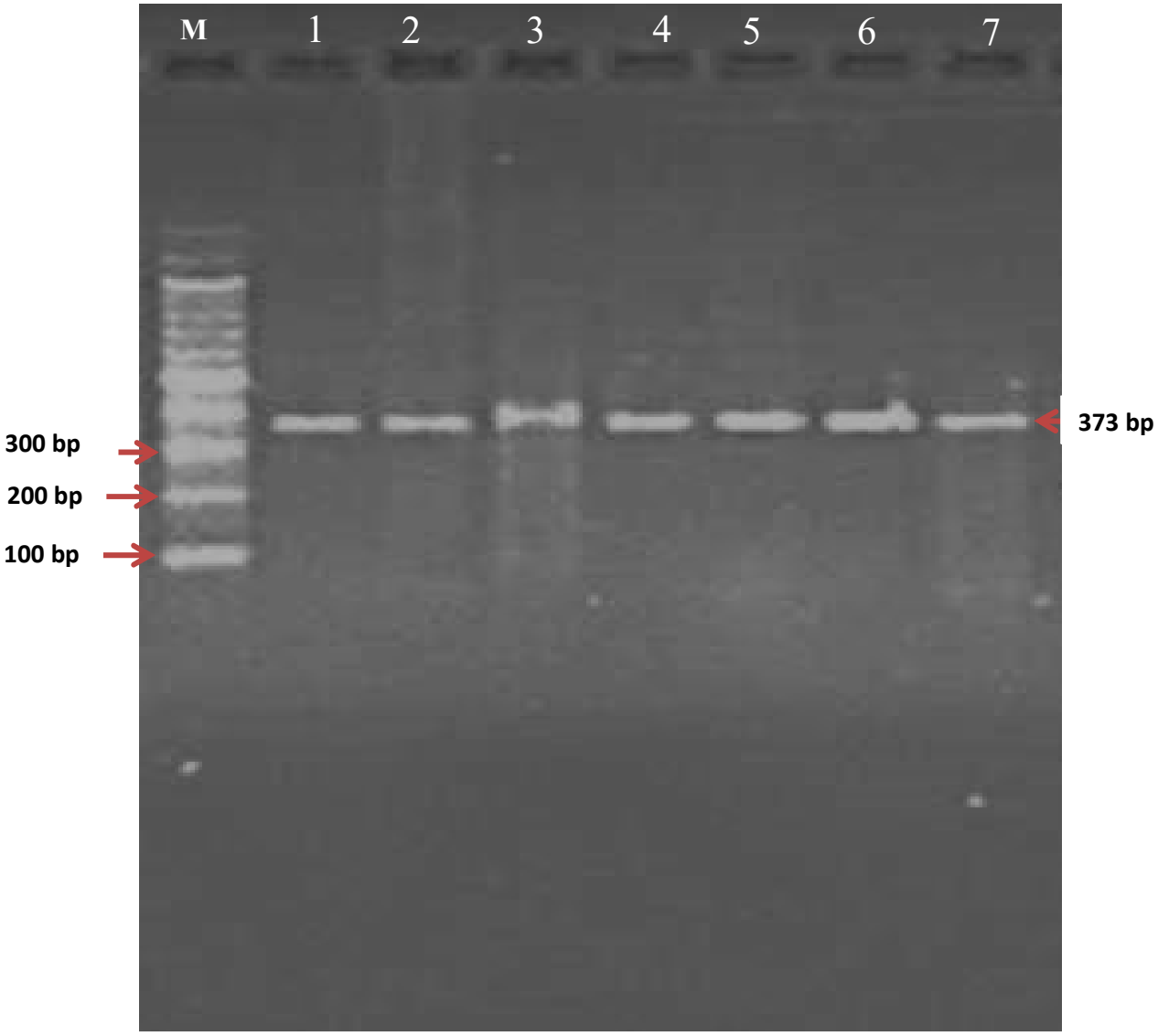

Plate 4. 1 Gel picture of PCR product of MSTN gene of indigenous chickens in Nigeria

M: 100 bp DNA marker

Lanes 1-7: Samples 1 to 7

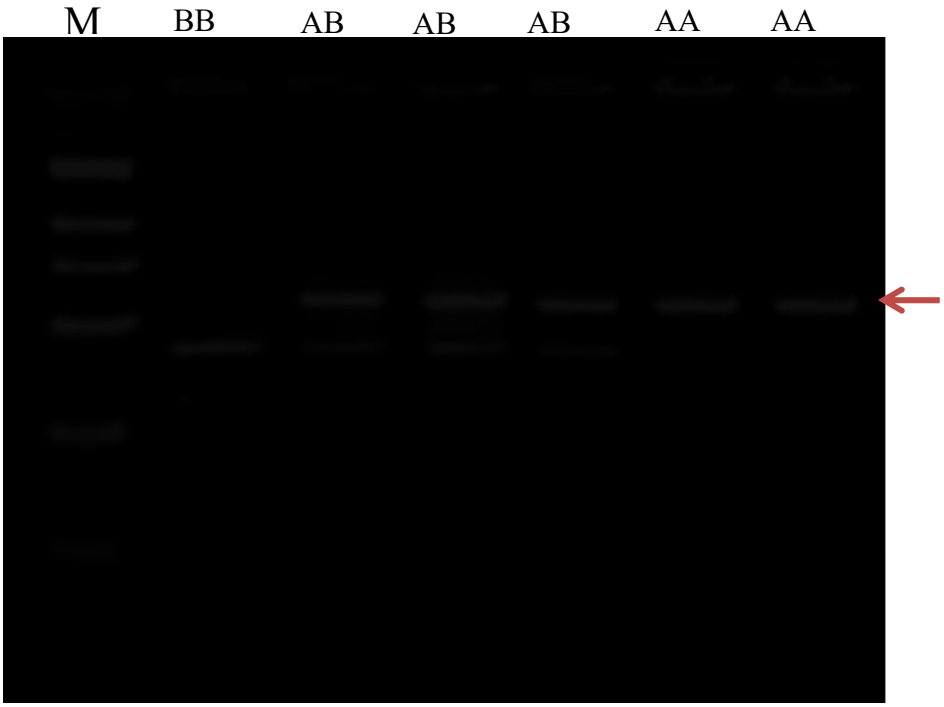

Plate 4. 2: PCR-RFLP pattern of MSTN gene in indigenous chickens in Nigeria

M: 100 bp DNA marker,

AA: Genotype AA

BB: Genotype BB

AB: Genotype AB

4.3.2 Allele, genotype and heterozygosity patterns of MSTNgene in Yoruba, Fulani and FUNAAB Alpha chickens

The single slower band was named A allele, while the fast band was named B allele. Individuals with single slower band were named homozygote AA, while those with combination of fast and slow alleles were named heterozygote AB. Thus, two co-dominant alleles (A and B) with corresponding three genotypes (AA, AB and BB) were obtained in all the three studied populations with differing frequencies (Table 4.3). Genotype AA had the highest genotype frequency value (0.7) while BB genotype had the least genotype frequency value (0.08). The chi-square values ranged from 7.82 to 13.55. Details of the MSTN/*Alu1* allele and genotype frequencies in Yoruba, Fulani and FUNAAB Alpha chickens are presented in Table 4.3. At MSTN locus, the observed heterozygosity values ranged from 21.4 (FUNAAB-Alpha) to 24.4% (Yoruba) chickens. However, FUNAAB-Alpha chickens were the least diverse, while Fulani chickens were the most diverse with 40.8% expected heterozygosity. Fulani and Yoruba chickens had the highest and closest Shannon's Information Index (59.8%); while the least Shannon's Information Index value was found in FUNAAB-Alpha chickens (48.7%) (Table 4.3). The heterozygosity were estimated using POPGENE version 1.32 software.

Table 4. 3 Allele and genotype frequencies of MSTN gene in Yoruba, Fulani and FUNAAB-Alpha Chickens

		Genotype frequency (%)			Allele frequency (%)		Heterozygosity(%)			
Ecotype	Sample size	AA	AB	BB	A	B	Observed	Expected	Shannon's Information Index	HWE (χ^2)
Yoruba	84	0.70	0.21	0.09	0.81	0.19	0.244	0.381	0.569	7.82*
Fulani	70	0.60	0.27	0.13	0.71	0.29	0.229	0.408	0.598	13.55*
FUNAAB-Alpha	82	0.62	0.24	0.14	0.74	0.26	0.214	0.308	0.487	10.62*

HWE: Hardy-Weinberg Equilibrium, * : Significant ($p<0.05$)

4.3.3Gene Flow and F-Statistics

Population differentiation examined by fixation indices (F_{IS}, F_{IT} and F_{ST}) for each of the five studied loci across the three Nigeria indigenous chicken populations is shown in Table 10. The global heterozygosity deficit (F_{IT}) was estimated at 0.145 and the within-breed deficit in heterozygote (F_{IS}) ranged from-0.265 (GH) to0.520 (HSP70) with an average of 0.137 for all loci. Global breed differentiation evaluated by F_{ST} was estimated at 0.009. The gene flow values for each of the studied loci ranged from 14.832 to 279.343, while the mean gene flow over all loci was recorded at 77.805 (Table 4. 4).

Table 4. 4F-Statistics and Gene Flow for five Loci in Nigeria Indigenous Chickens

Locus	Sample Size	F_{IS}	F_{IT}	F_{ST}	Nm*
MSTN	236	0.374	0.380	0.009	28.876
GH	195	-0.265	-0.244	0.017	14.832
PIT-1	214	-0.191	-0.185	0.005	48.976
GHRL	197	0.247	0.248	0.001	279.343
HSP70	207	0.520	0.527	0.014	16.999
Mean	210	0.137	0.145	0.009	77.805

*Nm = Gene flow estimated from $F_{ST} = 0.25(1- F_{ST})/ F_{ST}$.

4.3.4 BLASTn result showing the percentage similarity of the MSTN sequences of indigenous chickens in Nigeria and chicken MSTN sequences from the NCBI GenBank

The obtained sequences of MSTN gene from all the three studied chicken populations were compared with the reported chicken MSTN gene sequences in the NCBI database using BLASTn search. The BLASTn results showed 99% identity and zero expect-values with the *Gallus gallus* exon-1 MSTN gene. The details of the BLASTn results with the percent similarities values are presented in Table 4.5. The sequences were aligned using BioEdit 6.5 software.

Table 4. 5BLASTn result showing the percentage similarity of the MSTN sequences of indigenous chickens in Nigeria and chicken MSTN sequences from the NCBI Genbank

Accession	Description	Max score	Total score	E-Value	Max identity
AY670651.1	*Gallus gallus* myostatin (MSTN) gene, exon 1 and partial cds	684	684	0	99%
GU181323.1	*Gallus gallus* isolate 5671 myostatin (MSTN) gene, MSTN-C allele, exon 1 and partial cds	678	678	0	99%
GU181322.1	*Gallus gallus* isolate 5759 myostatin (MSTN) gene, MSTN-B allele, exon 1 and partial cds	684	684	0	99%
DQ912835.1	*Gallus gallu*s myostatin (GDF8) gene, complete cds	673	673	0	99%
AF346599.2	*Gallus gallus* myostatin (MSTN) gene, promoter region, exons 1, 2 and 3 and complete cds	667	667	0	99%
KT071543.1	*Gallus gallus* breed Type 3 Luqin myostatin gene, promoter region and partial cds	211	211	3.00E-53	98%

4.3.5 Phylogenetic tree and genetic distance derived from MSTN sequences from Yoruba, Fulani and FUNAAB-Alpha chickens

The phylogenetic tree constructed from the assembled nucleotide sequences of MSTN revealed that the Yoruba chickens are more closely related to the FUNAAB Alpha chickens than the Fulani chickens and AY670651(Figure 4.1). In addition, diversity study among the three studied populations and the sequences from the GenBank showed that highest genetic similarity exists between Yoruba and FUNAAB-Alpha chickens (99.50%) while Fulani and Yoruba chickens had the least genetic similarity value (99.43%). Table 4.6 presents genetic distance and similarity between the three studied chicken populations.

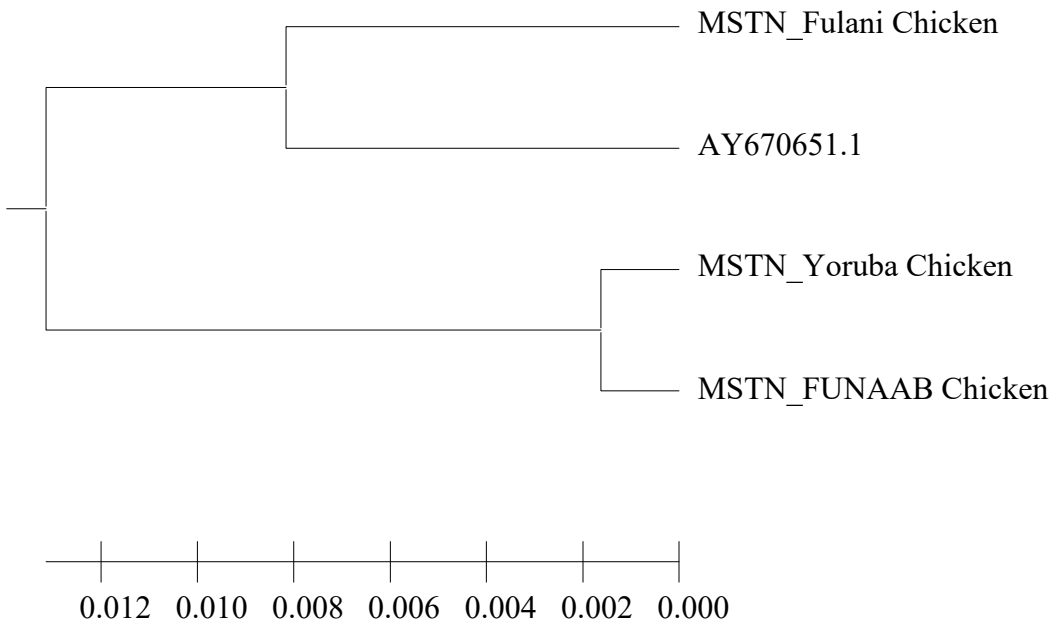

Figure 4.Phylogenetic tree derived from MSTN sequences of Yoruba, Fulani and

FUNAAB Alpha and chicken MSTN gene sequences (AY670651.1) from GenBank using UPGMA.

Table 4. 6 Genetic Distance and Percentage genetic similarity among indigenous chickens in Nigeria based on exon-1 of MSTN Gene

	Fulani (%)	Yoruba (%)	FUNAAB-Alpha (%)	AY670651.1 (%)
Fulani		97.43	97.7	98.73
Yoruba	2.57		99.50	98.22
FUNAAB-Alpha	2.30	0.50		98.22
AY670651.1	1.27	1.78	1.78	

Upper diagonal: Per cent genetic Similarity, Lower diagonal: Genetic Divergence

4.3.6 Effect of MSTN genotypes on growth and carcass traitsof Yoruba, Fulani and FUNAAB Alpha Chickens

The body weights of Fulani chickens with genotype AA were not significantly different from their counterparts with genotypes AB and BB throughout the study. Similarly, body weights of Yoruba chickens with AA of MSTN were not significantly different ($P>0.05$) from those of AB and BB from weeks 0 to 16. Yoruba chickens with AB had significantly higher ($P<0.05$) body weight (951.8±143.6) than BB (881.4±174.0) but similar to AA (896.4±146.1) at week 20. Myostatin genotypes had no significant effect on the obtained body weights of FUNAAB-Alpha chickens from week 0 to 8. However, FUNAAB-Alpha birds with AB had significantly higher ($P<0.05$) body weight than those of AA and BB genotypes at week 12 (Table 4.7). All the measured carcass traits of Yoruba chickens with AA were not significant different from those of AB and BB birds. The dressed and breast weights of Fulani chickens with genotype AB were significantly higher than those of AA and BB. Similarly, FUNAAB-Alpha chickens with AB had significantly higher dressed weight (1503.3±75.2) than AA (1413.8±103.9) and BB (1329.5±109.7). The thigh, breast and wing weight of FUNAAB-Alpha chickens with AA were not significantly different from those with AB and BB variants (Table 4.8).

Table 4. 7: Effect of MSTN Genotypes on Body Weight of Yoruba, Fulani and FUNAAB Alpha Chickens

Breed /Genotype	Body weight (g)						
	Week0	Week 4	Week 8	Week 12	Week 16	Week 20	Week 24
Yoruba							
AA	24.5±1.1	107.9± 13.7	265.2±30.5	472.5±72.9^{a}	695.3±118.4	896.4±146.1^{ab}	997.5±159.6^{b}
AB	24.7±1.1	111.6±16.4	267.5±29.7	469.9±60.5^{a}	677.8±116.9	951.8±143.6^{a}	1045.9±157.4^{ab}
BB	24.6±1.7	117.1±9.6	272.1±30.7	500.4±82.5^{a}	747.0±149.8	881.4±174.0^{b}	981.9±181.3^{b}
Fulani							
AA	26.6±2.4	165.5±21.5	353.4±44.4	582.4±80.8	891.5±135.9	1050.1±168.4	1145.9±184.1^{a}
AB	27.5±3.1	171.8±19.1	351.5±43.3	627.7±86.2	899.2±100.1	1134.7±151.2	1228.6±174.1^{a}
BB	26.3±3.1	158.8±20.8	341.2±40.8	576.0±51.7	838.4±133.4	1003.5±149.6	1096.2±168.4^{a}
FUNAAB-Alpha							
AA	34.4±2.0	319.9±59.8	766.6±127.5	1160.5±182.0^{b}	1508.4±222.6^{b}	2080.1±277.5^{a}	2306.3±252.1^{a}
AB	35.2±2.2	333.1±47.9	814.0±103.6	1257.9±145.1^{a}	1650.8±219.9^{a}	2180.5±289.6^{a}	2403.9±272.0^{a}
BB	34.0±1.8	289.2±44.6	731.3±125.0	1094.4±143.9^{b}	1573.9±240.7^{ab}	2166.9±322.9^{a}	2367.4±268.8^{a}

Means ± SD with different superscripts within breed along the columns differ significantly ($p<0.05$)

Table 4. 8 Effect of MSTN genotypes on carcass traitsof Yoruba, Fulani and FUNAAB Alpha Chicken

Breed/ Genotype	Dressed Weight (g)	Drum Stick (g)	Thigh (g)	Breast (g)	Wing (g)	Back (g)	Gizzard (g)
Yoruba							
AA	674.9±74.6^{a}	119.1±21.2	119.1±13.4	172.0±19.4^{a}	110.4±11.9	188.9±16.5	28.2±2.9
AB	680.7±64.5^{a}	125.1±11.8	120.4±13.3	181.9±19.3^{a}	104.0±13.0	184.9±13.3	28.2±3.2
BB	652.7±81.4^{a}	120.4±14.0	119.1±16.0	173.9±23.1^{a}	106.0±19.0	190.9±18.6	28.1±4.7
Fulani							
AA	792.1±108.4^{b}	125.3±17.7	137.9±20.5^{a}	200.8±27.7^{b}	122.9±6.3	208.7±26.4^{b}	28.5±1.2
AB	864.7±121.4^{a}	153.9±21.2	170.7±23.6^{a}	246.3±31.4^{a}	136.3±9.8	253.9±32.1^{a}	30.8±1.8
BB	777.7±69.5^{b}	125.7±12.6	134.4±13.5^{a}	197.2±15.5^{b}	119.0±6.3	204.4±12.2^{b}	27.8±1.9
FUNAAB-Alpha							
AA	1413.8±103.9^{b}	249.0±17.5^{ab}	274.7±19.3	299.1±22.2	168.5±11.8	296.0±23.4	36.5±2.7
AB	1503.1±75.2^{a}	261.4±12.8^{a}	289.2±14.2	318.9±17.1	176.8±8.5	321.7±16.7	38.2±1.8
BB	1329.5±109.7^{b}	233.8±16.8^{b}	259.0±18.0	280.5±25.0	158.3±11.0	282.5±8.5	34.8±1.7

Means ± SD with different superscripts within breed along the columns are significantly different ($p<0.05$)

4.3.7 Interaction Effects of Sex and MSTN genotype on growth and carcass traitsof Yoruba chickens

The body weight of Yoruba cocks were significantly higher ($p<0.05$) than that of the hens from weeks 16 and 24. At week 24, body weight of cocks ranged from 1142.8±14.84 (BB) to 1169.6±9.74 (AB) at MSTN locus (Table 4.9). More so, dressed weight of Yoruba cocks with AB of MSTN (721.9±44.2) was significantly higher than that of hens with BB (590.6±40.6) but similar to the dressed weight of hens with AA and AB as well as with cocks with AA and BB, respectively. Similarly, the wing weight of cocks with BB variant of MSTN (122.3±0.9) was significantly higher than that of hens with BB (89.6±17.4) but similar to their counterparts with AA and AB genotypes (Tables 4.10).

Table 4. 9 Interaction Effects of Sex and MSTN genotype on Body Weightof Yoruba Chickens

		Body weight (g)						
Sex	Genotype	Week0	Week 4	Week 8	Week 12	Week 16	Week 20	Week 24
Hen	AA	24.7±0.9	106.6±16.3	268.8±35.4	443.4±70.5	627.8±92.6^{b}	808.7±102.7^{b}	892.5±91.3^{b}
	AB	24.6±0.8	112.1±12.5	264.5±35.0	457.6±67.9	646.8±106.6^{b}	837.1±125.6^{b}	917.8±116.4^{b}
	BB	25.0±1.9	118.8±21.7	282.3±46.3	455.6±96.2	634.2±155.5^{b}	822.0±178.7^{b}	903.8±168.4^{b}
Cock	AA	24.1±1.3	110.0±7.9	259.5±20.0	519.3±49.2	801.3±64.1^{a}	1034.1±84.0^{a}	1162.7±85.7^{a}
	AB	24.8±1.6	150.6±16.4	268.0±7.5	532.3±18.1	806.3±21.5^{a}	1040.8±23.9 ^{a}	1169.6±18.7^{a}
	BB	24.0±1.0	119.0±1.7	258.7±1.2	513.3±12.9	791.2±24.7^{a}	1014.7±17.0^{a}	1142.8±25.7^{a}

Means ± SD with different superscript along the columns differ significantly ($p<0.05$)

Table 4. 10 Interaction Effects of Sex and MSTN genotype on Carcass traitsof Yoruba Chickens

Sex	Genotype	Dressed (g)	Drumstick (g)	Thigh (g)	Breast (g)	Wing (g)	Back (g)	Gizzard (g)
Hen	AA	615.0±85.6^{b}	100.3±10.9	106.1±11.4^{b}	153.2±16.5^{b}	100.5±12.4^{ab}	173.0±12.5	25.5±2.5
	AB	667.3±70.3^{ab}	105.9±9.8	115.8±14.0^{b}	167.2±20.2^{b}	108.3±13.0^{ab}	183.6±15.3	27.5±3.4
	BB	590.6±57.5^{b}	112.7±20.0	112.0±22.5^{b}	163.1±32.6^{b}	89.6±2.7^{b}	180.6±24.7	26.2±6.9
Cock	AA	703.0±29.6^{ab}	115.9±25.8	117.6±5.5^{b}	174.2±8.0^{ab}	116.8±5.5^{ab}	199.1±8.8	30.0±1.3
	AB	721.9±33.4^{a}	123.9±10.4	139.0±6.3^{a}	186.3±1.1^{a}	115.0±2.3^{ab}	188.7±3.9	30.4±0.9
	BB	714.0±23.6^{ab}	111.5±9.0	127.9±4.4^{ab}	180.6±6.4^{a}	122.3±1.3^{a}	201.2±2.1	30.1±1.5

Means ± SD with different superscript along the columns are significantly different ($p<0.05$)

4.3.8 Interaction Effects of Sex and MSTN genotype on growth and Carcass traitsof Fulani Chickens

The body weight of Fulani cocks was not significantly different from their hen counterparts between weeks 0 and 8. At week 12, hens with AB (MSTN) had significantly higher body weight (583.2±12.1) than BB (544.9±41.7) but similar to AA (551.1±7.49). While cocks with AB (MSTN) had significantly higher body weight (692.0±15.7) than cocks with AA (604.4±23.4). Similarly, Fulani cocks had significantly higher body weight than hens between week 16 and 24 across the three MSTN variants (Table 4.11).The dressed, drumstick, breast and thigh weights of Fulani cocks were significantly higher than that of the hens. However, all the measured carcass traits of Fulani cocks with AA of MSTN were not significantly different from cocks with AB and BB variants (Table 4.12).

Table 4. 11 Interaction Effects of Sex and MSTN genotype on Body Weightof Fulani Chickens

		Body weight (g)						
Sex	Genotype	Week0	Week 4	Week 8	Week 12	Week 16	Week 20	Week 24
Hen	AA	26.2±22.1	164.2±23.5	344.0±46.6	551.1±61.8^{b}	813.0±79.6^{b}	954.6±92.4^{b}	1036.6±87.8^{b}
	AB	28.0±4.3	166.5±17.9	349.3±41.5	583.2±59.1^{ab}	825.3±28.2^{b}	977.0±32.2^{b}	1042.9±39.1^{b}
	BB	27.4±3.8	170.2±23.6	319.7±37.3	544.9±41.7^{b}	728.2±59.2^{b}	857.6±65.5^{b}	932.98±70.1^{b}
Cock	AA	27.2±2.6	163.5±19.0	356.2±42.7	604.4±96.8^{ab}	943.0±79.9^{a}	1169.3±139.9^{a}	1282.7±152.2^{a}
	AB	27.1±1.9	175.9±20.1	353.1±47.1	692.0±25.7^{a}	1010.9±106.7^{a}	1257.4±58.2^{a}	1373.0±45.2^{a}
	BB	26.4±2.4	159.4±20.3	371.2±42.2	636.8±41.1^{ab}	992.7±127.1^{a}	1207.8±60.2^{a}	1324.6±71.7^{a}

Means ± SD with different superscript along the columns are significantly different ($p<0.05$)

Table 4. 12Interaction Effects of Sex and MSTN genotype on Carcass traitsof Fulani Chickens

Sex	Genotype	Dressed (g)	Drumstick (g)	Thigh (g)	Breast (g)	Wing (g)	Back (g)	Gizzard (g)
Hen	AA	698.4±33.1^{b}	109.9±8.4^{b}	119.9±6.7^{b}	177.0±9.4^{b}	119.9±5.6	187.3±14.0^{b}	28.5±1.3
	AB	708.5±22.0^{b}	119.8±6.4^{b}	126.8±4.3^{b}	180.8±6.4^{b}	120.3±4.2	187.2±5.9^{b}	28.7±1.0
	BB	675.9±12.2^{b}	112.4±4.0^{b}	114.7±2.4^{b}	172.0±3.9^{b}	114.8±1.6	181.9±6.4^{b}	27.8±1.6
Cock	AA	906.2±30.6^{a}	153.7±5.3^{a}	159.3±5.9^{a}	229.9±8.6^{a}	127.3±4.7	235.3±9.7^{a}	28.7±1.1
	AB	920.8±51.7^{a}	144.5±9.1^{a}	168.2±10.0^{a}	231.2±14.5^{a}	138.0±8.0	246.8±16.4^{a}	28.9±1.8
	BB	887.5±122.7^{a}	140.4±21.5^{a}	155.7±23.8^{a}	224.7±34.4^{a}	124.4±19.0	229.4±38.9^{a}	28.1±4.3

Means ± SD with different superscript along the columns are significantly different ($p<0.05$)

4.3.9 Interaction Effects of Sex and MSTN genotype on growth and Carcass traitsof FUNAAB-Alpha Chickens

The FUNAAB-Alpha cocks with AB (MSTN) had significantly higher ($p<0.05$) body weight (1771.6±34.9) than BB (1181.7±51.9) but similar to AA (1714.5±22.0) at week 12. Similarly, FUNAAB-Alpha hens with AB had significantly higher ($p<0.05$) body weight (1537.5±130.1) than AA (1377.9±34.2) at week 12. Generally, significantly higher body weight was observed between FUNAAB-Alpha cocks and hens at week 20 and 24 (Table 4.13). The dressed weight of FUNAAB-Alpha hens with BB was significantly lower ($p<0.05$) than both AA and AB individuals. While other measured carcass traits of FUNAAB-Alpha birds with AA were not significantly different from birds with AB and BB variants (Table 4.14).

Table 4. 13 Interaction Effects of Sex and MSTN genotype on Body Weight of FUNAAB-Alpha Chickens

		Body weight (g)						
Sex	Genotype	Week0	Week 4	Week 8	Week 12	Week 16	Week 20	Week 24
Hen	AA	34.4±2.3	306.8±43.1	739.4±123.4	1004.3±125.7^{c}	1377.9±171.5^{c}	1817.6±176.4^{b}	2071.0±175.6^{b}
	AB	34.7±2.3	301.2±39.9	791.8±118.3	1136.8±73.3^{bc}	1537.5±88.9^{b}	1850.5±85.4^{b}	2192.2 ±97.9^{b}
	BB	33.8±1.3	273.9±62.3	718.5±118.5	941.7±59.2^{c}	1394.8±260.2^{c}	1957.2±214.0^{b}	2081.9±179.4^{b}
Cock	AA	34.4±1.8	322.3±70.4	777.6±130.3	1294.1±96.7^{a}	1714.5±122.6^{ab}	2276.8±137.7^{a}	2481.0±119.8^{ab}
	AB	35.7±2.1	348.3±44.3	824.1±95.5	1316.5±136.9^{a}	1871.6±130.8^{a}	2337.1±194.1^{a}	2546.9±167.8^{a}
	BB	34.2±2.1	297.9±33.6	738.6±137.3	1181.7±89.8^{b}	1594.7±247.7^{b}	2286.70±323.7^{a}	2347.6±268.5^{b}

Means ± SD with different superscript along the columns are significantly different ($p<0.05$)

Table 4. 14 Interaction Effects of Sex and MSTN genotype on Carcass traitsof FUNAAB-Alpha Chickens

Sex	Genotype	Dressed (g)	Drumstick (g)	Thigh (g)	Breast (g)	Wing (g)	Back (g)	Gizzard (g)
Hen	AA	1386.9±122.8^{ab}	247.2±21.8^{ab}	272.3±23.9^{ab}	292.6±25.7^{ab}	167.4±14.7^{ab}	302.6±27.5	36.4±3.4
	AB	1453.0±46.7^{a}	257.0±8.5^{a}	284.5±9.2^{a}	305.0±9.9^{a}	174.0±5.7^{a}	318.0±9.9	38.5±0.8
	BB	1276.3±33.1^{b}	225.7±5.5^{b}	250.3±6.0^{b}	268.3±7.0^{b}	153.0±4.0^{b}	279.3±7.0	34.0±1.0
Cock	AA	1456.8±39.1^{a}	251.9±6.9^{a}	278.5±7.8^{ab}	309.6±8.6^{a}	170.2±4.7^{a}	285.5±7.8	36.6±1.1
	AB	1515.6±84.1^{a}	262.5±15.1^{a}	290.4±16.7^{a}	322.4±18.5^{a}	177.5±9.9^{a}	297.6±17.1	38.1±2.2
	BB	1489.0±16.3^{a}	258.0±2.8^{a}	285.0±3.7^{a}	317.0±4.1^{a}	174.0±4.3^{a}	292.0±6.4	37.0±0.6

Means ±SD with different superscript along the columns are not significantly different ($p<0.05$)

4.4.1 PCR and RFLP gel image of GH gene in Nigerian chickens

PCR Amplification of GH gene produced 195 bp fragments and subsequent restriction digestion of the amplicons with Van911restriction endonuclease yielded two alleles. A single slow band was designated 'A' while a single faster band was designated 'B' allele. Thus, individuals with only a single faster band were designated homozygote 'AA' while individuals with both fast and slow band were designated heterozygote 'AB' individuals. The gel pictures of GH gene amplicon and the RFLP pattern of GH/Van911of indigenous chickens in Nigerian are presented in Plate 4.3 and 4.4.

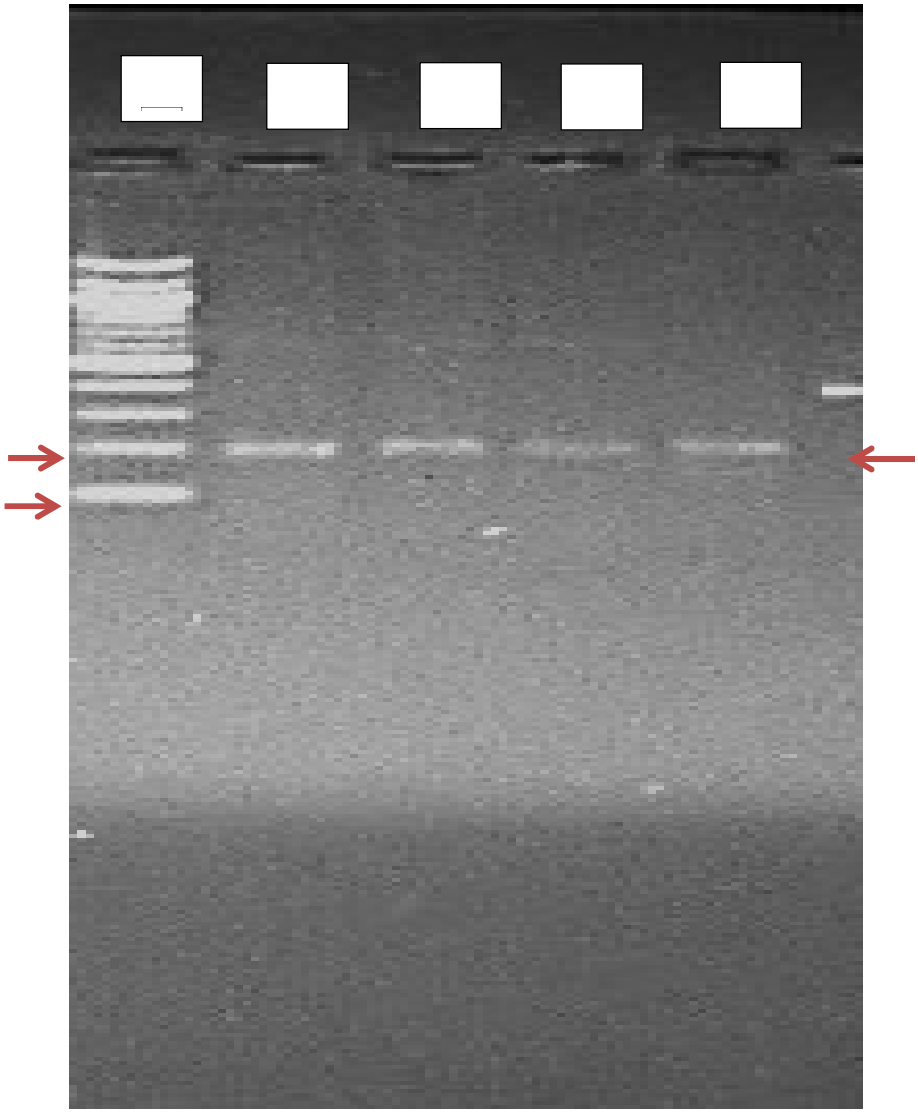

Plate 4. 3: Gel image of growth hormone gene in Nigerian chicken

M:100 bp DNA marker,

Lanes 1 – 4: Samples 1 to 4

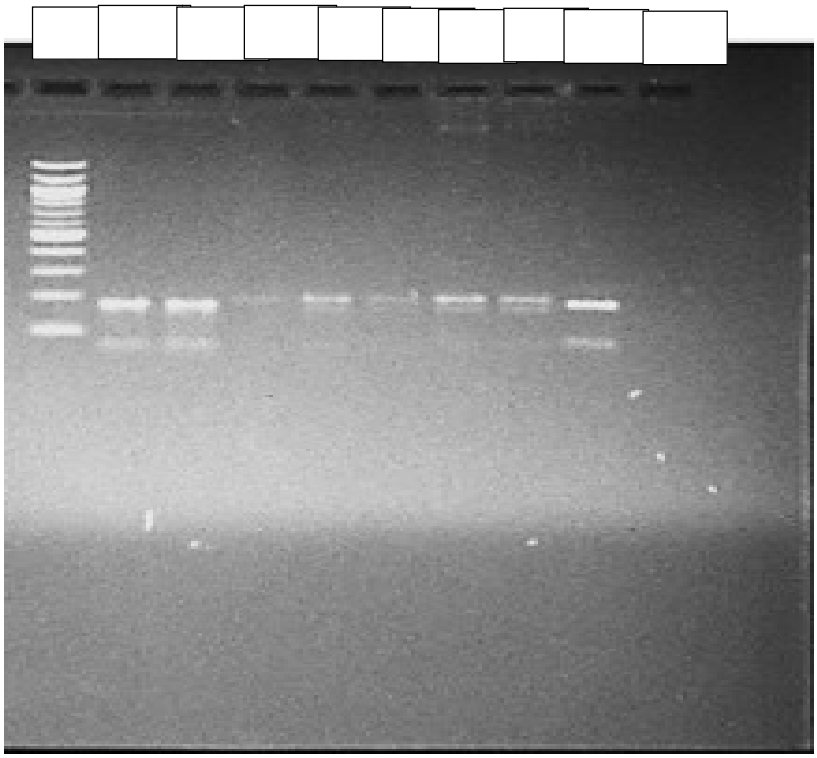

Plate 4. 4: RFLP gel image of GH gene in Nigerian chicken

M: 100 bp DNA marker,

AA: Genotype AA

AB: Genotype AB

-Ve: negative control

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4.4.2 Allele and genotype frequencies of GH gene in Yoruba, Fulani and FUNAAB-Alpha chickens

Growth hormone gene with product size of 195 bp produced two genotypes (AA and AB) after the digestion of the PCR products with Van911 enzyme. There were no birds with homozygous BB genotype in the three studied populations. Genotype AA had the highest genotype frequency value in all the three studied chicken populations. The least chi-square value was obtained from FUNAAB-Alpha chicken (2.72) while the highest value was obtained from Fulani chickens (4.083). Details of the allele and genotype frequencies are presented in Table 4.15.The observed heterozygosity values at growth hormone gene locus ranged between 31.1% (FUNAAB-Alpha chickens) and 53.6% (Yoruba chickens). Similarly, Fulani chickens had the highest expected heterozygosity value (39.2%) followed by Yoruba (27.5%) then FUNAAB-Alpha chickens (2.3%). The obtained values of Shannon's information indexfollowed similar trend with Fulani chickens having the highest values while the least values were obtained in FUNAAB-Alpha chickens. However, the observed fixation index values were negative for all the three studied chicken populations where Yoruba, Fulani and FUNAAB-Alpha chickens had -0.197, -0.366 and -0.184 respectively (Table 4.15).

Table 4. 15 Allele and genotype frequencies of GH gene in Yoruba, Fulani and FUNAAB-Alpha Chickens

		Genotype frequency (%)			Allele frequency (%)		Heterozygosity(%)				
Ecotype	N	AA	AB	BB	A	B	Observed	Expected	Shannon's Information Index	Fixation Index	HWE (χ^2)
Yoruba	53	0.87	0.13	0	0.75	0.25	0.329	0.275	0.447	-0.197	3.065 ^{ns}
Fulani	69	0.61	0.39	0	0.73	0.27	0.536	0.392	0.581	-0.366	4.083*
FUNAAB -Alpha	74	0.69	0.32	0	0.84	0.16	0.311	0.263	0.432	-0.184	2.72 ^{ns}

HWE: Hardy-Weinberg Equilibrium, * : Significant ($p<0.05$), ns : not significant ($p>0.05$)

4.4.3BLASTn result showing the percentage similarity of the GH sequences of indigenous chickens in Nigeria and chicken GH sequences from the NCBI GenBank

The BLASTn results of GH gene sequences derived from the Yoruba, Fulani and FUNAAB-Alpha chickens and the downloaded GH sequences from the NCBI GenBank are presented in Table 4.16. The GH sequences from this study produced multi-digit negative exponential expect-values which ranged from 1.00E-10 to 2.00E-98. The obtained sequences also had about 98 to 100 percent identity with the downloaded *Gallus gallus* GH sequences from the GenBank (Table 4.16). The obtained sequences were read using BioEdit 6.5 while multiple sequences alignments were generated using MEGA 7.0 software.

Table 4. 16BLASTn result showing the percentage similarity of the GH sequences of indigenous chickens in Nigeria and chicken GH sequences from the NCBI GenBank

Accession	Description	Max score	Total score	E-Value	Max identity
AY461843.1	*Gallus gallus* growth hormone gene, complete cds	361	361	2.00E-98	100%
D10484.1	*Gallus gallus* gene for growth hormone, complete cds	361	361	2.00E-98	100%
EF452679.1	*Gallus gallus* growth hormone gene, exon 1 and partial cds	76.8	76.8	6.00E-13	100%
AF404827.1	*Gallus gallus* growth hormone gene, exon 1 and partial cds	353	353	3.00E-96	99%
JN403373.1	*Gallus gallus* strain IC3 growth hormone gene, exon 1, complete sequence and intron 1, partial sequence	69.4	69.4	1.00E-10	98%
JN403372.1	*Gallus gallus* strain NG growth hormone gene, exon 1, complete sequence and intron 1, partial sequence	69.4	69.4	1.00E-10	98%

4.4.4 Phylogenic tree and genetic distance derived from GH sequences of Yoruba, Fulani and FUNAAB-Alpha chickens

Phylogenetic tree derived from sequences of Nigerian Chickens (Yoruba, Fulani and FUNAAB-Alpha) and GH sequences downloaded from the GenBank showed that Yoruba is more closely related to Fulani chickens while FUNAAB Alpha is more related to the downloaded GH sequence (MG906784.1) from the GenBank than the Yoruba and Fulani chickens (Figure 4.2). In addition, highest genetic similarity value was observed between the Yoruba and Fulani chickens (99.995), while the least percent similarity value was obtained between FUNAAB-Alpha and the downloaded cGH sequence (Table 4.17).

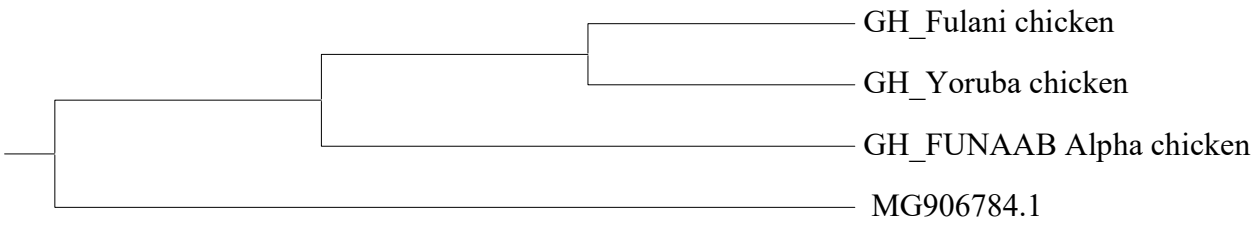

Figure 4.Phylogenetic tree derived from GH gene sequences of Yoruba, Fulani and FUNAAB-Alpha chickens and chicken GH gene sequences (MG906784.1) from the GenBank

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Table 4. 17 Genetic distance among Yoruba, Fulani and FUNAAB-Alpha Chickens based on exon-1 of GH gene

	MG906784.1(%)	FUNAAB-Alpha(%)	Fulani(%)	Yoruba(%)
MG906784.1		97.882	97.927	97.927
FUNAAB-Alpha	2.117		99.984	99.990
Fulani	2.073	0.016		99.995
Yoruba	2.073	0.010	0.005	

Upper diagonal: Per cent genetic similarity

Lower diagonal: Genetic Divergence

4.4.5 Effects of GH gene genotype on growth and carcass traits of Yoruba, Fulani and FUNAAB Alpha Chickens

The Fulani and Yoruba Chickens with heterozygote AB genotype of GH gene had higher mean body weight compared to birds with homozygote AA. The trend was however different in FUNAAB-Alpha birds as the homozygotes AA individuals appeared to have better growth performance than the heterozygotes AB of FUNAAB-Alpha chickens (Table 4.18). The obtained GH genotypes had no significant effects ($P<0.05$) on all the measured carcass traits across all the studied chicken populations (Table 4.19).

Table 4. 18 Effects of GH gene Genotype on Body Weight of Yoruba, Fulani and FUNAAB Alpha Chickens

Breed/	Body weight (g)						
Genotype	Week0	Week 4	Week 8	Week 12	Week 16	Week 20	Week 24
Yoruba							
AA	24.53±1.2	109.9±14.3	263.6±29.8	470.9±82.8	681.6±139.1	883.2±168.6	984.9±173.7
AB	24.41±1.0	108.8±15.9	267.0±35.1	482.3±49.1	725.6±80.5	929.9±101.6	1031.2±125.1
Fulani							
AA	26.01±2.1	155.9±16.1	341.7±44.7	579.7±74.0	870.0±126.2	1031.2±152.4	1129.2±166.7
AB	27.41±2.9	154.6±25.9	352.5±50.2	616.3±63.6	878.5±111.9	1083.3±161.7	1174.1±179.9
FUNAAB-Alpha							
AA	34.63±2.2	315.4±58.0	757.9 ±107.0	1162.9±176.8	1568.5±217.7	2070.0±265.6	2293.5±248.8
AB	34.31±1.7	310.3±47.4	762.6±131.6	1152.7±161.9	1539.1±234.7	2028.2±268.3	2272.4±246.6

Means ± SD with different superscripts within breed along the columns differ significantly ($p<0.05$)

Table 4. 19Effects of GH gene genotype on carcass traitsof Yoruba, Fulani and FUNAAB Alpha Chickens

Breed/Genotype		Dressed Weight (g)	Drum Stick (g)	Thigh (g)	Breast (g)	Wing (g)	Back (g)	Gizzard (g)
Yoruba								
	AA	673.4±79.2	105.6±22.2	118.8±14.5	171.5±20.9	108.0±13.7	186.8±16.7	28.0±3.3
	AB	680.7±56.6	109.1±8.3	123.2±10.9	175.6±15.7	110.0±11.0	191.2±13.9	28.8±2.4
Fulani								
	AA	815.94±118.8	129.7±19.0	141.7±23.4	207.1±30.9	125.2±7.2	214.5±30.0	29.0±1.6
	AB	773.60±111.0	122.9±18.4	135.1±20.0	196.2±28.1	119.5±9.2	204.3±28.2	27.9±1.8
FUNAAB-Alpha								
	AA	1438.4±109.2	252.7±17.3	278.8±19.1	305.0±23.9	170.9±11.6	297.2±18.1	37.0±2.5
	AB	1407.9±107.7	246.7±18.9	272.9±20.8	297.1±23.2	167.0±12.7	294.1±26.6	36.4±2.9

Mean ± SE with different superscripts within breed along the columns differ significantly ($p<0.05$)

4.4.5 Interaction Effects of Sex and GH genotype on growth and carcass traitsof Yoruba chickens

The sex and GH genotype had no significant interaction effect on the measured weekly body weight of Yoruba chickens. There was no significant difference ($P>0.05$) among all the obtained weekly body weight of Yoruba cocks and hens from weeks 0 to 8. However, Yoruba cocks had significantly higher ($p<0.05$) body weight compared to the hens from weeks 12 to 24. The weekly body weight of Yoruba cocks with AA (GH) was not significantly different from their AB counterparts. Similar trend was observed in hens with AA and AB of GH gene (Table 4.20). Yoruba cocks had significantly higher dressed weight compared to the hens. However, there was no significant difference in the obtained dressed weight of Yoruba cocks with AA of GH compared to the heterozygote AB cocks. Similarly, the dressed weight of Yoruba hens with AA of GH (626.2±22.4) was not significantly different ($p>0.05$) from those with AB (637.2±33.9). However, the drumstick and back weight were not significantly different for both the cocks and hens (Table 4.21).

Table 4. 20Interaction Effects of Sex and GH genotype on Body Weightof Yoruba Ecotype Chickens

		Body weight (g)						
Sex	Genotype	Week0	Week 4	Week 8	Week 12	Week 16	Week 20	Week 24
Cock	AA	24.1±1.5	118.8±10.2	263.8±9.4^{ab}	526.1±29.8^{a}	815.1±29.2^{a}	1053.4±40.1^{a}	1180.1±39.9^{a}
	AB	24.0±1.0	109.3±15.1	236.0±50.5^{b}	461.7±108.0^{b}	701.4±140.7^{b}	912.3±194.6^{b}	1030.4±190.6^{b}
Hen	AA	24.7±1.0	104.6±15.6	255.4±35.8^{ab}	421.4±78.8^{c}	603.8±116.4^{c}	784.3±131.8^{c}	877.9±116.3^{c}
	AB	24.8±1.0	114.8±16.3	285.3±28.7^{a}	489.9±38.1^{ab}	687.2±73.4^{bc}	879.0±88.4^{bc}	956.3±106.4^{bc}

Means ± SD with the different superscript along the columns differ significantly ($p<0.05$)

Table 4. 21 Interaction Effects of sex and GH genotype on carcass traitsof Yoruba Ecotype chickens

Sex	Genotype	Dressed Weight (g)	Drumstick (g)	Thigh (g)	Breast (g)	Wing (g)	Back (g)	Gizzard (g)
Cock	AA	723.9±25.0^{a}	108.42±30.1	129.6±4.7^{a}	187.1±6.7^{a}	118.5±4.3^{a}	198.6±9.5	30.5±1.1
	AB	697.2±25.1^{a}	105.88±8.6	124.6±4.7^{ab}	179.9±6.8^{ab}	114.4±6.3^{ab}	199.2±7.6	29.3±0.9
Hen	AA	626.5±83.9^{b}	99.1±10.3	108.7±13.1^{b}	156.9±19.0^{b}	98.3±12.0^{b}	175.9±14.4	25.6±2.9
	AB	637±75.8^{b}	111.4±8.2	113.2±14.7^{ab}	163.5±21.2^{ab}	102.0±12.9^{ab}	180.8±16.1	27.9±3.7

Means ± SD with the different superscript along the columns are significantly different ($p<0.05$)

4.4.6 Interaction Effects of Sex and GH genotype on growth and carcass traitsof Fulani chickens

The body weight of Fulani cocks and hens were not significantly different from weeks 0 to 4. However, Fulani cocks had significantly higher body weight compared to the hens from weeks 8 to 24. At week 8, the body weight of Fulani cocks with AB of GH (376.2±36.1) was significantly higher than the body weight of Fulani hens with AA (329.1±38.1) and AB (330.6±52.5) but similar to body weight of Fulani cocks with AA (560.1±48.2) (Table 4.22). The dressed, drumstick and breast weights of Fulani cocks were significantly higher than that of the Fulani hens (Table 4.23).

Table 4. 22 Interaction Effects of sex and GH genotype on Body Weightof Fulani Ecotype Chickens

		Body weight (g)						
Sex	Genotype	Week0	Week 4	Week 8	Week 12	Week 16	Week 20	Week 24
Cock	AA	26.1±2.0	157.1±16.1^{ab}	360.1±48.2^{ab}	623.1±66.3^{a}	978.7±101.0^{a}	1169.9±114.8^{a}	1288.7±122.8^{a}
	AB	26.6±1.5	144.6±27.4^{b}	376.2±36.1^{a}	650.2±70.3^{a}	945.3±113.8^{a}	1208.7±115.5^{a}	1322.0±108.2^{a}
Hen	AA	25.9±2.2	155.2±16.3^{ab}	329.1±38.1^{b}	550.3±64.6^{b}	796.1±79.7^{b}	937.0±89.6^{b}	1020.7±84.4^{b}
	AB	26.3±3.7	165.4±19.7^{a}	330.6±52.5^{b}	579.8±24.9^{b}	806.4±47.4^{b}	948.4±64.5^{b}	1044.8±66.1^{b}

Means ± SD with the different superscript along the columns differ significantly ($p<0.05$)

Table 4. 23 Interaction Effects of sex and GH genotype on carcass traitsof Fulani Ecotype Chickens

Sex	Genotype	Dressed Weight(g)	Drumstick(g)	Thigh(g)	Breast(g)	Wing(g)	Back(g)	Gizzard(g)
Cock	AA	922.5±39.9^{a}	146.6±7.0^{a}	162.5±7.7^{a}	234.5±11.2^{a}	129.8±6.2^{a}	240.5±12.7^{a}	29.3±1.4
	AB	874.6±71.6^{a}	138.2±12.5^{a}	153.2±13.9^{a}	221.1±20.0^{a}	122.4±11.1^{b}	225.3±22.7^{a}	27.6±2.5
Hen	AA	700.50±33.1^{b}	111.5±6.1^{b}	129.2±8.2^{b}	177.3±10.0^{b}	120.2±4.4^{b}	186.4±11.4^{b}	28.5±1.7
	AB	685.27±33.6^{b}	109.4±10.1^{b}	119.2±4.5^{b}	174.4±8.9^{b}	116.9±6.9^{b}	185.9±18.0^{b}	28.2±0.9

Means ± SD with the different superscript along the columns are significantly different ($p<0.05$)

4.4.7 Interaction Effects of Sex and GH genotype on growth and carcass traitsof FUNAAB-Alpha chickens

The body weight of FUNAAB-Alpha cocks was not significantly different from the body weight of the hens from weeks 0 to 8. However, FUNAAB-Alpha cocks had significantly higher body weight than the hens from week 12 to 24. The weekly body weight of cocks and hens with AA (GH) were not significantly different from body weight of the hens with AB (GH) (Table 4.24).The dressed weight of FUNAAB-Alpha cocks with AA (1509.1±14.6) was significantly higher ($p<0.05$) than the dressed weight of FUNAAB-Alpha hens with AA (1367.3±29.3) and AB (1393.3±49.4) but similar to the dressed weight of cocks with AB (1426.7±18.6). The drumstick, wing and gizzard weight of FUNAAB-Alpha chickens with AA of GH were not significantly different from FUNAAB-Alpha birds with AB of GH (Table 4.25).

Table 4. 24 Interaction Effects of Sex and GH genotype on Body Weight of FUNAAB-Alpha Chickens

		Body weight (g)						
Sex	Genotype	Week0	Week 4	Week 8	Week 12	Week 16	Week 20	Week 24
Cock	AA	34.6±2.0	330.9±65.4	777.7±113.1	1292.0±101.1[a]	1720.6±111.2[a]	2272.8±145.1[a]	2483.5±132.5[a]
	AB	34.6±1.8	311.5±49.8	750.8±105.8	1264.6±104.9[a]	1687.8±211.9[a]	2234.0±186.7[a]	2460.7±157.6[a]
Hen	AA	34.5±2.4	299.7±43.2	739.6±97.1	1004.1±121.6[b]	1407.0±177.0[b]	1849.5±164.2[b]	2092.2±157.1[b]
	AB	34.2±1.7	311.4±46.9	752.1±155.0	1030.1±134.6[b]	1416.3±161.6[b]	1858.1±173.4[b]	2119.3±174.1[b]

Means ± SD with the different superscript along the columns differ significantly ($p<0.05$)

Table 4. 25 Interaction Effects of sex and GH genotype on carcass traits of FUNAAB-Alpha chickens

Sex	Genotype	Dressed Weight(g)	Drumstick(g)	Thigh(g)	Breast(g)	Wing(g)	Back(g)	Gizzard(g)
Cock	AA	1509.4±52.6^{a}	261.3±9.5	289.1±10.4^{a}	321.1±11.5^{a}	176.6±6.3	296.2±10.7^{a}	38.0±1.4
	AB	1426.7±45.5^{ab}	246.7±8.1	272.5±9.2^{ab}	303.0±10.1^{ab}	166.7±5.4	279.5±9.2^{b}	35.7±1.0
Hen	AA	1367.3±105.6^{b}	244.0±19.2	268.5±20.5^{b}	278.8±22.1^{b}	165.2±13.0	298.2±23.7^{a}	35.9±2.9
	AB	1393.4±139.8^{b}	249.8±24.8	273.3±27.3^{ab}	292.6±29.5^{b}	177.3±16.6	305.0±30.6^{a}	36.9±3.8

Means ± SD with the different superscript along the columns are significantly different ($p<0.05$)

4.5.1 PCR and RFLP gel picture of GHRL gene in Nigerian chickens

The gel image of the PCR amplified 431 bp fragment of GHRL gene in indigenous chickens in Nigeria is presented in Plate 4.5. The amplified product of GHRL gene was digested with TspRI enzyme. The digested products yielded two alleles (A and B) and three genotypes (AA, AB and BB) in indigenous chickens in Nigeria. The gel image of the RFLP patterns of GHRL/ TspRI in indigenous chickens in Nigeria is presented in Plate 4.6.

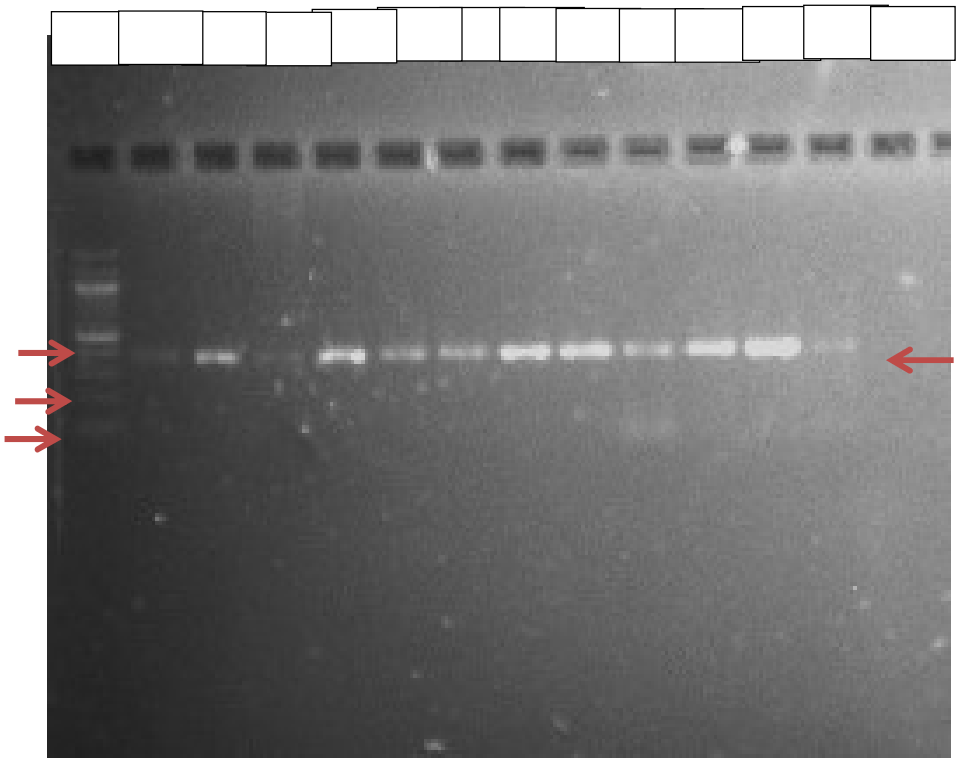

Plate 4. 5: PCR Gel image of GHRL gene of indigenous Chickens in Nigeria

M:100 bp DNA marker

Lane1-12: Samples 1-12

-Ve: negative control

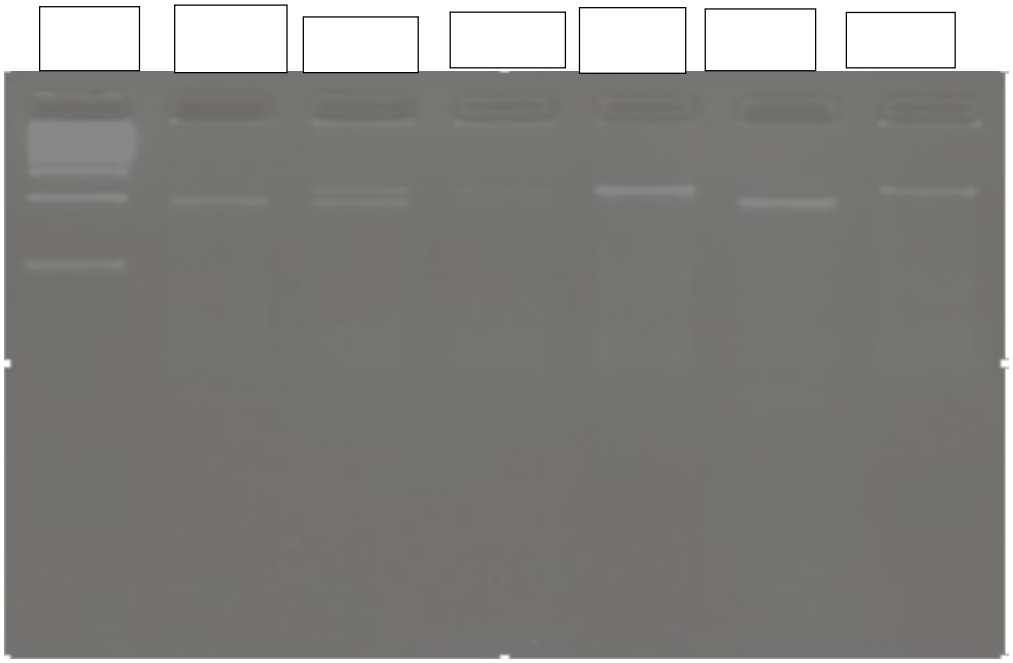

Plate 4. 6: RFLP gel image of GHRL in Yoruba, Fulani and FUNAAB-Alpha Chickens

AA: Genotype AA

BB: Genotype BB

AB: Genotype AB

4.5.2Allele and genotype frequencies of GHRL gene in indigenous chickens in Nigeria

The restriction digestion of PCR amplified product of GHRL gene in indigenous chickens in Nigeriawith TspRI enzyme produced two alleles (A and B) with corresponding three genotypes (AA, AB and BB). The fast band was designated A allele while the slower band was designated B allele. The genotype AA had the highest frequency in all the studied chicken populations. Fulani chickens had the highest heterozygote genotype AB (35%) while least heterozygote frequency was obtained from the FUNAAB-Alpha chickens (25.3%). The chi-square analysis of the observed and expected genotype frequencies showed that Fulani and Yoruba chickens GHRL locus are in conformity with Hardy-Weinberg principle with chi-square values 0.663 and 1.422 respectively. However, FUNAAB-Alpha chickens appeared to be deviated from Hardy-Weinberg principle at GHRL locus with the chi-square value 8.859 (Table 4.26).Two alleles were observed in all the three studied chicken populations at ghrelin locus. However, FUNAAB-Alpha chickens had the highest effective number of alleles (1.879) followed by Fulani chickens (1.609) then Yoruba chickens (1.524). Similarly, FUNAAB-Alpha chickens had the highest expected heterozygosity value (46.8%) while Yoruba had the least (34.4%). However, Yoruba and FUNAAB-Alpha chickens had the least and closest observed heterozygosity 29.4% and 29.6%, respectively. The highest Shannon's information index (66.1%) was obtained in FUNAAB-Alpha while Yoruba chickens had the least. In contrast, Fulani chickens had the least fixation index value while the highest fixation index was obtained in FUNAAB-Alpha chickens (Table 4.26).

Table 4. 26 Genotype and Allele frequencies of GHRL gene in Nigerian Chickens

		Genotype frequency (%)			Allele frequency (%)		Heterozygosity				
Ecotype	N	AA	AB	BB	A	B	observed	Expected	Shannon's Information Index	Fixation Index	HWE (χ^2)
Yoruba	68	0.63	0.29	0.07	0.78	0.22	0.294	0.344	0.528	0.145	1.422^{ns}
Fulani	71	0.61	0.25	0.14	0.68	0.32	0.362	0.379	0.566	0.043	0.663^{ns}
FUNAAB-Alpha	58	0.57	0.35	0.09	0.74	0.26	0.296	0.468	0.661	0.368	8.859*

N: Sample size, HWE: Hardy-Weinberg Equilibrium, * : Significant ($p<0.05$), ns: not significant ($P>0.05$)

4.5.3 BLASTn result showing the percentage similarity of the GHRL sequences of indigenous chickens in Nigeria and chicken GHRL sequences from the NCBI Genbank

The BLASTn results showed a very high homology values ranged 2.00E-116 to zero. The genetic distance analysis revealed 99 percent identity with the earlier reported *Gallus gallus* GHRL locus in the GenBank (Table 4.27).The obtained sequences had about 99 to 100 percent identity with the downloaded *Gallus gallus* GHRLsequences from the GenBank (Table 4.27). The derived sequences were read using BioEdit 6.5 while multiple sequences alignments were generated using MEGA 7.0 software.

Table 4. 27 BLASTn results showing the percentage identity of the exon-1 of the *Gallus gallus* GHRL locus of Fulani chickens

Accession	Description	Max score	Total score	E-Value	Max identity
KF976410.1	*Gallus gallus* breed Hubbard ghrelin (GHRL) gene, exons 1, 2 and partial cds	791	791	0	99%
KF976408.1	*Gallus gallus* breed Dokki-4 ghrelin (GHRL) gene, exons 1, 2 and partial cds	785	785	0	99%
AY303688.1	*Gallus gallus* ghrelin gene, complete cds	785	785	0	99%
EU477526.1	*Gallus gallus* ghrelin gene, complete cds	749	749	0	99%
AB158617.1	*Gallus gallus* ghre gene for ghrelin, complete cds	728	728	0	99%
BX931092.2	*Gallus gallus* finished cDNA, clone ChEST203g19	246	246	1.00E-63	99%
DQ458767.1	*Gallus gallus* preproghrelin (GHRL) gene, complete cds	422	422	2.00E-116	99%

4.5.4 Phylogenetic and genetic distance of Yoruba, Fulani and FUNAAB Alpha chickens at GHRL locus

The phylogenetic tree derived from GHRL sequences from this study revealed that FUNAAB-Alpha is more closely related with the downloaded GHRL sequence (KF976410.1) with 99.903% genetic similarity followed by Yoruba chickens (Figure 4.3) while Fulani chickens had the least percent similarity with the GHRL gene sequence from the GenBank (Table 4.28).

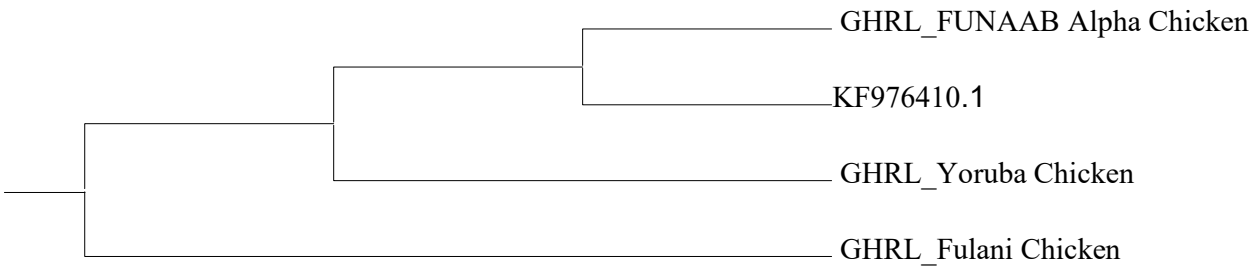

Figure 4. Phylogenetic tree derived from the sequences of Yoruba, Fulani and FUNAAB-Alpha and chicken GHRL gene sequence (KF976410.1) from the GenBank

Table 4. 28 Genetic distance among Yoruba, Fulani and FUNAAB-Alpha Chickens based on Ghrelin gene

	KF976410.1 (%)	FUNAAB Alpha (%)	Fulani (%)	(%)
KF976410.1 (cM)		99.903	99.828	99.997
FUNAAB-Alpha (cM)	0.097		99.795	99.99
Fulani (cM)	0.172	0.194		99.824
Yoruba (cM)	0.003	0.003	0.176	

Upper diagonal: Per cent Genetic Similarity

Lower diagonal: genetic divergence

cM: centimorgan

4.5.5 Effects of GHRL gene on growth and carcass traits of Yoruba, Fulani and FUNAAB-Alpha chickens

The obtained ghrelin genotypes (AA, AB and BB) were associated with weekly body weights of Yoruba, Fulani and FUNAAB-Alpha chickens. Fulani birds with homozygote BBof GHRL had significantly higher average body weight (618.41±24.10 g) than those with AA (607.3±9.19) but similar to AB(566.4±17.09) at Week 12 (Table 4.29). Similarly, the homozygote BB genotype birds had the highest weekly mean body weight in Yoruba chickens. The highest mean values of dressed weight, drumstick weight and wing weight were obtained from the Fulani birds with homozygote BB genotype. In addition, the BB genotype birds had the highest dressed weight, drumstick weight and wing weight. However, all the measured carcass traits of Yoruba and FUNAAB-Alpha birds with AA of GHRL were not significantly different ($P>0.05$) from birds with AA and BB variants (Table 4.30).

Table 4. 29 Effects of GHRL gene Genotype on Body Weightof Yoruba, Fulani and FUNAAB-Alpha Chickens

Breed/	Body weight (g)						
Genotype	Week0	Week 4	Week 8	Week 12	Week 16	Week 20	Week 24
Yoruba							
AA	24.5±1.2	106.8±16.4	257.8±32.4	454.5±77.4	670.0±121.8	868.9±146.8^{b}	924.1±155.3^{a}
AB	24.7±1.1	116.2±14.2	279.5±44.5	480.8±61.0	682.8±103.2	873.2±124.0^{b}	952.9±147.1^{a}
BB	24.4±1.1	113.2±10.7	271.5±27.2	511.8±74.0	744.3±143.6	963.8±180.4^{a}	1086.8±184.1^{a}
Fulani							
AA	27.1±2.5	133.5±21.6	351.7±46.3	607.3±57.4^{ab}	868.7±109.4^{a}	1006.3±149.6^{a}	1138.4±167.3^{a}
AB	26.0±2.0	144.4±19.4	331.9±56.4	566.4±83.7^{b}	874.8±207.2^{a}	1098.1±169.9^{a}	1120.8±170.9^{a}
BB	25.5±4.0	128.9±13.3	364.5±40.5	618.4±59.0^{a}	957.5±127.9^{a}	1176.3±170.3^{a}	1249.5±201.4^{a}
FUNAAB-Alpha							
AA	34.7±2.2	307.2±55.7	751.8±126.4	1131.4±162.3	1539.5±205.5	2033.4±262.5^{a}	2263.3±242.1^{a}
AB	34.6±1.6	331.6±52.4	740.0±106.0	1182.6±182.2	1562.2±286.9	2057.3±305.2^{a}	2294.7±296.9^{a}
BB	33.7±2.0	301.1±49.7	792.4±81.7	1253.6±160.3	1628.5±199.2	2145.6±231.4^{a}	2358.7±208.3^{a}

Means ± SD with different superscripts within breed along the columns differ significantly ($p<0.05$)

Table 4. 30Effects of GHRL genotypes on carcass traitsof Yoruba, Fulani and FUNAAB-Alpha Chickens

Breed/ Genotype	Dressed Weight (g)	Drum Stick (g)	Thigh (g)	Breast (g)	Wing (g)	Back (g)	Gizzard (g)
Yoruba							
AA	680.8±65.9[a]	104.1±10.5[a]	119.3±12.1	172.2±17.5	108.3±12.7	189.2±15.3	27.8±3.1
AB	615.9±72.5[a]	109.7±6.8[a]	109.4±13.6	157.9±19.6	103.5±13.7	177.1±15.3	26.9±2.3
BB	677.6±87.7[a]	102.2±38.2[a]	123.0±16.2	177.6±23.4	111.5±14.2	192.0±18.6	29.7±2.7
Fulani							
AA	781.2±114.0[b]	124.2±18.2[b]	136.0±21.1	198.3±28.7	1170.9±8.7[b]	206.1±28.5	27.9±1.7
AB	807.6±111.4[ab]	129.0±18.8[b]	142.0±22.0	207.3±29.3	125.5±4.9[ab]	214.2±27.9	28.8±1.2
BB	822.9±152.5[a]	138.3±23.5[a]	143.6±29.3	208.6±40.7	128.1±11.9[a]	218.4±40.1	30.2±1.9
FUNAAB-Alpha							
AA	1380.7±109.1[a]	250.2±17.8[a]	266.0±19.5	301.7±23.8	159.3±11.9[a]	294.8±20.2	36.6±2.6
AB	1418.3±116.5[a]	239.0±19.8[a]	275.8±21.8	289.0±25.2	168.6±13.4[a]	299.3±25.0	36.8±2.9
BB	1475.8±98.1[a]	256.8±17.2[a]	284.0±19.3	312.8±22.1	173.8±11.5[a]	297.8±23.5	37.5±2.5

Means ± SD with different superscripts within breed along the columns differ significantly ($p<0.05$)

4.5.6 Interaction Effects of Sex and GHRL genotype on growth and carcass traitsof Yoruba Chickens

The body weight of Yoruba chickens with AA of GHRL was not significantly different from their counterparts with AB and BB variants. Similar trend was observed in Yoruba hens with AA, AB and BB variants of GHRL. Generally, sex and obtained genotype of GHRL gene had no significant interaction effect ($p<0.05$) on all the measured growth and carcass traits in Yoruba, Fulani and FUNAAB-Alpha chickens (Tables 4.31 and 4.32).

Table 4. 31Interaction Effects of Sex and Ghrelin genotype on Body Weightof Yoruba Ecotype Chickens

		Body weight (g)						
Sex	Genotype	Week0	Week 4	Week 8	Week 12	Week 16	Week 20	Week 24
Cock	AA	24.0±1.4	111.3±10.9	264.6±10.7	530.4±26.3^{a}	814.5±33.0^{a}	1052.8±44.8^{a}	1179.5±42.6^{a}
	AB	24.0±0.7	92.0±6.4	248.0±59.4	440.0±177.5^{b}	740.0±189.1^{ab}	990.0±236.8^{ab}	1012.0±219.4^{ab}
	BB	24.6±1.5	112.4±8.0	262.4±5.8	514.2±20.6^{a}	803.5±27.5^{a}	1043.0±33.4^{a}	1165.3±31.1^{a}
Hen	AA	24.8±1.0	108.0±17.9	264.0±37.1	441.8±75.6^{b}	628.1±103.3^{b}	812.8±116.4^{c}	897.9±106.7^{b}
	AB	24.5±1.2	110.9±13.6	280.2±30.6	449.1±91.1^{b}	623.7±151.7^{b}	802.1±167.3^{c}	897.8±180.4^{b}
	BB	24.7±0.8	109.8±13.2	270.0±37.7	480.4±41.9^{b}	687.9±97.0^{ab}	879.3±114.2^{b}	968.9±146.9^{b}

Means ± SD with the different superscript along the columns differ significantly ($p<0.05$)

Table 4. 32 Interaction Effects of Sex and Ghrelin Genotype on Carcass Traitsof Yoruba Chickens

Sex	Genotype	Dressed Weight(g)	Drumstick(g)	Thigh(g)	Breast(g)	Wing(g)	Back(g)	Gizzard(g)
Cock	AA	709.1±22.0^{a}	110.5±8.5	126.8±4.1^{a}	183.1±5.9^{a}	117.2±5.1	198.4±8.5	29.8±1.0
	AB	602.8±23.7^{b}	101.0±1.4	122.3±4.4^{ab}	176.6±6.4^{ab}	113.3±7.8	197.7±11.3	28.8±1.0
	BB	735.9±33.5^{a}	99.2±48.9	131.8±6.3^{a}	190.4±9.1^{a}	119.2±5.8	201.9±9.1	31.0±1.5
Hen	AA	650.7±84.2^{ab}	98.0±9.1	111.2±13.0^{b}	160.6±18.8^{b}	108.4±11.1	178.7±14.2	25.7±3.1
	AB	580.4±44.2^{b}	107.1±16.4	108.3±17.9^{b}	156.4±25.8^{b}	98.5±15.3	175.4±19.6	27.6±3.1
	BB	685.2±76.6^{ab}	111.7±6.0	106.9±14.3^{b}	154.4±20.7^{b}	102.4±15.6	173.9±15.7	26.6±3.9

Means ± SD with the different superscript along the columns are significantly different ($p<0.05$)

4.5.7 Interaction Effects of Sex and GHRL genotype on growth and carcass traitsof Fulani Chickens

The body weight of Fulani cocks with BB of GHRL was significantly higher than body weight of hens with AB but similar with those with AA and BB at week 8. Further, the body weight of Fulani cocks was significantly higher than the body weight of hens from week 12 to 24 (Table 33). However, all the measured carcass traits were significantly higher for Fulani cocks than hens(Table 4.34).

Table 4. 33 Interaction Effects of Sex and Ghrelin genotype on Body Weightof Fulani Chickens

		Body weight (g)						
Sex	Genotype	Week0	Week 4	Week 8	Week 12	Week 16	Week 20	Week 24
Cock	AA	26.6±1.4	144.5±24.9	341.8±55.6^{ab}	653.9±64.5^{a}	949.4±116.1^{ab}	1208.4±104.1^{a}	1278.8±98.5^{a}
	AB	26.6±1.4	160.0±18.2	362.7±58.9^{ab}	605.6±74.6^{ab}	882.1±142.8^{ab}	1158.8±143.8^{a}	1189.6±286.6^{a}
	BB	26.4±3.7	159.5±7.1	384.4±51.1^{a}	668.1±31.4^{a}	1010.5±29.0^{a}	1267.7±23.8^{a}	1389.1±41.4^{a}
Hen	AA	26.9±3.0	168.8±16.2	344.5±37.1^{ab}	568.7±21.2^{b}	784.4±51.5^{b}	914.8±62.1^{b}	969.9±73.2^{b}
	AB	26.5±2.3	154.3±20.8	321.5±48.6^{b}	542.2±82.5^{b}	788.0±97.2^{b}	926.8±107.7^{b}	1006.2±91.3^{b}
	BB	27.4±4.6	160.0±18.1	359.4±17.3^{ab}	582.3±17.8^{b}	811.2±41.9^{b}	959.1±55.5^{b}	1039.7±54.2^{b}

Means ± SD with the different superscript along the columns are significantly different ($p<0.05$)

Table 4. 34 Interaction Effects o0f Sex and Ghrelin Genotype on Carcass Traits of Fulani Chickens

Sex	Genotype	Dressed Weight(g)	Drumstick(g)	Thigh(g)	Breast(g)	Wing(g)	Back(g)	Gizzard(g)
Cock	AA	901.7±78.6^{a}	142.9±13.7^{a}	158.4±15.2^{a}	228.7±22.0^{a}	126.6±12.2^{ab}	233.9±24.9^{a}	28.6±2.7
	AB	891.4±55.2^{a}	141.127±9.7^{a}	156.4±10.7^{a}	225.8±15.5^{a}	124.9±8.6^{ab}	230.6±17.5^{a}	28.2±1.9
	BB	915.6±21.7^{a}	145.3±3.8^{a}	161.1±4.2^{a}	232.6±6.1^{a}	128.7±3.4^{a}	238.3±6.9^{a}	29.1±0.8
Hen	AA	680.3±32.6^{b}	109.0±8.2^{b}	117.4±5.6^{b}	173.5±9.1^{b}	116.4±6.5^{b}	184.5±17.5^{b}	28.1±1.4
	AB	705.6±31.3^{b}	110.3±6.8^{b}	120.2±8.4^{b}	178.4±11.4^{b}	121.7±3.4^{ab}	186.7±10.7^{b}	28.4±1.6
	BB	715.3±26.9^{b}	117.2±7.8^{b}	123.1±6.6^{b}	179.8±3.9^{b}	120.7±3.5^{ab}	190.6±9.2^{b}	29.3±1.2

Means ± SD with the different superscript along the columns are significantly different ($p<0.05$)

4.5.8 Interaction Effects of Sex and GHRL genotype on growth and carcass traitsof FUNAAB-Alpha Chickens

The body weight of FUNAAB-Alpha cocks with AA of Ghrelin were not significantly different ($p<0.05$) from the body weight of AB and BB from week 0 to 24. However, the body weight of FUNAAB-Alpha hens with AB (970.6±29.3) was significantly lower than the body weight of AA (117.2±97.2) but similar to BB (1034.2±25.0) at week 12 (Table 4.35). All the measured carcass traits were not significantly influenced ($p<0.05$)by the interaction effect of sex and Ghrelin genotypes (Table 4.36).

Table 4. 35Interaction Effects of Sex and Ghrelin genotype on Body Weight of FUNAAB-Alpha Chickens

		Body weight (g)						
Sex	Genotype	Week0	Week 4	Week 8	Week 12	Week 16	Week 20	Week 24
Cock	AA	34.7±1.9	312.2±69.8^{ab}	738.5±86.8	1266.5±98.8^{a}	1691.6±132.1^{a}	2263.9±178.0^{a}	2473.8±158.6^{a}
	AB	34.9±1.3	346.1±55.9^{a}	772.4±104.7	1288.7±110.3^{a}	1703.2±223.8^{a}	2222.9±194.1^{a}	2456.5±182.7^{a}
	BB	33.8±2.3	310.0±44.3^{ab}	788.3±73.6	1312.1±125.7^{a}	1726.0±108.5^{a}	2268.8±118.5^{a}	2490.0±109.6^{a}
Hen	AA	33.4±2.4	280.2±43.1^{b}	802.0±127.7	970.6±71.6^{c}	1400.6±176.7^{ab}	1858.1±166.5^{b}	2098.9±161.6^{b}
	AB	33.8±2.0	302.6±30.5^{ab}	675.2±80.5	1034.2±168.3^{bc}	1280.2±162.7^{b}	1726.1±190.8^{b}	1970.9±194.1^{b}
	BB	34.6±1.4	302.0±65.7^{ab}	762.3±116.5	1117.2±124.9^{b}	1427.6±179.4^{a}	1863.8±146.9^{b}	2108.4±121.0^{b}

Means ± SD with the different superscript along the columns differ significantly ($p<0.05$)

Table 4. 36Interaction Effects of Sex and Ghrelin genotype on carcass traits of FUNAAB-Alpha chickens

Sex	Genotype	Dressed Weight (g)	Drumstick (g)	Thigh (g)	Breast (g)	Wing (g)	Back (g)	Gizzard (g)
Cock	AA	1485.7±57.0[a]	257.2±10.2	284.3±11.4	305.9±12.4	173.8±6.8	291.4±11.5	37.3±1.5
	AB	1471.3±45.8[a]	254.3±8.0	281.7±9.1	312.7±10.1	170.0±5.6	288.7±9.1	35.0±1.0
	BB	1584.7±118.2[a]	257.0±21.1	284.0±23.6	315.7±26.2	183.7±14.0	317.0±24.1	37.3±3.1
Hen	AA	1366.1±113.7[b]	243.7±20.9	268.3±22.1	288.5±24.2	165.1±14.0	297.9±25.5	36.0±3.1
	AB	1331.8±135.5[b]	246.3±24.0	272.8±26.4	292.2±28.4	166.8±16.2	304.7±29.4	33.7±3.6
	BB	1449.0±35.4[a]	256.0±6.4	284.0±7.1	304.0±7.8	174.0±4.2	317.0±7.8	38.0±1.4

Means ± SD with the different superscript along the columns are significantly different ($p<0.05$)

4.6.1 PCR and RFLP gel pictures of PIT-1 Gene

The PCR amplification of PIT-1 gene with the selected primers resulted in 243 bp size in indigenous chickens in Nigeria (Plate 4.7). The obtained RFLP pattern of PIT-1 gene is presented in Plate 4.8.

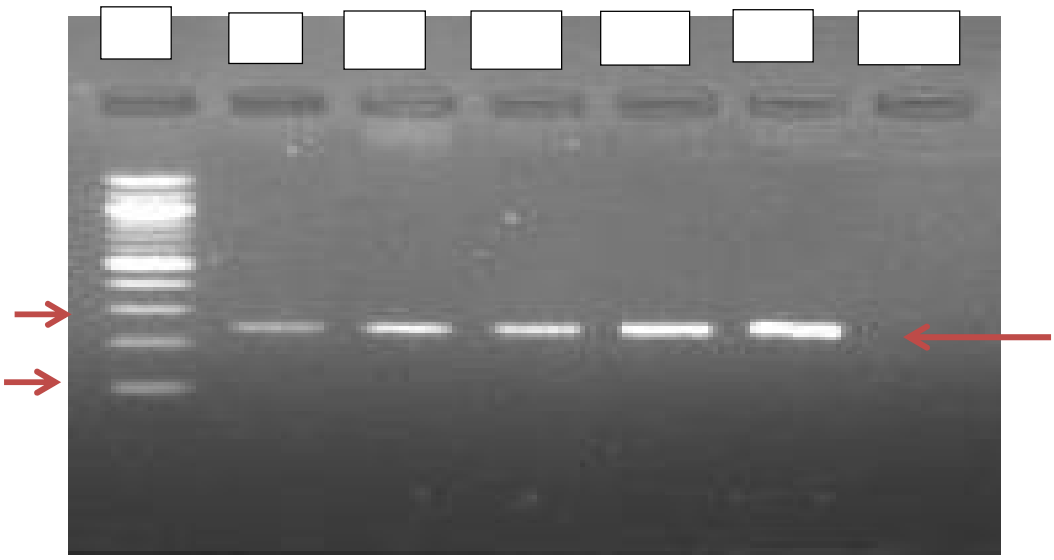

Plate 4. 7: A PCR product of PIT-1 gene in Nigeria indigenous chickens

M : 100 bp DNA marker

Lanes 1-5 : samples 1-5

-Ve : negative control

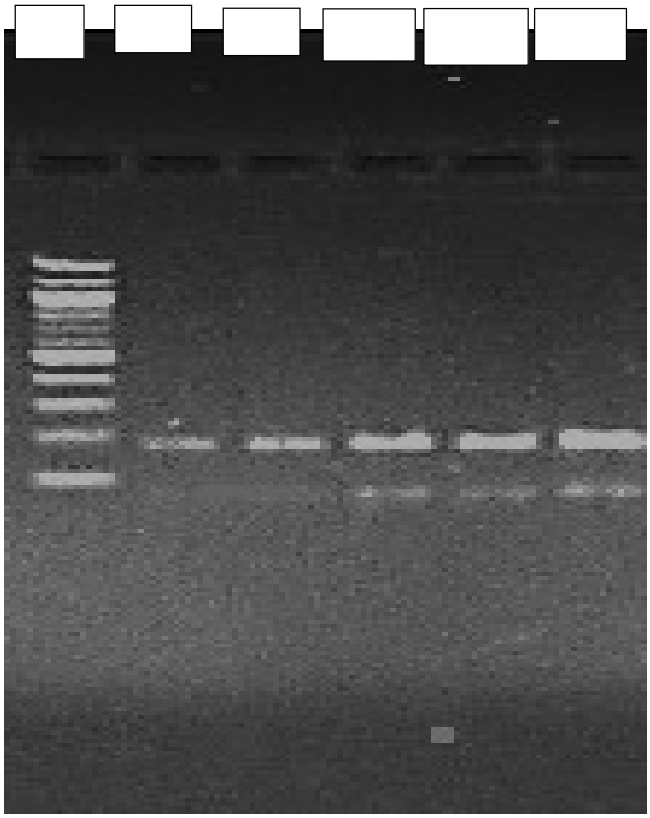

Plate 4. 8: RFLP pattern of PIT-1 gene in Nigeria indigenous chickens

M: 100 bp DNA marker

AA: Genotype AA

AB: Genotype AA

4.6.2 Allele and genotype frequencies of PIT-1 gene in Yoruba, Fulani and FUNAAB-Alpha chickens

The allele and genotype frequencies at PIT1/Hinf*I* locus are presented in Table 4.28. Two alleles (A and B) and two genotypes (AA and AB) were obtained from RFLP analysis of PIT-1 gene in Nigerian chickens. The A allele has higher allele frequency compared to the B allele. Consequently, AB genotype was less frequent compared to the AA genotype in all the three studied chicken populations. The comparison of the observed and expected genotype using Chi-square test ($P<0.05$) revealed that the genotype distributions of PIT-1 gene were in conformity with Hardy–Weinberg equilibrium in all the three studied chicken populations (Table 4.37). The obtained heterozygosity values at PIT-1 locus ranged between 26.9% (FUNAAB-Alpha) and 38.5% (Yoruba chickens). However, Fulani chickens were the most diverse with 31.1% expected heterozygosity. Similarly, Fulani had the highest Shannon's information index while FUNAAB-Alpha chickens had the least. All the threes studied chicken populations had negative fixation index values with FUNAAB-Alpha chickens having the highest value (Table 4.37). The heterozygosity were obtained using POPGENE 1.32 software.

Table 4. 37Allele and genotype frequencies of PIT-1 gene in Nigerian chickens

		Genotypefrequency (%)		Allele frequency(%)		Heterozygosity				
Ecotype	N	AA	AB	A	B	observed	Expected	Shannon's Information Index	Fixation Index	HWE (χ^2)
Yoruba	72	0.28	0.72	0.14	0.81	0.282	0.242	0.406	-0.164	1.91^{ns}
Fulani	61	0.40	0.60	0.20	0.80	0.385	0.311	0.490	-0.238	3.68^{ns}
FUNAAB -Alpha	70	0.27	0.73	0.13	0.87	0.269	0.233	0.395	-0.156	1.89^{ns}

HWE: Hardy-Weinberg Equilibrium, ns: not significant ($p>0.05$)

4.6.3 BLASTn result showing the percentage similarity of the PIT-1 sequences of indigenous chickens in Nigeria and chicken PIT-1 sequences from the NCBI GenBank

The BLASTn results of the derived PIT-1 gene sequences from this study and the available chicken PIT-1 gene sequences from the GenBank is presented in Table 4.38. The search revealed very high percent identity (99 to 100) with the *Gallus gallus* PIT-1 sequences in the GenBank (Table 4.38).

Table 4. 38BLASTn result showing the percentage similarity of the PIT-1 sequences of indigenous chickens in Nigeria and chicken PIT-1 sequences from the NCBI Genbank

Accession	Description	Max score	Total score	E-Value	Max identity
AY438373.1	*Gallus gallus* PIT-1 gene, exon 2 and partial cds	438	438	9.00E-122	100%
NM_204319.1	*Gallus gallus* POU class 1 homeobox 1 (POU1F1), mRNA	438	438	9.00E-122	100%
AJ222692.2	*Gallus gallus* mRNA for pituitary specific transcription factor, alternative transcript Pit1	438	438	9.00E-122	100%
HM590437.1	*Gallus gallus* strain PB-1 pituitary specific transcription factor (Pit-1) gene, Pit-1-P allele, exon 2 and partial cds	438	438	9.00E-122	100%
JN613457.1	*Gallus gallus* haplotype h24 pituitary specific transcription factor-1 (Pit-1) gene, complete cds	427	427	2.00E-118	99%
JN613456.1	*Gallus gallus* haplotype h23 pituitary specific transcription factor-1 (Pit-1) gene, complete cds	427	427	2.00E-118	99%

4.6.4 Phylogenetic tree derived from PIT-1 gene sequences derived from this study (Yoruba, Fulani and FUNAAB-Alpha chickens) and the downloaded PIT-1 sequences from the GenBank

Figure 4.4: shows the phylogenetic tree derived from PIT-1 gene sequences from this study (Yoruba, Fulani and FUNAAB-Alpha chickens) and the downloaded PIT-1 sequences from the GenBank**.** The phylogenetic tree revealed that Yoruba and Fulani chickens are more related at PIT-1 locus than the FUNAAB-Alpha chickens Figure 4.4.

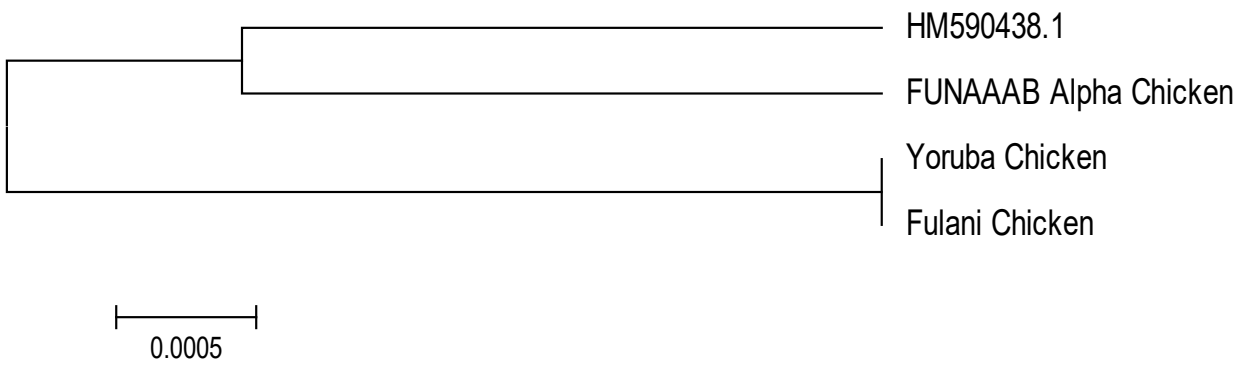

Figure 4. Phylogenetic tree derived from PIT-1 sequences of Yoruba, Fulani and FUNAAB Alpha and chicken PIT-1 gene sequences (HM590438.1) from the GenBank

4.6.5 Effect of PIT-1 gene genotype on growth and carcass traits of Yoruba, Fulani and FUNAAB-Alpha Chickens

Heterozygotes AB genotypes of PIT-1 gene had heavier weekly body weight in Fulani and FUNAAB Alpha chicken. In particular, PIT-1 AB genotype was observed to be significantly associated with body weight at week 16 and 12 in Fulani and FUNAAB Alpha chickens ($P<0.05$) respectively (Table 4.39). In turn, heterozygotes AB favour better dressing weight of Fulani and FUNAAB Alpha Chickens (Table 4.44). PIT-1 genotypes had no significant association with both the weekly body weight and the observed carcass traits of Yoruba chickens. Although chickens with heterozygotes AB had higher mean body weight compared to the AA genotypes (Table 4.40).

Table 4. 39 Effects of PIT-1 genotype on Body Weightof Yoruba, Fulani and FUNAAB Alpha Chickens

Breed/	Body weight (g)						
Genotype	Week0	Week 4	Week 8	Week 12	Week 16	Week 20	Week 24
Yoruba							
AA	24.4±1.2	109.5±13.7	261.3±30.0	472.2±75.9	688.2±127.7	891.0±154.8^{a}	987.5±163.2
AB	24.6±0.9	110.6±16.7	278.4±31.1	477.6±66.5	703.7±99.8	900.7±130.6^{a}	995.2±148.1
Fulani							
AA	26.4± 0.5	158.9±12.5	347.1±16.5	583.5±62.1	859.6±98.1^{b}	1012.2±124.4^{b}	1105.1±146.5^{a}
AB	26.8±0.4	158.2±13.0	356.1±19.6	619.2±52.3	906.4±82.2^{a}	1115.0±131.1^{a}	1209.5±135.2^{a}
FUNAAB-Alpha							
AA	34.6±2.1	315.9±52.7	758.5±104.0	1136.1±172.6^{b}	1511.9±226.0	2011.1±269.0^{a}	2250.1±255.7
AB	34.3 ±1.8	308.0±58.7	762.0±182.3	1214.9±182.3^{a}	1669.4±172.6	2161.8±228.9^{a}	2392.5±203.5

Means ± SD with different superscripts within breed along the columns differ significantly ($p<0.05$)

Table 4. 40 Effects of PIT-1 gene genotypes on carcass traits of Yoruba, Fulani and FUNAAB Alpha Chickens

Breed/ Genotype	Dressed Weight(g)	Drumstick(g)	Thigh(g)	Breast(g)	Wing(g)	Back(g)	Gizzard(g)`
Yoruba							
AA	672.8±70.1	104.4±20.1	119.3±12.6	172.3±18.3	109.8±11.7	188.9±16.3	28.1±2.7
AB	676.6±77.9	112.1±13.9	114.0±14.6	181.9±21.0	105.7±15.6	186.6±15.3	28.5±3.7
Fulani							
AA	789.2±109.4	124.8±18.3	137.3±20.8	200.1±28.1	122.8±6.2	208.9±27.1	28.5±1.1
AB	828.7±141.7	133.4±21.3	144.3±27.5	210.6±36.9	133.7±13.2	217.9±37.2	28.6±2.8
FUNAAB-Alpha							
AA	1411.1±97.6	248.0±15.6	273.6±17.0	298.9±21.7	173.7±10.5	304.0±16.0	37.8±2.2
AB	1486.3±112.9	256.6±19.7	284.0±21.7	309.8±24.3	167.8±13.1	292.7±27.3	36.3±3.0

Means ± SD with different superscripts within breed along the columns differ significantly ($p<0.05$)

4.6.6 Interaction Effects of Sex and PIT-1 genotype on growth and carcass traits (g) of Yoruba Ecotype Chickens

The body weight of Yoruba cocks with homozygote AA of PIT-1 were not significantly different ($P<0.05$) from the body weight of their counterparts with heterozygote AB variant. Similarly, the body weight of Yoruba hens with AA (PIT-1) was similar with the body weight of hens with AB from week 0-12. However, the body weight of Yoruba hens with AB (680.8±22.5) was significantly higher than the body weight of hens with AA (604.2±18.7) at week 16 (Table 4.41). The weight of all the measured carcass traits of Yoruba cocks and hens with AA of PIT-1 were not significantly different from the weight of Yoruba cocks and hens with heterozygote AB variant except the thigh and breast weight which were significantly lower ($P<0.05$) for hens with AA than those with AB (Table 4.42).

Table 4. 41 Interaction Effects of Sex and PIT-1 Genotype on Body Weight of Yoruba Ecotype Chickens

		Body weight (g)						
Sex	Genotype	Week0	Week 4	Week 8	Week 12	Week 16	Week 20	Week 24
Cock	AA	24.0±1.3	112.0±8.8	259.4±19.3	518.8±47.2[a]	794.1±62.2[a]	1025.2±81.1[a]	1152.5±81.9[a]
	AB	24.3±1.3	105.8±15.5	264.8±12.7	530.1±38.7[a]	806.8±28.8[a]	1080.7±28.7[a]	1176.3±38.4[a]
Hen	AA	24.7±1.1	107.5±16.4	262.9±36.6	435.2±74.4[b]	604.2±100.6[c]	784.6±109.3[b]	870.5±82.6[b]
	AB	24.6±0.9	105.8±17.2	281.4±33.3	465.9±66.3[b]	680.8±95.4[b]	865.2±115.9[b]	945.6±128.8[b]

Means ± SD with different superscripts within breed along the columns differ significantly ($p<0.05$)

Table 4. 42 Interaction Effects of Sex and PIT-1 Genotype on Carcass Traitsof Yoruba Ecotype Chickens

Sex	Genotype	Dressed Weight(g)	Drumstick(g)	Thigh(g)	Breast(g)	Wing(g)	Back(g)	Gizzard(g)
Cock	AA	705.8±24.3^{a}	103.6±25.4^{ab}	126.2±4.5^{a}	182.2±6.6^{a}	116.0±5.2^{a}	198.6±8.9	29.7±1.1
	AB	740.1±22.1^{a}	122.8±9.2^{a}	132.6±4.1^{a}	191.5±6.0^{a}	120.9±3.7^{a}	197.3±8.8	31.2±1.0
Hen	AA	620.5±82.0^{b}	100.5±10.4^{b}	106.2±10.8^{c}	153.4±15.5^{c}	100.2±11.9^{b}	173.2±11.8	25.2±4.4
	AB	644.9±79.9^{b}	105.5±12.2^{ab}	116.1±15.7^{b}	167.7±22.6^{b}	97.6±13.0^{b}	184.0±17.1	27.8±1.7

Means ± SD with different superscripts within breed along the columns differ significantly ($p<0.05$)

4.6.7 Interaction Effects of Sex and PIT-1 genotype on growth and carcass traitsof Fulani Ecotype Chickens

The body weight of Fulani cocks with AA of PIT-1 was not significantly different from those with heterozygote AB variant of PIT-1 from week 0 to 24. Similarly, the body weights of Fulani hens with AA of PIT-1 were not significantly different from those with heterozygote AB variant of PIT-1 from week 0 to 24. However, Fulani cocks had significantly higher body weight than the hens from week 16 to 24 (Table 4.43).The weights of all the measured carcass traits for Fulani cocks with AA were not significantly different from those with AB at PIT-1 locus. Similar trend was observed for all the measured carcass traits of Fulani hens with AA and AB variants (Table 4.44).

Table 4. 43Interaction Effects of Sex and PIT-1 genotype on growth traitsof Fulani Ecotype Chickens

		Body weight (g)						
Sex	Genotype	Week0	Week 4	Week 8	Week 12	Week 16	Week 20	Week 24
Cock	AA	26.0±0.7	163.7±13.3	352.9±40.2	640.5±71.4[a]	1007.6±112.1[a]	1185.2±144.3[a]	1286.2±158.4[a]
	AB	26.7±0.9	153.8±18.9	363.5±33.8	659.4±64.6[a]	971.9±130.8[a]	1236.1±150.0[a]	1352.4±163.3[a]
Hen	AA	26.6±1.0	156.5±19.4	344.2±38.4	555.0±62.8[b]	785.6±115.2[b]	925.7±147.8[b]	1007.6±157.3[b]
	AB	26.9±0.9	163.5±20.2	347.6±34.2	572.1±76.8[b]	830.1±99.9[b]	973.7±132.1[b]	1042.8±146.3[b]

Means ± SD with different superscripts within breed along the columns differ significantly ($p<0.05$)

Table 4. 44Interaction Effects of Sex and PIT-1 Genotype on Carcass traits of Fulani Chickens

Sex	Genotype	Dressed Weight(g)	Drumstick(g)	Thigh(g)	Breast(g)	Wing(g)	Back(g)	Gizzard(g)
Cock	AA	906.2±23.0[a]	143.7±4.0[a]	159.3±4.5[a]	229.9±6.4[a]	127.3±3.6	235.3±7.3[a]	28.7±0.8
	AB	910.8±96.6[a]	144.5±16.9[a]	160.2±18.7[a]	231.2±27.0[a]	128.0±15.0	236.8±30.6[a]	28.9±3.4
Hen	AA	698.4±35.4[b]	109.9±8.2[b]	119.9±6.9[b]	176.9±10.1[b]	119.9±5.9	187.3±1.4[b]	28.5±1.4
	AB	676.0±24.4[b]	112.4±7.9[b]	114.7±4.7[b]	172.0±7.7[b]	114.8±3.3	181.9±12.9[b]	27.8±1.9

Means ± SD with different superscripts within breed along the columns differ significantly ($p<0.05$)

4.6.8 Interaction Effects of Sex and PIT-1 genotype on growth and carcass traits (g) of FUNAAB-AlphaEcotype Chickens

The body weight of FUNAAB-Alpha cocks with AA of PIT-1 were not significantly different (P>0.05) from those with AB from week 0 to 24. However, the body weight of FUNAAB-Alpha hens with AB of PIT-1 was significantly higher (1507.8±46.3) than bodyweight of hens with AA (1364.9±30.7) at week 16 while the body weight of FUNAAB-Alpha hens with AA were consistently lower than the body weight of hens with AB from week 16 to 24 (Table 4.45). The dressed weight of FUNAAB-Alpha hens with AB (1454.5±69.13) was significantly higher than the dressed weight of hens with AA (1346.5±19.6) but similar to dressed weight of cocks with AA (1474.8±22.0) and AB (1494.9±17.3). The drumstick, thigh and breast weight of FUNAAB-Alpha cocks with AA of PIT-1 were not significantly different from those with AB (Table 4.46).

Table 4.45 Interaction Effects of Sex and PIT-1 genotype on Body Weightof FUNAAB-Alpha Chickens

		Body weight (g)						
Sex	Genotype	Week0	Week 4	Week 8	Week 12	Week 16	Week 20	Week 24
Cock	AA	34.9±1.8	331.9±57.5	778.1±121.1	1261.5±103.7^{a}	1675.8±163.4^{a}	2234.6±172.7^{a}	2465.0±151.9^{a}
	AB	34.0±1.9	311.1±66.6	752.1±87.9	1323.2±87.9^{a}	1773.3±94.1^{a}	2307.7±119.0^{a}	2496.6±115.5^{a}
Hen	AA	34.3±2.4	301.7±44.2	741.0±119.4	1023.7±115.0^{b}	1364.9±165.3^{c}	1810.7±158.0^{b}	2057.4±155.3^{c}
	AB	34.8±1.5	313.1±47.2	777.3±129.4	1046.5±162.4^{b}	1507.8±138.9^{b}	1934.7±160.8^{b}	2179.5±152.9^{b}

Means ± SD with different superscripts within breed along the columns differ significantly ($p<0.05$)

Table 4. 46Interaction Effects of Sex and PIT-1 genotype on Carcass traitsof FUNAAB-Alpha Chickens

Sex	Genotype	Dressed Weight(g)	Drumstick(g)	Thigh(g)	Breast(g)	Wing(g)	Back(g)	Gizzard(g)
Cock	AA	1462.8±72.8^{a}	245.3±13.1^{ab}	282.2±14.6^{a}	313.5±16.1^{a}	172.5±8.7^{ab}	289.3±14.8	37.0±1.9^{ab}
	AB	1494.9±48.9^{a}	258.6±8.7^{a}	286.1±9.6^{a}	317.9±10.6^{a}	174.9±5.8^{a}	293.3±9.9	37.6±1.3^{ab}
Hen	AA	1346.5±89.3^{b}	240.1±30.0^{b}	264.4±33.0^{b}	284.3±35.6^{b}	162.6±20.0^{b}	293.8±37.1	35.4±4.5^{b}
	AB	1454.5±76.0^{a}	257.5±14.5^{a}	285.2±14.8^{a}	305.3±16.5^{a}	174.5±9.9^{a}	318.2±17.2	38.5±2.1^{a}

Means ± SD with different superscripts within breed along the columns differ significantly ($p<0.05$)

GENETIC DIVERSITY OF MYOGENIN GENE IN YORUBA, FULANI AND FUNAAB-ALPHA CHICKENS

4.7.1 PCR Gel image of MYOG gene in Nigerian Chickens

A 152 bp fragment of exon-1 of myogenin gene was successfully amplified and sequenced using Sanger sequencer. The gel image showing the amplified product of 152 bp of Nigerian chicken is presented in Plate 4.9. Restriction digestion of the amplified products using Hinf1 and ECOR1 restriction endonucleases showed uncut single band in all the studied Nigerian chickens.

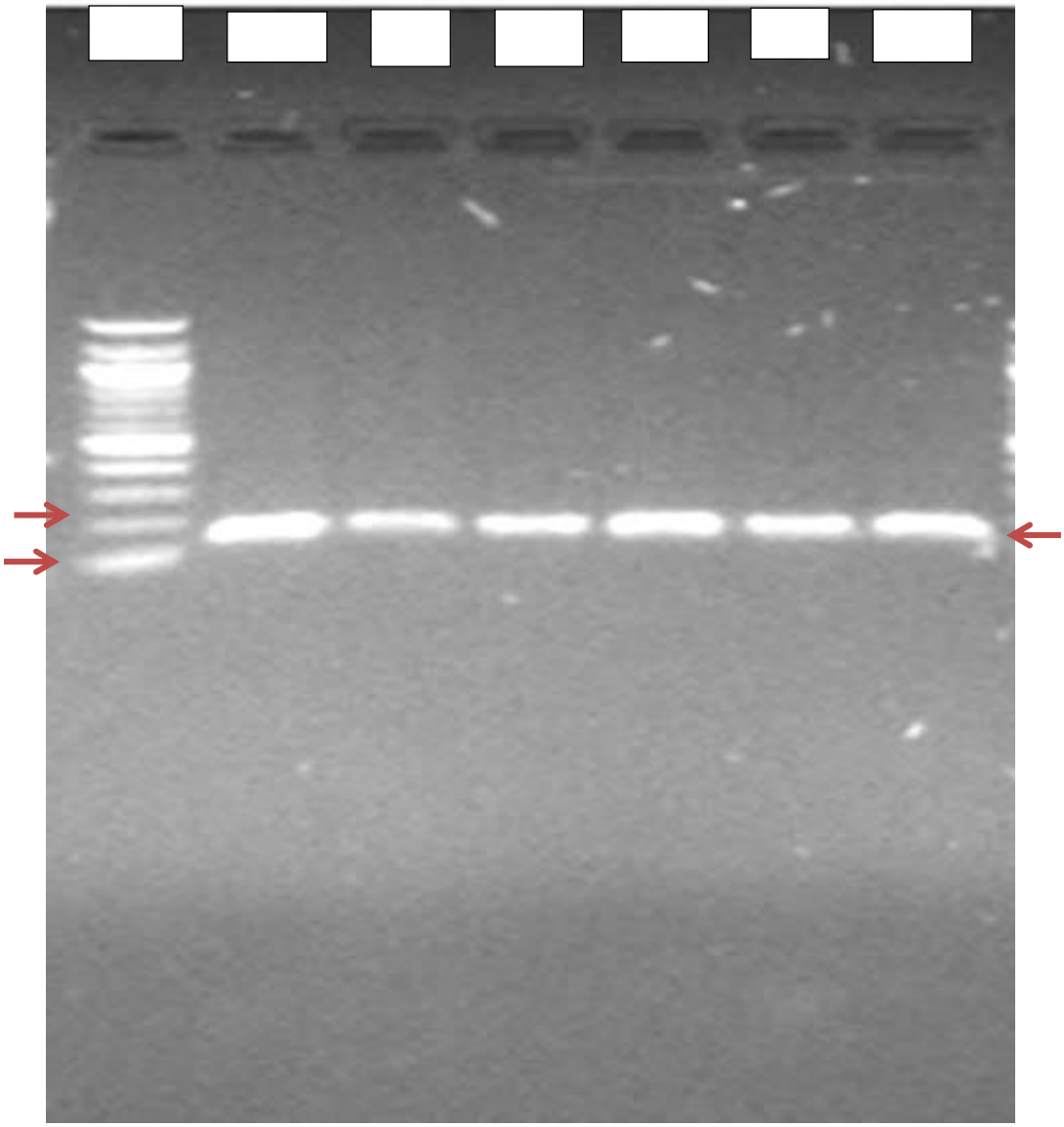

Plate 4. 9: PCR gel image of MYOG gene in Nigerian Chickens

M : 100 bp DNA marker

Lanes 1-6 : samples 1-5

4.7.2 BLASTn results showing the percentage identity of the *Gallus gallus* MYOG locus of Nigerian chickens

The BLASTn results of the derived MYOG sequences from Nigerian chickens (Yoruba, Fulani and FUNAAB-Alpha) had 96% identity and 2.00E-59 expect-values with the *Gallus gallus* MYOG gene from the GenBank. Further, the studied Nigerian chickens had 86%, 88%, 89% and 92% homology with goose, turkey and guinea fowl at MYOG locus respectively (Table 4.47).

Table 4. 47 BLASTn result showing the percentage of identity of the exon-1 of the Gallus Gallus GHRLlocus of Fulani chickens

Accession	Description	Max score	Total score	E-Value	Max identity
KJ130962.1	*Gallus gallus* myogenin (MyoG) mRNA, complete cds	232	232	2.00E-59	96%
FJ882411.1	*Gallus gallus* myogenin (MYOG) mRNA, complete cds	232	232	2.00E-59	96%
NM_204184.1	*Gallus gallus* myogenin (MYOG), mRNA	232	232	2.00E-59	96%
XM_021377425.1	*Numida meleagris* myogenin (MYOG), mRNA	204	204	1.00E-50	92%
NM_001303170.1	*Meleagris gallopavo* myogenin (LOC100303673), mRNA	186	186	3.00E-45	89%
XM_015884883.1	*Coturnix japonica* myogenin (myogenic factor 4) (MYOG), mRNA	185	185	3.00E-45	88%
KP893286.1	*Anser cygnoides* myogenin (MyoG) mRNA, complete cds	166	166	3.00E-39	88%
KT290042.1	*Anser cygnoides* myogenin (MyoG) gene, complete cds	171	171	7.00E-41	86%

4.7.3 Phylogenetic tree and genetic distance between Yoruba, Fulani and FUNAAB Alpha chickens

The phylogenetic tree constructed from obtained sequences of MYOG gene of Nigerian chickens (Yoruba, Fulani and FUNAAB-Alpha) and the reported MYOG sequences of the *Gallus gallus* from the GenBank revealed that; FUNAAB-Alpha chicken is more closely related to Yoruba chickens with 99.979 per cent genetic similarity than the Fulani chickens with 99.172 per cent similarity (Figure 4.5 and Table 4.48).

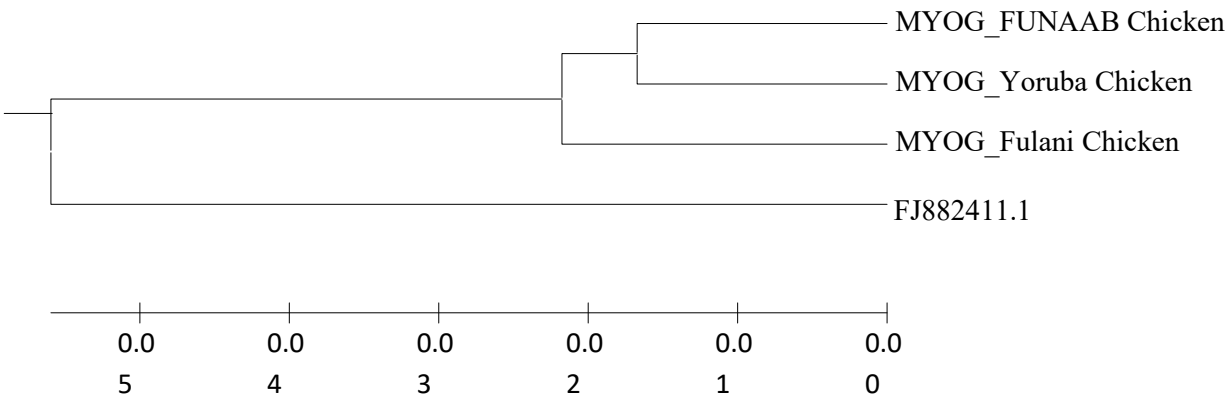

Figure 4. Phylogenetic tree derived from MYOG sequences of Yoruba, Fulani and FUNAAB Alpha and chicken MYOG gene sequences (FJ882411.1) from the GenBank

Table 4. 48Genetic Distance and Per cent Identity Between Nigerian Chickens Based On MYOG gene locus

	FJ882411.1(%)	FUNAAB Alpha(%)	Fulani(%)	Yoruba(%)
FJ882411.1		99.979	99.232	99.957
FUNAAB Alpha	0.021		99.168	99.979
Fulani	0.768	0.832		99.172
Yoruba	0.043	0.021	0.828	

Lower diagonal: Genetic divergence.

Upper diagonal: Per cent genetic similarity

ASSOCIATION OF HSP70 GENE POLYMORPHISM WITH GROWTH AND THERMAL-TOLERANCE TRAITS OF YORUBA, FULANI AND FUNAAB-ALPHA CHICKENS

4.8.1 PCR and RFLP gel image of HSP70 gene in Yoruba, Fulani and FUNAAB Alpha chicken

A 360 bp fragment of HSP70 gene was amplified in Nigerian chickens. Plate 4.10 showed the image of the PCR amplified HSP70 gene in Nigerian chickens. The obtained RFLP pattern of HSP70/Mme1 gene in the studied chickens is presented in Plate 4.11.

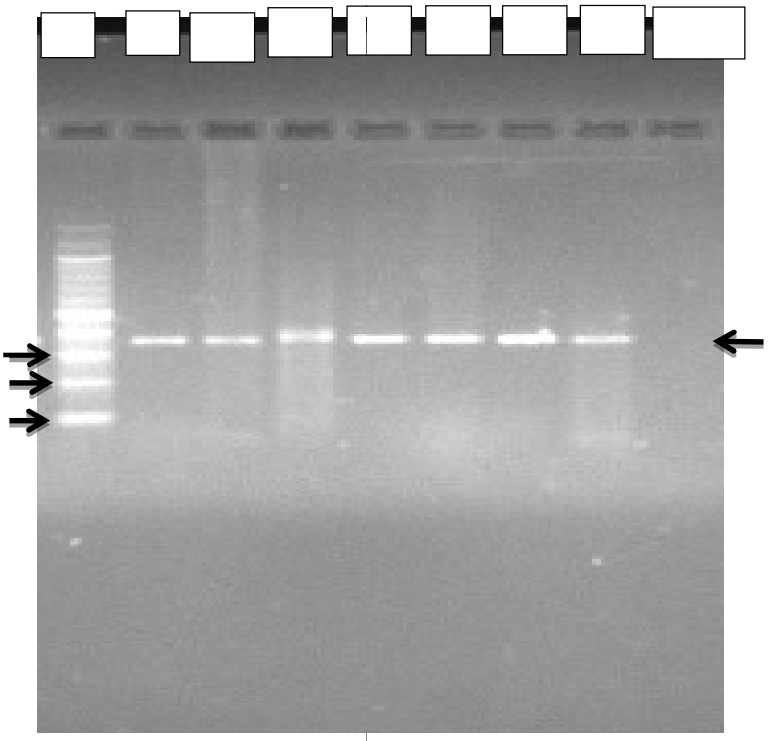

Plate 4. 10: Gel picture of PCR amplified HSP70 gene in Nigerian chickens

M:100 bp DNA marker

Lanes 1-7: samples 1-7

-Ve : negative control

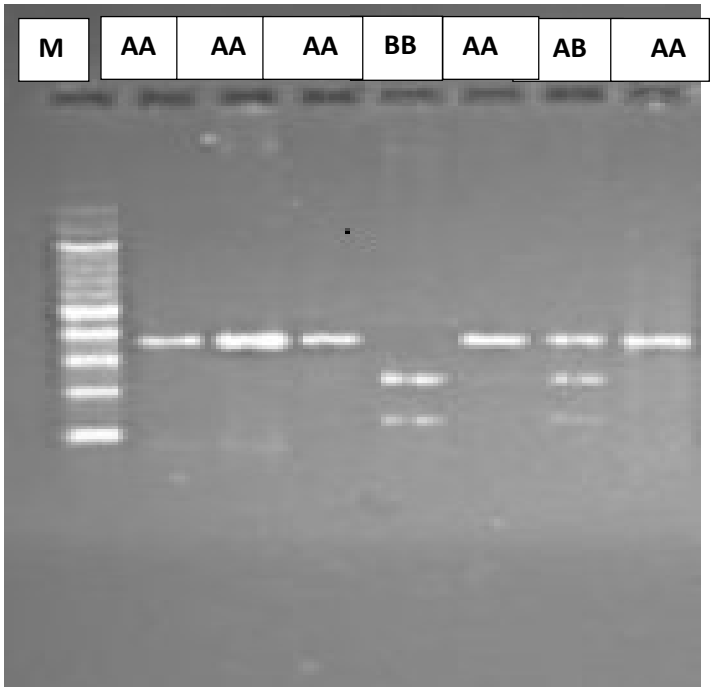

Plate 4. 11: RFLP gel image of HSP70 gene in Nigerian Chickens

M: 100 bp DNA marker

AA: Genotype AA

BB: Genotype BB

AB: Genotype AB

4.8.2 Genotype and Allele frequencies of HSP70 gene in Nigerian Chickens

The RFLP analysis of the amplified HSP70 gene digested with *Mme1*produced three genotypes (AA, AB and BB). AA genotypes had the highest genotype frequency while BB genotype was the least frequent in all the studied chicken populations (Table 4.49).Two alleles were observed in each of the three studied chicken populations at ghrelin locus. However, Fulani chickens had the highest effective number of alleles followed by Yoruba chickens then FUNAAB-Alpha chickens. Similarly, Fulani chickens had the highest expected heterozygosity value (46.9%) while FUNAAB-Alpha had the least (37.5%). However, Yoruba and FUNAAB-Alpha chickens had the highest and closest observed heterozygosity while Fulani chickens had the least value. The highest Shannon's information index (66.2%) was obtained in Fulani while FUNAAB-Alpha chickens had the least Shannon's information index value (56.2%). In contrast, Yoruba chickens had the least fixation index value (39.2%) while the highest fixation index value was obtained in Fulani chickens (Table 4.49).

Table 4. 49 Genotype and allele frequencies of HSP70 gene in Nigerian chickens

		Genotype frequency(%)			Allele frequency(%)		Heterozygosity				
Ecotype	N	AA	AB	BB	A	B	Observed	Expected	Shannon's Information Index	Fixation Index	HWE (χ^2)
Yoruba	67	0.62	0.24	0.15	0.73	0.27	0.239	0.393	0.582	0.392	10.31*
Fulani	68	0.56	0.26	0.18	0.69	0.31	0.132	0.469	0.662	0.718	9.82*
FUNAAB Alpha	72	0.65	0.19	0.15	0.75	0.25	0.222	0.375	0.562	0.407	16.69*

HWE: Hardy-Weinberg Equilibrium, * : Significant ($p<0.05$)

4.8.3 BLASTn result showing the percentage of identity of the exon-1 of the *Gallus gallus* HSP70 Locus of Nigerian chickens

The BLASTn results of derived HSP70 gene sequence from this study showed very high homology values (5.00E-165 to 1.00E-166) with the published HSP70 gene sequences from the GenBank. In addition, the genetic distance analysis revealed 97 to 100 percent genetic similarities with the published *Gallus gallus* heat shock protein 70 (HSP70) gene in the Genbank (Table 4.50).

Table 4. 50 BLASTn result showing the percentage of identity of the exon-1 of the *Gallus gallus* HSP70 Locus of Nigerian chickens

Accession	Description	Max score	Total score	E-Value	Max identity
JX827254.1	*Gallus gallus* heat shock protein 70 (HSP70) gene, complete cds	588	588	1.00E-166	100%
FJ217667.1	*Gallus gallus* heat shock protein 70 (HSP70) mRNA, complete cds	588	588	1.00E-166	100%
AY143691.1	*Gallus gallus* heat shock protein Hsp70 (hsp70) gene, hsp70-1 allele, complete cds	588	588	1.00E-166	100%
AY288303.1	*Gallus gallus* isolate Br-3-2 heat shock protein 70 (HSP70) gene, complete cds	588	588	1.00E-166	100%
AY143692.1	*Gallus gallus* heat shock protein Hsp70 (hsp70) gene, hsp70-3 allele, complete cds	582	582	5.00E-165	99%
AY288301.1	*Gallus gallus* isolate Br-2-2 heat shock protein 70 (HSP70) gene, complete cds	582	582	5.00E-165	99%
GU980869.1	*Gallus gallus* heat shock protein 70 mRNA, partial cds	532	532	5.00E-150	97%

4.8.4 Association of HSP70 gene with growth traits of Yoruba, Fulani and FUNAAB-Alpha chickens

The three obtained HSP70 genotypes (AA, AB and BB) were associated with the weekly body weights (0 - 24) of Yoruba, Fulani and FUNAAB-Alpha chickens. HSP70 genotypes had no significant effect ($P<0.05$) on the weekly body weights of all the studied chicken populations (Table 4.51)

Table 4. 51 Effects of HSP70 Genotype on Body Weightof Yoruba, Fulani and FUNAAB-Alpha chickens

Breed/ Genotype	Body weight (g)						
	Week0	Week 4	Week 8	Week 12	Week 16	Week 20	Week 24
Yoruba							
AA	24.6±0.20	109.7±2.52	264.4±5.24	466.1±12.14	679.4±19.50	879.2±23.79	976.9±25.25
AB	24.5±0.23	109.7±3.99	269.5±8.93	474.0±14.64	681.1±26.23	881.1±33.23	972.5±35.85
BB	24.6±0.34	107.6±3.15	268.4±5.83	473.7±21.55	703.6±37.22	893.4±45.50	993.9±51.87
Fulani							
AA	26.2±0.32	149.9±3.66	340.1±7.64	628.5±10.06	915.4±19.97	1140.8±24.37	1246.6±26.78
AB	26.6±0.36	150.5±4.00	342.9±13.29	583.7±14.86	921.2±31.29	1105.7±31.98	1203.7±34.46
BB	26.9±0.73	148.4±7.25	340.6±14.22	616.0±20.66	907.0±28.76	1099.7±34.58	1197.2±36.37
FUNAAB-Alpha							
AA	34.4±0.33	306.2±8.73	756.7±18.56	1156.9±23.79	1568.0±31.85	2063.2±37.70	2293.4±40.54
AB	34.8±0.46	330.9±12.45	735.6±25.86	1158.6±44.38	1563.0±67.43	2085.9±67.98	2312.0±73.96
BB	34.2±0.57	311.9±16.00	775.3±38.13	1153.3±69.91	1494.5±81.11	1965.5±87.47	2191.5±82.40

Means ± SD with different superscripts within breed along the columns differ significantly ($p<0.05$)

4.8.5 Effects of breed on thermal-tolerance and hematological traits of Nigerian chickens

The obtained respiratory rate values ranged between 38.72±0.32 and 41.39±0.28 before the exposure to acute heat stress (AHS) at 40±1 ^{o}C for one hour. Following the AHS, the RR values appeared to slightly increase across all the three studied populations. However, there was no significant difference ($P<0.05$) between the RR value of the acute heat stressed Fulani and FUNAAB-Alpha chickens (Table 4.52). Slight increase in rectal temperature and purse rate values were observed following the AHS exposure. Acute heat stressed FUNAAB-Alpha chickens had significantly higher ($p<0.05$) PR value (330.72±4.74) followed by Fulani (302.05±6.44) then Yoruba (270.80±11.34) chickens. This could be attributed to the higher body weights of FUNAAB Alpha chickens compared to the unimproved Yoruba and Fulani ecotypes. FUNAAB-Alpha chickens had the least heat stress index (1.08) prior to acute heat stress while Yoruba chickens had the highest value (1.22). Conversely, the highest heat stress index (HIS) value was observed in FUNAAB-Alpha chickens while there was no significant difference ($p<0.05$) between the HSI values of Yoruba and Fulani ecotype chickens (Table 4.58). In addition, Yoruba chickens had the least PCV value (31.23±0.27) while FUNAAB-Alpha and Fulani chickenshad similar PCV values. Lastly, the obtained values of heterophil, lymphocytes and heterophil/lymphocytes were similar before and after the acute heat stress exposure (Table 4.53).

Table 4. 52 Physiological responses of acute heat-stressed Yoruba, Fulani and FUNAAB-Alpha Chickens

Parameters		Yoruba	Fulani	FUNAAB-Alpha
RR (Beat/min)	0 hour	38.72±2.66^{b}	39.06±1.35^{b}	41.39±1.34^{a}
	1 hour	46.50±3.85^{b}	50.67±2.36^{a}	52.06±4.06^{a}
RT (^{O}C)	0 hour	40.47±0.72	40.14±0.53	40.26±0.56
	1 hour	40.84±0.73	40.65±0.41	40.57±1.38
PR (Breath/min)	0 hour	275.10±42.74^{b}	300.99±26.14^{a}	315.89±26.62^{a}
	1 hour	270.80±41.07^{c}	302.05±24.65^{b}	330.72±23.07^{a}
HIS	0 hour	1.22±0.17	1.12±0.11	1.08±0.10
	1 hour	1.36±0.18^{b}	1.46±0.09^{ab}	1.52±0.17^{a}

CT: Cloaca Temperature, RR: Respiration Rate, PR: Pulse Rate, HSI: Heat Stress Index

Means ± SD with different superscript along the rows are significantly different ($p<0.05$)

Table 4. 53 Hematological responses of acute heat-stressed Yoruba, Fulani and FUNAAB-Alpha chickens

Parameters		Yoruba	Fulani	FUNAAB-Alpha
PCV (%)	0 hour	31.00±0.70[b]	31.87±0.36[ab]	32.67±0.94[a]
	1 hour	31.23±1.15[b]	32.14±1.16[a]	32.55±1.57[a]
HB (g/dl)	0 hour	9.67±0.26[b]	9.89±0.27[b]	10.30±0.41[a]
	1 hour	8.56±0.48[a]	9.05±0.41[b]	9.59±0.53[c]
RBC ($x10^6$ Ul)	0 hour	2.75±0.49	2.85±0.36	3.02±0.34
	1 hour	2.65±0.52[b]	2.75±0.41[ab]	2.97±0.41[a]
Lymphocyte (%)	0 hour	63.67±4.28[a]	63.39±4.55[a]	59.67±6.51[b]
	1 hour	61.76±4.01	59.39±5.28	58.26±6.21
Heterophil (%)	0 hour	31.89±4.96	30.44±4.00	31.00±3.64
	1 hour	31.78±5.60	31.76±4.11	31.30±8.25
Het/ Lymph	0 hour	0.503±0.10	0.485±0.07	0.524±0.09
	1 hour	0.5200±0.12	0.536±0.07	0.538±0.14

Means ± SD with different superscript along the rows are significantly different ($p<0.05$)

PCV: Packed Cell Volume, HB: Hemoglobin, RBC: Red Blood Cell, Het/ Lymph: Heterophil/Lymphocyte Ratio

4.8.6 Effects of Hsp70 genotypes on physiological and hematological traits of Yoruba, Fulani and FUNAAB-Alpha chickens

The HSP70 genotype had no significant effects ($P<0.05$) on the average values of respiratory rate of Nigerian chickens prior to acute heat stress exposure (AHS). However, the homozygote BB birds had significantly higher respiratory rate values compared to the homozygote AA and heterozygote BB carrying individuals. Further, HSP70 genotype had no significant effects ($P<0.05$) on the average rectal temperature (RT) and purse rate (PR) values of the studied birds before and after the AHS. The birds with homozygote BB genotype of HSP70 gene had the least heat stress index values in all studied chicken populations while the homozygote AA birds had the highest HSI value (Table 4.54).There was a slight increase in the PCV values of birds with AB and BB genotypes following acute heat stress exposure. The highest PCV value was obtained from birds with genotype BB (32.91±0.26)while the homozygote AA birds had the least observed value (31.52±0.25). The HSP70 genotype had no significant effects ($P<0.05$) on the RBC, Lymphocytes, heterophil and heterophil/lymphocytes ratio of the studied birds prior to AHS. Following AHS however, the average RBC value of the homozygote BB birds was not significantly different from that of the heterozygote AB individuals just like the RBC values of the AA and AB genotype carrying birds. Similarly, the heterophil and heterophil/lymphocytes ratio values are not significantly different ($P<0.05$) following the AHS (Table 4.55).

Table 4. 54 Effects of HSP70 genotype on physiological traits of Yoruba, Fulani and FUNAAB-Alpha chickens

Parameters		AA	AB	BB
RR (Beat/min)	0 hour	39.33±3.21	39.56±1.98	40.28±1.41
	1 hour	47.76±6.76^{b}	49.13±3.48^{b}	52.33±4.74^{a}
CT (^{O}C)	0 hour	40.06±0.66^{b}	40.25±0.43^{ab}	40.56±0.64^{a}
	1 hour	40.59±0.48^{b}	41.11±1.06^{ab}	40.36±0.99^{a}
PR (Breath/min)	0 hour	271.19±29.33^{b}	300.45±21.21^{a}	320.34±37.65^{a}
	1 hour	274.12±32.93^{b}	308.53±29.54^{a}	320.91±37.89^{a}
HIS	0 hour	1.24±0.12	1.11±0.09	1.08±0.14
	1 hour	1.53±0.16^{b}	1.39±0.16^{a}	1.42±0.12^{b}

Means ± SD with different superscript along the rows are significantly different ($p<0.05$)

CT: Cloaca Temperature, RR: Respiration Rate, PR: Pulse Rate, HSI: Heat Stress Index

Table 4. 55 Effects of HSP70 gene genotype on haematological traits of Yoruba, Fulani and FUNAAB-Alpha chickens

Parameters		AA	AB	BB
PCV (%)	0 hour	31.52±1.05^{b}	31.66±0.87^{b}	32.36±0.84^{a}
	1 hour	31.08±2.14^{c}	31.92±1.40^{b}	32.91±1.12^{a}
HB (g/dl)	0 hour	9.75±0.41^{b}	9.90±0.26^{b}	10.21±0.43^{a}
	1 hour	8.64±0.61^{c}	9.06±0.63^{b}	9.51±0.65^{a}
RBC (x10^{6} Ul)	0 hour	3.02±0.39	2.79±0.44	2.81±0.39
	1 hour	2.59±0.43^{b}	2.77±0.50^{ab}	3.01±0.46^{a}
Lymphocyte (%)	0 hour	60.94±6.67	62.72±5.72	63.06±3.68
	1 hour	59.48±5.90	59.26±5.87	60.67±4.34
Heterophil (%)	0 hour	29.89±4.41^{b}	30.00±4.59^{b}	33.44±3.41^{a}
	1 hour	30.89±4.24	31.01±8.06	32.93±5.54
Het/ Lymph	0 hour	0.497±0.11	0.483±0.09	0.532±0.04
	1 hour	0.524±0.09	0.526±0.14	0.544±0.10

Means ± SD with different superscript along the rows differ significantly ($p<0.05$)

PCV: Packed Cell Volume, HB: Haemoglobin, RBC: Red Blood Cell, Het/ Lymph: Heterophil/Lymphocyte Ratio

4.8.7 Interaction effects of breed and HSP70 gene genotypes on physiological and haematological parameters of acute heat-stressed Yoruba, Fulani and FUNAAAB-Alpha chickens

The cloaca temperature (^{O}C)of Yoruba chickens with genotype BB of HSP70 gene (41.1±0.33) was significantly higher (P<0.05)than the cloaca temperature of their counterparts with AA (40.34±0.24) and AB (40.04±0.45) before the acute heat stress exposure. However, there was no significant difference (P>0.05) in the obtained values of cloaca temperature for the acute heat-stressed Yoruba chickens with AA, AB and BB of HSP70 gene. The highest value of pulse rate (Beat/min)was observed in FUNAAB-Alpha chickens which ranged (41.0±0.29 to 41.8±0.65) and 53.6±0.83 to 58.2±2.2) before and after acute heat stress exposure respectively. Acute heat-stressed FUNAAB-Alpha chickens with AA of HSP70 had the highest pulse rate value(Beat/min) while the least pulse rate value was obtained in birds with AB of HSP70. Similarly, acute heat-stressed Yoruba chickens with AA of HSP70 had the least Pulse rate value (262.2±17.93 beat/min) while FUNAAB-Alpha chickens with BB had the highest pulse rate value (344.5±7.15). The heat stress index value of Yoruba chickens with AA of HSP70 was not significantly different from that of AB and BB individuals. However, acute heat-stressed Fulani and FUNAAB-Alpha chickens with BB of HSP70 had the least value of heat stress index (1.32±0.05) and (1.35±0.03),respectively. However, the heat stress index values of acute heat-stressed Fulani and FUNAAB-Alpha chickens with BB of HSP70 were significantly lower than the heat stress index values of their counterparts with AA (1.48± 0.03) and (1.59±0.08) respectively (Table 4.56). The least value of PCV (%)was observed in the acute heat-stressed Yoruba chickens with AA of HSP70 (30.35±0.20%) while acute heat-stressed FUNAAB-Alpha chickens with BB of HSP70 had the highest value of PCV (33.94±0.44%). However, acute heat-stressed Yoruba chickens with AB had the least RBC value (2.54±0.28x 10^{6} ul) while FUNAAB-Alpha chickens with BB had the highest RBC value (3.304±0.16x 10^{6} ul). Similarly, the least (0.47±0.04) and highest (0.61±0.04) values of heterophil/lymphocyte ratio was obtained from the acute heat-stressed Yoruba chickens with BB of HSP70 and FUNAAB-Alpha with AA respectively (Table 4.57).

Table 4. 56 Interaction effect of HSP70 gene and breed on Physiological Parameters of Acute heat-stressed Yoruba, Fulani and FUNAAB-Alpha Chickens

	CT (^{O}C)		RR (Breath/min)		PR (Beat/min)		HSI	
YOR UBA	0 hour	1 hour	0 hour	1 hour	0 hour	1 hour	0 hour	1 hour
AA	40.3±0.82^{b}	40.8±0.45	35.8±1.37^{b}	43.7±3.93	239.0±47.75	262.2±22.07	1.30±0.19	1.44±0.51
AB	40.0±0.27^{b}	40.2±0.86	38.5±1.38^{ab}	46.8±3.54	295.5±30.96	302.7±46.09	1.13±0.12	1.37±0.26
BB	41.1±0.82^{a}	41.6±0.79	39.7±1.37^{a}	45.7±4.03	281.8±21.68	299.2±46.23	1.16±0.11	1.42±0.17
FULANI								
AA	40.3±0.82	40.6±0.29	38.5±1.05^{b}	49.0±2.45	273.1±18.22	287.3±28.05	1.22±0.08	1.48±0.10^{a}
AB	40.3±0.24	40.5±0.54	38.5±1.22^{b}	48.7±1.51	288.3±2.05	300.4±1.86	1.10±0.03	1.40±0.05^{ab}
BB	39.9±0.28	40.8±0.36	40.2±1.17^{a}	50.7±2.80	304.8±35.49	331.4±6.65	1.15±0.15	1.32±0.06^{b}
FUNAAB-Alpha								
AA	40.0±0.72	40.4±0.64	41.8±1.17	58.2±6.09	284.6±8.66	316.9±24.81^{b}	1.23±0.03	1.59±0.23^{a}
AB	40.5±0.58	41.7±1.29	41.7±1.37	53.6±2.93	296.9±23.22	319.8±23.84^{ab}	1.22±0.08	1.46±0.14^{ab}
BB	40.3±0.26	40.6±0.53	41.0±1.55	53.8±1.49	312.4±34.27	344.5±8.55^{a}	1.14±0.11	1.35±0.10^{b}

Means ± SD with the same superscript along the column are not significantly different (P<0.05)

CT: Cloaca Temperature, RR: Respiration Rate, PR: Pulse Rate, HSI: Heat Stress Index, 0 hour: before heat stress exposure, 1 hour: after heat stress exposure

Table 4. 57 Interaction effects of HSP70 gene and breed on Haematological Parameters of Acute heat-stressed Yoruba, Fulani and FUNAAB-Alpha Chickens

	PCV (%)		RBC (x 10^6 ul)		Heterophil (%)		Lymphocyte (%)		Het/Lymph	
YORUBA	0 hour	1 hour	0 hour	1 hour	0 hour	1 hour	0 hour	1 hour	0 hour	1 hour
AA	30.43±0.52	30.35±0.48	2.77±0.52	2.73±0.33	29.8±5.60	31.3±4.89	62.3±6.25	60.6±4.96	0.51±0.15	0.53±0.13
AB	30.87±0.48	31.57±1.63	2.68±0.61	2.54±0.69	28.3±4.32	35.0±6.07	64.5±1.55	61.8±2.86	0.46±0.07	0.57±0.11
BB	31.69±0.42	31.76±0.42	2.80±0.41	2.69±0.56	28.8±5.12	29.0±4.90	57.8±3.33	62.8±4.36	0.50±0.06	0.47±0.10
FULANI										
AA	31.59±0.31	32.80±1.57	3.19±0.14	2.47±0.45	30.0±5.46	32.8±3.87^{a}	64.0±4.62	30.0±5.12	0.48±0.12	0.55±0.07
AB	31.88±0.22	32.69±1.42	2.78±0.36	2.72±0.40	29.3±4.72	30.4±5.69^{ab}	66.6±4.04	29.3±6.74	0.46±0.06	0.52±0.09
BB	32.14±0.36	32.78±0.22	2.58±0.25	3.06±0.15	30.2±1.41	32.1±2.50^{a}	59.1±4.03	30.2±4.07	0.49±0.04	0.54±0.02
FUNAAB-Alpha										
AA	32.54±0.84	33.82±2.29	3.10±0.33	3.00±0.29	34.3±1.97	28.5±1.34^{b}	59.8±6.78	56.6±7.28	0.54±0.08	0.61±0.06
AB	32.23±1.05	32.71±0.96	2.92±0.35	2.87±0.36	31.8±6.53	27.7±10.91^{b}	61.7±1.97	56.8±6.94	0.51±0.13	0.53±0.22
BB	33.24±0.76	33.94±1.08	3.04±0.38	3.30±0.39	32.6±2.88	37.7±5.21^{a}	64.6±2.35	61.3±3.55	0.49±0.04	0.51±0.09

Means ± SD with the same superscript along the column are not significantly different (P<0.05)

PCV: Packed Cell Volume, RBC: Red Blood Cell, Het/Lymph: Heterophil/Lymphocyte Ratio, 0 hour: before heat stress exposure, 1 hour: after heat stress exposure

REGRESSION MODELS FOR DESCRIBING WEIGHT-AGE RELATIONSHIPS OF YORUBA CHICKENS

4.9.1 Observed and predicted bodyweight of Yoruba chickens

The highest observed asymptotic bodyweight in Yoruba chicken is 1029.71g at week 24. Logistic model predicted the highest initial bodyweight (27.99g) while the Mitscherlich model predicted the highest final bodyweight (1072.14g). The least (1029.71g) and the closest to the observed mean bodyweight were also predicted by the logistic model. The observed live weight and the predicted body weight by all the five fitted growth models to Yoruba chickens are presented in Table 4.58.

4.9.2 Parameter estimates and Goodness of fit for Yoruba chickens

The maturity ratevalue was similar for Gompertz and Von Bertalanffy while logistic model produced the highest maturing rate value. The R^2 value ranged from 0.987 to 0.999. Gompertz and Von Bertalanffy had the same and highest R^2 value (0.999)while the least R^2 value was observed in Mitscherlich model. However, the least AIC and BIC values was obtained in Gompertz model. Conversely, Mitscherlich had the least AIC and BIC values (Table 4.59). Overall, Gompertz model had the highest R^2 value and lowest AIC and BIC values. In contrast, Mitscherlich model had the least R^2 value (0.987) and highest AIC and BIC values (85.390 and 85.580 respectively). The growth curves of Yoruba chickens are presented in Figures 4.6 to 4.9.

Table 4. 58 Observed and predicted body weight values for non-linear models relating growth to age of Yoruba Chickens

Age (week)	Observed Body weight(g)	Logistic Predicted	Gompertz Predicted	Von bertalanffy Predicted	Mitscherlich Predicted
0	24.45	47.99	21.23	8.25	-69.62
2	49.68	74.53	50.35	39.47	25.91
4	109.69	114.18	99.83	96.83	121.37
6	177.61	171.43	171.71	176.64	216.76
8	264.92	250.22	263.9	272.75	312.09
10	344.51	351.71	370.98	272.75	407.34
12	482.59	471.89	485.91	489.12	502.52
14	621.99	600.8	601.78	599.36	597.63
16	716.14	725.18	712.92	706.22	692.67
18	819.32	833.53	815.39	807.46	787.64
20	923.9	919.83	906.96	901.68	882.55
22	991.53	983.79	986.76	988.15	977.38
24	1029.71	1028.69	1054.96	1066.61	1072.14

Table 4. 59Parameter estimates (g) and standard errors (SE) of Logistics, Gompertz, Von bertalanffy and Mitscherlich functions of regression of body weight on age of Yoruba Chickens

	Models			
Parameters	Logistics	Gompertz	Von Bertalanffy	Mitscherlich
A	111.4.547	1365.605	500.906	1350708.147
B	22.223	4.171	4.161	135077.739
K	0.233	0.116	0.126	0.00
R^2	0.998	0.999	0.999	0.987
AIC	62.71	60.480	85.360	85.390
BIC	60.890	58.670	83.550	85.580
SE	15.932	33.872	12.461	0.007

A=Asymptotic weight, B=Constant of integration, K=Maturity rate, R^2=Coefficient of determination, AIC=Akaike' Information Criterion, BIC=Bayesian Information Criterion, SE=Standard Error.

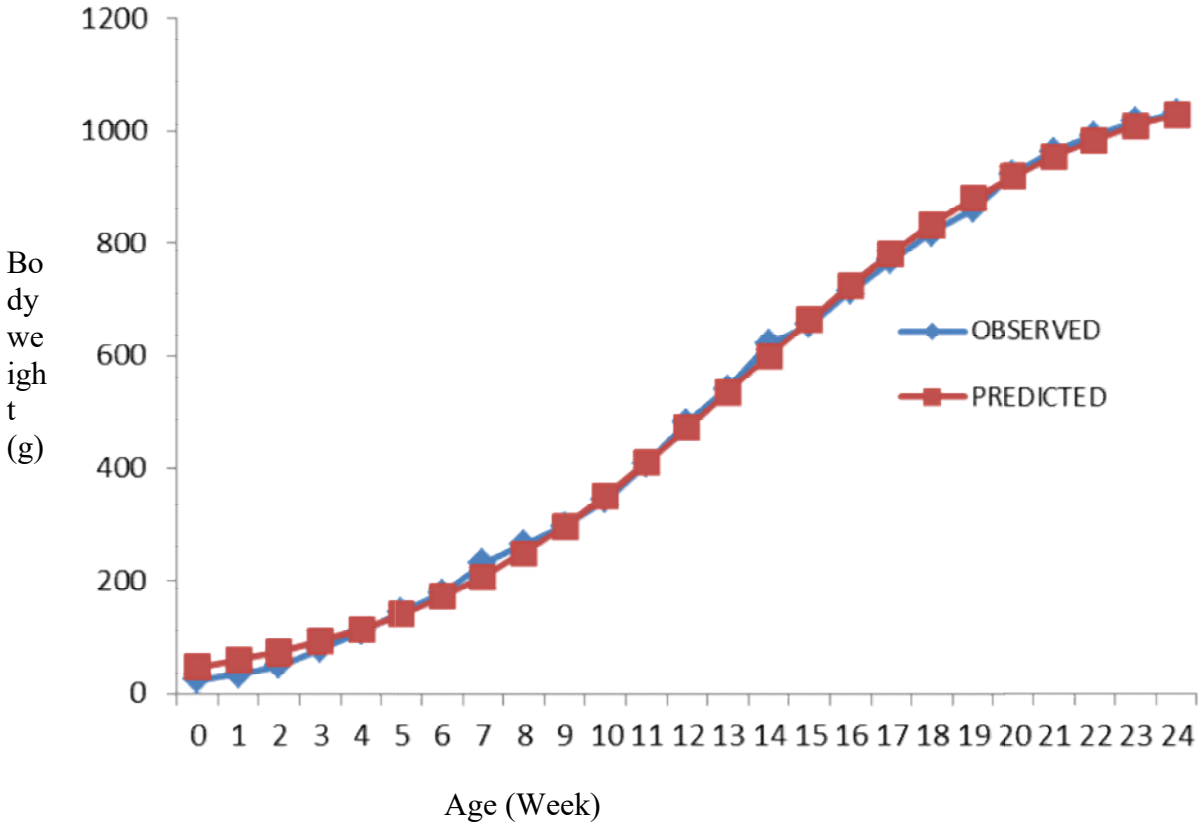

Figure 4. Logistic Growth curve for Yoruba Ecotype Chickens

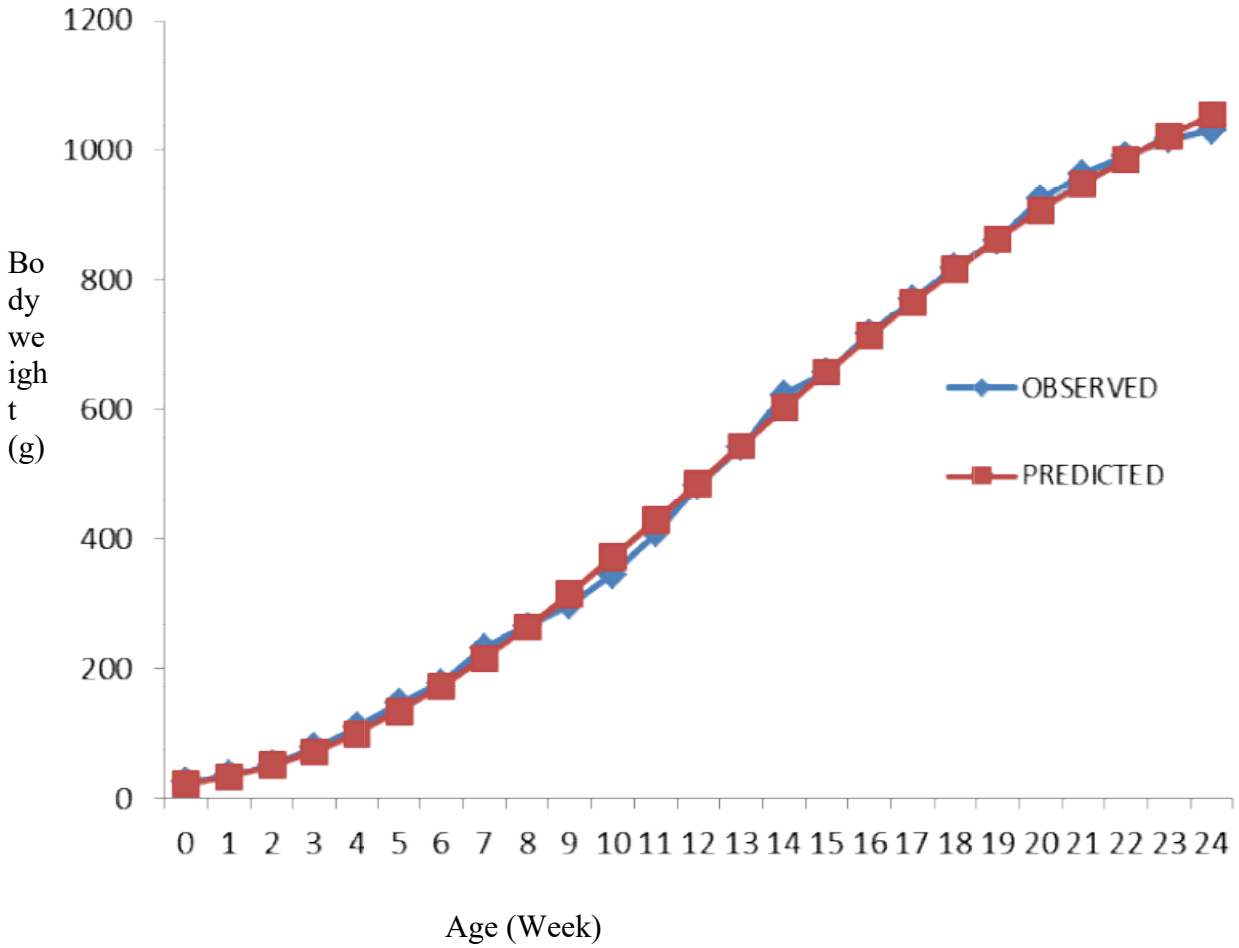

Figure 4.Gompertz Growth curve for YorubaEcotypeChickens

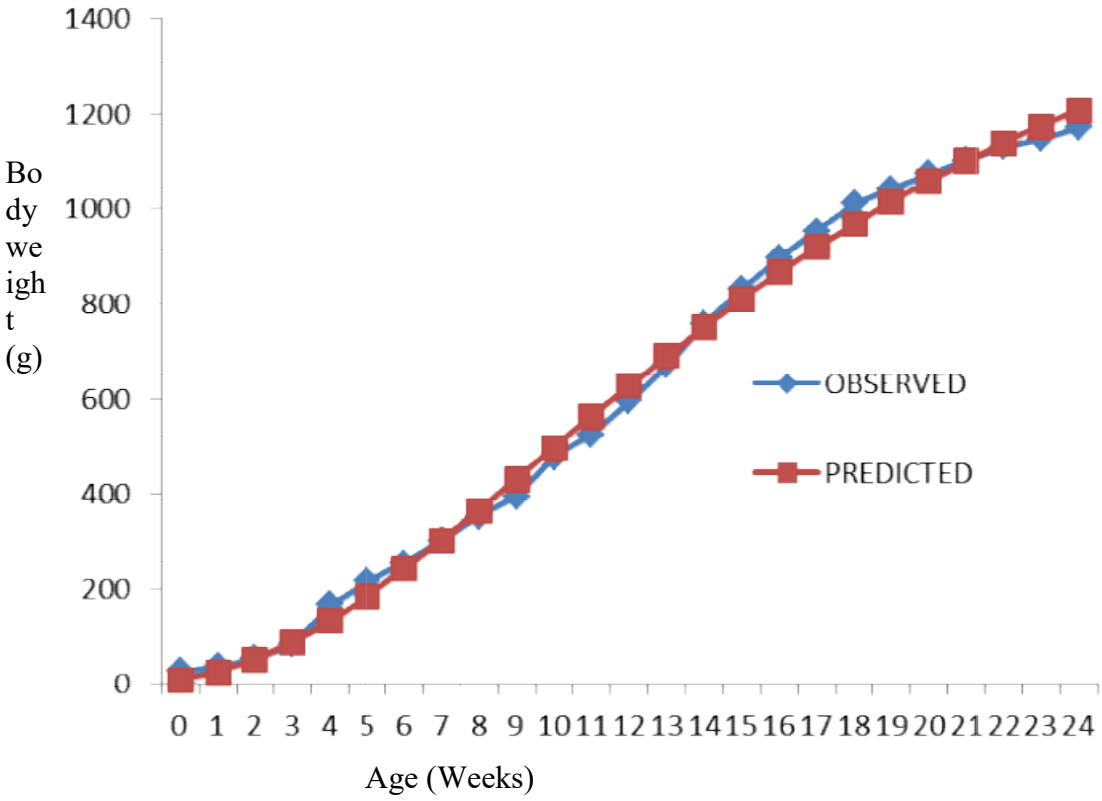

Figure 4. Von Bertalanffy Growth curve for Yoruba Ecotype Chickens

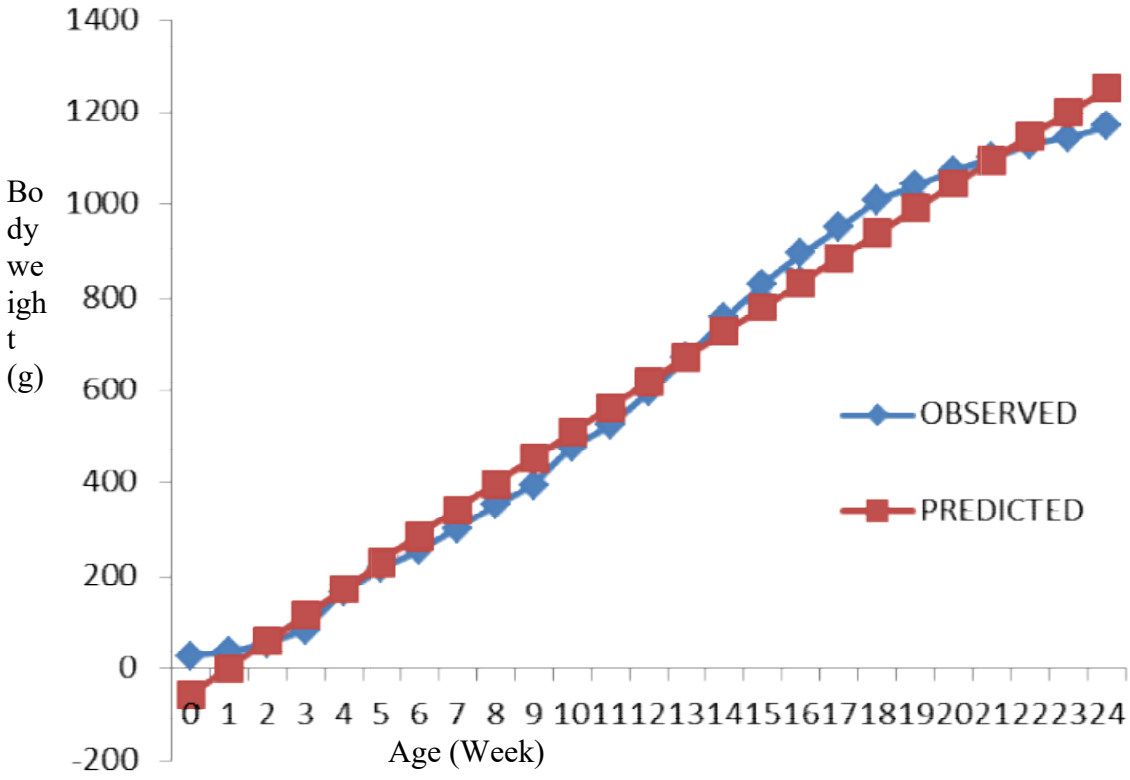

Figure 4. Mitscherlich Growth curve for Yoruba Ecotype Chickens

REGRESSION MODELS FOR DESCRIBING WEIGHT-AGE RELATIONSHIPS OF FULANI CHICKEN

4.10.1 Observed and predicted Bodyweight of Fulani Chickens

The initial observed body weight of Fulani chickens was 26.76g. Logistic model overestimated the initial body weight with a predicted value of 59.64g while, Mitscherlich model underestimated the initial weight value (-57.63g). However, Mitscherlich gave the highest predicted value at week 24. The observed live weight and the predicted body weight by all the five fitted growth models to Fulani chickens are presented in Table 4.60.

4.10.2 Parameter Estimates and Goodness of Fit for Fulani Chickens

Logistic model gave the highest mature weight value (1222.749g) and the highest maturity rate (0.248) while Mitscherlich model produced the least maturity rate (0.05). Details of the parameter estimates in Fulani chickens are presented in Table 4.61. The obtained R^2 values ranged from 0.987 to 0.997. The highest R^2 value was observed in Logistic and Gompertz model (0.997) while Mitscherlich model gave the least value of R^2 (0.987). Further, Logistic model had the least AIC and BIC values 71.0 and 69.01 respectively. However, Mitscherlich produced the highest AIC and BIC. The growth curves of Fulani chickens are presented in Figures 4.10-4.13.

Table 4. 60Observed and predicted body weight values for non-linear models relating growth by age for Fulani chickens

Age (weeks)	Observed Body weight(g)	Logistic Predicted	Gompertz predicted	Von bertalanffy Predicted	Mitscherlich Predicted
0	26.76	59.64	25.84	9.23	-57.63
2	52.89	94.97	65.73	52.24	57.96
4	165.83	148.54	134.44	132.38	172.31
6	253.48	226.26	232.66	240.79	285.44
8	351.37	332.09	354.22	365.92	397.37
10	476.59	464.34	488.84	497.5	508.1
12	595.09	613.01	625.73	627.73	617.65
14	757.17	761.49	756.06	751.36	726.03
16	895.18	893.25	874.04	865.22	833.25
18	1010.42	998.46	976.78	967.78	939.33
20	1072.77	1075.61	1063.62	1058.59	1044.28
22	1129.59	1128.72	1135.36	1137.95	1148.1
24	1169.13	1163.71	1193.6	1206.59	1250.82

Table 4. 61Parameter estimates (g) and Model selection criteria for Logistics, Gompertz, Von bertalanffy and Mitscherlich functions of regression of body weight on age of Fulani Chickens

	Models			
Parameters	Logistics	Gompertz	Von Bertalanffy	Mitscherlich
A	1222.749	1406.47	1575.96	10772.917
B	19.501	3.997	0.820	10830.544
K	0.248	0.133	0.094	0.005
R^2	0.997	0.997	0.996	0.987
AIC	71.0	71.530	75.68	88.720
BIC	69.01	69.720	73.870	86.910
SE	17.821	36.784	70.099	14418.794

AIC=Akaike's Information Criterion, BIC=Bayesian Information Criterion, SE=Standard Error. A=Asymptote weight, B=Constant of integration, K=Maturity rate, R^2=Coefficient of determination

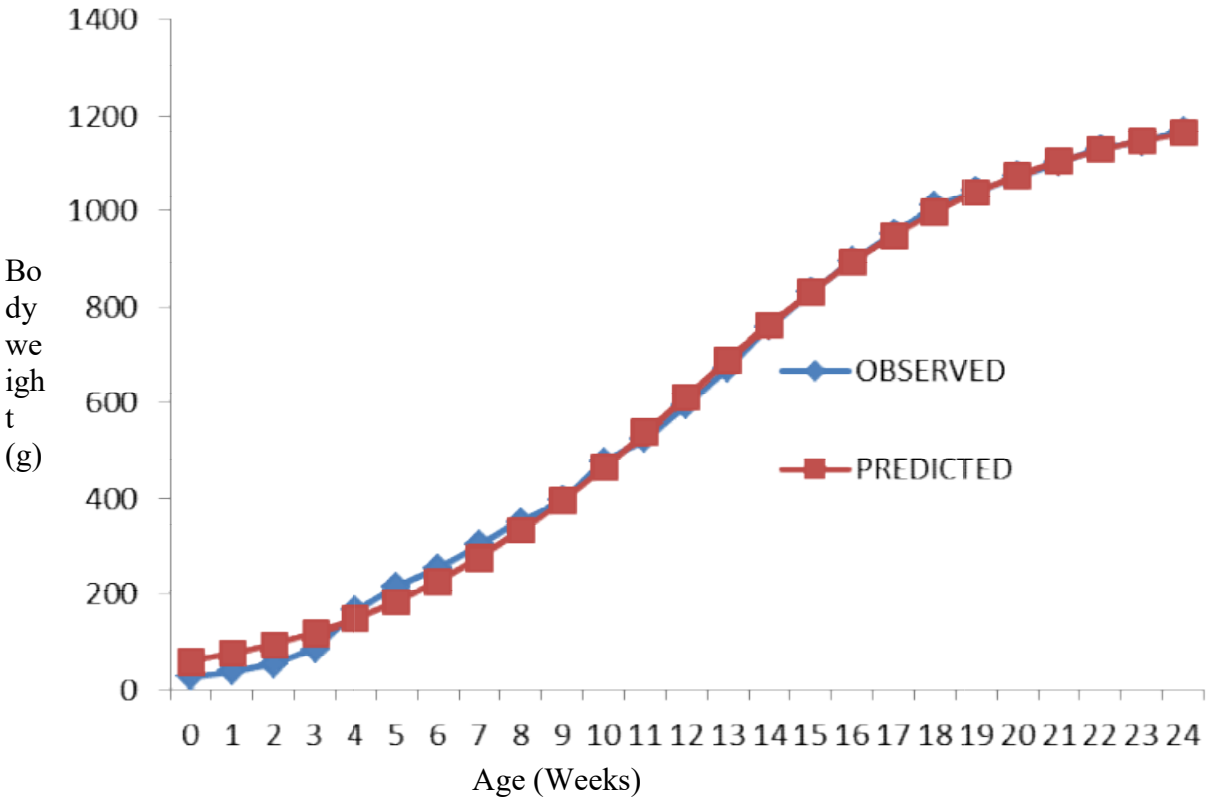

Figure 4.Logistic Growth curve for Fulani chickens

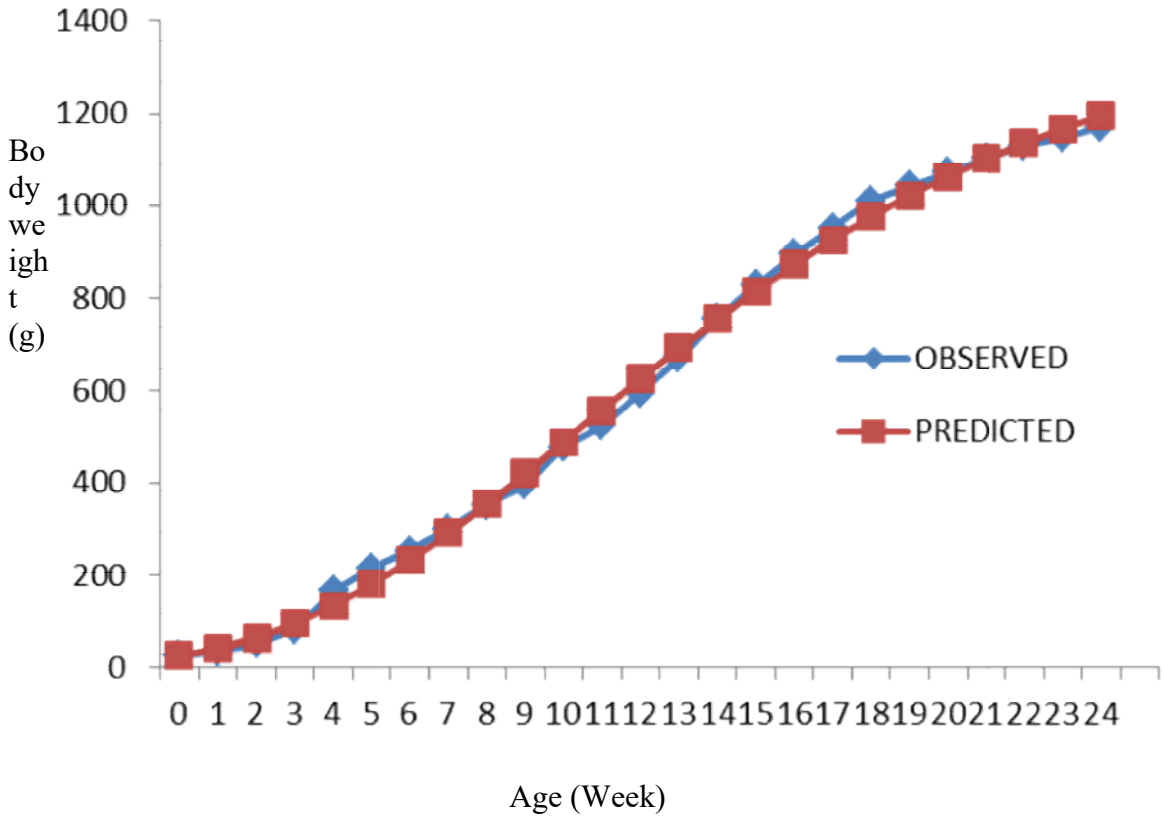

Figure 4. Gompertz Growth curve for Fulani EcotypeChickens

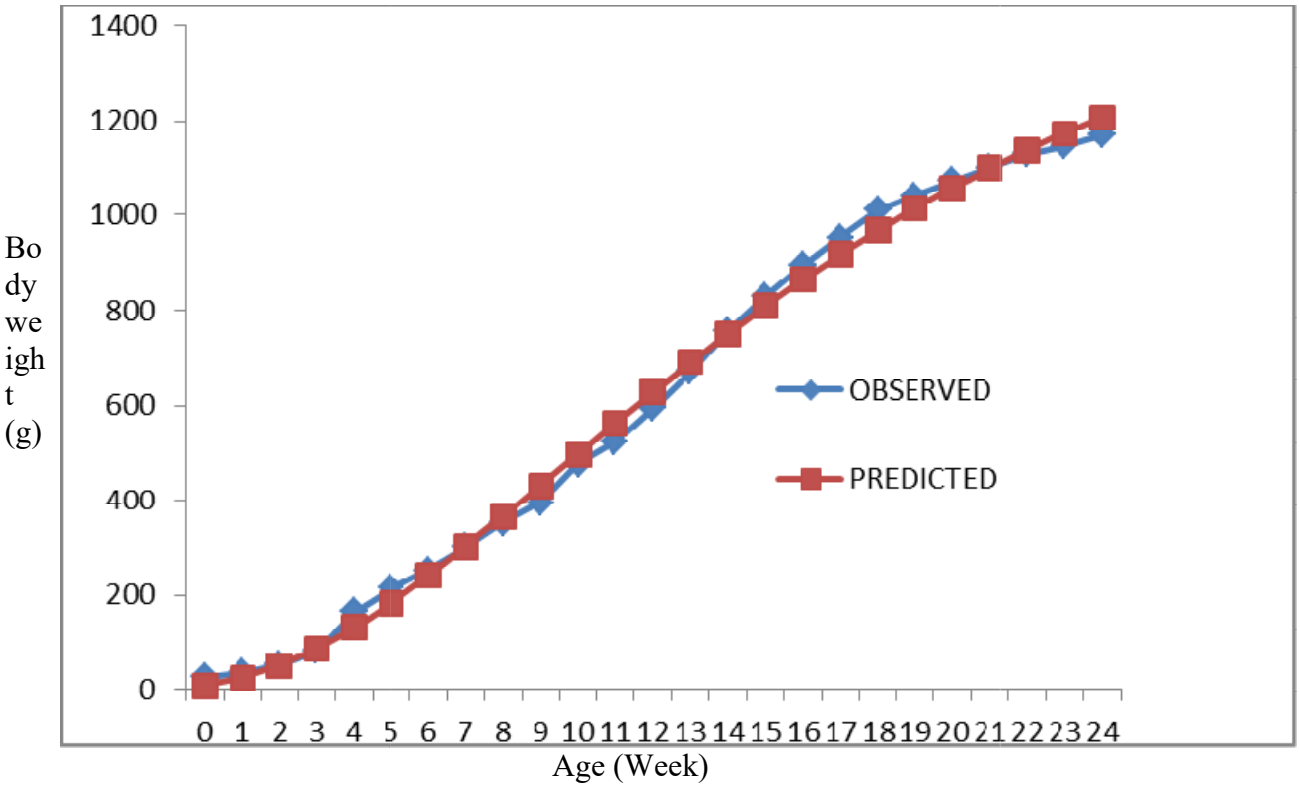

Figure 4. Von Bertalanffy Growth curve for Fulani EcotypeChickens

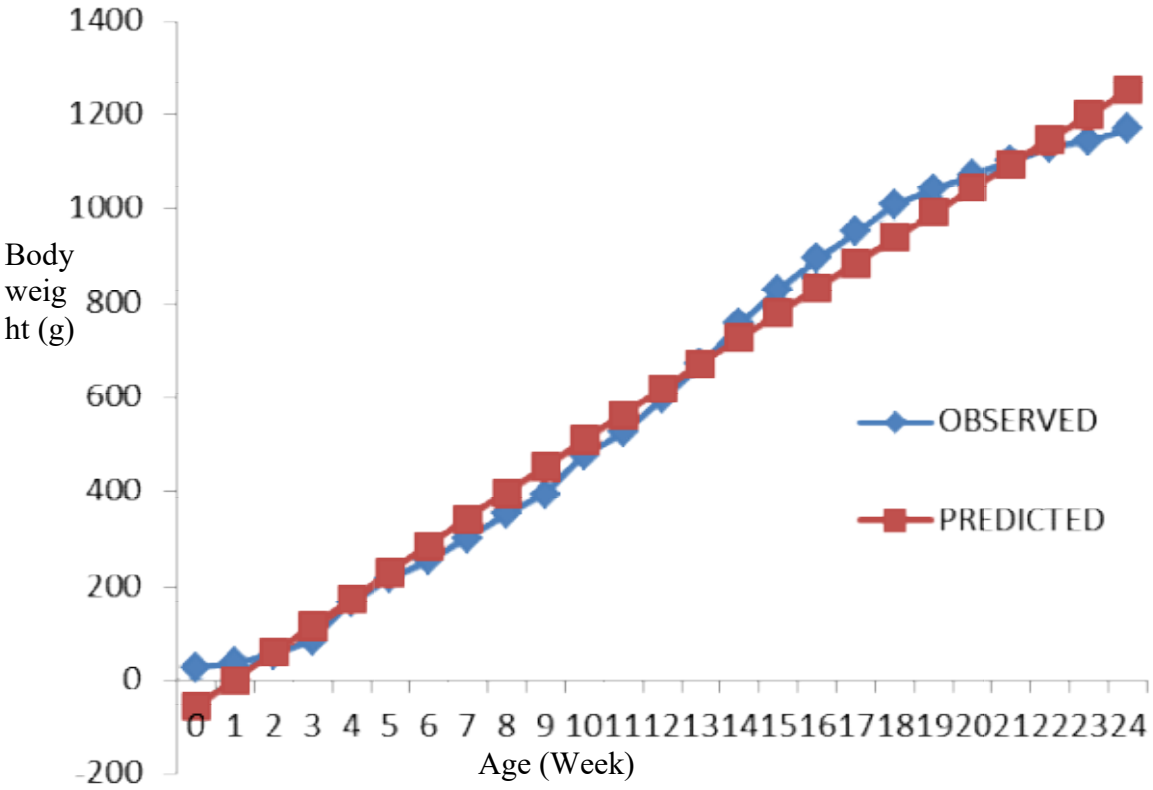

Figure 4.13 Mitscherlich Growth curve for Fulani EcotypeChickens

REGRESSION MODELS FOR DESCRIBING WEIGHT-AGE RELATIONSHIPS OF FUNAAB-ALPHA CHICKENS

4.11.1 Observed and predicted Bodyweight of FUNAAB-Alpha Chickens

All the four fitted models overestimated the initial bodyweight of FUNAAB-Alpha chickens with exception of Brody model. Brody model underestimated the hatch weight value (-54.20). The highest observed body weight at week 24 is 2291.63g. Brody model produced the highest predicted body weight (2392.47g) at week 24. While the least and closest to the observed value (2278.73) was predictedby the logistic model. The observed and predicted bodyweight for the growth of FUNAAB-Alpha chickens are presented in Table 4.62.

4.11.2 Parameter Estimates and Goodness of Fit for FUNAAB-Alpha Chickens

Logistic model produced the least mature body weight (2512.764) while the highest value of mature body weight was observed from Mitscherlich model. Conversely, the least integration parameter and maturity rate values were produced by Mitscherlich model while the highest values of integration parameter and maturity rate were gotten from the Logistic model. Details of the parameter estimates are presented in Table 4.63.The obtained R^2 values ranged from 0.992 to 0.997. The Mitscherlich model had the highestR^2value while the least was produced by Logistic model. In addition, the least AIC (83.72) and BIC (81.91) values were obtained from Von Bertalanffy model. The growth curves of FUNAAB-Alpha chickens are presented in Figures 4.14 to 4.17. Richard model (a four parameter model) failed to converge when fitted to the obtained growth data of Yoruba, Fulani and FUNAAB-Alpha ecotype chickens. Surprisingly, it produced no meaningful interpretable parameter estimate at an iteration rate up to 1200.

Table 4. 62Observed and predicted body weight values for non-linear models relating growth by age for FUNAAB-Alpha Chickens

Age (weeks)	Observed Body weight(g)	Logistic Predicted	Gompertz predicted	Von bertalanffy predicted	Brody predicted
0	34.58	169.13	99.84	127.83	-54.2
2	134.8	246.15	192.59	212.95	151.71
4	314	352.98	327.27	335.01	357.25
6	563.18	496.02	502.06	497.06	562.42
8	767.25	678.8	709.16	696.22	767.22
10	962.57	898.92	937.11	923.44	971.65
12	1157.05	1145.82	1173.5	1165.34	1175.72
14	1421.71	1401.64	1407.1	1407.32	1379.42
16	1556.23	1645.81	1629.12	1636.6	1582.76
18	1794.12	1861.26	1833.6	1844.05	1785.74
20	2067.22	2038.59	2017.2	2024.73	1988.34
22	2214.96	2176.38	2178.69	2177.28	2190.59
24	2291.63	2278.73	2318.4	2302.91	2392.47

Table 4. 63Parameter estimates (kg) and standard errors (SE) of Logistics, Gompertz, Von bertalanffy and Brody functions of regression of body weight on age of FUNAAB-Alpha Chickens

	Models			
Parameters	Logistics	Gompertz	Von Bertalanffy	Mitscherlich
A	2512.764	3006.709	2758	11480.859
B	13.857	3.405	1.884	1.00
K	0.204	0.107	0.140	0.001
R^2	0.992	0.996	0.995	0.997
AIC	97.470	88.150	83.720	86.890
BIC	95.66	86.350	81.910	85.080
SE	88.649	122.748	105.210	462668.370

A=Asymptote weight, B=Constant of integration, K=Maturity rate, R^2=Coefficient of determination, AIC=Akaike' Information Criterion, BIC=Bayesian Information Criterion, SE=Standard Error.

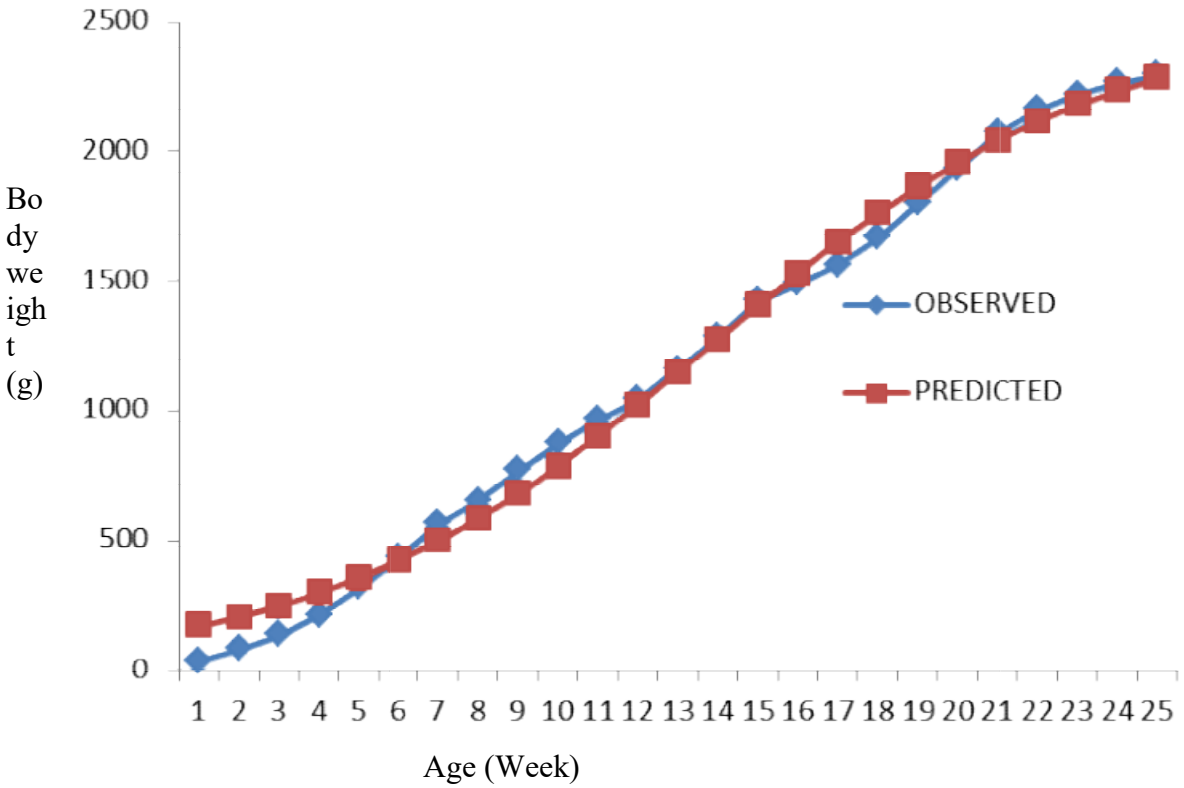

Figure 4. Logistic Growth curve for FUNAAB Alpha chickens

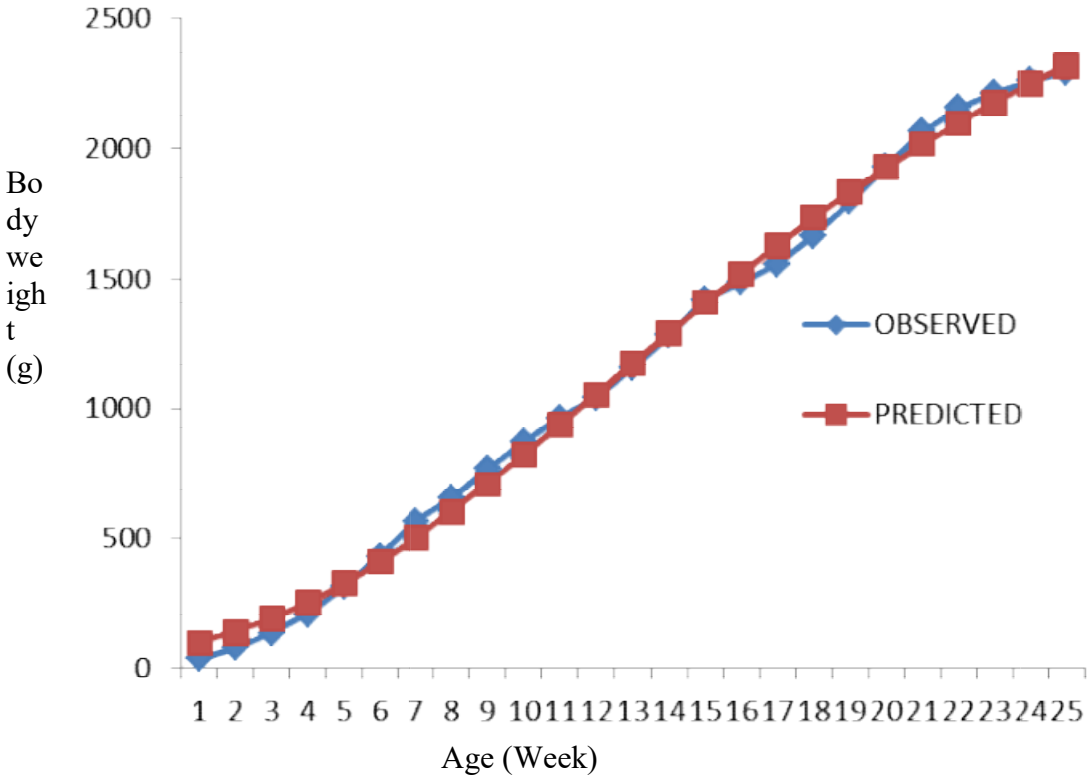

Figure 4. Gompertz Growth curve for FUNAAB Alpha chickens

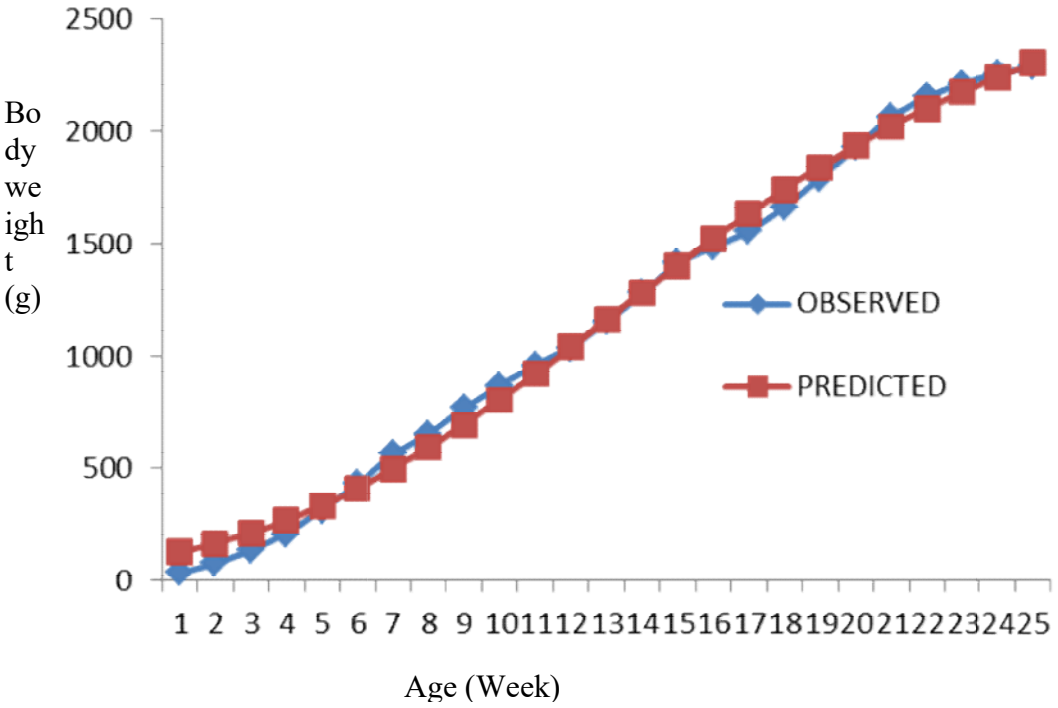

Figure 4. Von Bertalanffy Growth curve for FUNAAB Alpha chickens

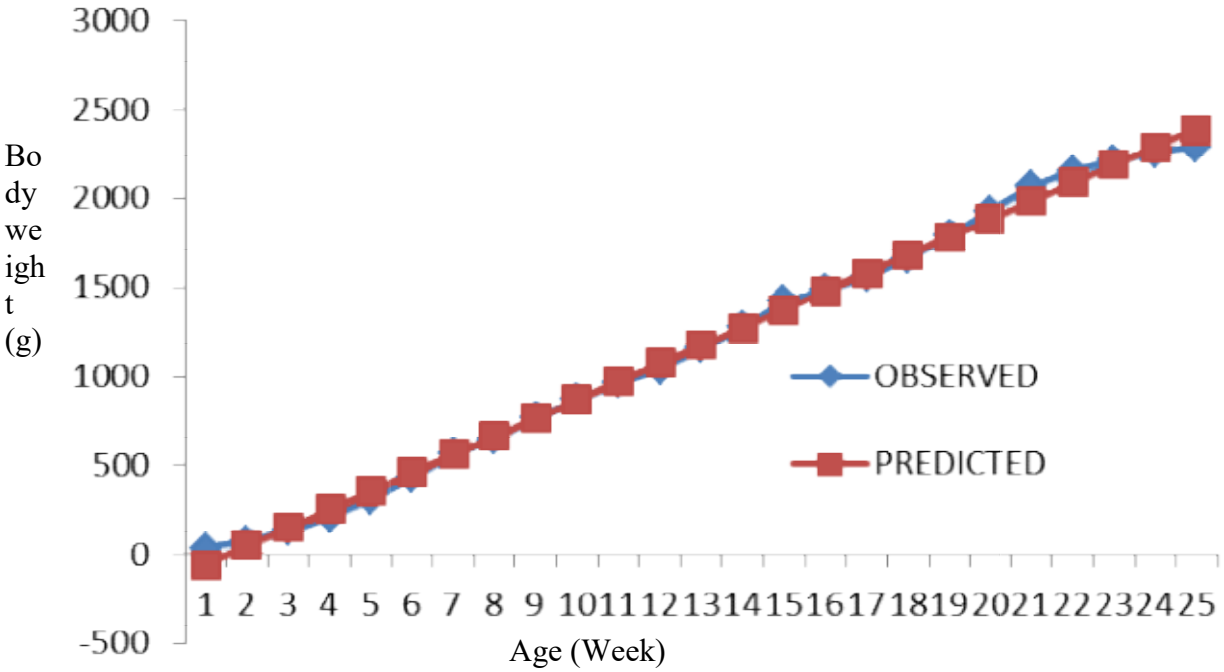

Figure 4. Brody Growth curve for FUNAAB Alpha chickens

CHAPTER FIVE

5.0 DISCUSSION

5.1 The growth-influencing genes

Myostatin gene

Myostatin (MSTN) is a multifunctional cytokines; highlyexpressed in the skeletal muscles of vertebrates with diverse effects on cell proliferation, growth and proper functioning (Piek *et al.,* 1999). The PCR amplification at 51^{O}C annealing temperature produced 373 bp of exon-1 MSTN gene in Nigerian chickens. The sizes of the single compact bands produced were estimated by comparing them with a molecular marker of 100 bp size. This is in accordance with the findings of Bhattacharya and Chatterjee, (2013) who also amplified 373 bp of MSTN gene in Indian chicken lines using the same set of primers that was used in this study. The observed heterozygosity values ranged from 21.4 (FUNAAB-Alpha) to 24.4% (Yoruba) chickens. However, the highest value of expected heterozygosity was recorded in Fulani chickens while FUNAAB-Alpha had the least value suggesting that; FUNAAB-Alpha chickens are the least diverse at MSTN locus, while Fulani chickens were the most diverse with 40.8% expected heterozygosity.

The highest and closest Shannon's Information Index values were obtained in the unimproved populations (Fulani and Yoruba chickens), while the least Shannon's Information Index value was found in FUNAAB-Alpha chickens. The obtained sequences of MSTN gene in Yoruba, Fulani and FUNAAB-Alpha chickens were compared with the available MSTN sequences at the NCBI GenBank. In all, the sequences showed 99 per cent homology to the exon-1 of *Gallus gallus* MSTN gene (Table 4.4). It thus confirmed that the amplified region is part of exon-1 of MSTN locus. The zero Expect-value obtained from the BLASTn search indicated that the match between the query sequence and the sequence from the data base was due to homology rather than chance. The 373 nucleotide sequences that were generated from this study were used to calculate the genetic distance among the three studied Nigerian chickens using the Neighbor-Joining

method.The 373 nucleotide sequences that were generated from this study were used to calculate the genetic distance among the three studied Nigerian chickens using the Neighbor-Joining method. Among the three studied chicken populations, the percent genetic similarity between the Yoruba and FUNAAB-Alpha was the highest (99.50%) while the least per cent similarity was observed between Yoruba and Fulani chickens (99.43%). Thus, the genetic distances revealed that the Yoruba chickens are more closely related to FUNAAB-Alpha than the Fulani chickens at MSTN locus.

The negative values of F_{IT} observed at two out of the studied loci with corresponding negative values of F_{IS} in the three studied chicken population showed the deficiency of homozygotes in the population (Table 4.5). This observed excess of heterozygotes could be due to non-random mating and genetic exchange between the populations. A large part of the total genetic diversity can be explained by the observed variation within breeds (0.137) and to a smaller extent by the variation among breeds (0.009).Restriction digestion of the amplified fragment of MSTN gene in all the three studied populations produced three genotypes AA, AB and BB (Plate 4.2). Genotype AA had the highest genotype frequency whileBB had the least genotype frequency (Table 4.3). Comparison of the observed and expected genotypes using chi-square test revealed that the studied populations deviate from Hardy-Weinberg equilibrium principle at MSTN locus. Deviation from the Hardy-Weinberg equilibrium implies that one or more assumptions of the Hardy-Weinberg equilibrium have been violated and that MSTN locus has been significantly affected by factors such as mutation, migration, non-random mating, selection *etc*. This was in agreement with the findings of Zandi *et al.,* (2013) in their study on MSTN gene in Western Azerbaijan Native Chickens.

Yoruba chickens with heterozygote AB of MSTN had higher bodyweight than their counterparts with homozygotes AA and BBat week 20. The body weight of Yoruba cocks (g) ranged from 1142.8±14.84g (BB) to 1169.6±9.74 (AB) at week 24. The dressed weight of Yoruba cocks with AB of MSTN (721.9±44.2g) was significantly higher than that of hens with BB (590.6±40.6g) though similar to dressed weight of hens with AA and AB as well as with cocks with AA and BB, respectively. This suggests that MSTN influence on Yoruba chicken growth and carcass development were not sex specific. Fulani hens with AB (MSTN) had significantly higher body weight (583.2±12.1g) than

BB (544.9±41.7g) but similar to AA (551.1±7.49g) at week 12. While cocks with AB (MSTN); had significantly higher body weight (692.0±15.7g) than cocks with AA (604.4±23.4g). Similarly, Fulani cocks had significantly higher body weight than hens from weeks 16 to 24 across all identified MSTN variants. The obtained higher body weight of both Fulani cocks and hens with AB (MSTN) indicates that AB-myostain could be a potential marker for selection of Fulani chickens for early growth rate. The dressed, drumstick, breast and thigh weights of Fulani cocks were significantly higher than those of their hen counterparts. This could be attributed to the sexual dimorphism in chicken which favor higher body size and consequently higher carcass cuts in cocks than hens.The FUNAAB-Alpha cocks with AB of MSTN had higher bodyweight (1771.6±34.9g) than BB (1181.7±51.9g) but similar to AA (1714.5±22.0g) at week 12. Similarly, the bodyweight of FUNAAB-Alpha hens with AB (1537.5±130.1g) was higher than those with AA (1377.9±34.2g). Significantly higher body weight was also observed between FUNAAB-Alpha cocks and hens at week 20 and 24.

The dressed weight of FUNAAB-Alpha hens with BB was significantly lower than those of AA and AB individuals. While other measured carcass traits were not significantly influenced by the MSTN variants.This indicates that MSTN/Alu1 could further be explored for its amenability as maker for selection of FUNAAB-Alpha chickens for early maturing rate. Success in this direction will aid selection of FUNAAB-Alpha chicken as meat-type line. Further studies may however be needed to validate this observation by using larger sample size since statistical significance is sample size sensitive. In a related study, the homozygote BB(MSTN) genotype chickens had significantly lower bodyweights than both the AA and AB genotype birds from Week 1 to 3 in Bian Chicken (Zhang *et al.,* 2011).Zhang *et al.,* (2012) also observed that chickens with both homozygote AA and heterozygote AB genotypes of MSTN gene had higher body weight values (from 6 to 18 weeks of age) than the homozygotes GG genotype in Bian chicken.

Bhattacharya and Chatterjee, (2013) reported significant association of some haplotypes of the MSTNgene with growth traits of three chicken populations (two broiler lines and a layer chickens). The PCR-SSCP analysis of MSTN gene in three Indian chicken lines (PD-1, Control Layer and Aseel) revealed a very high polymorphism and

significant association with both growth and carcass traits (Dushyanth *et al.,* 2016). In turn, Dushyanth *et al.,*(2016) reported that body weight at 4 and 6 weeks in PD-1 line were associated with the observed haplogroups and the measured carcass traits (carcass weight, leg weight, breast weight*etc.*) in both PD-1 and Aseel chicken lines. The observed association of the heterozygote AB genotype of MSTN gene to the growth traits of Nigerian chickens was also in agreement with the earlier findings of Paswan *et al.,* (2013) in broiler line where a significant association of MSTN genotypes with body weights at Week 4, 5 and 6 was reported.However, findings from this current study were slightly different from the submission of Gu *et al.,* (2004).

Gu *et al.,* (2004) observed that broiler chickens with homozygote BB and AA genotype of MSTN had the highest body weight at weeks 1 and 6. Also, Ye *et al*., (2007) reported the associations of MSTN gene polymorphisms with the performance and mortality traits in broiler chickens. The authors found that SNPs in MSTN were significantly associated with the growth and hen resistance to infectious bursal disease in three commercial broiler lines.Goats with heterozygote AB genotype had superior bodyweight at birth, 90 and 300 days of age (Zhang *et al.,* 2012).Similarly, Zhu *et al.,*(2007) found significant association between single nucleotide polymorphism in exon-1 of MSTN and Wenling grass-chicken carcass traits including: dressed, breast muscle and abdominal fat weight.

The obtained significant association of AB (MSTN) with some carcass traits of indigenous chickens in Nigeria were not consistent with the findings of (Gu *et al.,* 2004) in F2 crosses between broiler and Silky chickens. The authors found that birds with AA genotype showed higher breast muscle percentage than the AB genotype. The differences observed could be attributed to the genetic breed effect of the studied indigenous chickens in Nigeria and the reported breeds by the earlier authors. The repeated significantly higher bodyweight of chickens with AB (MSTN) in both cocks and hens across the threes studied population suggest that MSTN influence on growth and carcass traits are not sex specific.

Growth hormone gene

Growth hormone gene stimulates growth and development of chicken via the regulation of ovarian folliculogenesis, proliferation and differentiation of follicular cells, as well as

oocyte maturation (Khoa *et al.*, 2013). The PCR amplification of GH at 51 ^{o}C annealing and 5 minutes final extension produced 195 bp size fragment size contrary to the 56 ^{o}C and 15 minutes final extension earlier reported by Su *et al.,* (2014) in Qingyuan partridge and recessivewhite chickens using the same set of primers that was used in this study. These variations may be attributable to differences in the chicken population studied.

The RFLP analysis of GH with Van911 produced two alleles (A and B) and two genotypes (AA and AB). The frequency of A allele was the highest (0.84) compared to the B allele (0.16). This observation was consistent with the findings of Enayati and Rahimi-Mianji, (2009). The authors reported the dominance of A allele (with 0.99 allele frequency) in Mazandaran native breeder fowls of Iran.Prevalence of homozygote AA against heterozygote AB in the studied Nigerian chickens might be influenced by some factors such as genetic drift, natural selection, population size. There were no birds with homozygote BB in this present study. Similarly, Tanmankaur *et al*.(2008) and Mohsen, (2011) found no homozygote BB (at GH/MspI locus) in both Indian New genotype chickens and Punjab broiler respectively. Absence of genotype BB at GH locus would have undoubtedly favoured the incidence of other genotypes in the studied indigenous chickens in Nigeria. Selection for a trait of interest over generations (which could be natural) is also known to lead to an increase in the incidence of the candidate allele/genotype at the detriment of others. In contrast to the obtained result from this study, Enayati and Rahimi-Mianji, (2009) found 3% of the Mazandaran native breeder fowls population as homozygote BB genotypes individuals.

Singh *et al*.,(2008) observed the BB genotype with least frequencies in their studied chicken populations. Based on the obtained allele and genotype frequencies from this study; Alleles A and B of cGH/Van911 locus can be safely classified as polymorphic alleles. Previously, polymorphism of different regions of growth hormone gene have been reported in both indigenous (Enayati and Rahimi-Mianji, 2009) and broiler chickens (Mohsen, 2011). The chi-square test analysis of the expected and observed genotype frequencies showed that the population conformed to the Hardy-Weinberg Equilibrium (HWE) at GH locus. Conformity of the population to HWE at GH locus indicates that; the population has not been significantly affected by selection, mutation or genetic drift.

Phylogenetic tree derived from sequences of Nigerian Chickens (Yoruba, Fulani and FUNAAB-Alpha) and GH sequences downloaded from the GenBank showed that Yoruba ecotypechickens are more closely related to Fulani chickens while FUNAAB-Alpha is more related to the downloaded GH sequence (MG906784.1) than the Yoruba and Fulani chickens. Thus, highest genetic similarity value was observed between the Yoruba and Fulani chicken ecotypes (99.995), while the least percent genetic similarity value was obtained between FUNAAB-Alpha and the downloaded GH sequence (99.927) from the GenBank.The obtained genetic similarity values in this study is closer to the per cent similarity values reported by Mohsen, (2011) among the four studied Indian chicken populations (99.2 - 99.7).

Genetic homology study (using BLASTn tools) between Yoruba, Fulani, FUNAAB-Alpha chickens and the reference sequence from the GenBank revealed that sequences obtained from Nigerian chickens (Yoruba, Fulani and FUNAAB-Alpha chickens) has a multi-digit negative exponential expect-values and 100% homology with the *Gallus gallus* reference data from the GenBank. This confirmed that the amplified region is exon-1 chicken GH region and that the obtained match occurred due to homology rather than chance. Similarly, Mohsen, (2011) also found 99% homology between the studied Indian chickens and the reference sequence from the GenBank at this same locus. The present result indicates that the growth hormone gene sequence is conserved among the different breeds/strains of chicken.

Fulani and Yoruba Chickens ecotype chickens with heterozygote AB genotype of GH gene had heavier mean body weight while the homozygotes AA individuals appeared to have better growth performance than the heterozygotes AB in FUNAAB-Alpha chickens. However, there was no significant difference between the measured body weight and the GH genotypes in all the three studied chicken populations. This implies that GH genotypes had no significant influence on the growth traits of the studied chicken populations. Further studies may however be needed to validate this observation since an increase or decrease in sample size often influence the level of significance. Similarly, Bingxue *et al.,*(2003) and Mohsen, (2011) reported that GH genotypes had no significant effects on body weight of New Genotype chickens and Red Cornish chickens at 0-6 week and F2 crosses between broilers and silky chickens respectively. In contrast, Mohsen,

(2011) found significant effects of GH genotypes on the sixth week body weight of the Indian University Male line chickens and Punjab Broiler strains.

Recently, Nguyen *et al.,* (2015) reported an association between GH genotype and live body weight of Thai native chickens from 0-16 weeks. More so, relationship between the cGHgenotype and body weight at hatching up till 10 weeks of age was reported in Thai broilers (Anh *et al.,* 2015).In this study, the observed GH genotypes have no significant effects on all the measured carcass traits across the three studied chicken populations (Table 4.18). This is in agreement with the report ofBingxue *et al.,* (2003). The authors reported no significant effects of GH genotype on all the measured live weight and carcass traits with the exception of breast muscle and abdominal fat. This is not unexpected since no significant association was found between the obtained genotypes of GH/Van911 and the growth traits of the studied birds. Conversely, Nie *et al.,* (2005) found significant association between GH genotype and carcass traits of F2 reciprocal cross between the WRR and X Chinese chicken breeds. Overall, sex and GH genotype had no significant interaction effect on all the measured growth and carcass traits in this study (Table 4.20).

Ghrelin gene

Ghrelin gene (GHRL) is a key factor in the hypothalamic melanocortin system which is involved in various bioactivities of animals including poultry. Primarily, ghrelin gene plays a fundamental role in feed intake and energy balance in birds (Rossi *et al.,* 2008). The RFLP analysis of the amplified 373bp of GHRL gene using *TspR I* resulted in three restriction patterns which correspond to AA, AB and BBgenotypes in all the three studied chicken populations. The A allele had the highest frequency compared to the Ballele. Overall, AA genotype constituted 60% of all the studied birds, followed by AB genotype (29.8%) while BB genotype had the least genotype frequency in the studied Nigerian chickens. Polymorphism of different regions of ghrelin gene have been reported in 12 Chinese indigenous chickens (Li *et al.,* 2006), four Indian chicken strains (Mohsen, 2011), Botswana indigenous chickens and broiler chickens (Sharifinejad*et al.,* 2015).The chi-square test of the observed and expected genotype frequencies showed that Yoruba and Fulani Chickens were in Hardy-Weinberg equilibrium at GHRL locus. It also implies that

Yoruba and Fulani chickens have not been significantly affected by external factors such as non-random, inbreeding or selection. This agrees with the findings of (Nie *et al.,* 2004) in Xinghua, Beijing Fat, Recessive White and Silky chickens at this same locus. In contrast, chi-square test results of observed and expected genotype in FUNAAB-Alpha chickens from this study deviated from the Hardy-Weinberg equilibrium principle. This could be attributed to thesignificant impacts of selection, inbreedingor non-random mating of FUNAAB-Alpha chickens at GHRL locus.

The BLASTn results showed a very high expect values ranged 2.00E-116 to zero. The genetic distance analysis revealed 98 to 100 percent genetic similarity with the *Gallus gallus* GHRL locus in the GenBank. The observed zero and negative multi-digit expect value indicate that the obtained high homology value (99%) was truly due to homology rather than any other external influence such as mutation. The phylogenetic tree derived from GHRL sequence from this study revealed that FUNAAB-Alpha is more closely related with the downloaded GHRL sequence (99.903) followed by Yoruba chickens, while Fulani chickens had the least percent similarity with the GHRL from the GenBank (99.824). Similarly, Mohsen, (2011) observed high homology of more than 99 per cent between New Genotype, Red Cornish and Panjab Broiler strains and that of the available GHRL sequences from the GenBank.

The three obtained Ghrelin genotypes (AA, AB and BB) in this study were associated with weekly body weights of Yoruba, Fulani and FUNAAB-Alpha chickens. A significant difference in body weight was observed in Fulani chicken at Week 12. Birds with homozygote BB was the heaviest (618.41±24.10 g) compared to AA and AB genotypes. Similarly, Yoruba chickens with BB genotypes had higherweekly mean body weight at Week 20. Polymorphisms at different regions of GHRL gene have been reported to have significant effects on growth and carcass traits of chickens. For instance, significant association were reported between:haplotype genotype of GHRL gene and body weight of Tibetan chicken and Recessive White chicken breeds at week 16 (Li *et al.,* 2006), GHRL genotype and six week body weight of NG and PB1 birds, where GHRL genotype VV had heavier body weight compared to LL homozygote (Mohsen, 2011), 8 bp indel of *cGHRL* gene and body weight of F2 crosses of White Recessive Rock and Xinghua chickens at different ages (Fang *et al.,* 2006).

In addition, association of GHRL genotypes with the eight measured carcass traits in this study showed that; BB genotype birds had significantly higher dressed weight, drumstick weight and wing weight compared to AA and AB genotypes in Fulani chickens. However, the GHRL genotype had no significant effects on all the measured carcass traits in Yoruba and FUNAAB-Alpha birds. Related studies revealed significant association between 8 bp indel of chicken*GHRL* gene and carcass traits of F2 crosses of White Recessive Rock and Xinghua chickens (Fang *et al.,* 2006), three SNPs of the *cGHRL* gene (C-2047G, A-2220C and A-2355C) and abdominal fat weight (Nie *et al.,* 2009). More so, polymorphism in *GHRL* and *GHSR* genes have been associated with abdominal fat weight and leg muscle protein in chicken as well as ducks (Nie *et al.,* 2009). Recently, a significant association between T1857C polymorphism of ghrelin receptor and the carcass traits of F2 *Kampung* x broiler cross was reported (Khaerunnisa *et al.,* 2017).

The body weights of Yoruba cocks were superior to their hen counterparts. However, weekly body weights of Yoruba cocks with genotype AA were similar to those with AB and BB variants except at week 12, where birds with homozygote BB genotype had higher body weights compared with cocks with AA genotype. Similarly, the body weights of FUNAAB-Alpha hens with BB were higher than those with AA but similar to those BB at week 12 (Table 4.35). In contrast, the body weights of Yoruba hens with genotype BB were not different from those with genotypes AB and AA. In turn, the dressed weight of Yoruba cocks with AA were similar with those with BB but higher than those with AB. However, all other measured carcass traits were not influenced by ghrelin genotypes in both the Yoruba cocks and the hens. This suggests that the expression and influence of ghrelin gene were not sex specific.

The body weight of Fulani cocks with BB of GHRL was heavier thanthose of hens with genotype AB but similar with those with AA and BB at week 8. Further, the body weight of Fulani cocks was higher than the body weight of their hen counterparts from weeks 12 to 24. However, all the measured carcass traits were significantly higher for Fulani cocks than hens. This observation could be adduced to the sexual dimorphism which usually favors the cocks than the hens (Momor *et al.,* 2010). Generally, sex and GHRL genotypes in this study had no significant interaction effect on all the measured growth and carcass traits (Table 4.26 and 4.27). This implies that the effects of the

polymorphic variants of GHRL/*TspR I* gene on the studied birds' growth and carcass traits were not sex specific. However, there is no information in the accessible literature to compare this observation. Thus, further study using larger sample size and or gene expression study would be needed to validate this observation.

Pituitary transcription factor-1 gene

Pituitary transcription factor (PIT-1) is involved in muscle growth by regulating the expression of other key growth-correlated genes such as prolactin, thyrotropin *etc* in the pituitary gland(Renaville *et al.,* 1997).In this study, a 243 bp fragment of PIT-1 gene was PCR-amplified using the previously designed primer sets by Nie *et al.,* (2005). Two alleles with corresponding two genotypes (AA and AB)were observed following the RFLP analysis of PIT-1 gene using *Hinf1* restriction endonuclease. Allele A had the highest allele frequency compared to the allele B. This is similar to the findings of Nie *et al.,* (2008) in the cross between White Recessive Rock and Xinghua chickens. Chi-square analysis revealed that the observed and expected genotypes frequencies from this study conformed to the Hardy-Weinberg equilibrium. The conformity of the studied populations at PIT-1 locus to the Hardy –Weinberg equilibrium indicated that the observed PIT-1 gene frequency was not significantly different from the expected genotype frequencies. This agrees with the submissions of Bhattacharya *et al.,* (2012) in chicken at PIT-1 locus.

The BLASTn search of the NCBI database produced a multi-digit negative exponential expect-values and very high percent genetic identity (99 to 100) with the *Gallus gallus* PIT-1 sequences from the NCBI GenBank. These results indicate that the high matches between the derived sequences from this study and the available sequences from the database were neither due to mutation nor chance. Thus, the BASTn results confirmed that amplified region is part of exon-2 of *Gallus gallus* PIT-1 gene. This agrees with the earlier reported sequences by Nie *et al.,* (2005). The derived Phylogenetic tree obtained from sequences from the Yoruba, Fulani and FUNAAB-Alpha chickens and the downloaded PIT-1 sequences from the NCBI GenBank showed that Yoruba and Fulani chickens are more closely related at PIT-1 locus than the FUNAAB-Alpha chickens.

The body weight of Yoruba, Fulani and FUNAAB-Alpha cocks with homozygote AA of PIT-1 were not significantly different from the body weight of their counterparts

with heterozygote AB variant from weeks 0 to 24. Similarly, the body weight of Fulani and Yoruba hens with AA of PIT-1 were not significantly different from those with heterozygote AB variant of PIT-1 from weeks 0 to 24 except at week 16 where the body weight of Yoruba hens with AB (680.8±22.5g) was significantly higher than the body weight of hens with AA (604.2±18.7g). The weight of all the measured carcass traits of Yoruba and Fulani cocks and hens with AA of PIT-1 were not significantly different from the weight of Yoruba cocks and hens with heterozygote AB variant except the thigh and breast weight of Yoruba cocks which were significantly lower for hens with AA than those with AB. However, the dressed weight of FUNAAB-Alpha hens with AB (1454.5±69.13g) was significantly higher than the dressed weight of hens with AA (1346.5±19.6g) but similar to dressed weight of cocks with AA (1474.8±22.0g) and AB (1494.9±17.3). In contrast, Jiang *et al.,* (2004) found significant association between PIT-1 gene polymorphism and body weight at week 4, 6, 8, 12, average daily weight gain and other measured body conformation traits in chickens.

McElroy *et al.,* (2006) reported quantitative trait loci for body weight in chicken at a very close location to PIT-1 gene. Further investigation however revealed that SNPs in PIT-1 gene played crucial roles on chicken growth (Nie *et al.,* 2008). These among other findings led to the final submission of Nie *et al.,* (2008)that PIT-1gene polymorphism significantly influences chicken growth traits. Contrary to the findings from this study, Rodbari*et al.,* (2011) reported that chickens with the homozygote BB genotype of Pit1-*Taq1* loci have significantly higher body weight at Week6. The discrepancies could be due to differences in the chicken breeds studied or the restriction enzyme used; since restriction enzymes are known to be restriction site specific.Similarly, Rodbari*et al.,* (2011) reported significant association of Pit-1loci polymorphism with higher breast muscle, leg muscle, wing and back weight in chickens. In Yoruba chickens, PIT-1 genotypes had no significant association with all the measured carcass traits in this study. Although heterozygotes AB carrying chickens appeared to have higher mean carcass traits compared to the AA genotypes. This is consistent with the reports of Nie *et al.,* (2008). The authors found no significant association between PIT-1gene polymorphism and all the measured carcass and fatty traits in Indian chickens.

Myogenin gene (MYOG)

Myogenic regulatory factors (MRFs: Myogenin, MyoD, Myf5 and MRF4) are specific transcription factors that essentially regulate muscle formation, growth and regeneration. The MRFs aid initiation of the conversion of muscle satellite cells to muscle stem cell. The myogenin gene controls the differentiation of myoblast to myotubes (Naka *et al.,* 2013).In this study, A 152 bp fragment of exon-1 of myogenin gene was successfully amplified and sequenced using the earlier reported primers by Wei *et al.,* (2016). The RFLP analysis resulted in uncut single band in all the studied birds. *Hinf1* and *EcoR1* were found unsuitable for RFLP analysis of exon-1 of MYOG gene in Yoruba, Fulani ecotypes and FUNAAB Alpha Chickens. This was adduced to the lack of restriction site for the enzymes used in this study. Previously, polymorphisms and mutations in MYOG gene have been reported and possible effects of the obtained SNPs have been investigated in farm animals. Wang *et al.,* (2008) detected three mutations in seven chicken breeds at the MYOGgene region in seven chicken breeds.

The PCR-SSCP analysis showed four mutations (T/C in locus A, and T/A, T/C and A/G in locus B) in MYOG gene promoter region (Wang *et al.,* 2008). Studies of the effects of MYOG polymorphisms on growth, carcass and meat quality traits revealed significantly higher body weight at week 6-10 in Bian chickens with FF genotype of MYOG than EE birds (Wei *et al.,* 2016). Related association study on pig showed significant differences in the birth weight and the backfat thickness among the different myogeningenotypes. However, their weaning weight and mature body weight of those pigs were similar (Hui-Liang and Zhong-Xiao,2006). Similarly, Verner *et al.,* (2007) reported significant differences among different genotypes of MYOG gene and some of the studied pig carcass traits including but not limited to eviscerated yield. A significant effect of MYOG genotypes on the carcass and meat quality characteristics of three crossbred pigs has been established. However, there was no correlation between the MYOG gene and the pH value, and intramuscular fat of the studied pork (Kapelanski *et al.,*2005).

In addition, bioinformatic analysis showed that MYOG sequence from this study was highly similar to the exon-1 of the chicken MYOG region. Similarly, the obtained result from the analysis of the MYOG gene sequence from this study and the retrieved

chicken sequences from the GenBank proved that Yoruba chicken had the highest genetic similarity (99.979 %) with FUNAAB Alpha chickens.

Heat shock protein 70 gene

Heat stress is among the most challenging environmental conditions affecting poultry growth and production, particularly in the tropical regions. The indigenous poultry species are exposed to the influence of high ambient temperatures and high relative humidity that characterize the tropical climate because they are reared mainly under the extensive management system (Ogundipe, 1990). Unlike the intensive commercial chicken management system where measures are always in place against adverse effects of heat stress, smallholder chicken producers usually provide temporary light shade and radiation shield which are often grossly inadequate to ward off the heat stress effects (Adedokun and Sonaiya, 2001). Recommended measures against effects of heat stress are not only often unaffordable to the smallholder farmers but also practically difficult to implement by smallholder chicken producers that dominate rural sub-Sahara Africa. Thus, studies that focus on the selection of heat-tolerant chicken genotypes with concomitant better growth performance are imperative.

The PCR amplification of HSP70 gene generated a 360 bp fragment size in Nigerian chickens (Plate 4.10). The RFLP analysis of HSP70 with *Mme1*restriction endonuclease produced two alleles (A and B) and three genotypes (AA, AB and BB). Overall, AA genotype had the highest genotype frequency while BB genotype was the least frequent in all the studied chicken populations (Table 4.36). Thus, HSP70/*Mme1* gene can be safely concluded to be polymorphic in the studied Nigeria chickens. Similarly, Mahmoud, (2000) obtained three different allelic fragments for the chicken HSP70gene when *PstI* was used as restriction enzyme. In another similar study, Mazzi *et al.,* (2003) reported two polymorphic sites (A258G and C276G) in different broiler chickens. Recently, three genotypes (AA, AB and BB) and three single nucleotide polymorphisms (A258A, A258G, and G258G) were found in Taiwan Native chickens using single-strand conformation polymorphism analysis (Liang *et al.,* 2016). The highest Shannon's information index was obtained in Fulani while FUNAAB-Alpha chickens had the least Shannon'sIndex value. In contrast, Yoruba chickens had the least fixation index

value (39.2%) while the highest fixation index value was obtained in Fulani chickens.In addition, the chi-square analysis of the observed and expected genotype frequencies showed deviation from the Hardy-Weinberg equilibrium. This implies that, HSP70 locus in the studied birds has not been significantly affected by factors such non-random mating, mutation, genetic drift and or selection which could either be natural or deliberate selection for the trait of interest by man.

The HSP70 genotypes had no effect on the weekly bodyweights (from week 0 to 24) of Yoruba, Fulani and FUNAAB-Alpha chickens. Similarly, HSP70 gene genotypes had no significant effectson egg weight at first egg and the number of eggs laid until 40 weeks of age in Taiwan native chickens (Liang *et al.,* 2016). However, in contrast to the obtained findings from this study, Liang *et al.,* (2016) reported significant effects of the PCR-SSCP detected chicken HSP70 genotypes on body weight at 0–16 weeks and body weight at first egg of Taiwan native chickens. The authors recorded highest body weight at week 1 and lowest body weight at first egg in the BB genotype birds compared to birds that carried other genotypes. These discrepancies could be due to either difference in chicken breeds studied in both studies or different molecular approaches used as the authors utilised PCR-SSCP as against PCR-RFLP that was used in this study.

Rectal temperature (RT), pulse-rate (PR) and respiratory rate (RR) are the most important measure of poultry response or adaptation to the heat stress. Following acute heat stress exposure, the observed average respiratory rate (RR) from this study ranged between 42.5 and 52.06 beat/min. FUNAAB-Alpha chickens had the highest average respiratory rate (52.06beat/min) while the least RR value was obtained from Yoruba chickens. This is closer to the earlier reported average RR value (44.6 and 51.07beat/min) by Isidahomen *et al.,* (2012) and Adedeji *et al.,* (2015) in the exotic and indigenous Nigerian chickens under heat stress. The higher RR value in FUNAAB-Alpha agreed with the reports of Robert (1994) that the body size of the animal affects the respiratory rate. Similar observation was also made by Isidahomen *et al.,* (2012) where the heavier chicken breed had the highest respiratory rate. In addition, Defra (2003) submitted that body weight, species and breed affected the heat production by poultry, thus, increase in ambient temperature led to increase panting rate consequently increases in respiratory rates.

The average observed rectal temperature value from this study (40.69^{o}C) was slightly higher than the average value reported by Isidahomen *et al.,* (2012) in Nigerian chickens. Breed has no significant effects on the observed rectal temperature of the acute heat stressed chickens studied. Conversely, Iheukwumereand Herbert, (2003) observed significant difference in rectal temperature values among the chicken breeds. The respiratory rate of FUNAAB-Alpha chickens with AB and BB genotype of HSP70 genewere not significantly differentfrom theFulani chickens with BB genotype. More so, Yoruba chickens with AA and AB genotype of HSP70 gene are not significantly different from Fulani AA and AB genotypes. The obtained values of the rectal temperature ranged from 39.68 to 41.66 ^{o}C. The observed changes in physiological and hematological parameters by the acute heat stressed birds could indicate an attempt to maintain thermal equilibrium.

The PCR-SSCP derived HSP70genotypes had no significant effects on respiration rate and CT of acute heat stressed Taiwan indigenous chickens (Liang *et al.,* 2016). Similar submission was made by Nascimento *et al.,* (2012) when exotic and Nigerian locally adapted chickens were subjected to acute heat stress.The highest pulse rate (PR) value was observed in FUNAAB-Alpha chickens (330.72±4.74 breath/min) followed by the Fulani (302.05±6.44 breath/min) while the least was found in Yoruba chickens. This is in line with submission of Yalcin *et al.,* (1997) that body size of chicken influences the respiratory and pulse rate.The heat stress index value of the acute heat stressed Nigerian chickens ranged 1.36±0.06 to 1.52±0.02. The highest heat stress index was observed in FUNAAB-Alpha while Yoruba chickens had the least heat stress index value. Thus, FUNAAB-Alpha chickens could be the most affected by the acute heat stress compared to Yoruba and Fulani chickens since higher heat stress index indicates higher severity of the heat stress (Isidahomen *et al.,*2012).This observation could be due to higher body size of FUNAAB-Alpha birds and or their genetic makeup. Body size affects tolerance to heat stress and the exotic chickens are less tolerant to heat stress than the tropically adapted chickens. More so, crossbreeding might have led to reduced toleranceof the FUNAAB-Alpha chickens.

The homozygote BB genotype of HSP70 gene had the least heat stress index values in all studied chicken populations with the exception of Yoruba chickens where

heat stress index between the BB and AB genotypes were not statistically different ($P<0.05$). Thus, HSP70/*Mme1* genotypes influenced heat tolerant traits of the studied chickens and BB genotypes appeared to be more heat-tolerant compared to other genotypes. In contrast with this observation, Tamzil*et al.,* (2014)reported that chickens with heterozygote (AD) genotype of HSP70 as the most heat-tolerant and homozygote (BB) as the least heat-tolerant. The discrepancies between the observed values from this study and reports of Tamzil*et al.,* (2014) could be due to differences in chicken breeds studied or different methodology used; as the author utilised PCR-SSCP as against the PCR-RFLP that was used in this study.

There was a slight increase in the PCV values of birds with AB and BB genotypes following acute heat stress exposure. The highest PCV value was obtained from birds with genotype BB (32.91±0.26%)while the homozygote AA birds had the least observed value (31.52±0.25). Noticeable differences were also reported by Isidahomen *et al.,*(2012) between the PCV values of heat stressed frizzle feathered, naked neck and normal feathered chickens of Nigeria. The authors ascribed the decreased PCV values to adverse climatic condition. While, the frizzle feathered birds were reported to have the highest PCV values compared to the naked neck and normal feathered chickens. In this study, the HSP70 genotype had no significant effects on the RBC, Lymphocytes, heterophil and heterophil/lymphocytes ratio of the studied birds prior to acute heat stress exposure.

Following acute heat stress, the average RBC value of the homozygote BB birds were not significantly different from those of the heterozygote AB individuals.The obtained RBC values of the AA and AB genotype carrying birds followed similar trend. Similarly, the heterophil and heterophil/lymphocytes ratio values are not significantly different following the acute heat stress exposure.Yoruba chickens had the least PCV (31.23±0.27%) and Hb (8.56±0.11) value while there was no significant difference among the obtained PCV values for FUNAAB-Alpha and Fulani chickens. The obtained values of PCV and Hb are in agreement with the findings of Clubb and Schubot (1991). The authors attributed higher PCV and Hb values to a higher weight gain in the studied population. Similar submission was made by Oke *et al.,* (2007) where the reported highest PCV and Hb values of naked neck chickens was adduced to its comparatively higher body weight compared to the Normal feathered genotype. However, breed, environment and

season have been reported to have significant effects on chicken haematological parameters (Isidahomen *et al.,* 2012). The observed values of heterophil, lymphocytes and heterophil/lymphocytes are not significantly different across the three acute heat-stressed chicken populations. This observation implies that acute heat stress has no significant effect on the heterophil, lymphocytes and heterophil/lymphocytes values of the studied birds. The significant effects of breed on the physiological and haematological responses of the studied birds to acute heat stress corroborated the submission of Adedeji *et al.,* (2015).The packed cell volume and haemoglobin values were significantly influenced by the HSP70 genotypes.

Birds with homozygote BB genotype had the highest average PCV and Hb values while the least was observed in Yoruba chickens. However, there was no significant difference in the obtained values of RBC, Lymphocytes, heterophil and heterophil/lymphocytes ratio in all the studied chicken populations. These observations slightly disagreed with the findings of Tamzil*et al.,* (2014)**.** The authors reported significant increaseinthe percentage of heterophil, basophil, lymphocyte and heterophil/lymphocyte ratio with the exception of eosinophil and monocyte which were not statistically different in the acute heat stressed Arabic, kampong and commercial chickens.

5.2 Growth Models

Growth modelsconveniently summarise the information provided on animals intoconciseinterpretable biologicalparameters that can be used to describe animal growth over time and to estimate the expected weight of animal at a specific age.The hatching weight of FUNAAB-Alpha (34.58g) was the highest compared to the Yoruba (24.45g) and Fulani (26.76g) chickens. The average body weight at day-old in this study (24.45g - 26.76g) are within the range (24.27 – 30.2g) that was reported by Osaiyuwu *et al.,* (2009) in Yoruba and Fulani chickens. However, the average hatching weight of FUNAAB-Alpha chicken (34.58g) was significantly higher than those of Yoruba (24.45g) and Fulani (26.76g) chickens. This higher average hatching weight above those of the unimproved stocks could be adduced to the successful genetic gain on FUNAAB-Alpha chicken improvement project. This is because higher body weight at day-old has been found to have positive association with superior mature weight in animals including poultry.

FUNAAB-Alpha chickens had the highest mature bodyweight (2291.63g) compared to the Fulani and Yoruba chickens at Week 24. FUNAAB-Alpha chicken was over 1000g higher than the both Fulani and Yoruba chickens at Week 24. However, the mature body weight of FUNAAB-Alpha was lower than 2115g that was reported by Olawumi and Fagbuaro, (2011) in commercial broilers at week 12. The higher hatching and mature bodyweight recorded in FUNAAB-Alpha chickens (above the average of obtained body weight of Yoruba and Fulani chickens) indicated that they have truly been genetically improved.Momoh *et al.,* (2010) comparatively evaluated the growth traits of Nigerian local chickens and their crossbred. The authorsreported that crossbreeding could potentially increase the currently obtainable mature body weight of the former and that the obtained mature body weights of the Fulani ecotype (heavy ecotype) chickens were not significantly differentfrom those of the crossbred groups as from weeks 8-20. The crossbred groups (heavy and light ecotype: Fulani X Yoruba ecotype) grew consistently and significantly higher than the straight crossbreds (light and light ecotype: Yoruba X Yoruba ecotype) during the weeks 12-20 (Momoh *et al.,* 2010).

In Yoruba Chickens,comparisons of asymptotic weight obtained with all the four successfully converged models showed that Gompertz (1365.605g) and Logistic (111.4.547g) models are better than those of Mitscherlich and Von Bertalanffy based on the predicted weights. This is in agreement with the report of (Kucuk and Eyduran 2009) that ranked Gompertz ahead of Logistic models in terms of asymptotic weight. Similarly, Narinc *et al.,* (2010) ranked Gompertz first followed by Richards then Logistic model. Furthermore, Gompertz and Von Bertalanffy had the same rate of maturing (k) value (0.116) while the highest maturing rate 'k' value was found in Logistic model.The coefficient of determination (R^2) was generally high for all the four fitted models in Yoruba chickens (0.987 – 0.999). This is similar to the reported R^2 values of 0.985 - 0.999 by Narinc *et al.,* (2010) in chicken. The generally high observed R^2 values in this study indicate that all the models adequately described the observed Yoruba chickens growth data. Comparatively, the Mitscherlich model had the poorest fit (highest BIC and AIC values and lowest R^2 value) while the Gompertz model best described the Yoruba growth data (lowest BIC and AIC values and highest R^2). Similarly, Hruby *et al.,* (1996) observed that the Gompertz gave the best description of broiler growth compared to the Logistic

and linear models. Aggrey, (2002) also opined that Gompertz growth model is one of the best models for describing chicken growth data.

Recently, Selvaggi *et al.,* (2015) found that Gompertz model fitted live weight data of Italian chicken better than the Logistic and Richards growth curve models. This claim was supported by the submissions of Teleken *et al.,* (2017) thatGompertz model was the best model for chickens with the highest R^2 and lowest AIC and BIC values in Athens-Canadian chickens. However, Ersoy *et al.,* (2006) ranked Richard ahead of Gompertz model as the best descriptor of chicken, turkey and ostrich growth data. The observed discrepancies in the submissions of the authors of the accessible literature could be adduced to factors such as breed of the chicken studied or other environmental factors such as the feeding regime.

In Fulani chickens, Mitscherlich model produced the highest asymptotic weight (10772.917g) while the least value was observed in Logistic model (1222.749g). Conversely, the least rate of maturing parameter 'k' value (0.005) was observed in Mitscherlich model while Logistic model produced the highest k value (0.248). Similar to the observation from this study, Narinc *et al.,* (2010) reported varied A, B and k values from their studies when some non-linear models were fitted to the same chicken growth data. This submission; in line with the observation from this study suggests that; the model of choice affects parameter estimates. In Fulani chickens, the highest R^2 value was observed in Logistic and Gompertz model (0.997) while Mitscherlich model gave the least value(0.987). Further, Logistic model had the least AIC (71.0) and BIC (69.21) values while, Mitscherlich produced the highest AIC and BIC value. Comparatively, Logistic model produced the best fit to the Fulani chicken growth while Mitscherlich produced the poorest fit. This agrees with the submission of Va´zquez *et al.,* (2012) that ranked the Logistic equation as the best model to describe the chicken growth data particularly by using AIC and BIC values as selection criteria. In addition, Ozkan and Kocabas,(2004) found that the Logistic model gave the best fit to unselected quail populations when compared with the Exponential, Bertalanffy, Gompertz and Brody models.

In FUNAAB-Alpha Chickens, Logistic model produced the least asymptotic weight 'a' value (2512.764) while the highest value of asymptotic weight 'a' was

observed from Mitscherlich model. Conversely, the least rate of maturity value 'k' and parameter 'B' value were produced by Mitscherlich model while the highest values of parameter 'B' and 'k' were obtained from the Logistic model. The asymptotic weights estimated in this study were closer to the values reported by Selvaggi, *et al.,* (2015) and Teleken *et al.,* (2017) in Italian chicken breed and Athens-Canadian chickens, respectively.The observed R^2 values in FUNAAB-Alpha ranged 0.992 to 0.997. These R^2 values fall within the reported range (0.938 – 0.982) by Hruby *et al.,* (1996) in an unselected quail population. The generally high R^2 for all models indicates that they adequately described FUNAAB-Alpha growth data. However, the Von Bertalanffy model best described the live weight data of FUNAAB-Alpha (highest R^2value and lowest BIC and AICvalues). Similarly, Gbangboche *et al.,* (2008) ranked the four fitted models to West African Dwarf sheep in the following order Von Bertalanffy, Gompertz, Brody then Logistic. Overall, Aggrey, (2002) and Roush *et al*., (2006) reported the appropriateness of Gompertz curve to describe the growth of chickens, Zhang *et al*., 2008 and Osei-Amponsah *et al*., (2014) reported the superiority of Richards model while; Yang *et al*., (2006) suggested the correctness of Bertalanffy equation. All these differences may be due to breed or population structure, feeding and other environmental conditions.

5.3 Summary, Conclusion and Recommendation

5.3.1 Summary

FUNAAB Alpha chickens had the highest weekly mean body weight from hatch to maturity compared to Yoruba and Fulani ecotype chickens. In addition, the mature body weight and other carcass traits such as dressing weight, breast muscle weight, thigh weight, and drum stick weight of FUNAAB Alpha was the highest followed by the Fulani chickens. Sexual dimorphism was not noticeable in the studied birds until about 12 weeks of age and persisted until maturity.Fulani birds with homozygote BB genotype of GHRL gene had significantly higher bodyweight at Week 12. In addition, BB genotype was significantly associated with dressed, drum stick and wing weights of Fulani chickens ($P<0.05$). However, GHRL genotypes had no significant association with all the observed values of growth and carcass traits in Yoruba chickens.The heterozygote genotype AB of PIT-1gene was observed to be significantly associated with body weight at Week 12 and

16 of FUNAAB-Alpha and Fulani chickens respectively. In turn, heterozygotes AB favor better dressing weight of Fulani and FUNAAB Alpha Chickens. The RFLP analysis of MYOG gene using *Msp1* and *EcoR1*restriction endonucleases showed uncut single band in all the studied birds. Thus; *Msp1* and *EcoR1*were found unsuitable for RFLP analysis of exon-1 of MYOG gene in Yoruba, Fulani and FUNAAB Alpha Chickens. This was adduced to the lack of restriction site for the enzymes used in this study. Further, search of the NCBI database showed that MYOG sequence from this study was highly similar to the exon-1 of the chicken MYOG gene region. Three genotypes (AA, AB and BB) were obtained from RFLP analysis of HSP70/Mme1 gene in all the studied chicken breeds. The homozygote BB genotype of HSP70 gene had the least heat stress index values in all the studied chicken populations with the exception of Yoruba chickens where HSI of the BB were similar to the AB genotypes. Thus, HSP70/Mme genotypes influenced heat tolerant traits of the studied chickens and BB genotypes appeared to be more heat-tolerant compared to other genotypes.

Generally, all the successfully sequenced gene regions showed varied degree of genetic distance and similarity values. Comparatively; the highest genetic distance value (2.57) was observed between Yoruba and Fulani chickens at exon-1 of MSTN gene locus, while the least genetic distance value (0.003) among the three studied Nigerian chickens was found between Yoruba and FUNAAB-Alpha chickens at GHRL locus.Of all the five fitted asymptotic growth models, the Gompertz, Logistic and Von Bertalanffy model best described the growth patterns of Yoruba, Fulani and FUNAAB-Alpha chickens respectively.

5.3.2 Conclusion

The weekly body weights of FUNAAB Alpha ecotype chickens were consistently heavier than those of Yoruba and Fulani ecotype chickens.Indicating that the former has been truly genetically improved. Myostatin, ghrelin, heat shock protein-70 and pituitary transcription factor-1 genes were polymorphic in all the three studied populations. Genotypes (AA and AB) of Growth hormone gene did not have significant effect on growth and carcass traits of indigenous chickens in Nigeria.The observed variants of GHRL, MSTN and PIT-1 genes were found to be associated with early growth traits of the

studied chickens and hence could be potential markers for selection for body weight in the production of broiler lines. The homozygote BB genotypes of HSP70/Mme gene appeared to be more heat-tolerant compared to others. Similarly, FUNAAB-Alpha chickens had the highest heat stress index value. The derived phylogenetic trees suggest that the studied birds are genetically distinct chicken populations.

5.3.3 Recommendation

Future assessment of genetic variation within/among different breeds/ecotypes is imperative in order to provide basis for improvement or further improvement of Nigeria indigenous chickens. More so, genetic variation between breeds/populations for important genes and their frequencies can help to understand dynamics of genetic change owing to factors such as natural selection, breeding strategies and genotype x environment interaction. Thus, the observed variations in the studied genes and variants of the investigated genes that were associated with superior growth and carcass traits needs to be further studied; in order to validate and establish them as marker for selection of Nigeria indigenous chickens for improved growth performance.

5.4 Contributions to Knowledge

1. Myostatin, ghrelin, heat shock protein-70 and pituitary transcriptic factor-1 genes were observed to be polymorphic while myogenin was monomorphic which imply that EcoR1 restriction enzyme was not suitable for restriction digestion of exon-1 of chicken myogenin gene.
2. The average body weight of FUNAAB-Alpha chickens was about 1000g heavier than those of Yoruba and Fulani chickens from weeks 20 to 24.
3. The growth and carcass traits of indigenous chickens in Nigeria with genotype AB-myostatin were superior to their counterparts with AA-myostatin and BB-myostatin genotypes.
4. The hematological responses of acute heat stressed indigenous chickens (40±1°C for one hour)were similar. However, acute heat-stressed Fulani and FUNAAB-Alpha chickens with genotype BB-HSP70 had significantly lower heat stress index than those of AA-HSP70 and AB-HSP70 which indicates higher thermo-tolerance.

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