ANALYSES OF INTERFERON REGULATORY FACTOR-5 GENE AND ITS ASSOCIATION WITH PRODUCTIVE AND ADAPTIVE TRAITS IN NIGERIAN INDIGENOUS CHICKENS

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DECLARATION

I hereby declare that this thesis was written by me and is a correct record of my own research work. It has not been presented in any previous application for any degree of this or any other University. All citations and sources of information are clearly acknowledged by means of references.

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CERTIFICATION

We certify that this Thesis entitled "Analyses of Interferon Regulatory Factor-5 Gene and its Association with Productive and Adaptive Traits in Nigerian Indigenous Chickens" is the outcome of the research carried out by S.O. Durosaro in the Livestock Science and Sustainable Environment Programme, Federal University of Agriculture, Abeokuta.

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ABSTRACT

Interferon regulatory factor-5 (IRF-5) gene is involved in immune defence against virus, stress response, cell differentiation and growth. This study was conducted to analyse IRF-5 gene and associate its haplotypes with productive and adaptive traits in Nigerian indigenous chickens (NICs). Three hundred and thirty-two chickens (111 Normal Feather, 112 Naked Neck and 109 Frizzle Feather) were used for the experiment which lasted for 20 weeks. The birds comprised 147 males and 185 females. Productive traits (body weight, linear body parameters and feed efficiency) and adaptive traits (thermotolerance, immune response to Newcastle disease (ND) and survivability) were measured weekly, except the immune response to ND which was measured twice (24 and 91 days of age). Blood samples were collected from the birds for the determination of immune response to ND and deoxyribonucleic acid extraction. Exons 3, 4, 5 and 7 of IRF-5 gene were amplified and sequenced. Data collected on productive and adaptive traits were subjected to General Linear Model. Single nucleotide polymorphisms (SNPs) present in exons 3, 4, 5 and 7 of IRF-5 gene were identified and analysed using ClustalW, DnaSp and SNAP[2] software packages. Diversity and evolution of the regions were determined using DnaSp and MEGA6 softwares, respectively. Association analysis was done using mixed model procedure of SAS (Version 9.0). Body weight, breast girth and all the thermotolerance traits were significantly ($p<0.05$) affected by genotype of the birds in all the weeks considered. The highest ($p<0.05$) breast girth was recorded in Normal Feather chickens at weeks 16 and 20. Maternal immune response to ND was also significantly ($p<0.05$) affected by genotype of the birds with naked neck chickens generating highest immune response. Frizzle feather chickens had the lowest survivability in all the weeks. Four SNPs, 33A>G, 48G>A, 57T>C and 174T>C, were identified in exon 3 of IRF-5 gene

in all the three genotypes. Exon 4 of the gene was conserved while three of the SNPs (164T>C, 170C>T and 347G>A) identified in exon 7 were shared among the three genotypes. Linkage disequilibrium of 1.00 existed between 33A>G and 57T>C polymorphisms identified in exons 3 of normal feather and frizzle feather chickens. Most of the SNPs identified exons 3, 5 and 7 were synonymous and singletons which could not be used for association study. Phylogenetic analyses based on IRF-5 gene revealed that Red Jungle Fowl formed the ancestral lineage of NICs. Prediction of selective events revealed that both adaptive and purifying selective forces were acting on IRF-5 gene of NICs. Haplotypic differences in exon 3 of IRF-5 gene accounted for feed utilisation and thermotolerance while haplotypic differences in exon 7 of IRF-5 gene accounted for body weight and feed utilisation in NICs. The study recommended that haplotypes identified in exons 3 and 7 of IRF-5 gene can be used in marker-assisted selection when improving feed utilisation and thermotolerance in Nigerian indigenous chickens.

DEDICATION

This Thesis is dedicated to my wife, Mrs. Durosaro, Titilope and to my son, Durosaro, David Oluwafikayomi, for their love and encouragement.

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TABLE OF CONTENTS

Content **Page**

LIST OF TABLES

Table **Page**

LIST OF FIGURES

Figure **Page**

LIST OF PLATES

Plate **Page**

CHAPTER ONE

1.0 INTRODUCTION

Chickens are most widely known for their uses as food (meat and eggs), commercial products (feather products and vaccines) and experimental animals in developed countries (Dessie *et al*., 2011). However, chickens in developing countries have more diverse uses and benefits to households. In the tropics, indigenous chickens are kept for socio-cultural and religious functions such as entertainment, funeral right, spiritual cleansing, biological clock, pest control, ingredient of health dishes, gift and dowry (Njenga, 2005; Dessie *et al*., 2011).

Indigenous chickens have evolved through adaptation to various agro-climatic conditions. They possess gene combinations and special adaptation not found in other improved modern breeds (Egahi *et al*., 2010). They are found in all developing countries and play a vital role in many poor rural households (Alders, 2004; Alexander *et al*., 2004). They provide scarce animal protein in the form of meat and egg which are sold to meet essential family needs such as medicine, clothes and payment of school fees (Bagnol, 2001). Indigenous chickens are generally owned and managed by women in Africa, so they are essential elements of female-headed households (Gueye, 2000; Bagnol, 2001). Also, indigenous chickens have an inherent scavenging and nesting behaviour. Years of natural selection, under scavenging conditions, have made them robust and tolerant/resistant to various diseases. They have a better survival rate than commercial hybrid strains under local production conditions (Minga *et al*., 2004).

The Nigerian indigenous chicken (NIC) is a dual purpose bird that is raised for meat and egg production in the rural and peri-urban areas of the country (Sonaiya and Olori, 1990). They constitute about 80% of the 120 million birds found in rural areas of

Nigeria (Oke, 2011). These native chickens play major roles not only in Nigerian rural economies, but also contribute substantially to the Gross National Product (Momoh *et al*., 2007). They are kept in small flocks and feed on household refuse, homestead pickings, crop residues, herbage, seeds, grasses, earthworms, insects and small amount of supplements offered by the flock owners. They contain a highly conserved genetic system with high level of heterozygosity (Wimmers *et al*., 2000). They also have a better flavour of meat and are reared predominantly in the villages because of their inherent advantages over their exotic breed contemporaries. They are productive and well adapted to the adverse climatic conditions of the tropical environment and low management inputs (Egahi *et al*., 2010).

Productivity is an important indicator of the overall performance of an animal. The productive traits of an animal include growth, reproductive performance and product quality (Adedeji, 2009; Burrow, 2014; Burrow and Henshall, 2014). Improving the productivity of livestock owned by large number of people living in tropical and subtropical regions is of high priority because those livestock comprise a major part of household income for some of the world's poorest people (Burrow and Henshall, 2014).

Tropical adaptation is defined as an animal's ability to survive, grow and reproduce in the presence of endemic stressors of tropical environment. The concept of adaptability implies that phenotypic performance expresses animal's true genetic capability in its ability to cope with environmental stresses such as heat stress and disease parasites (Adedeji, 2009). Disease tolerance, heat tolerance, water metabolism, feed utilization and survivability are important adaptive traits of an animal (Adedeji, 2009). The economic implications for livestock production systems that lack adaptation include production losses, mortalities, treatment costs where treatment is feasible and potential

losses of markets (Burrow *et al*., 2001; Prayaga *et al*., 2006). The unique adaptive features of the Nigerian indigenous chicken allowing it to adapt to the local environment have been reported by several authors (Adebambo *et al*., 1999; Yunis and Cahaner, 1999; Ikeobi *et al*., 2001). These adaptive features include relatively small adult body size, flighty nature, thick egg shell, grey or black skin colour and presence of some major genes affecting their feather structure and distribution (Egahi *et al*., 2010). The two major genes of frizzling and naked neck have been implicated in heat tolerance of Nigerian indigenous chickens (Yunis and Cahaner, 1999).

The host defence system (immunity) is categorised into non-specific or innate immunity and specific immunity. Host immune response represents a highly regulated yet integrated interaction between different types of cells that respond to eliminate foreign invaders (pathogens) (Pal *et al*., 2011). It is the expression of the self for its own wellbeing carried out via an array of several interacting molecules. Interferon regulatory factor molecules are one of such molecules, conferring self-defence to the host against various pathogens. They are mostly found on various differentiated cell types present in the body (Tamura *et al*., 2008).

The interferon regulatory factor (IRF) gene family encodes transcription factors with multiple biological functions, which include immune defence against virus, stress response, cell differentiation, reproduction, growth and development (Chen and Royer, 2010). The IRFs regulate the expression of interferons and interferon-stimulated genes by binding to specific elements in their promoters (Taniguchi *et al*., 2001). All IRFs share significant homology in the N-terminal 115 amino acids, which contains the DNA-binding domain and is characterized by five well-conserved tryptophan repeats (Tamura *et al*., 2008). The DNA-binding domain forms a helix-turn-helix structure and recognises a DNA sequence known as interferon-stimulated response elements

(ISRE) (Darnell *et al*., 1994) which is characterized by the consensus, 5'-AANNGAAA-3' (Fuji *et al*., 1999). The C-terminal region of IRFs is less well conserved and mediates the interactions of a specific IRF with other family members, transcriptional factors or cofactors, so as to confer specific activities upon each IRF (Meraro *et al*., 1999). The IRF family consists of nine members in chicken and they include: IRF-1, IRF-2, IRF-4, IRF-5, IRF-6, IRF-7, IRF-8 IRF-9 and IRF-10. The first member, IRF-1, was discovered in 1998. The IRF-10 has been identified in chickens, but it is absent in mammals (Nehyba *et al*., 2002).

Interferon regulatory factor-5 is involved in activation of type I interferon genes, inflammatory cytokines and chemokines (Sigurdsson *et al*., 2005). The IRF-5 gene is also involved in apoptosis and immune response to pathogens (Paun and Pitha, 2007). It is also an important player in macrophage polarization, regulation of B-cell differentiation (Lien *et al*., 2010) and tumour necrosis factor (Krausgruber, 2011).

Chicken IRF-5 gene has been mapped to chromosome 1: 664,415-677,898 in the forward strand. It is organised into nine exons and eight introns. Eight of the nine exons are coding exons while exon 1 is a non-coding exon. Exons 1 to 9 are 37 bp, 290 bp, 184 bp, 68 bp, 31 bp, 237 bp, 408 bp, 119 bp and 622 bp, respectively. Exons 2 and 9 are not totally translated to protein while exons 3, 4, 5, 6, 7, 8 are totally translated to protein. The gene has a transcript length of 1996 bp and a translation length of 472 residues (Ensembl Chicken Gallus_gallus 5.0).

Scanty reports are available on chicken IRF-5. So far, no report is available on molecular analysis and characterization of the gene in Nigerian indigenous chickens. Keeping the aforementioned facts in mind, the present study was planned to clone and sequence exons 3, 4, 5 and 7 of the gene in Nigerian indigenous chickens, identify and

analyse polymorphisms in the gene, study the evolution of the gene, predict selective forces acting on the gene, analyse gene-derived peptide using bioinformatics tools and associate the identified haplotypes with productive and adaptive traits.

1.1 Justification

This study derived its justification from the following:

- The Nigerian indigenous chickens manifest a great deal of variation which is due to genetic and environmental factors (Sonaiya and Olori, 1990) hence, they are reservoir of genetic materials for genetic studies, improvement, preservation and conservation.
- Currently, there is a general consensus that introducing high yielding livestock breeds developed under specialized modes of production into traditional and extensive production systems can lead to loss of genetic diversity in indigenous animals (Sere and Steinfeld, 1996). It is therefore important to evaluate the existing chicken genetic resources using genomic approaches. This needs to be done for sustainable use of the existing chicken genotypes that are adapted to the production environment in which they are maintained (Dessie *et al*., 2011).
- Improvement of productivity and adaptation of Nigerian indigenous chickens should not only be done with existing technologies such as crossbreeding. New technology like marker assisted selection should also be applied.
- Previous researches have focused on the functions of individual interferon regulatory factors in mammals (Fujita *et al*., 1988; Klein *et al*., 2006; Honda and Taniguchi, 2006). However, the composition and function of these interferon regulatory factor family members are less investigated in other classes of vertebrates like chickens (Dougherty *et al*., 2009).

- Chickens exhibit a robust induction of type I interferons in response to infections by a variety of viruses (Kim and Zhou, 2015) such as Newcastle disease virus. Therefore, gene involved in transcription of interferons such as IRF-5 should be studied in chickens to elucidate their roles in Newcastle disease.
- To date, nine IRF homologues have been identified in chickens, though their functions are not yet well defined (Nehyba *et al*., 2002). This study elucidated the functions of IRF-5 in chicken growth, immunity and response to environmental stress.
- Despite the importance of IRF-5 and the effects of its polymorphisms in human and cattle, there is no report of sequence variations of this gene in Nigerian indigenous chickens and their possible effects on productive and adaptive traits.
- Sequence variations identified in chicken IRF-5 is useful for analysis of the evolutionary history, development, assessment of biodiversity and association study in NICs.
- Previous studies showed that there is a relationship between immunocompetence and productivity in animals (Molee *et al*., 2016). This study described the relationship between immunocompetence and productive adaptability in NICs using IRF-5 as gene of interest.

1.2 Objectives

1.2.1 Broad objective

To analyse exons 3, 4, 5 and 7 of IRF-5 gene in Nigerian indigenous chickens and associate them with productive and adaptive traits.

1.2.2 Specific objectives

i. To determine the effect of genotype and sex on productive (growth traits and feed efficiency) and adaptive (thermotolerance, survivability and antibody response to Newcastle disease) traits in Nigerian indigenous chickens.

ii. To identify single nucleotide polymorphisms (SNPs) in exons 3, 4, 5 and 7 of IRF-5 gene in Nigerian indigenous chickens.

iii. To determine the diversity and haplotype distribution of exons 3, 4, 5 and 7 of IRF-5 gene in Nigerian indigenous chickens.

iv. To determine the evolutionary relationship among exons 3, 4, 5 and 7 of IRF-5 gene in Nigerian indigenous chickens and other chicken genotypes.

v. To predict the likely selective forces acting on exons 3, 4, 5 and 7 of IRF-5 gene in Nigerian indigenous chickens.

vi. To predict the protein structure, motif and domain architecture of IRF-5 (exons 3, 4, 5 and 7) in Nigerian indigenous chickens.

vii. To determine the effect of identified haplotypes on productive (growth traits and feed efficiency) and adaptive (thermotolerance and antibody response to Newcastle disease) traits in Nigerian indigenous chickens.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Domestication of chicken

The domestic chicken is believed to have originated from the tropical jungle fowl of the genus *Gallus.* The four recognised wild species of genus *Gallus,* which may have contributed to today's domesticated fowl are the red jungle fowl (*G. gallus*), the grey jungle fowl (*G. sonnerrati*), the Ceylon jungle fowl (*G. lafayettei*) and the green jungle fowl (*G. varius*). Although no subspecies were recognised for the grey, Ceylon or green jungle fowls; the red jungle fowl, *G. gallus*, includes five subspecies: *G. g. gallus*, *G. g. spadiceus*, *G. g. bankiva*, *G. g. murghi* and *G. g. jabouillei* (Horst, 1989; Crawford, 1990).

Whether chickens were domesticated from one or all of these species remains an open question. Taking into account the geographic range of the species (Crawford, 1990), archaeological discoveries (West and Zhou, 1988), protein polymorphism and morphological characteristics (Moiseyeva *et al*., 2003), it has been suggested that domestic chickens were derived predominantly from the red jungle fowl. However, questions still linger on whether only one or the five sub-species of red jungle fowl contributed to the genetics of domestic chickens. In a series of studies that analysed 400 base pairs of the mitochondrial DNA D-loop region of the four species of genus *Gallus* (*G. gallus*, *G. varius*, *G. lafayettei* and *G. sonnerati*), three sub-species of *G. gallus* (*G. g. gallus*, *G. g. spadiceus* and *G. g. bankiva*), nine domestic breeds of chicken from South Asia, South East Asia, Japan and Europe, Akishinonomiya *et al*. (1994, 1996) presented evidence which suggested that domestic chickens are derived from a single continental population of *G. g. gallus*. However, in a separate study, Liu

et al. (2006) demonstrated that besides *G. g. gallus*, several other sub-species of the red jungle fowl were also involved in the genesis of modern chickens.

Apart from the red jungle fowl, other wild species of jungle fowl might have also contributed to the genetics of modern chickens. A study by Nishibori *et al*. (2005) revealed genetic evidence for hybridization of species in the genus *Gallus* which suggests multiple species origins of domestic fowls. Erikson *et al*. (2008), by examining the origins of skin colour variations in domestic chickens, revealed that although the white skin allele in modern chickens is derived from the red jungle fowl, the most likely origin of the yellow skin gene is the grey jungle fowl (*G. sonnerati*).

Crawford (1990) proposed that the domestication of chickens took place in the Indus valley around 2500-2100 BC. However, archaeological discoveries in 16 Neolithic sites along the Huang He (Yellow River valley) in Northeast China indicated that domestication of chickens may have taken place as early as 6000 BC (West and Zhou, 1988). Based on the fact that the conditions around the 16 Chinese Neolithic sites were not typical of the natural environment for jungle fowls, West and Zhou (1988) proposed that domestication may have taken place in Southeast Asia and birds taken to China by humans.

2.1.1 Evolution of chicken domestication

Archaeological and historical records showed that domestic chickens were first used in religion, decorative art and entertainment and much later as a source of human food (Crawford 1990). Skinner (1974) and Crawford (1984, 1990) proposed that the process of chicken domestication followed four distinct stages. The first evolutionary stage involved the use of chickens for religious, cultural and traditional purposes, which resulted in active selection for morphological features, such as particular

plumage colour. This is unlike the case of other animals, which were domesticated from the beginning as sources of food and/or primarily served as work animals. The second stage is characterised by the dispersal of chickens from centres of domestication to adjacent countries and continents with different environment and cultures. These are believed to have led to gradual genetic differentiation of the chicken populations in different parts of the world. According to Clutton-Brock (1999), the current genetically diverse populations are the result of this long term evolutionary process, which have led to adaptations to different environmental conditions and to a wide range of human needs. In each case, the primary factors contributing to today's populations were complex and included founder effects, migration, mutation, natural selection and selection by humans. The third stage was epitomed by the 'hen craze' of the 19th century when most of the indigenous pure breeds and varieties in Europe and the USA were developed. The fourth stage involved the period in the 20th century when the modern chicken meat and egg industry was developed (Crawford, 1990).

2.1.2 Introduction of chicken to Africa

The introduction of the domesticated chicken into Africa is not well documented. Clutton-Brock (1993) summarised the archaeological findings on the domestic fowl in Africa: the earliest evidence being a sketch of a cockerel on an ostracon from the tomb of Ramses IX (1156-1148 BC). Chickens were not common in Egypt until the Ptolemaic period (332-330 BC). In West Africa, they were excavated from the Iron Age site of Jenne-jalo in Mali, dating from 500-800 AD. In East Africa they were found in two Iron age sites in Mozambique and other sites in South Africa from the 18th century. Plug (1996) confirmed that chickens were found in early Iron Age sites,

but apparently not very common.

As suggested by MacDonald (1992), it is likely that chickens were present in Africa well before the earliest date yet determined by archaeological findings. Williamson (2000) concluded that there is a basic conflict between archaeological findings to date and the apparently deep embedding of chicken in many African cultures as well as the linguistic and ethnographic evidence which suggest presence of chicken in Africa at much earlier dates. As was the case with other livestock species, the domesticated chicken could have been introduced into Africa through the Isthmus of Suez in Egypt, the horn of Africa and through direct sea trading between Asiatic countries and coastal eastern Africa (Dessie *et al*., 2012).

2.2 Nigerian indigenous chickens

Nigerian indigenous chickens are generally hardy, adaptive to rural environment, survive on little or no inputs and adjust to fluctuations in feed availability (Gueye, 2003). They constitute 80% of the 120 million poultry type raised in the rural areas in Nigeria (RIM, 1992). They are self-reliant birds with the capacity to withstand harsh weather condition and adapted to adverse environment. They possess the ability to hatch their own eggs, brood and scavenge for major parts of their feed. They also possess immunity from endemic diseases. Their products are preferred by many Nigerians because of the pigmentation, taste, leanness and suitability for special dishes (Horst, 1989).

In Nigeria, indigenous chickens were characterized along genetic lines of feather (normal and frizzle feather), body structure (heavy ecotype and dwarf types) and feather colour (black, white, brown and mottle). The frequency distribution of the normal feather chicken was about 91.8% while that of frizzle and naked neck were

5.2% and 3.0%, respectively in Bayelsa state of Nigeria (Oke, 2011). Adebambo *et al*. (2015) found the frequency distribution of normal to be 76.8% while that of naked and frizzle were 23.2% in Ogun, Oyo, Lagos and Osun States of Nigeria.

Classification of indigenous chicken has also been done on location. There are various ecotypes of local chickens in different agro-ecological zones of Nigeria. Most of the classification by the different agro ecological zones considered mainly the normal feather indigenous chickens because they are the most prominent whereas the frizzle feather and naked are rare. Olori (1992) noted two ecotypes characterized as Fulani and Yoruba ecotypes. Recent work revealed that the different ecotypes can be grouped into two major categories on the basis of body size and body weight as heavy ecotype and light ecotype (Momoh *et al*., 2007). The heavy ecotype (also called Fulani ecotype) is found in the dry savannahs (Guinea and Sahel), montane regions and cattle Kraals of the North. The heavy ecotype weighs about 0.9 to 2.5 Kg when matured. The light ecotype is found in swamp, derived Savannah and rainforest. The light ecotype weighs about 0.68-1.5kg when matured (Momoh *et al*., 2007).

2.2.1 Major genes in Nigerian indigenous chickens

Certain major genes have been found to be relevant to the indigenous chicken breeds in the tropical environment which is characterized by stress factor (Mathur and Horst, 1990). The feather distribution gene, naked gene (*Na*) and the feather structure gene, frizzle (*F*) are among these major genes. These major genes are economically important in modern breeding systems as they act as sex marker gene and disease resistant factors. These major genes cause reduction in tropical heat stress by improving the breed's ability for convection, resulting in feed conversion and better performance (Oke, 2011).

Horst (1989) stated that the *Na* and *F* gene confer superiority in some production characters in the tropics. Mathur and Horst (1990) further showed that indigenous chickens with *F* and *Na* genes both singly and in combination were superior to birds with normal feathering for egg number, egg mass/weight and forty week body weight in tropical environment. Ibe (1993) observed that naked neck and frizzle genes are associated with early sexual maturity in tropical environment.

2.2.2 Productive performance of Nigerian indigenous chickens

Productivity is an important indicator of the overall performance of an animal. The productive traits of chickens include growth, reproductive performance and product quality (Adedeji, 2009; Burrow, 2014; Burrow and Henshall, 2014).

2.2.2.1 Growth of Nigerian indigenous chickens

Animal growth involves increase in size and changes in functional capabilities of the various tissues and organs of animals from conception till maturity (Kor *et al*., 2006). Growth is normally accompanied by an orderly sequence of maturational changes and involves accretion of proteins. It involves an increase in length and size, not just an increase in body weight alone. Growth in livestock may be evaluated with body components such as live weight and other body parameters (Wolanski *et al*., 2006).

Oke (2011) observed significant effect of genotype on growth traits of local pullets at weeks 16 and 24. The highest body weight (714.00±15.03 g) was observed in frizzle feather chickens while the smallest body weight (477.67±34.52) was observed in naked neck chickens at 16 weeks of age.

Fadare (2014) observed significant effect of genotype on morphometric and growth performance of pure and crossbred indigenous chickens. The highest body weight

(1022.52±42.18 g) was observed in naked neck chicken at week 12 while the least weight (992.93±41.30 g) was observed in frizzle feather at week 12. Fadare (2014) also observed no significant difference in body length of normal feather (22.25±0.32 cm) and frizzle feather (22.45±0.28 cm) at week 12. The highest keel length (10.22±0.24 cm) was observed in Giriraja while no significant difference was observed in keel length of normal feather and naked neck chickens.

2.3 Adaptation in chickens

Tropical adaptation is defined as an animal's ability to survive, grow and reproduce in the presence of endemic stressors of tropical environment (Burrow *et al*., 2001; Prayaga *et al*., 2006). The concept of adaptability implies that phenotypic performance expresses animal's true genetic capability in its ability to cope with environmental stresses such as heat stress and disease parasites. Disease tolerance, heat tolerance, water metabolism, feed utilization and survivability are important adaptive traits of an animal (Adedeji, 2009).

2.3.1 Heat stress in Nigerian indigenous chickens

In Nigeria, period of high temperatures have a negative effect on the health and performance of the birds. The effect of stress caused by elevated temperatures can result in heavy domestic losses from increased mortality and reduced productivity. For birds to perform at their optimum capacity, they need to be in homeostasis with their environment through the maintenance of thermobalance (Zerjal *et al*., 2013).

Heat stress is experienced whenever the ambient temperature of the birds exceeds the zone of comfort. Birds produce heat to maintain a relatively constant body temperature and may permit certain variations within their temperature range without significant

perturbation. The chicken's body temperature is around 40-41.5^{o}C, but will fluctuate depending on the temperature of the environment. Altan *et al*. (2003) reported that high ambient temperature and relative humidity increase heat stress and are responsible for the increase in rectal temperatures of birds. Heat stress may also result when the birds are not able to dissipate the metabolically generated heat (Adedeji *et al*., 2015).

Birds gain heat from the environment and their metabolism. They regulate the balance between heat production and heat loss to maintain their deep body temperature at around 40-41.5^{o}C. Birds transport the generated heat to the body surface to allow sensible heat loss from the surfaces such as combs, shanks, wattles and unfeathered parts because they don't have sweat glands (Azoulay *et al*., 2011). The sensible heat loss is effective when the environmental temperature is below or within the thermoneutral zone ranging from 13-24^{o}C (Schmidt-Nielsen, 1997). Heat loss mechanism begins to shift to panting when the environmental temperature exceeds 25^{o}C. Birds have to increase their evaporative losses to maintain body temperature and therefore start to breath more rapidly and panting normally occurs at about 30^{o}C (Azoulay *et al*., 2011).

Heat stress is considered to be one of the most important variables affecting feed intake, body weight gain, mortality rate and profitability of chickens, particularly in the hot climate (Deeb and Cahaner, 2001) like Nigeria. An increase in respiratory rate is the first visible sign and spectacular response that birds make in response to heat stress. Changes in rectal temperature, pulse rate and respiratory rate have been frequently used as physiological indices of heat stress (Adedeji *et al*., 2015).

Isidahomen *et al*. (2012) carried out an experiment on heat tolerance traits among local and exotic chickens in Southern Nigeria. They observed a significant effect of genotype on rectal temperature, pulse rate and respiratory rate. The highest rectal temperature, respiratory rate and pulse rate was observed in naked neck chickens while the lowest rectal temperature, respiratory rate and pulse rate was observed in normal feather chickens.

Adedeji *et al*. (2015) observed no significant genotype effect on rectal temperature of Nigerian indigenous chickens. They observed a mean rectal temperature of 41.04±0.04 ^{o}C for Nigerian indigenous chickens. Pulse rate, respiratory rate and heat stress index were observed to be significantly affected by genotype and sex. The highest pulse rate and respiratory rate was observed in frizzle feather chickens while the least respiratory rate was observed in normal feather chickens. Adedeji *et al*. (2015) also observed the highest pulse rate, respiratory rate and heat stress index in female chickens.

2.4 Newcastle disease in chicken

The first outbreaks to be recognised and termed Newcastle disease (ND) occurred in poultry in 1926, in Java, Indonesia (Kraneveld, 1926) and in Newcastle-upon-Tyne, England (Doyle, 1927). However, there are earlier reports of similar disease outbreaks in Central Europe before this date (Halasz, 1912). In particular, Macpherson (1956) attributed the death of all chickens in the Western Isles of Scotland in 1896 as being due to Newcastle disease. It is possible therefore, that ND did occur in poultry before 1926, but its recognition as a specifically defined disease of viral aetiology was from the outbreak in Newcastle-Upon-Tyne.

The name, Newcastle disease, was coined after the first geographical location of the first outbreak and was coined by Doyle as a temporary measure because he wished to

avoid descriptive name that might be confused with other diseases (Doyle, 1935). The name has however continued to be used although when referring to the disease, the synonym “avian paramyxovirus type 1” (APMV-1) is now often used (Alexander *et al*., 2004).

The pattern of outbreak which are due to virulent NDV throughout the world suggest that several panzootics have occurred in poultry since 1926. The first outbreak appeared to have spread very slowly across the globe. It took over 20 years to become a true panzootics and never reached poultry in some continents. The beginning of the second ND panzootic was first recognised at the end of 1960s and within four years had reached all corners of the earth (Hanson, 1972). The reasons for the different spreading rates of the two panzootics appear to be the development of the world poultry industry and commercialization of poultry food production, both of which led to greater contact among farms because poultry delivery vehicles move from one farm to another farm. Another factor is the revolution that occurred in world transport. Air transportation especially has led to a huge and growing trade in captive caged birds. There is no doubt that imported caged birds were responsible for introducing the panzootic virus into birds in different countries (Hanson, 1972; Francis, 1973).

Antigenic and genetic evidence has indicated that there was probably a worldwide spread of a third virulent virus during the late 1970s, the start and the spread of which is unclear, presumably due to the masking of the disease by the almost universal use of vaccines since the mid-1970s (Herczeg *et al*., 2001).

2.4.1 Aetiology of Newcastle disease

The three virus families *Rhabdoviridae*, *Filoviridae* and *Paramyxoviridae* form the order *Mononegavirales*. Newcastle disease is caused by avian paramyxovirus serotype

1 (APMV-1) viruses, which, with viruses of other eight APMV serotypes (APMV-2 to APMV-9), have been placed in genus *Avulavirus*, sub-family *Paramyxovirinae*, family *Paramyxoviridae*, in the current taxonomy (Lamb *et al*., 2000).

Antigenic variation of the ND virus PMV-1 was demonstrably different from standard strains in haemaglutination inhibition tests, but not sufficiently different antigenically that conventional ND vaccines were not protective (Alexander and Parsons, 1986). In recent years, antigenic variations detected by monoclonal antibodies and genetic variations detected by nucleotide sequencing of the virus genome have proved valuable in understanding the epidemiology of ND (Herczeg *et al*., 2001).

2.4.2 Pathogenicity of Newcastle disease in birds

The clinical signs seen in birds infected with ND virus vary widely and are dependent on factors such as the virus, host species, age of the host, infection with other organisms, environmental stress and immune status. In some circumstances, infection with the extremely virulent viruses may result in sudden, high mortality with comparatively few clinical signs. Although none of the variable clinical signs can be regarded as pathognomonic, certain signs do appear to be associated with particular viruses. This has resulted in the grouping of viruses into five pathotypes on the basis of the predominant signs in affected chickens. These five pathotypes are: viscerotropic velogenic, neurotropic velogenic, mesogenic, lentogenic and asymptomatic enteric (Beard and Hanson, 1984).

Viscerotropic velogenic are viruses responsible for disease characterized with acute lethal infections, usually with haemorrhagic lesions in the intestine of dead birds. Neurotropic velogenic are viruses causing death characterized with high mortality which follows respiratory and neurological disease but where gut lesions are usually

absent. Mesogenic are viruses that cause clinical signs consisting of respiratory and neurological signs, with low mortality. Lentogenic viruses cause mild infections of the respiratory tract while asymptomatic enteric are viruses causing avirulent infections in which replication appears to be primarily in the gut (Alexander and Allan, 1974).

The ND may consist of signs of depression, diarrhoea, prostration, oedema of the head and wattles, nervous signs such as paralysis and torticollis, and respiratory signs. As with clinical signs, no gross or microscopic lesions can be considered pathognomonic for any form of ND. Carcasses of birds dying as a result of virulent ND usually have a fevered and dehydrated appearance. Gross lesions may vary with the infecting virus. Virulent panzootic ND viruses typically cause haemorrhagic lesions of the intestinal tract (McFerran and McCracken, 1998).

2.4.3 Serological diagnosis of Newcastle disease

In the absence of vaccination, the presence of specific antibodies against the ND virus indicates that the bird has been infected by the virus at some time, but not necessarily that it was suffering from the disease at the time of sampling. In practice, a high antibody titre is indicative of a recent infection. Two methods are used to measure antibody titre: the haemaglutination inhibition (HI) test and the enzyme-linked immunosorbent assay (ELISA) (Alders and Spradbrow, 2001).

The HI test is based on the principle that the haemagglutin on the viral envelope can bring about the agglutination of chicken red blood cells and that this can be inhibited by specific antibodies (Allan and Gough, 1974). V-bottomed micro-titration plates are used in HI test. The HI titre is the reciprocal of the highest dilution of the serum which completely inhibits haemaglutination and is usually and most conveniently expressed as the logarithm to base 2. The HI titre gives an indication of the immune status of the

bird. A titre of $\log_2 3$ is indicative of protection and a titre of $\log_2 6$ or more suggests a recent infection by the virus (Maas *et al*., 1998)

The ELISA works on the principle of recognition of anti-NDV antibodies, attached to a plate coated with viral antigen, by antibodies produced in another species against chicken antibodies. This anti-chicken antibody is conjugated to an enzyme that catalyses a reaction, causing a change of colour which can then be read quantitatively on a photo spectrometer designed to read micro-titration plates (Bell *et al*., 1991).

2.4.4 Newcastle disease in chickens raised in Nigeria

Okwor *et al*. (2010) reported the antibody profile in Shaver brown layers vaccinated with Newcastle disease vaccine (LaSota). The mean antibody titres for the laying birds before vaccination, 4 weeks post vaccination and 12 weeks post vaccination were observed to be 12.1, 299.0, 84.4 and 37.0, respectively.

Adeleke *et al*. (2015) carried out a research on genotype effect on distribution pattern of maternally derived antibody against Newcastle disease in Nigerian local chickens and Anak Titan. The mean titres for the parent stocks observed were 413.33±68.85, 426±33±121.95, 481.67±145.45 and 1148.33±532.84 for frizzle feather, normal feather, naked neck and Anak Titan, respectively. The mean titres obtained in chicks by Adeleke *et al*. (2015) were 398.80±43.87, 400.00±53.95, 427.00±89.35 and 398.80±57.59 for frizzle feather, normal feather, naked neck and Anak Titan chicks. They also observed that the maternally derived antibody transfer rate against Newcastle disease differed for different genotypes. The transfer rates were 96.4, 93.8, 88.7 and 34.7% for frizzle feather, normal feather, naked neck and Anak Titan, respectively.

2.5 Single nucleotide polymorphisms

DNA based markers have many advantages over phenotypic markers in that they are highly heritable, relatively easy to assay and are not affected by the environment (Duran *et al*., 2009).

The most abundant source of genetic polymorphism is single nucleotide polymorphism, representing a single base change between two individuals at a defined location. There are three different categories of SNPs: transitions (C/T or G/A), transversions (C/G, A/T, C/A, and T/G) and small insertions/deletions (indels). Single nucleotide polymorphisms at any particular site could in principle be bi-, tri- or tetra-allelic. However tri- and tetra-allelic SNPs are rare, and in practice SNPs are usually biallelic (Doveri *et al*., 2008). This disadvantage, when compared with multiallelic markers such as Simple Sequence Repeats (SSRs), is compensated by the relative abundance of SNPs, which can provide a high density of markers near a locus of interest. Single nucleotide polymorphisms are evolutionarily stable, not changing significantly from generation to generation. This low mutation rate makes SNPs excellent markers for studying complex genetic traits and as a tool for understanding genome evolution (Syvanen, 2001). Single nucleotide polymorphisms are direct markers as the exact nature of the allelic variants is provided by the sequence information. This sequence variation can have a major impact on how the organism develops and responds to the environment. Single nucleotide polymorphisms are becoming increasingly used in animal breeding, with particular success being derived from the bovine HapMap project (Khatkar *et al*., 2007).

Single nucleotide polymorphisms markers which can be rapidly and cheaply identified through bioinformatics have many uses in genetics, such as the detection of alleles

associated with disease, genome mapping, association studies, genetic diversity, paternity assessment, forensics and inferences of population history (Brumfield *et al*., 2003). Single nucleotide polymorphisms within specific genes or genomic regions have also been used to infer phylogenetic relationships between species (Duran *et al*., 2009).

2.6 Linkage disequilibrium

Linkage disequilibrium (LD) is a property of SNPs on a contiguous stretch of genome sequence that describes the degree to which an allele of one SNP is inherited or correlated with an allele of another SNP within a population. The term linkage disequilibrium was coined by population geneticists in an attempt to mathematically describe changes in genetic variation within a population over time (Bush and Moore, 2012). It is a non-random association of alleles at two or more loci that gained interest in genetics when its usefulness in gene mapping became evident and large scale surveys of closely linked loci became feasible (Slatkin, 2008).

Linkage disequilibrium is of importance in evolutionary genetics because it is affected by so many factors. It provides information about the past events and it also constrains the potential response to both natural and artificial selection. LD throughout the genome reflects the population history, the breeding system and pattern of geographic subdivision. The linkage disequilibrium in each genomic region reflects the history of natural selection, gene conversion, mutation and other forces that cause gene-frequency evolution (Slatkin, 2008).

Many measures of LD have been proposed (Devlin and Risch, 1995), though all are ultimately related to the difference between the observed frequency of co-occurrence for two alleles and the frequency expected if the two markers are independent. The

two commonly used measures of LD are D' and r^2 (International Hapmap Consortium, 2005). D' is a population genetics measure that is related to recombination events between markers and is scaled between 0 and 1. A D' value of 0 indicates complete linkage equilibrium, which implies frequent recombination between the two markers and statistical independence under principles of Hardy-Weinberg equilibrium. A D' value of 1 indicates complete LD, indicating no recombination between the two markers within the population. For the purpose of genetic analysis, LD is generally reported in terms of r^2, a statistical measure of correlation. High r^2 values indicate that two SNPs convey similar information, as one allele of the first SNP is often observed with one allele of the second SNP, so only one of the two SNPs needs to be genotyped to capture the allelic variation (Bush and Moore, 2012). There are dependencies between these two statistics; r^2 is sensitive to the allele frequencies of the tow markers and can only be high in regions of high D'.

The presence of LD creates two possible positive outcomes from a genetic association study. In the first outcome, the SNP influencing a biological system that ultimately leads to the phenotype is directly genotyped and found to be statistically associated with the trait. This is called direct association and the genotyped SNP is referred to as functional SNP. The second possibility is that the influential SNP is not directly typed, but instead a tag SNP in high LD with the influential SNP is typed and statistically associated to the phenotype. This is referred to as indirect association (Hirschhorn and Daly, 2005).

2.7 Chicken interferons

Interferons (IFNs) are a multigene family of inducible cytokines (Blatt *et al*., 1996) that possess antiviral activity (Stark *et al*., 1998). They are classified into three types

in chickens. The three classes are type I IFN, type II IFN and type III IFN. Interferons that bind IFN-A receptor 1 and IFN-A receptor 2 are type I, while those that interact with receptor complexes of IFN-G receptor 1 and IFN-G receptor 2 are type II. Type III IFN interacts with hereodimeric receptor complex of IL-28RA and IL-10RB (Santhakumar *et al*., 2017).

Two serological distinct Type I IFNs have been identified in chickens and they are IFN-A and IFN-B. These two type I interferons are intronless, acid and heat stable. They are located on short arm of Z chromosome (Sick *et al*., 1996). All type I IFN are involved in induction of antiviral state, inhibition of cell proliferation, modulation of cell fate and mediation of cell differentiation (Hertzog and Williams, 2013). Type I interferons work by binding to their respective receptors (Guo *et al*., 2014). Type I interferons ameliorate avian viral diseases such as Marek, infectious bursal disease and infectious bronchitis (Mo *et al*., 2001; Pei *et al*., 2001).

IFN-G is the only member of type II IFN in avian species and serves as a bridge between adaptive and innate immunity. IFN-G is involved in regulation of maturation and differentiation of several immune cells such as T cells (Fensterl and Sen, 2009). Chicken IFN-G is sensitive to low pH and heat (Digby and Lowenthai, 1995). It has adjuvant properties against viral diseases like Newcastle disease and influenza (Sawant *et al*., 2011; Susta *et al*., 2013). Chicken IFN-G also induces MHC class I and II molecules and assists in the production of nitric oxides which are important inhibitory mechanisms for viruses (Mallick *et al*., 2011).

Avian type III interferon are functionally conserved compared to mammalian species, playing a predominant role in the antiviral defence of epithelial barriers (Zhang *et al*., 2015)

2.8 Activation of interferon pathways

One of the most essential events in host-virus battle is the activation of interferon pathway. Three classes of nucleic acid receptors are involved in activation of interferon pathways. The first category is the family of retinoic acid-inducible gene I (RIG-I)-like helicases (RLH), which includes RIG-I, melanoma differentiation-associated gene 5 (MDA5) and laboratory of genetics and physiology 2 (LGP2) (Fensterl and Sen, 2009). The second class is the family of Toll-like receptors, TLRs 3, 7 and 9, which senses extracellular, phagosomal or endosomal pathogen-associated molecular patterns (Randall and Goodbourn, 2008). The third category is the family of DNA sensors, which include absent in melanoma 2 (AIM2) and cyclic GMP-AMP synthetase (cGAS) (Rathinam and Fitzgerald, 2011). Upon activation, these receptors recruit downstream signalling molecules and result, directly or indirectly, in the activation of interferon regulatory factor genes, as well as activating protein 1 (AP-1) and nuclear factor Kappa-B transcription factors (Randall and Goodbourn, 2008). These are the minimally essential events to initiate transcription of type I IFN genes and establishment of an antiviral state by expressing hundreds of IFN-stimulated genes (ISGs) (Fensterl and Sen, 2009) in infected cells.

2.9 Interferon regulatory factors

The interferon regulatory factors are transcription mediators of virus-, bacteria- and interferon-induced signalling pathways and so they play a crucial role in antiviral defence, immune response and cell growth regulation (Barnes *et al*., 2002). All interferon regulatory factors share significant homology in the N-terminal 115 amino acid residues, which contains the DNA-binding domain and it is characterized by five tryptophan repeats called IRF tryptophan pentad repeats. Three of these repeats

contact DNA with specific recognition of the GAAA and AANNNGAA sequences present in interferons (Escalante *et al*., 1998). The function of a particular IRF is determined by a combination of cell-type-specific expression, its transactivation potential and its ability to interact with other IRF members (Taniguchi *et al*., 2001).

The IRF-1 and IRF-2 were identified through their ability to bind to the positive regulatory domain in the virus responsive elements present in 5'region of IFN-B. They were originally thought to function as activators and repressors of IFN-B, respectively (Fujita *et al*., 1989). It was later shown by Kimura *et al*. (1994) that IRF-1 is involved in many antiviral defence mediated by IFN-G. The IRF-1 has essential roles in the development and activation of various immune cells. It also plays a critical role in the inducible expression of MHC class I and programmed cell death (Reis *et al*., 1994). In humans, variations in IRF-1 have been associated with asthma in infants (Wang *et al*., 2006) while deletion in IRF-1 has been associated with blood cancer (Willman *et al*., 1993).

IRF-2 binds to the to the same recognition site as IRF-1. Over-expression of IRF-2 in NIH/3T3 cells resulted in tumour in these cells. IRF-2 has also been shown by Vaughan *et al*. (1995) to activate transcription of histone-4 gene and inhibit N-Ras. The N-Ras functions as growth inhibitor and over-expression of IRF-2 in a myeloid cell reversed N-Ras-induced growth suppression (Passioura *et al*., 2005). IRF-2 deficient mice exhibit natural killer cell deficiency and IRF-2 deficient natural killer cells show an immature phenotype and compromised receptor expression, indicating that IRF-2 deficiency results in a defect in the later stages of natural killer cells maturation (Taki *et al*., 2005). IRF-2 gene has also been discovered to play a major role in myeloid dendritic cells (Honda *et al*., 2004). It also works with STAT1 gene to stimulate expression of antigenic peptides to MHC Class I (Rouyez *et al*., 2005).

Expression of IRF-3 is activated in infected cells upon recognition of double stranded RNA which is the common feature of virus infected cells. Toll-like receptor 3 and RIG-I/MDA5 assist in phosphorylation of IRF-3 at the C terminal region (McWhirter *et al*., 2004). The phosphorylated IRF-3 homo or heterodimerizes with IRF-7 and stimulates the transcription of IFN-B, as well as other interferon stimulated genes such as ISG54 and RANTES (Lin *et al*., 1999).

IRF-4 is expressed majorly in macrophages, dendritic cells, lymphocytes and B cells (Eisenbeis *et al*., 1995). It has weak DNA binding affinity which can only be increased by interaction with other transcription factors (Tailor *et al*., 2006) such as PU.I (Eisenbeis *et al*., 1995). IRF-4 biding is strengthened upon heterodimerization with PU.I and this heterodimer binds with IgG enhancer and activates expression of immunoglobulin light chains in B cells (Eisenbeis *et al*., 1995). IRF-4 is also a natural antagonist of both IRF-1 and IRF-5 transactivation by antagonising TRAIL promoter in IRF-1 and competing for binding to MyD88 in IRF-5 (Yoshida *et al*., 2005). IRF-4 has a crucial role in maturation of B and T cells as IRF-4 null mice have deficiency in mature T and B cells (Lu *et al*., 2003).

IRF-6 unlike other IRFs is involved in morphogenesis and development. It is a major regulator of the switch from keratinocyte proliferation to differentiation (Richardson *et al*., 2006). Mice that are deficient in IRF-6 are embryonic lethal and showed abnormal external morphology such as short forelimbs, lack of ears and tails. Human IRF-6 gene is localised in the critical region of Van der Woulde syndrome locus which is a disease associated autosomal dominant form of cleft lip and palate (Mostowska *et al*., 2005). Mutations in IRF-6 are also associated with Popliteal pterygium syndrome which is a disorder characterized by similar orofacial phenotype skin lesions and genital abnormalities in human (Kondo *et al*., 2002)

IRF-7 was identified as a transcription factor binding to the Oq promoter of Epstein Barr virus and its splice variant was recognised as a player in induction of IFN-A gene (Zhang and Pagano, 1997). The expression of IRF-7 can be induced by Type I IFN and TNFA (Lu *et al*., 2002). The gene is constructively expressed in some lymphoid cells, especially pDC, which are high producers of IFNA in response to TLRs-7, 8 and 9 activations (Izaguirre *et al*., 2003). IRF-7 has been shown to have a short half-life which may be significant in the regulation of transient expression of IFN-A (Sato *et al*., 2000). Expression of IRF-7 has a role in differentiation of monocytes to macrophages (Lu and Pitha, 2001) while expression of constitutively active IRF-7 in macrophages increased tumouricidal activities of these macrophages (Romieu-Mourez *et al*., 2006).

IRF-8, like IRF-4 is also expressed primarily in macrophages, B cells, DC and lymphocytes (Eisenbeis *et al*., 1995). It also shares many attributes with IRF-4. It binds DNA after interaction with the transcription factors of the IRF family members which are IRF-1, IRF-2, IRF-4 as well as PU.1 and E47 (Politis *et al*., 1992). IRF-8/IRF-4 heterodimer activates transcription of ISG15 (Meraro *et al*., 2002) while IRF-8/IRF-1 complex induces many genes that are involved in macrophage differentiation and macrophage-induced inflammation (Holtschke *et al*., 1996). IRF-8 deficient mice showed high susceptibility to infections due to defect in Th1 immune response and inability to express IL-12 gene as IRF-8 stimulates the transcription of IL-12 (Meraro *et al*., 2002)

IRF-9 plays an important role in antiviral effect of type I IFN and it is an essential component of the tertiary complex, ISGF3, which is formed in IFN-treated cells and binds to the ISRE elements of ISG, stimulating their transcription (Improta *et al*., 1994)

2.9.1 Interferon regulatory factor-5

IRF-5 is implicated in immune response to pathogens and apoptosis. It is also implicated in the innate inflammatory responses (Takaoka *et al*., 2005). It is distinct from IRFs-3 and 7 by containing two nuclear localization signals with one present in IRFs-3 and 7. Activation and phosphorylation of IRF-5 by viral infection can be detected in cells infected with ND virus and vesicular stomatitis virus but not in cells infected with Sendai virus which is an indication of virus specific activation (Barnes *et al*., 2002). Type I interferons such as IFN-A, IFN-B and IFN-G; and viral infection stimulate expression of IRF-5 gene (Mancl *et al*., 2005). It can also be induced by tumour suppressor, p53 (Mori *et al*., 2002). The gene stimulates cyclin-dependent kinase inhibitor p21 and represses cyclin B1. It also stimulates the expression of proapoptotic genes such as Bak1, Bax, Caspase 8 and DAP kinase 2 (Barnes *et al*., 2002). It also promotes cell cycle arrest and apoptosis independently of p53 (Hu and Barnes, 2006). Over-expression of IRF-5 gene up-regulates many early inflammatory genes such as RANTES, I-309, MCP-1 and IL-8, which is an indication that IRF-5 has an important role in the transcriptional regulation of the early inflammatory cytokines and chemokines (Barnes *et al*., 2004).

There is also a link between IRF-5 expression, IFN-A production and autoimmunity as mutations in human IRF-5 gene confers predisposition to systemic lupus erythromatosis which is an autoimmune disease characterized by constitutive IFN-A production (Graham *et al*., 2006).

2.9.2 Organization of chicken IRF-5 gene

Chicken IRF-5 gene has been mapped to chromosome 1: 664,415-677,898 in the forward strand. It is organised into nine exons and eight introns (Table 1). Eight of the

nine exons are coding exons while exon 1 is a non-coding exon. Exons 1 to 9 are 37 bp, 290 bp, 184 bp, 68 bp, 31 bp, 237 bp, 408 bp, 119 bp and 622 bp, respectively. Exons 2 and 9 are not totally translated to protein while exons 3, 4, 5, 6, 7, 8 are totally translated to protein. 107 bp are not translated in exon 2 while 189 bp are translated in exon 9 of the gene. Introns 1 to 8 are 1482 bp, 2474 bp, 194 bp, 108 bp, 398 bp, 5051 bp, 1159 bp and 622 bp, respectively. The gene has a transcript length of 1996 bp and a translation length of 472 residues. The gene has been annotated with 19 domains and features, majority of which are IRF DNA-binding domain, IRF-3, SMAD/FHA domain and winged helix-turn-helix DNA-binding domain. Seventy-one variations have also been reported in the gene (Table 2) (Ensembl Chicken Gallus_gallus 5.0).

Table 1: Organization of chicken IRF-5 gene

Region	Length (bp)	Start	End	Translation
Exon 1	37	664,415	664,451	Not translated
Intron 1-2	1,482	664,452	665,933	Not translated
Exon 2	290	665,934	666,223	Partially translated
Intron 2-3	2,474	666,224	668,697	Not translated
Exon 3	184	668,698	668,881	Fully translated
Intron 3-4	194	668,882	669,075	Not translated
Exon 4	68	669,076	669,143	Fully translated
Intron 4-5	108	669,144	669,251	Not translated
Exon 5	31	669,252	669,282	Fully translated
Intron 5-6	398	669,283	669,680	Not translated
Exon 6	237	669,681	669,917	Fully translated
Intron 6-7	5,051	669,918	674,968	Not translated
Exon 7	408	674,969	675,376	Fully translated
Intron 7-8	1,159	675,377	676,535	Not translated
Exon 8	119	676,536	676,654	Fully translated
Intron 8-9	622	676,655	677,276	Not translated
Exon 9	622	677,277	677,898	Partially translated

Source: Ensembl Chicken Gallus_gallus 5.0

Table 2: Single nucleotide polymorphisms reported in chicken IRF-5 gene

Region	Number reported	SNP identifier
Exon 1	0	
Exon 2	16	rs735649686, rs737030659, rs732291695, rs734374398, rs736455128, rs733086953, rs733822034, rs741380658, rs731173907, rs734597544, rs741486236, rs731279318, rs738841291, rs740892510, rs732068170, rs738276389
Exon 3	8	rs317511101, rs737812399, rs312902332, rs315149141, rs739303538, rs735923203, rs737981373, rs739389464
Exon 4	5	rs793891433, rs733641399, rs730970842, rs738551822, rs740638934
Exon 5	0	
Exon 6	3	rs1058494410, rs740155218, rs313129419
Exon 7	12	rs735591820, rs737706780, rs732919639, rs734331790, rs736423928, rs733072359, rs733727437, rs741250292, rs734554215, rs740736761, rs1059098103, rs1059453342
Exon 8	3	rs732576768, rs733988429, rs741559832
Exon 9	24	rs739306233, rs735933534, rs736654274, rs1058109259, rs733265721, rs734663053, rs736781258, rs733398796, rs317283670, rs315960147, rs741586168, rs731447421, rs734855756, rs741077011, rs732236144, rs737755648, rs739818864, rs730975564, rs738535222, rs794030492, rs739268371, rs794229979, rs735855521, rs737952197

Source: Ensembl Chicken Gallus_gallus 5.0

2.10 Selective forces acting on avian immune genes

Immune genes are under acute selective pressure in order to resist pathogenic attacks (Downing *et al*., 2009). There exist two contrasting views about selective forces acting on immune genes. The first view opines that immunity is ancient and crucial, then selection had sufficient time to fix the most effective alleles and as such there is no tolerance for new alleles, so purifying selection or negative selection is major selective force acting on immune genes (Mukherjee *et al*., 2009). The other view opines that given the rapid rate of pathogen evolution, selection for high allelic diversity enhances the flexibility and broad spectrum antipathogenic activity that characterises the immune system, therefore, diversifying or positive selection are the main selective drivers (Ferrer-Admetlla *et al*., 2008).

It is not really clear the type of selective force that act on immune genes because diverse pathogen load and host population density. So, different selective forces act on different immune genes with different functional properties. Afferent (sensing) immune genes involved in pathogen recognition and signalling must recognise many pathogens and thus, positive selection may be observed in them. Efferent (eliminating) immune genes may be more constrained and display low tolerance for new genetic variants, and therefore be maintained by purifying selection. Also, selective patterns can also be shaped by coevolution between gene and pathogen. For example, TLRs are signalling and receptor molecules that have two major domains which are leucine rich repeats (LRR) and toll interleukin receptor (TIR) domains. LRR domain is responsible for recognition and binding of pathogen ligands and this domain is under balancing selection. TIR domain is involved in signalling to other components of the immune system cascades and under purifying selection (Alcaide and Edwards, 2011).

Therefore, selective forces can vary, even across small genomic portions, when the functional properties of immune genes differ (Chapman *et al*., 2016).

Chapman *et al*. (2016) predicted purifying selection to be the major selective force acting on ancient B-Defensin gene in Waterfowl and they also predicted balancing selection as the major selective force acting on recently duplicated B-Defensin gene in Waterfowl. Downing *et al*. (2009) predicted purifying selection to be the major force acting on 26 immune genes out of 64 immune genes they examined in chicken.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental site

The experiment was carried out at the Poultry Breeding Unit of the Directorate of University Farms, Federal University of Agriculture, Abeokuta, Alabata, Ogun State, Nigeria. Alabata (latitude $7^0$10'N and longitude $3^0$2'E) is in Odeda Local Government Area of Ogun State, Nigeria. The area which lies in the South Western part of Nigeria has a prevailing tropical climate with a mean annual rainfall of about 1037 mm. The mean ambient temperature ranges from 28^{o}C in December to 36^{o}C in February with a yearly average humidity of about 82%. The vegetation represents an interphase between the tropical rainforest and the derived savannah (Google Earth, 2018)

3.2 Source, sample size and management of experimental birds

The experimental birds were generated from mating of parent stocks of indigenous chickens available on the farm through artificial insemination. Three hundred and thirty-two (332) birds (111 Normal Feather, 112 Naked Neck and 109 Frizzle Feather chickens) were used for the experiment which lasted for 20 weeks. The birds comprised 147 males and 185 females. The experimental birds were raised under intensive management system. The chicks were brooded in deep litter pen at the brooding stage. All birds were wing-tagged for proper identification and subjected to the same management practices throughout the experimental period. Commercial feeds were provided for the birds *ad libitum*. Chick starter mash containing 23% crude protein and 11.1MJ/kg metabolizable energy was fed to the birds from 0 to 8 weeks of age. Grower mash containing 18% crude protein and 10.48MJ/kg metabolizable energy was fed to the birds from 9-20 weeks of age. Clean water was provided for the

birds *ad libitum*. Vaccination schedule for chicken was strictly adhered to and adequate sanitation was practised to prevent occurrence of diseases.

3.3 Data collection

3.3.1 Meteorological data

Ambient temperature and relative humidity of the pen were measured daily, both in the morning and evening, using a digital device that contains both thermometer and hygrometer. Daily temperature and relative humidity were measured for 20 weeks.

3.3.2 Growth traits

Growth parameters of each bird belonging to different genotypes were measured. The growth data were measured on weekly basis on each bird for 20 weeks. The body weight of the birds was measured using ATOM-A120 weighing scale of 0.05g sensitivity. The body weight of the birds was measured in the morning before feeding. The linear body parameters were measured in centimetre using tailor's tape rule as follows:

Breast girth: It was measured as the circumference of the breast around the deepest region of the breast.

Keel length: It was measured as the length region of the sternum.

Shank length: It was measured as the distance between the hock joint and the tarso-metatarsus of the left leg.

Thigh length: It was measured as the distance between the patella and the posterior end of the tibia joining the tarso-metatarsus.

Wing length: It was measured as the distance between the tip of the phalanges and the coracoid-humerus joint of the left wing.

Wing span: It was measured as the distance between the tip of left phalanges and the tip of the right phalanges.

3.3.3 Feed efficiency

3.3.3.1 Feed intake and weight gain

The quantity of left over feed was weighed and subtracted from the amount of feed given to determine the daily feed intake of the birds per day as follows:

Feed intake (g) = feed supplied - left over feed.

The weight gain of the birds was measured as final weight minus initial weight as follows:

Weight gain (g) = final weight - initial weight

The feed efficiency of each bird was calculated as the ratio of weight gain to feed intake as follows:

$$\text{Feed efficiency} = \frac{\text{Weight gain (g)}}{\text{Feed intake (g)}}$$

3.3.4 Heat tolerance traits

Rectal temperature, pulse rate and respiratory rate of all the birds were measured early in the morning between 7:00 am and 8.00am before the rising of the sun and again between 2.00pm and 3.00pm of the same day.

3.3.4.1 Rectal temperature

This was measured in each animal using a digital thermometer which was inserted into the rectum at the display of L^0C (meaning, lowest readable temperature by the thermometer) and was removed after the sound of the alarm signal.

3.3.4.2 Respiratory Rate

This was determined by counting the number of movement of the vent per minute using stopwatch.

3.3.4.3 Pulse Rate

This was determined for each bird by placing the fingertips under the wing vein and counting the number of pulses per minute using stop watch.

3.3.4.4 Heat stress index

The heat stress index was calculated from the relationship between pulse rate and respiratory rate together with their normal average values using the formula below:

H= (AR/AP)* (NP/NR)

H= Heat stress index

AR= Average respiratory rate

AP= Average pulse rate

NP= Normal pulse rate

NR= Normal respiratory rate (Oladimeji *et al*., 1996)

290 beats/min was used as normal pulse rate while 32 breaths/min was used as normal respiratory rate as reported by Dagoon (1989).

3.3.5 Mortality and survivability

The initial numbers of chicks stocked was recorded for each genotype. The number of mortality per genotype as well as number that survived was recorded for each genotype.

3.4 Blood collection

About 2 ml of blood was collected from brachial vein of each bird using needle and syringe. 1 ml of blood was deposited in plain bottles for serum preparation while the remaining 1 ml was deposited in EDTA bottle for DNA extraction.

3.5 Serum preparation

The blood was allowed to clot by leaving it undisturbed at room temperature for 30 minutes. The blood was later centrifuged at 5000 revolution per minute for 5 minutes in Thermo Scientific refrigerated centrifuge at 4^0C.

3.6 Antibody response to Newcastle disease virus

The serum of the chickens was collected at 24 days of age to evaluate the baseline antibody titre to Newcastle disease virus. The chickens were immunized with a commercial Newcastle disease virus vaccine of the LaSota strain, using the standard dose given in the instruction of the vaccine at 25 days of age and at 50 days of age respectively. At 41 days after second immunization (91 days of age), serum samples were collected from the chickens for evaluation of another antibody titre.

The antibody response to Newcastle disease virus was determined using haemaglutination inhibition test as described: 25 µl of phosphate buffer saline was dispensed into each well of microwell plates. The serum samples were shaken and 25 µl of each chicken sample was dispensed into the first well and the last (control) well of a row of the microwell plate. Two-fold serial dilution was made along the row until the second to the last well. 25 µl of 4HA (standard amount of Newcastle virus used in HI tests) of Lasota Vaccine was added to each well excluding the control well. The sides of the microwell plates were gently tapped to mix the reagents. The plates were covered with a lid and allowed to stand for 30 minutes at room temperature. 25 µl of

1% washed red blood cell was added to each well including the control. The sides of the microwell plates were gently tapped in order to mix the reagents. The plates were covered with a lid and allowed to stand at room temperature for 45 minutes. The settling pattern for each serum was observed and antibody level for each serum was recorded. In the wells where antibodies were produced, the red blood cells settled as button while the red blood cells agglutinate where antibodies were not produced. The end point was determined as the number of the well where there was complete inhibition of haemaglutination.

The end point for each chicken sample was converted to geometric mean titre (GMT) using the formula:

GMT = Antilog ((A-1) × logB + logC) (Villegas and Purchase, 1989)

Where:

A is the average end point well number

B is factor 2.0

C is the reciprocal of the first dilution (2.0)

3.7 DNA extraction

Genomic DNA was extracted at Biotechnology laboratory of the Department of Animal Breeding and Genetics, Federal University of Agriculture, Abeokuta from 90 birds using Zymo research quick-gDNA^{TM} miniprep kit (catalogue number: D3024) as described: 400 µl of genomic lysis buffer containing beta-mercaptoethanol was added to 100 µl of whole blood. The mixture was vortexed for 6 seconds and allowed to stand at room temperature for 10 minutes. The mixture was transferred to Zymo-Spin^{TM} column in a collection tube and centrifuged for one minute at 10,000 ×g. The collection tube was discarded with the flow-through and the Zymo-Spin^{TM} column

was transferred to a new collection tube. 200 µl of DNA pre-wash buffer was added to the spin column and centrifuged for one minute at 10,000 ×g. 500 µl of g-DNA wash buffer was added to the spin column and centrifuged for one minute at 10,000 ×g. The spin column was transferred into an eppendorf tube and 50 µl of DNA elution buffer was added to the spin column. The mixture was incubated for 5 minutes at room temperature and centrifuged at 16,000 ×g for 30 seconds to elute the DNA. The eluted DNA was stored immediately for molecular based applications at -4^0C.

3.8 DNA quantification

The extracted gDNA was quantified for concentration and purity using Nanodrop spectrophotometer using the protocol described by Desjardins and Conklin (2010). The integrity of the gDNA was also checked using gel electrophoretic method by running 1 µl of each gDNA sample on 1.5% agarose gel at 120 V for 20 minutes.

3.9 Primer design and amplification of chicken IRF-5 gene

Primers that can amplify exons 3-5, 7 and the surrounding introns of chicken IRF-5 gene were designed at Stab Vida genetics Laboratory situated in Monte da Caparica, Portugal using Primer Blast of National Centre for Biotechnology information (NCBI) database, United States of America. The primer sequences, lengths, annealing temperatures and product sizes of the amplicons are shown in Table 3. For amplification, 1 µl of genomic DNA (~10-15 ng) was added to a reaction mixture containing 16.8 µl of nuclease free water, 2.5 µl of 10× PCR buffer, 1.5 µl of 25mM $MgCl_2$, 1 µl of 5mM dNTP, 1 µl of 10 UM forward primer, 1 µl of 10UM reverse primer and 0.2 µl of 10U/ µl surf Hot Taq. The PCR conditions included initial denaturation at 96^0C for 15 minutes, 35 cycles of final denaturation at 95^0C for 30

seconds, annealing at 62^0C (exons 3-5) and 58^0C (exon 7) for 30 seconds, extension at 70^0C for 1 minute and final extension at 70^0C for 5 minutes.

3.10 Polymerase chain reaction purification and Sanger sequencing

Polymerase chain reaction (PCR) purification was done with Magnetic Beads Carboxylate (MCLab, USA). Sequencing of PCR products was done using the primers used for amplification (Table 3) with BigDye Terminator v. 3.1 using the instrument 3730 XL following the supplier's protocol.

Table 3: Primer sequences, annealing temperatures and product sizes of the amplicons

Primer	Sequence	PCR size (bp)	Annealing temperature (^{0}C)
Exons 3-5	Fwd 5'-TAACCACAACCCAATGATGC-3' Rev 5'-ATTCCCCCATAAAACACCC-3'	742	62
Exon 7	Fwd 5'-CGGAGCGATATGGAGTG-3' Rev 5'-TTCTACCTGATGTCCCCC-3'	747	58

3.11 Trimming and cleaning of sequences

The nucleotide sequences were trimmed and edited using BIOEDIT and MEGA 6 software to remove noises in the sequences.

3.12 Multiple sequence alignment

The sequences obtained for exons 3-5 and 7 were aligned with individual reference exons 3, 4, 5 and 7, respectively to cut out the individual exons so as to do the analyses for each exon separately. The alignment was carried out on all the nucleotide sequences using Clustal W software (Thompson *et al*., 1994) incorporated inside MEGA 6 software.

3.13 Identification and analyses of single nucleotide polymorphisms

The SNPs present in exons 3, 4, 5 and 7 of IRF-5 gene in Nigerian indigenous chickens were identified by aligning each exon with the reference exons downloaded from Ensembl database using Clustal W (Thompson *et al*., 1994). The SNPs were also confirmed using DnaSP (Librado and Rozas, 2009).

Allele frequency of each SNP was determined by dividing the frequency of each allele with total sample size for each genotype. The genotype of each SNP was determined by manual inspection of the electrophoregram.

Heterozygosity of the SNPs was calculated using the formula proposed by Guo and Elston (1999):

Heterozygosity (H_e) = $1-(p^2+q^2)$

Polymorphism information content (PIC) of the SNPs was calculated using the formula proposed by Botstein *et al*. (1980):

$PIC = H_e - 2p^2q^2$

Where p is the major allele frequency and q is the minor allele frequency.

The linkage disequilibrium among the SNPs was determined by pairwise comparison of r^2 using DnaSP (Librado and Rozas, 2009).

The resultant amino acid variation of each SNP was determined using codoncode aligner software. The effect of the amino acid variation on the protein function was predicted using $SNAP^2$ software.

3.14 Diversity of exon 3, 4, 5 and 7 of IRF-5 gene in Nigerian indigenous chickens

Diversity indices such as number of haplotype, haplotype diversity, average number of nucleotide differences, nucleotide diversity, singleton variable site and parsimony informative site were estimated for exons 3, 4, 5 and 7 of IRF-5 gene in Nigerian indigenous chickens using DnaSP (Librado and Rozas, 2009).

3.15 Haplotype distribution

Haplotypes present in exons 3, 4, 5 and 7 of IRF-5 gene in Nigerian indigenous chickens were reconstructed using DnaSP (Librado and Rozas, 2009). DnaSP uses algorithms provided by PHASE, fastPHASE and HAPAR software in haplotype reconstruction. PHASE, fastPHASE and HAPAR use coalescent-based Bayesian, patterns of linkage disequilibrium and pure parsimony approaches, respectively to infer haplotypes.

3.16 Evolutionary analysis.

The frequency of nucleotides present in exons 3, 4, 5 and 7 of IRF-5 gene in Nigerian indigenous chickens was determined using MEGA 6 software.

The minimum number of recombination events (Hudson and Kaplan, 1985) in exons 3, 4, 5 and 7 of IRF-5 gene in Nigerian indigenous chickens was determined using DnaSP (Librado and Rozas, 2009).

MEGA6 software was used to determine the phylogenetic relationship among exons 3, 4, 5 and 7 of IRF-5 in Nigerian indigenous chickens and other chicken genotypes (Leghorn with accession NM001031587.1 and Red Jungle Fowl with accession number XM015281939.1). The phylogenetic tree was inferred using unweighted pair group method with arithmetic mean based on Jones-Taylor-Thornton matrix-based model. The reliability of the inferred phylogenetic tree was evaluated using bootstrap analysis of 1000 replications.

3.17 Selection analysis

Tajima's D, Fu's Fs, Fu, Li's D* and Fu and Li's F* tests were performed to test exons 3, 4, 5 and 7 of IRF-5 gene in Nigerian indigenous chickens deviation from neutrality using DnaSP (Librado and Rozas, 2009).

Mean non-synonymous substitutions per non-synonymous site (dN) and mean synonymous substitutions per synonymous site (dS) were calculated for exons 3, 4, 5 and 7 of IRF-5 gene in Nigerian indigenous chickens to predict likely selective force acting on the exons using HyPhy software implemented inside MEGA6 software. Positive dN-dS value suggests positive selection while negative dN-dS suggest negative selection.

3.18 Protein structure prediction

The nucleotide sequences of exons 3, 4, 5 and 7 of IRF-5 gene in Nigerian indigenous chickens were translated to amino acid sequences using NCBI Open Reading Frame finder. Secondary structures of the proteins were predicted using GORIV software (Garnier *et al*., 1996). The tertiary structures were predicted using Phyre2 software (Kelley and Sternberg, 2009) and viewed using Rasmol software.

3.19 Prediction of motifs and domain architecture

Motifs in the amino acid sequences of exons 3, 4, 5 and 7 of IRF-5 gene in Nigerian indigenous chickens were predicted using PROSITE software (De Castro *et al*., 2006) while the domain structure was predicted using SMART (Letunic *et al*., 2009).

3.20 Statistical analyses

3.20.1 Effect of genotype and sex on productive and adaptive traits

Survivability data was subjected to descriptive statistics (bar charts) using Excel (2007). The antibody titre was converted to geometric mean titre to normalize this data as it doesn't follow normal distribution.

All other productive and adaptive data collected were subjected to general linear model (GLM) procedure of SAS (2002) using the model below:

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

Where:

Y_{ijkl} is the dependent variable such as growth traits, feed efficiency, heat tolerance traits, antibody response to Newcastle disease virus.

μ is the population mean

G_i is the fixed effect of i^{th} genotype (i=1-3)

S_j is the fixed effect of j^{th} sex (j=1-2)

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

e_{ijkl} is the random residual error

3.20.2 Association analysis

Preliminary analysis of the SNPs revealed that the identified SNPs are synonymous and the ones that are not synonymous are singletons which cannot be used for association study. So the association study was done based on haplotypes identified using Proc Mixed of SAS (2002) with the model stated below:

$Y = X\beta + \gamma + \varepsilon$

Where:

Y is the dependent variable such as growth traits, feed efficiency, heat tolerance traits, antibody response to Newcastle disease virus.

X is the candidate haplotype genotype.

β is the effect size of candidate haplotype.

γ is the effect of sex and genotype

ε is the random residual error

Haplotypes shared by the three genotypes were only used for the association analysis. Also, haplotypes with frequency of 1 were not used for the analysis.

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CHAPTER FOUR

4.0 RESULTS

4.1 Effect of genotype, sex and their interaction on productive traits

4.1.1 Effect of genotype on body weight and feed efficiency

The effect of genotype on body weight and feed efficiency of Nigerian indigenous chickens is presented in Table 4. Genotype had significant ($p<0.05$) effect on body weight in all the weeks considered. At week 4, highest body weight value of 480.65±10.64 g was observed in frizzle feather chickens, while the least was recorded in naked neck chickens. There was however no significant ($p>0.05$) difference in the body weight of normal feather and naked neck chickens at weeks 8 and 16. At week 20, there was also no significant ($p>0.05$) difference in the body weight among the three genotypes.

Feed efficiency was significantly ($p<0.05$) affected by genotype of the birds in all the weeks except at week 16. The feed efficiency of the birds reduced with age except in frizzle feather chickens at week 20. At weeks 1, 8 and 12, the highest feed efficiency was observed in normal feather chickens whereas there was no significant ($p>0.05$) difference in the feed efficiency of normal feather and naked neck chickens at weeks 4 and 20.

Table 4: Effect of genotype on body weight and feed efficiency of Nigerian indigenous chickens

Age in weeks	Genotype	N	Body weight (g)	Feed efficiency (%)
1	Normal feather	111	88.42±1.51[a]	63.69±1.78[a]
	Naked neck	112	81.66±1.50[b]	54.02±1.76[b]
	Frizzle feather	106	81.71±1.52[b]	56.43±1.77[b]
4	Normal feather	111	400.84±9.01[b]	45.90±3.17[b]
	Naked neck	112	375.81±8.95[c]	41.89±2.04[b]
	Frizzle feather	77	480.65±10.64[a]	53.71±2.11[a]
8	Normal feather	110	896.05±45.79[b]	43.86±2.34[a]
	Naked neck	111	853.94±40.64[b]	36.87±2.37[b]
	Frizzle feather	75	942.45±54.76[a]	28.95±1.88[c]
12	Normal feather	108	1260.95±18.18[a]	20.67±1.03[a]
	Naked neck	111	1155.18±18.01[b]	12.67±1.31[b]
	Frizzle feather	73	1140.74±21.98[b]	9.04±1.25[c]
16	Normal feather	108	1317.76±11.94[a]	6.74±0.85
	Naked neck	111	1286.89±11.82[a]	7.44±0.52
	Frizzle feather	72	1235.62±14.56[b]	6.84±0.54
20	Normal feather	107	1491.19±16.05[a]	6.66±0.56[b]
	Naked neck	110	1444.13±15.95[a]	6.43±0.44[b]
	Frizzle feather	71	1427.18±19.69[a]	9.54±0.77[a]

N: number of observation

[a,b,c]Means in the same column with different superscripts for the same age are significantly different ($p<0.05$)

4.1.2 Effect of sex on body weight and feed efficiency

Sex effect on body weight and feed efficiency of Nigerian indigenous chickens is as shown in Table 5. Sex significantly ($p<0.001$) affected body weight at weeks 16 and 20. Significantly ($p<0.05$) higher body weights were observed in males at weeks 16 and 20. Sex however, had no significant ($p>0.05$) effect on feed efficiency of the Nigerian indigenous chickens except at week 16. Significantly ($p<0.05$) higher feed efficiency was observed in male chickens (9.40±0.42%) compared with their female counterparts (4.61±0.62%) at week 16.

Table 5: Effect of sex on body weight and feed efficiency of Nigerian indigenous chickens

Age in weeks	Sex	N	Body weight (g)	Feed efficiency (%)
1	Male	146	83.62±1.30	57.83±1.52
	Female	183	84.24±1.17	58.27±1.37
4	Male	132	413.80±8.12	46.30±1.99
	Female	168	424.40±7.49	48.03±2.08
8	Male	131	995.35±41.30	38.54±1.78
	Female	165	932.94±38.54	34.58±1.83
12	Male	131	1199.69±15.50	14.70±0.99
	Female	161	1171.56±16.30	13.55±0.97
16	Male	131	1394.97±10.70[a]	9.40±0.42[a]
	Female	160	1165.21±10.26[b]	4.61±0.62[b]
20	Male	130	1501.60±14.39[a]	8.12±0.45
	Female	158	1316.74±13.89[b]	6.97±0.54

N: number of observation

[a,b]Means in the same column with different superscripts for the same age are significantly different ($p<0.05$)

4.1.3 Genotype by sex effect on body weight and feed efficiency of Nigerian indigenous chickens

Genotype by sex effect on body weight and feed efficiency of Nigerian indigenous chickens is shown in Table 6. Body weight and feed efficiency were not significantly ($p>0.05$) affected by interaction between genotype and sex except at week 16. Feed efficiency at week 16 ranged from 3.92±0.79% to 10.96±0.66%. Also, the significantly ($p<0.05$) highest feed efficiency (10.96±0.66%) was observed in male naked neck chickens while the significantly ($p<0.05$) least values were observed in female normal feather, female naked neck and female frizzle feather chickens at week 16.

Table 6: Effect of interaction between genotype and sex on body weight and feed efficiency of Nigerian indigenous chickens

Age in weeks	Genotype	Sex	N	Body weight (g)	Feed efficiency (%)
1	Normal feather	Male	45	86.47±2.33	62.03±2.73
	Normal feather	Female	66	90.38±1.93	65.35±2.27
	Naked neck	Male	46	81.54±2.31	52.95±2.70
	Naked neck	Female	66	81.77±1.93	55.09±2.25
	Frizzle feather	Male	54	82.86±2.11	54.35±2.56
	Frizzle feather	Female	52	80.55±2.19	58.50±2.45
4	Normal feather	Male	45	391.07±13.89	51.78±2.70
	Normal feather	Female	66	410.62±11.47	55.63±3.24
	Naked neck	Male	46	377.37±13.74	38.05±2.61
	Naked neck	Female	66	374.25±11.47	45.73±3.13
	Frizzle feather	Male	41	472.97±14.55	42.74±4.33
	Frizzle feather	Female	36	488.32±15.53	49.07±4.42
8	Normal feather	Male	45	888.92±70.39	30.54±2.92
	Normal feather	Female	65	903.19±58.57	27.36±2.37
	Naked neck	Male	45	963.91±70.39	46.33±3.14
	Naked neck	Female	66	923.98±58.12	41.40±3.47
	Frizzle feather	Male	41	963.22±73.74	41.93±3.60
	Frizzle feather	Female	34	921.67±80.98	31.81±3.09
12	Normal feather	Male	45	1301.52±23.47	19.40±1.55
	Normal feather	Female	63	1220.38±27.78	21.94±1.35
	Naked neck	Male	45	1164.42±27.78	7.79±1.77
	Naked neck	Female	66	1125.94±22.93	10.28±1.77
	Frizzle feather	Male	41	1151.60±32.94	13.45±1.74
	Frizzle feather	Female	32	1129.89±29.10	11.89±1.95
16	Normal feather	Male	45	1394.54±18.24^{a}	8.55±0.74^{b}
	Normal feather	Female	63	1240.98±15.41^{b}	5.13±0.78^{c}
	Naked neck	Male	45	1423.07±18.24^{a}	10.96±0.66^{a}
	Naked neck	Female	66	1150.71±15.05^{c}	3.92±0.79d^{c}
	Frizzle feather	Male	41	1367.31±19.11^{ab}	8.69±0.77^{b}
	Frizzle feather	Female	31	1150.94±21.97^{c}	4.79±1.51^{c}
20	Normal feather	Male	45	1529.70±24.44	7.04±0.66
	Normal feather	Female	62	1452.68±20.82	5.82±0.59
	Naked neck	Male	44	1456.71±24.72	7.16±0.85
	Naked neck	Female	66	1331.55±20.18	6.17±0.72
	Frizzle feather	Male	41	1488.38±25.60	10.17±0.79
	Frizzle feather	Female	30	1365.98±29.93	8.91±1.32

N: number of observation,

^{a,b,c}Means in the same column with different superscripts for the same age are significantly different ($p<0.05$)

4.1.4 Effect of genotype on linear body measurements

The effect of genotype on linear body measurements of Nigerian indigenous chickens is as shown in Table 7. Genotype significantly ($p<0.05$) affected breast girth in all the weeks studied. Thigh length, shank length and keel length were also significantly ($p<0.05$) affected by genotype at weeks 1 and 4. Also, genotype effect was significant ($p<0.05$) on wing span in all the weeks except at week 1. All the linear body measurements increased with age of the birds. At week 20, there was no significant ($p>0.05$) difference in the breast girth of Naked Neck and Frizzle Feather chickens as well as their wing length and wing span.

Table 7: Effect of genotype on linear body measurements of Nigerian indigenous chickens

Age in weeks	Genotype	N	BG (cm)	TL (cm)	SL (cm)	KL (cm)	WL (cm)	WS (cm)
1	NF	111	10.70±0.08^{a}	4.91±0.05^{b}	3.28±0.03^{a}	3.92±0.05^{a}	7.63±0.09	17.48±0.17
	NN	112	10.65±0.08^{a}	4.81±0.05^{b}	3.03±0.03^{c}	3.47±0.05^{c}	7.63±0.09	17.26±0.16
	FF	106	10.20±0.08^{b}	5.14±0.06^{a}	3.13±0.03^{b}	3.71±0.05^{b}	7.65±0.09	17.23±0.16
4	NF	111	18.00±0.15^{b}	9.21±0.11^{b}	5.25±0.06^{b}	6.85±0.09^{b}	12.36±0.18^{b}	27.77±0.34^{c}
	NN	112	17.61±0.15^{b}	8.85±0.11^{a}	5.28±0.06^{b}	6.71±0.09^{b}	12.84±0.18^{b}	28.77±0.34^{b}
	FF	77	18.50±0.18^{a}	11.37±0.13^{c}	6.07±0.08^{a}	8.49±0.10^{a}	14.98±0.21^{a}	35.67±0.40^{a}
8	NF	110	22.11±0.19^{b}	15.70±0.12	7.44±0.05^{b}	10.09±0.10	16.60±0.13	41.52±0.25
	NN	111	22.91±0.18^{a}	15.99±0.12	7.62±0.05^{a}	10.29±0.10	16.85±0.13	42.05±0.25
	FF	75	22.59±0.22^{ba}	15.79±0.15	7.44±0.06^{b}	10.09±0.12	16.49±0.16	42.18±0.30
12	NF	108	24.58±0.16^{b}	16.66±0.09	9.05±0.07^{a}	10.88±0.11	23.25±0.15^{b}	47.35±0.29^{b}
	NN	111	24.76±0.16^{ba}	16.49±0.09	9.11±0.06^{a}	10.95±0.11	23.98±0.15^{a}	48.28±0.29^{a}
	FF	73	25.26±0.20^{a}	16.33±0.11	8.77±0.08^{b}	10.70±0.14	21.57±0.18^{c}	44.02±0.35^{c}
16	NF	108	25.98±0.16^{a}	17.30±0.11	9.19±0.06	12.35±0.09	25.91±0.17^{a}	50.51±0.20^{a}
	NN	111	25.39±0.16^{b}	17.25±0.11	10.01±0.06	12.51±0.09	25.02±0.17^{b}	50.14±0.20^{a}
	FF	72	25.34±0.19^{b}	17.40±0.13	9.04±0.07	12.63±0.11	24.79±0.21^{b}	49.46±0.24^{b}
20	NF	107	27.94±0.16^{a}	18.33±0.09	10.38±0.06	14.33±0.10	29.79±0.17^{a}	54.97±0.20^{a}
	NN	110	27.29±0.16^{b}	18.14±0.09	10.22±0.06	14.45±0.10	29.00±0.17^{b}	54.63±0.20^{ba}
	FF	71	27.36±0.19^{b}	18.06±0.11	10.25±0.07	14.60±0.12	28.80±0.21^{b}	54.10±0.25^{b}

NF: Normal Feather, NN: Naked Neck, FF: Frizzle Feather, N: number of observation, BG: breast girth,

TL: thigh length, SL: shank length, BL: body length, KL: keel length, WL: wing length, WS: wing span

^{a,b,c}Means in the same column with different superscripts for the same age are significantly different ($p<0.05$)

4.1.5 Effect of sex on linear body measurements

The effect of sex on linear body measurements of Nigerian indigenous chickens is shown in Table 8. The linear body measurements of the birds were not significantly ($p>0.05$) affected by their sex except for thigh length at weeks 1, 8 and 12; keel length and wing length at week 16 and wing span at week 20. There was however no significant ($p>0.05$) difference in the thigh length of male and female birds at week 8.

Table 8: Effect of sex on linear body measurements of Nigerian indigenous chickens

Age in weeks	Sex	N	BG (cm)	TL (cm)	SL (cm)	KL (cm)	WL (cm)	WS (cm)
1	Male	146	10.46±0.07	5.02±0.05[a]	3.14±0.03	3.72±0.04	7.63±0.08	17.39±0.14
	Female	183	10.58±0.06	4.89±0.04[b]	3.15±0.02	3.68±0.04	7.64±0.07	17.26±0.13
4	Male	132	17.98±0.14	9.71±0.10	5.51±0.06	7.28±0.08	13.45±0.16	30.48±0.31
	Female	168	18.09±0.13	9.91±0.09	5.55±0.05	7.42±0.07	13.34±0.15	30.98±0.28
8	Male	131	22.50±0.17	16.00±0.11[a]	7.50±0.05	10.19±0.09	16.54±0.11	41.89±0.23
	Female	165	22.57±0.16	15.66±0.11[a]	7.49±0.05	10.12±0.09	16.76±0.11	41.94±0.21
12	Male	131	25.06±0.14	16.63±0.08[a]	9.05±0.06	10.91±0.10	23.08±0.13	46.68±0.25
	Female	161	24.68±0.15	16.35±0.07[b]	8.91±0.06	10.77±0.10	22.78±0.13	46.41±0.26
16	Male	131	25.64±0.14	17.39±0.09	9.09±0.05	12.61±0.09[a]	25.51±0.15[a]	50.18±0.17
	Female	160	25.49±0.13	17.24±0.10	9.07±0.05	12.38±0.08[b]	24.97±0.14[b]	49.89±0.18
20	Male	130	28.95±0.14	20.13±0.08	11.28±0.05	16.44±0.09	30.17±0.16	54.84±0.17[a]
	Female	158	27.71±0.14	18.21±0.08	10.28±0.05	14.49±0.08	29.22±0.15	54.28±0.18[b]

N: number of observation, BG: breast girth, TL: thigh length, SL: shank length, BL: body length, KL: keel length,

WL: wing length, WS: wing span

[a,b]Means in the same column with different superscripts for the same age are significantly different ($p<0.05$)

4.1.6 Genotype by sex effect on linear body measurements

The genotype by sex effect on linear body measurements of Nigerian indigenous chickens is shown in Table 9. All the linear body measurements were not significantly ($p>0.05$) affected by interaction between genotype and sex except breast girth at week 4 and wing length at week 16.

At week 16, the highest wing length (26.62±0.26 cm) was observed in male normal feather chickens while there was no significant ($p>0.05$) difference in wing length of other groups.

Table 9: Effect of interaction between genotype and sex on linear body measurements of Nigerian indigenous chickens

Age in weeks	N	Genotype	Sex	BG (cm)	TL (cm)	SL (cm)	KL (cm)	WL (cm)	WS (cm)
1	45	Normal feather	Male	10.64±0.13	4.94±0.08	3.28±0.05	3.93±0.08	7.66±0.14	17.63±0.25
	66	Normal feather	female	10.77±0.10	4.89±0.07	3.27±0.04	3.90±0.07	7.59±0.11	17.32±0.21
	46	Naked neck	Male	10.68±0.13	4.84±0.08	2.98±0.04	3.42±0.08	7.63±0.14	17.38±0.25
	66	Naked neck	Female	10.62±0.10	4.77±0.07	3.07±0.04	3.52±0.07	7.64±0.11	17.15±0.21
	54	Frizzle feather	Male	10.06±0.11	5.28±0.07	3.16±0.04	3.80±0.07	7.61±0.12	17.16±0.23
	52	Frizzle feather	Female	10.34±0.12	5.00±0.08	3.11±0.04	3.61±0.07	7.70±0.13	17.29±0.23
4	45	Normal feather	Male	17.84±0.24^{bc}	9.10±0.17	5.23±0.10	6.74±0.14	12.54±0.27	27.43±0.53
	66	Normal feather	female	18.16±0.20^{ba}	9.32±0.14	5.26±0.08	6.96±0.11	12.17±0.22	28.10±0.44
	46	Naked neck	Male	17.90±0.23^{b}	8.90±0.16	5.30±0.10	6.79±0.14	12.89±0.27	28.67±0.52
	66	Naked neck	Female	17.32±0.20^{c}	8.80±0.14	5.26±0.08	6.63±0.11	12.78±0.22	28.86±0.44
	41	Frizzle feather	Male	18.21±0.25^{ba}	11.13±0.17	6.00±0.10	8.30±0.14	14.91±0.28	35.34±0.55
	36	Frizzle feather	Female	18.79±0.26^{a}	11.61±0.19	6.13±0.11	8.67±0.15	15.05±0.31	35.99±0.59
8	45	Normal feather	Male	22.05±0.28	15.73±0.19	7.40±0.08	10.20±0.16	16.55±0.20	41.30±0.39
	65	Normal feather	female	22.17±0.24	15.67±0.16	7.47±0.07	9.99±0.13	16.66±0.17	41.74±0.32
	45	Naked neck	Male	22.84±0.28	16.16±0.16	7.65±0.08	10.30±0.16	16.72±0.20	42.18±0.39
	66	Naked neck	Female	22.98±0.23	15.81±0.19	7.58±0.07	10.27±0.13	16.98±0.17	41.92±0.32
	41	Frizzle feather	Male	22.61±0.30	16.45±0.20	7.45±0.09	10.08±0.17	16.34±0.21	42.19±0.41
	34	Frizzle feather	Female	22.54±0.33	15.13±0.22	7.43±0.09	10.10±0.18	16.65±0.23	42.17±0.45
12	45	Normal feather	Male	24.60±0.21	16.89±0.13	9.08±0.08	11.05±0.15	23.28±0.19	47.45±0.44
	63	Normal feather	female	24.55±0.25	16.42±0.11	9.03±0.10	10.71±0.18	23.21±0.23	47.24±0.37
	45	Naked neck	Male	24.91±0.20	16.59±0.13	9.20±0.08	11.06±0.14	24.04±0.17	48.50±0.37
	66	Naked neck	Female	24.61±0.25	16.39±0.11	9.02±0.10	10.83±0.18	23.91±0.23	48.05±0.44
	41	Frizzle feather	Male	25.65±0.29	16.92±0.14	8.86±0.11	10.77±0.18	21.91±0.26	44.31±0.53
	32	Frizzle feather	Female	24.87±0.26	16.24±0.16	8.67±0.10	10.62±0.21	21.23±0.24	43.73±0.46

Table 9 (Cont'd): Effect of interaction between genotype and sex on linear body measurements of Nigerian indigenous chickens

Age in weeks	N	Genotype	Sex	BG (cm)	TL (cm)	SL (cm)	KL (cm)	WL (cm)	WS (cm)
16	45	Normal feather	Male	26.11±0.24	17.22±0.16	9.26±0.10	12.48±0.14	26.62±0.26[a]	50.13±0.31
	63	Normal feather	female	25.85±0.20	17.37±0.14	9.11±0.08	12.22±0.12	25.19±0.22[b]	50.89±0.26
	45	Naked neck	Male	25.44±0.24	17.10±0.16	9.56±0.08	12.65±0.14	25.12±0.26[b]	50.02±0.31
	66	Naked neck	Female	25.34±0.20	17.40±0.13	9.52±0.08	12.37±0.12	24.92±0.21[b]	50.25±0.25
	41	Frizzle feather	Male	25.37±0.25	17.40±0.17	9.40±0.10	12.71±0.15	24.78±0.27[b]	49.51±0.32
	31	Frizzle feather	Female	25.31±0.29	17.41±0.19	9.10±0.11	12.54±0.17	24.79±0.31[b]	49.41±0.37
20	45	Normal feather	Male	28.20±0.20	18.35±0.13	10.40±0.08	14.40±0.12	29.85±0.23	55.49±0.26
	62	Normal feather	female	27.68±0.24	18.30±0.11	10.35±0.09	14.26±0.15	29.72±0.27	54.45±0.31
	44	Naked neck	Male	27.57±0.20	18.25±0.11	10.29±0.09	14.53±0.12	29.04±0.27	54.77±0.25
	66	Naked neck	Female	27.02±0.24	18.02±0.14	10.15±0.08	14.37±0.15	28.97±0.22	54.48±0.31
	41	Frizzle feather	Male	27.36±0.24	18.08±0.16	10.29±0.11	14.67±0.15	28.85±0.33	54.28±0.37
	30	Frizzle feather	Female	27.36±0.29	18.03±0.14	10.21±0.10	14.53±0.18	28.74±0.28	53.92±0.32

N: number of observation, BG: breast girth, TL: thigh length, SL: shank length, BL: body length, KL: keel length, WL: wing length,

WS: wing span

[a,b,c]Means in the same column with different superscripts for the same age are significantly different ($p<0.05$)

4.2 Effect of genotype, sex and their interaction on adaptive traits

4.2.1 Effect of genotype on heat tolerance traits of Nigerian indigenous chickens

Temperature and relative humidity values during experimental period are presented in Table 10 while the effect of genotype on heat tolerance traits of Nigerian indigenous chickens is shown in Table 11. All the heat tolerance traits measured were significantly ($p<0.05$) affected by genotype in all the weeks.

The highest rectal temperature (41.86±0.03 ^{0}C) was observed in naked neck chickens at week 16. The significantly highest ($p<0.05$) respiratory rate was observed in all the three chicken genotypes at week 4. There was also no significant ($p>0.05$) difference in the respiratory rate of normal feather (30.46±0.78 breaths/min) and frizzle feather (29.99±0.95 breaths/min) chickens at week 16. The significantly highest ($p<0.05$) pulse rate of 271.01±1.23 beats/min was observed in frizzle feather chickens at week 20.

Table 10: Temperature and relative humidity during experimental period

Month	Corresponding age of bird in weeks	Temperature (^{0}C)	Relative humidity (%)
January	Week 4	30.40	77.00
February	Week 8	34.00	81.00
March	Week 12	29.30	96.00
April	Week 16	32.60	77.00
May	Week 20	35.10	58.50

Table 11: Effect of genotype on heat tolerance traits of Nigerian indigenous chickens

Age in weeks	N	Genotype	Rectal temperature (^{0}C)	Respiratory rate (breaths/min)	Pulse rate (beats/min)	Heat stress index
4	111	NF	41.44±0.05^a	72.75±1.02^b	183.31±2.51^b	3.64±0.07^a
	112	NN	41.16±0.05^b	76.64±1.02^a	205.29±2.50^a	3.47±0.07^a
	77	FF	41.34±0.06^a	46.31±1.21^c	206.24±2.97^a	2.10±0.08^b
8	110	NF	41.48±0.04^a	38.27±0.53^a	221.10±1.92^a	1.58±0.03^b
	111	NN	41.25±0.04^b	36.74±0.52^b	220.35±1.91^a	1.52±0.03^b
	75	FF	41.49±0.04^a	34.54±0.63^c	176.67±2.29^b	1.83±0.03^a
12	108	NF	41.20±0.03^b	37.11±0.65^a	170.98±2.59^b	2.01±0.04^a
	111	NN	41.66±0.03^a	34.79±0.65^b	211.14±2.56^a	1.53±0.04^b
	73	FF	41.64±0.04^a	33.24±0.79^b	207.14±3.13^a	1.47±0.05^b
16	108	NF	41.75±0.03^b	30.46±0.78^b	223.02±1.84^a	1.25±0.03^b
	111	NN	41.86±0.03^a	42.18±0.77^a	228.62±1.82^a	1.68±0.03^a
	72	FF	41.70±0.03^b	29.99±0.95^b	208.98±2.24^b	1.30±0.04^b
20	107	NF	41.38±0.03^{ba}	32.06±0.51^b	260.07±1.00^c	1.12±0.02^b
	110	NN	41.45±0.03^a	34.61±0.51^a	265.14±0.99^b	1.19±0.02^a
	71	FF	41.35±0.03^b	31.24±0.62^b	271.01±1.23^a	1.05±0.02^c

N: number of observation

NF: Normal Feather, NN: Naked Neck, FF: Frizzle Feather

^{a,b,c}Means in the same column with different superscripts for the same age are significantly different ($p<0.05$)

4.2.2 Effect of sex on heat tolerance traits of Nigerian indigenous chickens

Effect of sex on heat tolerance traits of Nigerian indigenous chickens is shown in Table 12. Rectal temperature was not significantly ($p>0.05$) affected by sex except at week 16 while the only significant ($p<0.05$) effect of sex on respiratory rate was observed at week 20. All the heat tolerance traits of female birds were significantly ($p<0.05$) higher than that of males in all the weeks except pulse rate at weeks 8 and 20 as well as heat stress index at weeks 4 and 12.

Table 12: Effect of sex on heat tolerance traits of Nigerian indigenous chickens

Age in weeks	N	Sex	Rectal temperature (^{0}C)	Respiratory rate (breaths/min)	Pulse rate (beats/min)	Heat stress index
4	132	Male	41.32±0.04	66.30±0.92	190.43±2.27^b	3.23±0.06^a
	168	Female	41.31±0.04	64.14±0.85	206.13±2.09^a	2.91±0.05^b
8	131	Male	41.42±0.03	36.13±0.47	212.20±1.73^a	1.58±0.03^b
	165	Female	41.39±0.03	36.91±0.44	199.88±1.61^b	1.71±0.02^a
12	131	Male	41.52±0.03	35.81±0.58	193.08±2.32^b	1.74±0.04^a
	161	Female	41.47±0.03	34.29±0.56	199.77±2.21^a	1.60±0.03^b
16	131	Male	41.72±0.02^b	34.62±0.70	218.87±1.65	1.43±0.03
	160	Female	41.82±0.02^a	33.80±0.67	221.54±1.58	1.39±0.03
20	130	Male	41.39±0.02	31.76±0.46^b	268.55±0.90^a	1.07±0.02^b
	158	Female	41.40±0.02	33.52±0.44^a	262.27±0.87^b	1.16±0.02^a

N: number of observation

^{a,b}Means in the same column with different superscripts for the same age are significantly different ($p<0.05$)

4.2.3 Genotype by sex effect on heat tolerance traits of Nigerian indigenous chickens

Genotype by sex effect on heat tolerance traits of Nigerian indigenous chickens is as presented in Table 13. The interaction between genotype and sex significantly ($p<0.05$) affected rectal temperature at weeks 16 and 20, respiratory rate at week 20, pulse rate at week 8 and heat stress index at week 8. At week 8, the highest heat stress index was observed in female frizzle feather chickens.

Table 13: Effect of interaction between genotype and sex on heat tolerance traits of Nigerian indigenous chickens

Age in weeks	N	Genotype	Sex	Rectal temperature (^{0}C)	Respiratory rate (breaths/min)	Pulse rate (beats/min)	Heat stress index
4	45	Normal feather	Male	41.47±0.07	74.38±1.58	177.53±3.88	3.84±0.10
	66	Normal feather	female	41.41±0.06	71.12±1.30	189.09±3.20	3.44±0.08
	46	Naked neck	Male	41.20±0.07	77.52±1.56	199.35±3.84	3.61±0.10
	66	Naked neck	Female	41.12±0.06	75.76±1.30	211.23±3.20	3.33±0.08
	41	Frizzle feather	Male	41.30±0.08	47.00±1.65	194.42±4.06	2.26±0.11
	36	Frizzle feather	Female	41.39±0.08	45.61±1.76	218.06±4.34	1.95±0.11
8	45	Normal feather	Male	41.44±0.06	37.62±0.81	221.78±2.95^{a}	1.55±0.04^{c}
	65	Normal feather	female	41.51±0.05	38.92±0.67	220.42±2.45^{a}	1.61±0.04^{cb}
	45	Naked neck	Male	41.28±0.06	36.27±0.81	221.49±2.95^{a}	1.49±0.04^{c}
	66	Naked neck	Female	41.22±0.05	37.21±0.67	219.21±2.43^{a}	1.54±0.04^{c}
	41	Frizzle feather	Male	41.54±0.06	34.49±0.85	193.34±3.09^{b}	1.71±0.05^{b}
	34	Frizzle feather	Female	44.44±0.06	34.59±0.93	160.00±3.39^{c}	1.96±0.05^{a}
12	45	Normal feather	Male	41.20±0.05	38.00±1.00	168.93±3.95	2.09±0.06
	63	Normal feather	female	41.20±0.04	36.22±0.84	173.03±3.34	1.93±0.05
	45	Naked neck	Male	41.68±0.05	35.38±1.00	208.51±3.95	1.58±0.06
	66	Naked neck	Female	41.64±0.04	34.20±0.82	213.77±3.26	1.48±0.05
	41	Frizzle feather	Male	41.70±0.05	34.05±1.04	201.78±4.14	1.56±0.07
	32	Frizzle feather	Female	41.58±0.06	32.44±1.18	212.50±4.69	1.38±0.07
16	45	Normal feather	Male	41.75±0.04^{b}	30.73±1.19	221.64±2.28	1.26±0.05
	63	Normal feather	female	41.76±0.04^{b}	30.19±1.00	224.40±2.38	1.23±0.04
	45	Naked neck	Male	41.85±0.04^{b}	41.64±1.19	228.76±2.81	1.65±0.05
	66	Naked neck	Female	41.87±0.03^{ab}	42.71±0.98	228.49±2.32	1.71±0.04
	41	Frizzle feather	Male	41.58±0.04^{c}	31.49±1.24	206.22±2.94	1.38±0.05
	31	Frizzle feather	Female	41.82±0.05^{b}	28.48±1.43	211.74±3.39	1.22±0.06

Table 13 (Cont'd): Effect of interaction between genotype and sex on heat tolerance traits of Nigerian indigenous chickens

Age in weeks	N	Genotype	Sex	Rectal temperature (^{0}C)	Respiratory rate (breaths/min)	Pulse rate (beats/min)	Heat stress index
20	45	Normal feather	Male	41.30±0.04^{b}	30.93±0.77^{d}	264.24±1.52	1.06±0.03
	62	Normal feather	female	41.47±0.04^{a}	33.19±0.66^{ca}	255.90±1.30	1.18±0.02
	44	Naked neck	Male	41.47±0.04^{a}	34.77±0.78^{a}	267.91±1.54	1.18±0.03
	66	Naked neck	Female	41.43±0.03^{a}	34.46±0.64^{ba}	262.38±1.26	1.19±0.02
	41	Frizzle feather	Male	41.39±0.04^{ba}	29.59±0.81^{d}	273.49±1.60	0.99±0.03
	30	Frizzle feather	Female	41.31±0.05^{b}	32.90±0.95^{da}	268.53±1.87	1.11±0.03

N: number of observation

^{a,b,c}Means in the same column with different superscripts for the same age are significantly different ($p<0.05$)

4.2.4 Effect of genotype and sex on survivability of Nigerian indigenous chickens

Effect of genotype on survivability of Nigerian indigenous chickens is as shown in Figure 1. Survivability of 100% was recorded in normal feather and naked neck chickens at weeks 1 and 4. Also, survivability of 97%, 71%, 69%, 67%, 66% and 65% was recorded in frizzle feather chickens at weeks 1, 4, 8, 12, 16 and 20, respectively.

Effect of sex on survivability of Nigerian indigenous chickens is presented in Figure 2. Survivability of 99%, 90%, 89%, 89%, 89% and 88% was recorded in male chickens at weeks 1, 4, 8, 12, 16 and 20, respectively while survivability of 99%, 91%, 89%, 87%, 87% and 85% was recorded in female birds at weeks 1, 4, 8, 12, 16 and 20, respectively.

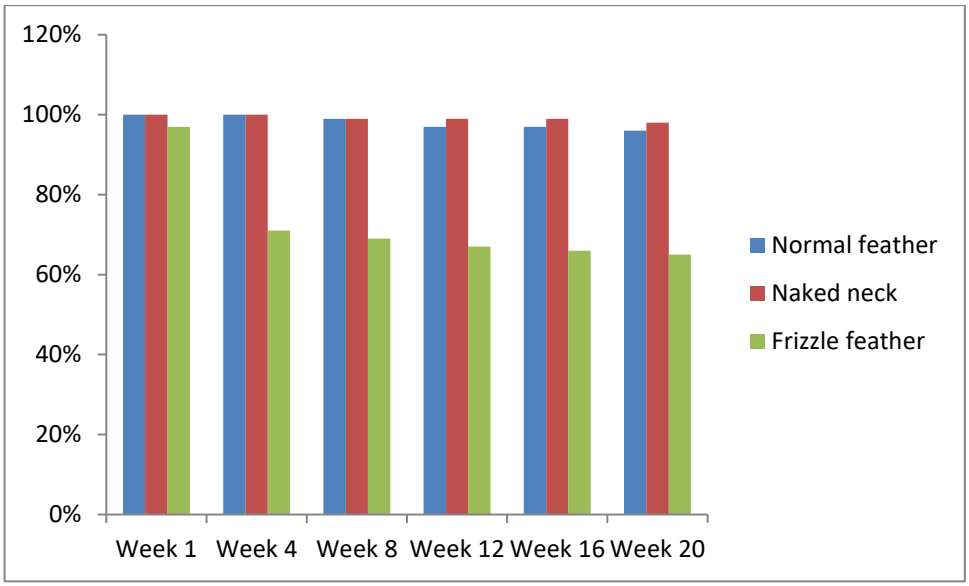

Figure 1: Percentage survivability of Nigerian indigenous chickens with different genotypes

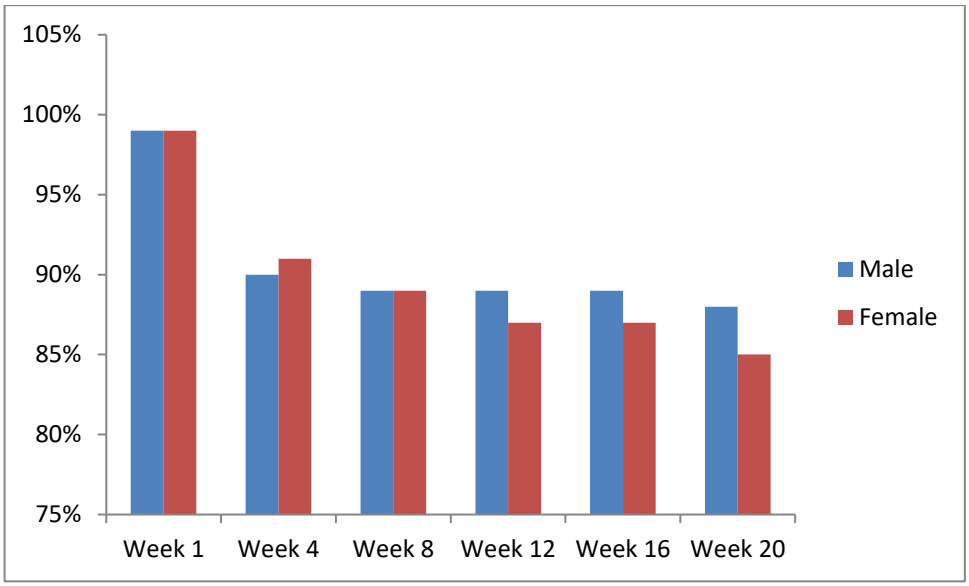

Figure 2: Percentage survivability of male and female Nigerian indigenous chickens

4.2.5 Effect of genotype, sex and their interaction on antibody response to Newcastle disease before vaccination in Nigerian indigenous chickens

The effect of genotype, sex and their interaction on antibody response (GMT) to Newcastle disease before vaccination is shown in Table 14. Genotype significantly ($p<0.001$) affected antibody response to Newcastle disease in the birds. Naked neck chickens generated the highest pre vaccination immune response. There was however no significant ($p>0.05$) difference in the antibody response of normal feather and frizzle feather chickens.

Sex also significantly ($p<0.001$) affected antibody response to Newcastle disease. A significantly ($p<0.05$) higher antibody titre was recorded in female chicks (6.13±2.15) when compared to the male chicks (1.00±0.00).

Genotype by sex effect was significant ($p<0.001$) on antibody response to Newcastle disease before vaccination. The highest immune response to Newcastle disease was observed in female naked neck chicks while there was no difference in the immune response of other groups

Table 14: Effect of genotype, sex and their interaction on antibody response to Newcastle disease before vaccination in Nigerian indigenous chickens

Genotype	N	Geometric mean titre
Normal feather	60	1.23±0.08[b]
Naked neck	63	7.86±2.94[a]
Frizzle feather	56	1.00±0.00[b]
Sex		
Male	92	1.00±0.00[b]
female	87	6.13±2.15[a]
Genotype × sex		
Normal feather × Male	29	1.00±0.00[b]
Normal feather × Female	31	1.45±0.15[b]
Naked neck × Male	33	1.00±0.00[b]
Naked neck × Female	30	15.40±5.92[a]
Frizzle feather × Male	30	1.00±0.00[b]
Frizzle feather × Female	26	1.00±0.00[b]

N: number of observations

[a,b]Means in the same column with different superscripts are significantly different ($p<0.05$)

4.2.6 Effect of genotype, sex and their interaction on antibody response to Newcastle disease after vaccination in Nigerian indigenous chickens

The effect of genotype, sex and their interaction on antibody response (GMT) to Newcastle disease after vaccination is shown in Table 15. Antibody response to Newcastle disease was significantly ($p<0.001$) affected by genotype of the birds. Normal feather chickens generated the least immune response to Newcastle disease. There was however no significant ($p>0.05$) difference in the antibody response of naked neck and frizzle feather chickens.

Similarly, antibody response to Newcastle disease was significantly ($p<0.001$) affected by sex of the birds. A significantly ($p<0.05$) higher antibody titre of 101.52±8.03 was recorded in females when compared to males (32.28±8.19).

Also, antibody response to Newcastle disease was significantly ($p<0.05$) affected by interaction between genotype and sex. There was however no significant ($p>0.05$) difference in the antibody response of male normal feather, female normal feather, male naked neck and male frizzle feather chickens.

Table 15: Effect of genotype, sex and their interaction on antibody response to Newcastle disease after vaccination in Nigerian indigenous chickens

Genotype	N	Geometric mean titre
Normal feather	60	35.10±10.06[b]
Naked neck	65	92.06±9.67[a]
Frizzle feather	60	73.53±10.08[a]
Sex		
Male	91	32.28±8.19[b]
female	94	101.52±8.03[a]
Genotype × sex		
Normal feather × Male	29	22.21±14.46[b]
Normal feather × Female	31	48.00±13.99[b]
Naked neck × Male	34	42.71±13.36[b]
Naked neck × Female	31	141.42±13.99[a]
Frizzle feather × Male	28	31.93±14.72[b]
Frizzle feather × Female	32	115.12±13.77[a]

N: number of observations

[a,b]Means in the same column with different superscripts are significantly different ($p<0.05$)

4.3 Quality of genomic DNA and optimisation of exons 3-5 and 7

4.3.1 Quality and quantity of chicken genomic DNA

Chicken genomic DNA was successfully isolated from blood samples. The gel electrophoresis pattern of different samples and integrity of the genomic DNA checked visually on 1.5% agarose gel electrophoresis is presented in Plate 1.

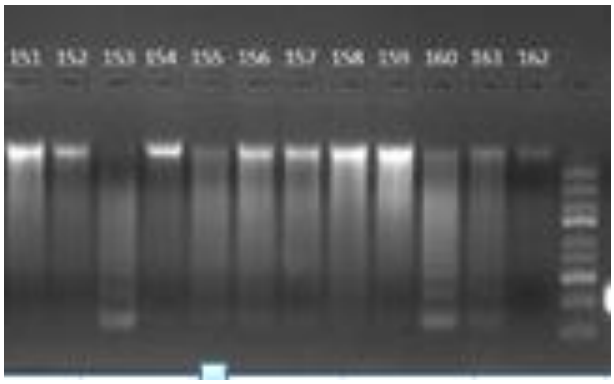

Plate 1: Genomic DNA isolated from Nigerian indigenous chickens after electrophoresis

Lanes 151-162: DNA from different samples

Last lane: 100 bp DNA ladder

4.3.2 Optimization of chicken exons 3-5 and exon 7

Amplification of the 742 bp fragment (corresponding to exons 3-5 with their surrounding introns) of chicken IRF-5 gene is presented in Plate 2 while amplification of 747 bp fragment (corresponding to exon 7 and its surrounding introns) of the chicken IRF-5 gene is shown in Plate 3.

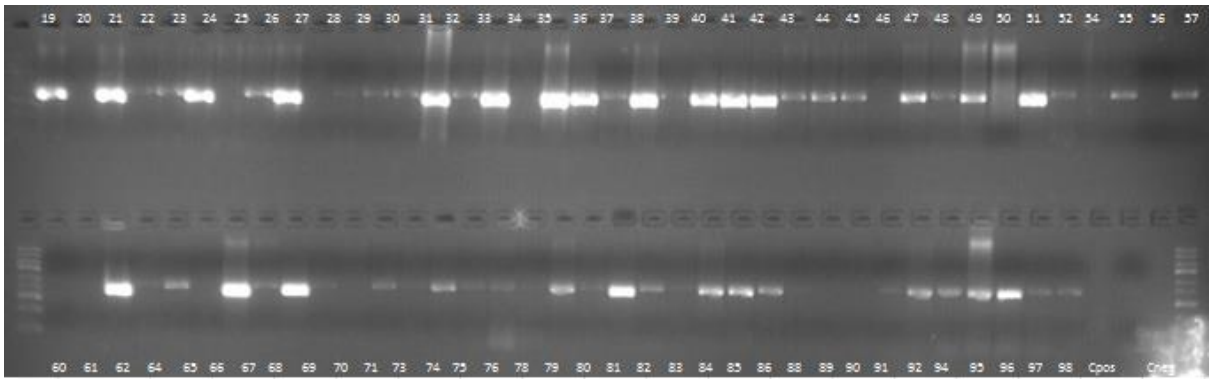

Plate 2: Amplification of the 742 bp fragment (corresponding to exons 3-5 with their surrounding introns) of chicken IRF-5 gene.

Lane 19-98: Amplified product of 742 bp fragment

Last lane: 200 bp DNA ladder

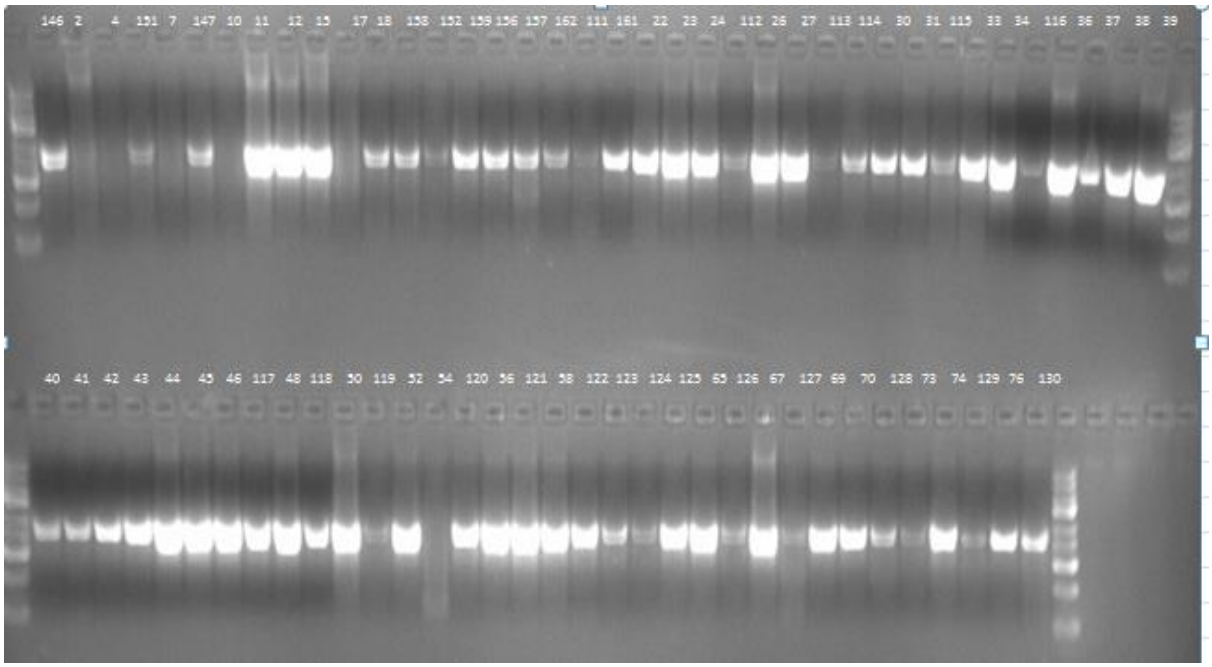

Plate 3: Amplification of the 747 bp fragment (corresponding to exon 7 with their surrounding introns) of chicken IRF-5 gene.

All the lanes with numbers: Amplified product of 747 bp fragment

First and last lanes: 200 bp DNA ladder

4.4 Identified polymorphisms in exons 3, 4, 5 and 7 of IRF-5 gene in Nigerian indigenous chickens and characteristics of these polymorphisms

4.4.1 Polymorphisms identified in exons 3, 4, 5 and 7 of interferon regulatory factor-5 gene in Nigerian indigenous chickens

The single nucleotide polymorphisms identified in exons 3, 4, 5 and 7 of interferon regulatory factor-5 gene in Nigerian indigenous chickens are as presented in Table 16. Four single nucleotide polymorphisms, 33A>G, 48G>A, 57T>C and 174T>C, were identified in exon 3 of interferon regulatory factor-5 gene in Nigerian indigenous chickens. The SNPs observed in exon 3 of interferon regulatory factor-5 gene are transitions and were present in normal feather, naked neck and frizzle feather chickens. The four transitions (33A>G, 48G>A, 57T>C and 174T>C) identified in exon 3 of interferon regulatory factor-5 gene in Nigerian indigenous chickens have been previously reported in database of single nucleotide polymorphism (dbSNP) and have identifiers: rs317511101, rs312902332, rs315149141 and rs739389464, respectively.

Single nucleotide polymorphisms were not identified in exon 4 of interferon regulatory factor-5 gene in Nigerian indigenous chickens. However, six polymorphisms (two transitions and four transversions) were identified in exon 5 of interferon regulatory factor-5 gene in normal feather chickens. The SNPs present in exon 5 of interferon regulatory factor-5 gene in normal feather chickens have not been previously reported in dbSNP. Polymorphisms were however not identified in exon 5 of interferon regulatory factor-5 gene of naked neck and frizzle feather chickens.

Five SNPs with genomic locations 1:674974, 1:675081, 1:675132, 1:675138 and 1:675315 were identified in exon 7 of interferon regulatory factor-5 gene in Nigerian

indigenous chickens. The five SNPs included: 6G>A, 113G>C, 164T>C (rs736423928), 170C>T and 347G>A (rs740736761). Polymorphisms, 6G>A and 113G>C, were specific to exon 7 of interferon regulatory factor-5 gene in naked neck chickens while the other three polymorphisms were present in the three genotypes.

Overall, nearly 67% of the SNPs observed in exons 3, 4, 5 and 7 of interferon regulatory factor-5 gene in Nigerian indigenous chickens were transitions.

Table 16: Polymorphisms identified in exons 3, 4, 5 and 7 of interferon regulatory factor-5 gene in Nigerian indigenous chickens

Region	SNP[a]	Genomic location[b]	Type of mutation	Genotype where SNP occurs	dbSNP ID
Exon 3	33A>G	1: 668,730	Transition	NF, NN and FF	rs317511101
	48G>A	1: 668,745	Transition	NF, NN and FF	rs312902332
	57T>C	1: 668,754	Transition	NF, NN and FF	rs315149141
	174T>C	1: 668,871	Transition	NF, NN and FF	rs739389464
Exon 4	Not present				
Exon 5	6G>T	1: 669,257	Transversion	NF	FKR
	14T>A	1: 669,265	Transversion	NF	FKR
	17C>T	1: 669,268	Transition	NF	FKR
	19C>T	1: 669,270	Transition	NF	FKR
	20T>A	1: 669,271	Transversion	NF	FKR
	23G>C	1: 669,274	Transversion	NF	FKR
Exon 7	6G>A	1: 674,974	Transition	NN	FKR
	113G>C	1: 675,081	Transversion	NN	FKR
	164T>C	1: 675,132	Transition	NF, NN and FF	rs736423928
	170C>T	1: 675,138	Transition	NF, NN and FF	FKR
	347G>A	1: 675,315	Transition	NF, NN and FF	rs740736761

[a] Exact position of each SNP on each exon based on Ensembl Chicken Gallus_gallus 5.0 with transcript identity: ENSGALT00000068201.1

[b] Location of the SNP on chromosome 1 based on Ensembl Chicken Gallus_gallus 5.0

dbSNP ID: identity number of the SNP in single nucleotide polymorphism database

NF: normal feather chicken, NN: naked neck chicken, FF: frizzle feather chicken

FKR: First known report

4.4.2 Major allele frequency, heterozygosity and polymorphism information content of SNPs identified in exons 3, 4, 5 and 7 of IRF-5 gene in normal feather chickens

The major allele frequency, heterozygosity and polymorphic information content of SNPs identified in exons 3, 4, 5 and 7 of IRF-5 gene in normal feather chickens are presented in Table 17. The major allele frequency of polymorphisms identified in exon 3 of IRF-5 gene in normal feather chickens ranged from 0.67 to 0.93 with mutation 174T>C having the lowest value. All the SNPs identified in exon 5 of IRF-5 gene in normal feather chickens have a major allele frequency of 0.96. The major allele frequency of SNPs observed in exon 7 of IRF-5 gene in normal feather chickens ranged from 0.52 to 0.64 with SNP 170C>T having the highest major allele frequency.

The heterozygosity of the SNPs observed in exon 3 of IRF-5 gene in normal feather chickens ranged from 0.14 to 0.44. A heterozygosity value of 0.38 was observed in SNPs 33A>G and 57T>C. All the SNPs in exon 5 of IRF-5 gene in normal feather chickens have an heterozygosity value of 0.07 while a range of 0.46 to 0.50 was observed in exon 7 with SNPs 164T>C and 347G>A having the same heterozygosity value of 0.50.

A polymorphism information content of 0.31 was observed for mutations 33A>G and 57 T>C in exon 3 of IRF-5 gene in normal feather chicken. All the SNPs present in exon 5 of IRF-5 gene in normal feather chickens had a PIC value of 0.07. The PIC of SNPs observed in exon 7 of IRF-5 gene in normal feather chicken ranged from 0.36 to 0.38 with mutation 170C>T having the lowest value.

Table 17: Major allele frequency, heterozygosity and polymorphism information content of SNPs identified in exons 3, 4, 5 and 7 of IRF-5 gene in normal feather chickens

Region	SNP	Major allele frequency	Heterozygosity	Polymorphism information content
Exon 3	33A>G	0.74	0.38	0.31
	48G>A	0.93	0.14	0.13
	57T>C	0.74	0.38	0.31
	174T>C	0.67	0.44	0.35
Exon 4	Not present			
Exon 5	6G>T	0.96	0.07	0.07
	14T>A	0.96	0.07	0.07
	17C>T	0.96	0.07	0.07
	19C>T	0.96	0.07	0.07
	20T>A	0.96	0.07	0.07
	23G>C	0.96	0.07	0.07
Exon 7	164T>C	0.55	0.50	0.37
	170C>T	0.64	0.46	0.36
	347G>A	0.52	0.50	0.38

4.4.3 Major allele frequency, heterozygosity and polymorphism information content of SNPs identified in exons 3, 4, 5 and 7 of IRF-5 gene in naked neck chickens

The major allele frequency, heterozygosity and polymorphism information content of SNPs identified in exons 3, 4, 5 and 7 of IRF-5 gene in naked neck chickens are presented in Table 18. The major allele frequency of polymorphisms identified in exon 3 of IRF-5 gene in naked neck chickens ranged from 0.53 to 0.73 with mutation 33A>G having the least major allele frequency. Also, the major allele frequency of SNPs identified in exon 7 of IRF-5 gene in naked neck chickens ranged from 0.61 to 0.97. Major allele frequency of 0.97 was observed in both 6G>A and 113G>C polymorphisms observed in exon 7 of IRF-5 gene in naked neck chickens.

The heterozygosity of SNPs present in exon 3 of IRF-5 gene in naked neck chickens ranged from 0.39 to 0.50 with mutation 33A>G having the highest heterozygosity value. Heterozygosity value of 0.06 was observed for both mutations 6G>A and 113G>C in exon 7. The highest heterozygosity value of 0.48 was observed for 164T>C in exon 7 of Naked Neck chickens.

A polymorphism information content of 0.37 was observed for mutations 33A>G and 57T>C in exon 3 of IRF-5 gene in naked neck chickens. The PIC value of SNPs present in exon 7 of IRF-5 gene in naked neck chickens ranged from 0.06 to 0.31. Also, mutations 6G>A and 113G>C in exon 7 of IRF-5 gene in naked neck chickens had a PIC value of 0.06.

Table 18: Major allele frequency, heterozygosity and polymorphism information content of SNPs identified in exons 3, 4, 5 and 7 of IRF-5 gene in naked neck chickens

Region	SNP	Major allele frequency	Heterozygosity	Polymorphism information content
Exon 3	33A>G	0.53	0.50	0.37
	48G>A	0.73	0.39	0.32
	57T>C	0.60	0.48	0.37
	174T>C	0.69	0.43	0.34
Exon 4	Not present			
Exon 5	Not present			
Exon 7	6G>A	0.97	0.06	0.06
	113G>C	0.97	0.06	0.06
	164T>C	0.61	0.48	0.36
	170C>T	0.77	0.35	0.29
	347G>A	0.74	0.38	0.31

4.4.4 Major allele frequency, heterozygosity and polymorphism information content of SNPs identified in exons 3, 4, 5 and 7 of IRF-5 gene in frizzle feather chickens

The major allele frequency, heterozygosity and polymorphism information content of SNPs identified in exons 3, 4, 5 and 7 of IRF-5 gene in frizzle feather chickens are presented in Table 19. The major allele frequency of the four SNPs identified in exon 3 of IRF-5 gene in frizzle feather chickens ranged from 0.56 to 0.89. Major allele frequency of 0.56 was observed in 33A>G, 57T>C and 174T>C. A major allele frequency of 0.74 was also observed in 164T>C and 347G>A out of the three SNPs identified in exon 7 of IRF-5 gene in frizzle feather chickens.

The heterozygosity of the SNPs identified in exon 3 of IRF-5 gene in frizzle feather chickens ranged from 0.20 to 0.49 with 33A>G, 57T>C and 174T>C having the same heterozygosity value. Single nucleotide polymorphisms 164T>C and 347G>A in exon 7 also had the same heterozygosity value.

The polymorphism information content of SNPs observed in exon 3 of IRF-5 gene in frizzle feather chickens ranged from 0.18 to 0.37 with mutations 33A>G, 57T>C and 174T>C having the same PIC value. The PIC of SNPs observed in exon 7 of IRF-5 gene in frizzle feather chickens ranged from 0.25 to 0.31 with mutation 170C>T having the least value.

Table 19: Major allele frequency, heterozygosity and polymorphism information content of SNPs identified in exons 3, 4, 5 and 7 of IRF-5 gene in frizzle feather chickens

Region	SNP	Major allele frequency	Heterozygosity	Polymorphism information content
Exon 3	33A>G	0.56	0.49	0.37
	48G>A	0.89	0.20	0.18
	57T>C	0.56	0.49	0.37
	174T>C	0.56	0.49	0.37
Exon 4	Not present			
Exon 5	Not present			
Exon 7	164T>C	0.74	0.39	0.31
	170C>T	0.83	0.29	0.25
	347G>A	0.74	0.39	0.31

4.4.5 Amino acid variations and predicted effects of SNPs identified in exons 3, 4, 5 and 7 of IRF-5 gene in Nigerian indigenous chickens

The resultant amino acid variations and predicted effects of SNPs identified in exons 3, 4, 5 and 7 of IRF-5 gene in Nigerian indigenous chickens is presented in Table 20. All the SNPS identified in exon 3 of IRF-5 gene in Nigerian indigenous chickens were synonymous SNPs and were predicted to have neutral effect on the resultant protein function with accuracy of 97%.

All the SNPs identified in exon 5 of IRF-5 gene in normal feather chickens were predicted to have neutral effect on the resultant protein function except mutation 14T>A. Also, mutation 6G>A identified in exon 7 of IRF-5 gene in naked neck chickens was predicted to have effect on the resultant protein function with accuracy of 71%.

Table 20: Resultant amino acid variations and predicted effects of SNPs identified in exons 3, 4, 5 and 7 of IRF-5 gene in Nigerian indigenous chickens

Region	SNP	Codon change	Amino acid variation	Type of mutation	Predicted effect	Score	Accuracy (%)
Exon 3	33A>G	GC**A**→GC**G**	Alanine→Alanine	Synonymous	Neutral	-99	97
	48G>A	CC**G**→CC**A**	Proline→Proline	Synonymous	Neutral	-99	97
	57T>C	GC**T**→GC**C**	Alanine→Alanine	Synonymous	Neutral	-99	97
	174T>C	GC**T**→GC**C**	Alanine→Alanine	Synonymous	Neutral	-99	97
Exon 4	Not present						
Exon 5	6G>T	CA**G**→CA**T**	Glutamine→Histidine	Nonsynonymous	Neutral	-75	87
	14T>A	A**T**G→A**A**G	Methionine→Lysine	Nonsynonymous	Effect	5	53
	17C>T	T**C**T→T**T**T	Serine→Phenylalanine	Nonsynonymous	Neutral	-27	61
	19C>T	**C**TG→**T**TG	Leucine→Leucine	Synonymous	Neutral	-99	97
	20T>A	C**T**G→C**A**G	Leucine→Glutamine	Nonsynonymous	Neutral	-45	72
	23G>C	A**G**T→A**C**T	Serine→Threonine	Nonsynonymous	Neutral	-59	78
Exon 7	6G>A	**G**AC→**A**AC	Aspartic acid→Asparagine	Nonsynonymous	Effect	40	71
	113G>C	CC**G**→CC**C**	Proline→Proline	Synonymous	Neutral	-99	97
	164T>C	CC**T**→CC**C**	Proline→Proline	Synonymous	Neutral	-99	97
	170C>T	CC**C**→CC**T**	Proline→Proline	Synonymous	Neutral	-99	97
	347G>A	CG**G**→CG**A**	Arginine→Arginine	Synonymous	Neutral	-99	97

Positions where the SNPs occurred were in bold.

4.4.6 Linkage disequilibrium among SNPs identified in exon 3 of IRF-5 gene in Nigerian indigenous chickens

Linkage disequilibrium among SNPs identified in exon 3 of IRF-5 gene in Nigerian indigenous chickens is shown in Table 21. Linkage disequilibrium among the SNPs present in exon 3 of IRF-5 gene in normal feather chickens ranged from 0.028 to 1.000. A very low LD of 0.028 was observed between 33A>G and 48G>A as well as between 48G>A and 57T>C. A very high LD of 1.000 was observed between 33A>G and 57T>C in normal feather chickens.

The linkage disequilibrium among SNPs identified in exon 3 of IRF-5 gene in naked neck chickens ranged from 0.164 to 0.762 with the highest value observed between 33A>G and 57T>C. A high significant ($p<0.001$) LD of 0.640 was observed between 33A>G and 174T>C in exon 3 of IRF-5 gene in frizzle feather chickens.

Table 21: Linkage disequilibrium (based on r^2 statistics) among SNPs identified in exon 3 of IRF-5 gene in Nigerian indigenous chickens

Genotype	Site 1	Site 2			
		33A>G	48G>A	57T>C	174T>C
Normal feather	33A>G		0.028^{NS}	1.000***	0.701***
	48G>A			0.028^{NS}	0.160^{NS}
	57T>C				0.701***
	174T>C				
Naked neck		33A>G	48G>A	57T>C	174T>C
	33A>G		0.214**	0.762***	0.283***
	48G>A			0.242**	0.164**
	57T>C				0.301***
	174T>C				
Frizzle feather		33A>G	48G>A	57T>C	174T>C
	33A>G		0.100^{NS}	1.000***	0.640**
	48G>A			0.100^{NS}	0.100^{NS}
	57T>C				0.640**
	174T>C				

**significant at p<0.01, ***significant at p<0.001, ^{NS}not significant

4.4.7 Linkage disequilibrium among SNPs identified in exon 5 of IRF-5 gene in Nigerian indigenous chickens

Linkage disequilibrium among SNPs identified in exon 5 of IRF-5 gene in Nigerian indigenous chickens is presented in Table 22. Very high linkage disequilibrium of 1.00 was observed among all the SNPs identified in exon 5 of IRF-5 gene in normal feather chickens. There were no pairwise comparisons for naked neck and frizzle feather chickens as they contained no single nucleotide polymorphisms.

Table 22: Linkage disequilibrium (based on r^2 statistics) among SNPs identified in exon 5 of IRF-5 gene in Nigerian indigenous chickens

Genotype	Site 1	Site 2				
	6G>T	14T>A	17C>T	19C>T	20T>A	23G>C
Normal feather	6G>T	1.00*	1.00*	1.00*	1.00*	1.00*
	14T>A		1.00*	1.00*	1.00*	1.00*
	17C>T			1.00*	1.00*	1.00*
	19C>T				1.00*	1.00*
	20T>A					1.00*
	23G>C					
Naked neck	No pairwise comparison					
Frizzle feather	No pairwise comparison					

*significant at $p<0.05$

4.4.8 Linkage disequilibrium among SNPs identified in exon 7 of IRF-5 gene in Nigerian indigenous chickens

Linkage disequilibrium among SNPs identified in exon 7 of IRF-5 gene in Nigerian indigenous chickens is shown in Table 23. A significant ($p<0.001$) moderate LD of 0.369 was observed between 170C>T and 347G>A in exon 7 of IRF-5 gene in normal feather chickens.

Linkage disequilibrium among SNPs present in exon 7 of IRF-5 gene in naked neck chickens varied from 0.001 to 0.546. A very low LD of 0.001 was observed between 6G>A and 113G>C while LD of 0.546 was observed between 170C>T and 347G>A. linkage disequilibrium of 0.596 was observed between 164T>C and 170C>T as well as 170C>T and 347G>A in exon 7 of IRF-5 gene in frizzle feather chickens.

Table 23: Linkage disequilibrium (based on r^2 statistics) among SNPs identified in exon 7 of IRF-5 gene in Nigerian indigenous chickens

Genotype	Site 1			Site 2		
				164T>C	170C>T	347G>A
Normal feather	164T>C				0.202*	0.270**
	170C>T					0.369***
	347G>A					
		6G>A	113G>C	164T>C	170C>T	347G>A
Naked neck	6G>A		0.001^{NS}	0.053^{NS}	0.010^{NS}	0.012^{NS}
	113G>C			0.021^{NS}	0.010^{NS}	0.012^{NS}
	164T>C				0.184*	0.220*
	170C>T					0.546***
	347G>A					
				164T>C	170C>T	347G>A
Frizzle feather	164T>C				0.596**	0.601**
	170C>T					0.596***
	347G>A					

*significant at p<0.05, **significant at p<0.01, ***significant at p<0.001, ^{NS}not significant

4.5 Diversity of exons 3, 4, 5 and 7 of IRF-5 gene in Nigerian indigenous chickens

The diversity of exons 3, 4, 5 and 7 of IRF-5 gene in Nigerian indigenous chickens is shown in Table 24. Four segregating and parsimony informative sites were present in exon 3 of IRF-5 gene in Nigerian indigenous chickens. Exon 3 of IRF-5 in naked neck chickens had the highest number of haplotypes (6), haplotype diversity (0.71) and average number of nucleotide differences (1.838).

Exon 4 of IRF-5 gene in Nigerian indigenous chickens had no segregating sites and had a conservation value of 1.000. Six segregating sites were observed in exon 5 of IRF-5 gene in normal feather chickens whereas the two other genotypes (naked neck and frizzle feather chickens) had no segregating sites.

Exon 7 of normal feather and frizzle feather chickens had 4 segregating sites. Singleton variable sites were absent in exon 7 of IRF-5 gene in normal feather and frizzle feather chickens. Also, exon 7 of IRF-5 gene in Nigerian indigenous chickens had four parsimony informative sites and high sequence conservation of 0.985 to 0.990

Table 24: Diversity of exons 3, 4, 5, 7 of IRF-5 gene in Nigerian indigenous chickens

		Diversity indices								
Region	**Gen**	**N**	**LSA**	**S**	**H**	**Hd**	**Pi**	**K**	**SVS**	**PIS**	**C**
Exon 3	NF	27	184	4	3	0.50	7.62×10^{-3}	1.402	0	4	0.978
	NN	45	184	4	6	0.71	9.99×10^{-3}	1.838	0	4	0.978
	FF	18	184	4	3	0.63	9.66×10^{-3}	1.778	0	4	0.978
Exon 4	NF	27	68	0	1	0.00	0.00	0.000	0	0	1.000
	NN	45	68	0	1	0.00	0.00	0.000	0	0	1.000
	FF	18	68	0	1	0.00	0.00	0.000	0	0	1.000
Exon 5	NF	27	31	6	2	0.07	1.43×10^{-3}	0.444	6	0	0.806
	NN	45	31	0	1	0.00	0.00	0.000	0	0	1.000
	FF	18	31	0	1	0.00	0.00	0.000	0	0	1.000
Exon 7	NF	33	408	4	11	0.81	5.06×10^{-3}	2.064	0	4	0.990
	NN	31	408	6	9	0.85	4.99×10^{-3}	2.034	2	4	0.985
	FF	23	408	4	6	0.75	4.32×10^{-3}	1.763	0	4	0.990

Gen: Genotype, NF: normal feather, NN: naked neck, FF: frizzle feather, N: number of sequences analysed, LSA: length of sequence analysed in base pairs, S: segregating sites, H: number of haplotype, Hd: haplotype diversity, Pi: nucleotide diversity, K: average number of nucleotide differences, SVS: singleton variable sites, PIS: parsimony informative site, C: sequence conservation

4.6 Haplotype distribution of exon 3, 4, 5 and 7 of IRF-5 gene in Nigerian indigenous chickens

4.6.1 Haplotypes present in exon 3 of IRF-5 gene in Nigerian indigenous chickens

Haplotypes present in exon 3 of IRF-5 in Nigerian indigenous chickens are as shown in Table 25. Six haplotypes were present in exon 3 of IRF-5 in Nigerian indigenous chickens. Haplotypes 1, 2 and 3 were shared among the three chicken genotypes while haplotypes 4, 5 and 6 were specific to naked neck chickens.

Table 25: Haplotypes present in exon 3 of IRF-5 gene in Nigerian indigenous chickens

Haplotype	Haplotype sequence	Genotype			
		Normal feather	Naked neck	Frizzle feather	Total
1	GGCC	7 (21.21%)	18 (54.55%)	8 (24.24%)	33
2	AGTT	18 (46.15%)	13 (33.33%)	8 (20.51%)	39
3	AATC	2 (13.33%)	11 (73.33%)	2 (13.33%)	15
4	GGTC	0 (0.00%)	1 (100.00%)	0 (0.00%)	1
5	GGTT	0 (0.00%)	1 (100.00%)	0 (0.00%)	1
6	GATC	0 (0.00%)	1 (100.00%)	0 (0.00%)	1

The percentage of each haplotype is presented in the parenthesis

4.6.2 Haplotypes present in exon 5 of IRF-5 gene in Nigerian indigenous chickens

Haplotypes present in exon 5 of IRF-5 gene in Nigerian indigenous chickens are shown in Table 26. Two haplotypes were identified in exon 5 of IRF-5 gene in Nigerian indigenous chickens. Haplotype 1 was shared by the three genotypes with naked neck chickens having the highest frequency of 50.56%. Haplotype 2 was specific to normal feather chickens.

Table 26: Haplotypes present in exon 5 of IRF-5 gene in Nigerian indigenous chickens

Haplotype	Haplotype sequence	Genotype			
		Normal feather	Naked neck	Frizzle feather	Total
1	GTCCTG	26 (29.21%)	45 (50.56%)	18 (20.23%)	89
2	TATTAC	1 (100.00%)	0 (0.00%)	0 (0.00%)	1

The percentage of each haplotype is presented in the parenthesis

4.6.3 Haplotypes present in exon 7 of IRF-5 gene in Nigerian indigenous chickens

Haplotypes present in exon 7 of IRF-5 gene in Nigerian indigenous chickens are shown in Table 27. Thirteen haplotypes were identified in exon 7 of IRF-5 gene in Nigerian indigenous chickens. Haplotypes 1, 2, 3 and 5 were shared by the three genotypes. Haplotypes 4 and 8 were specific to naked neck chickens. Haplotype 12 and 13 were specific to normal feather chickens while haplotypes 6, 7 and 9 were shared between normal feather and naked neck chickens.

Table 27: Haplotypes present in exon 7 of IRF-5 gene in Nigerian indigenous chickens

Haplotype	Haplotype sequence	Genotype			
		Normal feather	Naked neck	Frizzle feather	Total
1	GGTCGG	12 (44.44%)	8 (29.62%)	7 (25.92%)	27
2	GGCCTG	1 (11.11%)	7 (77.77%)	1 (11.11%)	9
3	GGTCTG	1 (7.69%)	3 (23.07%)	9 (69.23)	13
4	GCCCTG	0 (0.00%)	1 (100.00%)	0 (0.00%)	1
5	GGCTAA	8 (44.44%)	6 (33.33%)	4 (22.22%)	18
6	GGCCTA	2 (50.00%)	2 (50.00%)	0 (0.00%)	4
7	GGCCGG	1 (33.33%)	2 (66.66%)	0 (0.00%)	3
8	AGTCGG	0 (0.00%)	1 (100.00%)	0 (0.00%)	1
9	GGCTGG	1 (50.00%)	1 (50.00%)	0 (0.00%)	2
10	GGCCGA	1 (50.00%)	0 (0.00%)	1 (50.00%)	2
11	GGTCGA	2 (66.66%)	0 (0.00%)	1 (33.33%)	3
12	GGCCAA	1 (100.00%)	0 (0.00%)	0 (0.00%)	1
13	GGTTGA	3 (100.00%)	0 (0.00%)	0 (0.00%)	3

The percentage of each haplotype is presented in the parenthesis

4.7 Evolution of exons 3, 4, 5 and 7 of IRF-5 gene in Nigerian indigenous chickens

4.7.1 Frequency of nucleotides present in exons 3, 4, 5 and 7 of IRF-5 gene in Nigerian indigenous chickens

The frequency of nucleotides present in exons 3, 4, 5 and 7 of IRF-5 gene in Nigerian indigenous chickens is shown in Table 28. Exon 3 of IRF-5 gene in normal feather and naked neck chickens had the same adenine content of 19.46%. Also, exon 4 of IRF-5 in all the chicken genotype had the same adenine, cytosine, guanine and thymine contents. Exons 3, 4 and 7 of IRF-5 gene in Nigerian indigenous chickens had a guanine-cytosine content of 65.64-65.94%, 61.77% and 69.65-69.72%, respectively. Exon 5 had higher adenine-thymine content (58.06-58.42%) than guanine-cytosine content (41.58-41.94%).

Table 28: Frequency of nucleotides present in exons 3, 4, 5 and 7 of IRF-5 gene in Nigerian indigenous chickens

Region	Genotype	Purines		Pyrimidines			
		Adenine (%)	Guanine (%)	Cytosine (%)	Thymine (%)	GC (%)	AT (%)
Exon 3	NF	19.46	35.97	29.67	14.90	65.64	34.36
	NN	19.46	35.98	29.94	14.63	65.92	34.08
	FF	19.38	36.05	29.89	14.67	65.94	34.06
Exon 4	NF	26.47	47.06	14.71	11.76	61.77	38.23
	NN	26.47	47.06	14.71	11.76	61.77	38.23
	FF	26.47	47.06	14.71	11.76	61.77	38.23
Exon 5	NF	26.05	25.57	16.01	32.38	41.58	58.42
	NN	25.81	25.81	16.13	32.26	41.94	58.06
	FF	25.81	25.81	16.13	32.26	41.94	58.06
Exon 7	NF	15.88	30.66	38.99	14.47	69.65	30.35
	NN	15.80	30.65	39.07	14.47	69.72	30.28
	FF	15.79	30.67	38.99	14.55	69.66	30.34

NF: Normal Feather, NN: Naked Neck, FF: Frizzle Feather

GC: guanine-cytosine content, AT: adenine-thymine content

4.7.2 Minimum number of recombination events in exons 3, 4, 5 and 7 of IRF-5 gene in Nigerian indigenous chickens

Minimum number of recombination events in exons 3, 4, 5 and 7 of IRF-5 gene in Nigerian indigenous chickens is presented in Table 29. Exon 3 of IRF-5 gene in normal feather and frizzle feather chickens had no recombination events whereas their naked neck chicken counterpart had one recombination event between sites 33 and 44.

Minimum number of recombination events was not estimated for exon 4 of IRF-5 gene in Nigerian indigenous chickens as they contained no sequence variation. One specific minimum number of recombination event between sites 164 and 170 occurred in exon 7 of IRF-5 gene in normal feather chickens while normal feather and naked neck chickens shared a common minimum recombination event occurring between sites 170 and 347. A specific recombination between sites 164 and 347 was also observed in exon 7 of IRF-5 gene in frizzle feather chickens.

Table 29: Minimum number of recombination events in exons 3, 4, 5 and 7 of IRF-5 gene in Nigerian indigenous chickens

	Genotype		
Region	**Normal feather**	**Naked neck**	**Frizzle feather**
Exon 3	0	1 (33, 44)	0
Exon 4	NE	NE	NE
Exon 5	0	NE	NE
Exon 7	2 (164, 170) (170, 347)	1 (170, 347)	1 (164, 347)

NE: not estimated, values in parenthesis are sites where recombination events occur

4.7.3 Phylogenetic relationship between exons 3, 4, 5 and 7 of IRF-5 gene in Nigerian indigenous chickens and other chicken genotypes

Phylogenetic relationship between exon 3 of IRF-5 gene in Nigerian indigenous chickens and other chicken genotypes is shown in Figure 3. Two clades were formed with exon 3 of naked neck and frizzle feather chickens forming sister taxa in one clade while normal feather and red jungle fowl formed another sister taxa in another clade.

Figure 4 shows the phylogenetic relationship among exon 4 of IRF-5 gene in naked neck, normal feather, frizzle feather, leghorn and red jungle chickens. Exon 4 of leghorn, red jungle and naked neck chickens formed one clade while exon 4 of normal feather and frizzle feather chickens formed another clade.

Phylogenetic relationship between exon 5 of IRF-5 gene in Nigerian indigenous chickens and other chicken genotypes is shown in Figure 5. Two clades were also formed with exon 5 of frizzle feather, red jungle and normal feather chickens forming one clade in which frizzle feather and red jungle fowl also forming sister taxa.

Figure 6 shows the phylogenetic relationship among exon 7 of IRF-5 gene in Nigerian indigenous chickens and two other chicken genotypes. Exon 7 of IRF-5 gene in Nigerian indigenous chickens and leghorn were in the same lineage while exon 7 of red jungle fowl formed the ancestral lineage.

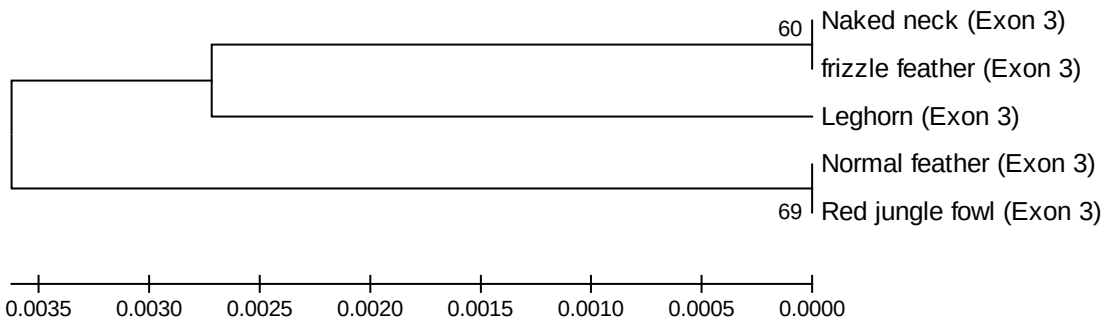

Figure 3: Phylogenetic relationship between exon 3 of IRF-5 gene in Nigerian indigenous chickens and other chicken genotypes.

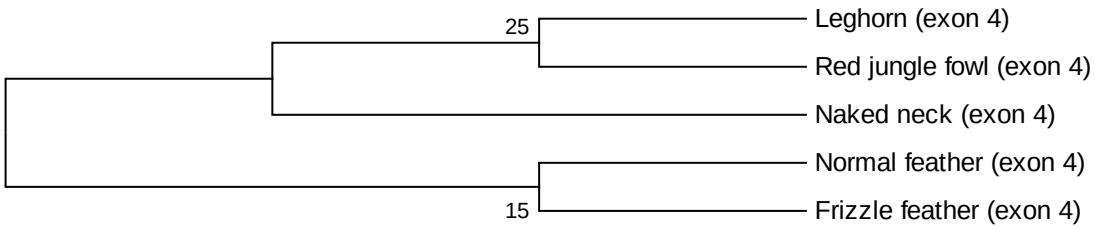

Figure 4: Phylogenetic relationship between exon 4 of IRF-5 gene in Nigerian indigenous chickens and other chicken genotypes.

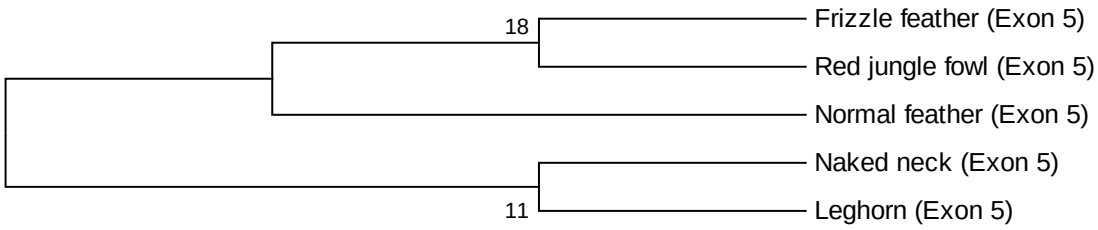

Figure 5: Phylogenetic relationship between exon 5 of IRF-5 gene in Nigerian indigenous chickens and other chicken genotypes.

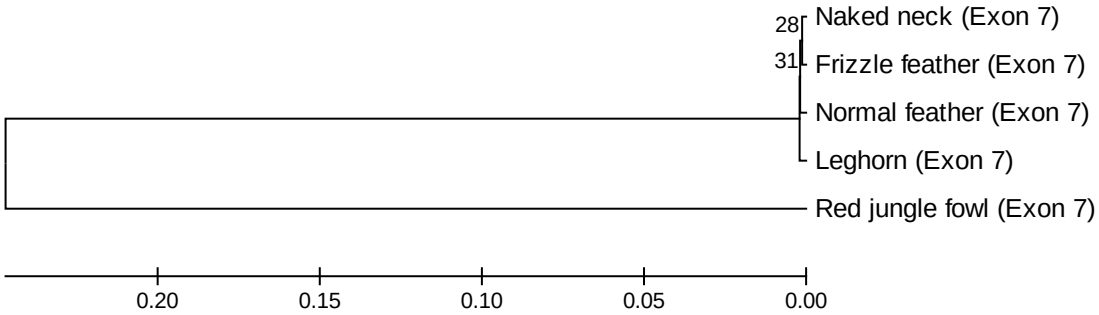

Figure 6: Phylogenetic relationship between exon 7 of IRF-5 gene in Nigerian indigenous chickens and other chicken genotypes.

4.8 Selection analyses of exons 3, 4, 5 and 7 of IRF-5 gene in Nigerian indigenous chickens

4.8.1 Test of deviation of exons 3, 4, 5 and 7 from neutrality

The test of deviation of exons 3, 4, 5 and 7 from neutrality is shown in Table 30. Significant ($p<0.05$) Tajima's D value of 2.35 was estimated for exon 3 of IRF-5 gene in naked neck chickens. All the test of neutrality indices obtained for exon 3 of IRF-5 gene in Nigerian indigenous chickens were greater than 1 except Tajima's D value of normal feather chickens (0.93) and Fu's Fs value of naked neck chickens (0.71). The neutrality indices for exon 4 of IRF-5 gene in Nigerian indigenous chickens and exon 5 of IRF-5 gene in naked neck and frizzle feather chickens were not estimable as the exons did not contain any variation.

Negative Fu's Fs values were observed in exon 7 of IRF-5 gene in all the three chicken genotypes while positive Tajima's D values were observed in the three chicken genotypes with significant ($p<0.01$) deviation observed in normal feather chickens.

Table 30: Test of deviation of exons 3, 4, 5 and 7 from neutrality

Region	Genotype	Tajima's D	Fu's Fs	Fu and Li's D*	Fu and Li's F*
Exon 3	NF	0.93^{NS}	2.48	1.07^{NS}	1.19^{NS}
	NN	2.35*	0.71	1.02^{NS}	1.66^{NS}
	FF	1.59^{NS}	2.65	1.12^{NS}	1.44^{NS}
Exon 4	NF	NE	NE	NE	NE
	NN	NE	NE	NE	NE
	FF	NE	NE	NE	NE
Exon 5	NF	-2.09*	1.35	-3.52**	-3.44**
	NN	NE	NE	NE	NE
	FF	NE	NE	NE	NE
Exon 7	NF	2.74**	-3.77	1.05^{NS}	1.80*
	NN	1.00^{NS}	-2.03	-0.35^{NS}	0.07^{NS}
	FF	1.74^{NS}	-0.39	1.09^{NS}	1.47^{NS}

NF: Normal Feather, NN: Naked Neck, FF: Frizzle Feather, NS: Not significant, *significant at $p<0.05$, **significant at $p<0.01$, NE: not estimated

4.8.2 Selective forces acting on exons 3, 4, 5 and 7 of IRF-5 gene in Nigerian indigenous chickens

Selective forces acting on exons 3, 4, 5 and 7 of IRF-5 gene in Nigerian indigenous chickens are presented in Table 31. Mean non-synonymous substitutions per non-synonymous site value of 0.00 was estimated for exon 3 of IRF-5 gene in all the three genotypes. Negative selection occurred in exon 3 of IRF-5 gene in the three chicken genotypes while positive selection occurred in exon 7 of IRF-5 gene in the three genotypes.

Table 31: Selective forces acting on exons 3, 4, 5 and 7 of IRF-5 gene in Nigerian indigenous chickens

Region	Genotype	dN	dS	dN-dS	Type of selection
Exon 3	NF	0.00	4.00	-4.00	Negative selection
	NN	0.00	5.00	-5.00	Negative selection
	FF	0.00	4.00	-4.00	Negative selection
Exon 4	NF	NE	NE	NE	ND
	NN	NE	NE	NE	ND
	FF	NE	NE	NE	ND
Exon 5	NF	1.70	0	1.70	Positive selection
	NN	NE	NE	NE	ND
	FF	NE	NE	NE	ND
Exon 7	NF	5.23	0.00	5.23	Positive selection
	NN	4.59	2.60	1.99	Positive selection
	FF	3.38	0.00	3.38	Positive selection

NF: Normal Feather, NN: Naked Neck, FF: Frizzle Feather, dN: Mean non-synonymous substitutions per non-synonymous site, dS: Mean synonymous substitutions per synonymous site, NE: not estimated, ND: not determined

4.9 Protein structure and motif of IRF-5 (exons 3, 4, 5 and 7) in Nigerian indigenous chickens

4.9.1 Protein structure of IRF-5 (exons 3, 4, 5 and 7) in Nigerian indigenous chickens

The secondary protein structures of different regions of IRF-5 in Nigerian indigenous chickens are shown in Table 32. Prediction of secondary structure revealed that exons 3 and 7 contained alpha helices, extended strands and random coils. Exon 4 contained just only random coils while exon 5 contained 60% extended strands and 40% random coils. The modelled tertiary protein structure of exon 3 of IRF-5 in Nigerian indigenous chickens is shown in Plate 4 while that of exon 7 was presented in Plate 5. The tertiary protein structures of exons 4 and 5 could not be predicted because they have less than 30 amino acid residues. The α-helix was depicted as pink coloured spiral sheet, the beta sheet was depicted as yellow coloured spiral sheet, random coil as blue coloured strand and extended strands as white coloured strands.

4.9.2 Motifs predicted in IRF-5 (exons 3, 4, 5 and 7) of Nigerian indigenous chickens

Motifs predicted in IRF-5 of Nigerian indigenous chickens are shown in Table 33. IRF tryptophan pentad repeat DNA-binding domain profile was predicted to be present in exon 3. Casein kinase II phosphorylation site was predicted to be present in exon 4 while none was predicted in exon 5. Casein kinase II phosphorylation, amidation, cAMP- and cGMP- independent protein kinase phosporylation and N-myristoylation sites were predicted in exon 7.

Table 32: Protein structure (secondary structure) of IRF-5 of Nigerian indigenous chickens

Region	Genotype	Alpha helix (%)	Extended strand (%)	Random coil (%)
Exon 3	Normal feather	19.67	24.59	55.74
	Naked neck	19.67	24.59	55.74
	Frizzle feather	19.67	24.59	55.74
Exon 4	Normal feather	0.00	0.00	100.00
	Naked neck	0.00	0.00	100.00
	Frizzle feather	0.00	0.00	100.00
Exon 5	Normal feather	0.00	60.00	40.00
	Naked neck	0.00	60.00	40.00
	Frizzle feather	0.00	60.00	40.00
Exon 7	Normal feather	28.68	16.91	54.41
	Naked neck	28.68	16.91	54.41
	Frizzle feather	28.68	16.91	54.41

Table 33: Motifs predicted in IRF-5 (exons 3, 4, 5 and 7) of Nigerian indigenous chickens

Region	Motif	Sequence	Position	Genotype where the motif occurs
Exon 3	IRF tryptophan pentad repeat DNA-binding domain profile	AWATETGKFLAGRDEPDPAKWKATLR CALNKSREFRLRYDGTRAVPPRPYKVY EVCG	1-57	NF, NN, FF
Exon 4	Casein kinase II phosphorylation site	TGDD	4-7	NF, NN, FF
Exon 5	No motif found			
Exon 7	Casein kinase II phosphorylation site	TDLE	2-5	NF, NN, FF
	Amidation site	RGRR	11-14	NF, NN, FF
	cAMP- and cGMP- independent protein kinase phosporylation site	RRVS	13-16	NF, NN, FF
	cAMP- and cGMP- independent protein kinase phosporylation site	RRYT	66-69	NF, NN, FF
	N-myristoylation site	GCRLSH	25-30	NF, NN, FF

NF: Normal Feather chickens, NN: Naked Neck chickens, FF: Frizzle Feather chickens

Plate 4: Tertiary protein structure of exon 3 of IRF-5 in Nigerian indigenous chickens

Plate 5: Tertiary protein structure of exon 7 of IRF-5 in Nigerian indigenous chickens

4.10 Effects of haplotype identified in exons 3 and 7 of IRF-5 gene on body weight and feed efficiency of Nigerian indigenous chickens

4.10.1 Effect of haplotype identified in exon 3 of IRF-5 gene on body weight and feed efficiency of Nigerian indigenous chickens

Effect of haplotype identified in exon 3 of IRF-5 gene on body weight and feed efficiency of Nigerian indigenous chicken is shown in Table 34. Body weight of the birds was not significantly ($p>0.05$) affected by the haplotype. However, feed efficiency of the birds was significantly ($p<0.05$) affected by the haplotype in all the weeks considered except at week 1. The highest feed efficiency was recorded in birds carrying haplotype GGCC at week 4 while the lowest feed efficiency was recorded in birds carrying haplotype AGTT at week 20.

Table 34: Effect of haplotype identified in exon 3 of IRF-5 gene on body weight and feed efficiency of Nigerian indigenous chickens

Age in weeks	Haplotype	N	Body weight (g)	Feed efficiency (%)
1	GGCC	33	87.33±5.73	60.53±4.95
	AGTT	39	87.88±6.07	65.00±4.11
	AATC	15	74.77±7.31	49.89±6.00
4	GGCC	33	457.42±40.00	83.80±1.81[a]
	AGTT	39	402.67±41.30	45.14±5.07[b]
	AATC	15	370.57±46.49	36.91±4.25[b]
8	GGCC	33	945.78±38.47	29.04±1.32[b]
	AGTT	39	854.73±43.34	17.08±1.01[c]
	AATC	15	885.41±57.50	41.02±5.37[a]
12	GGCC	33	1165.11±47.41	11.39±0.67[c]
	AGTT	39	1244.08±50.40	44.11±3.98[a]
	AATC	15	1170.72±61.43	20.36±0.93[b]
16	GGCC	33	1218.62±96.35	2.96±0.21[c]
	AGTT	39	1300.16±97.68	3.55±0.29[b]
	AATC	15	1266.70±103.44	8.28±0.82[a]
20	GGCC	33	1509.24±107.24	11.24±1.54[a]
	AGTT	39	1410.48±109.16	2.87±0.26[c]
	AATC	15	1439.08±117.49	7.00±0.36[b]

N: number of observation

[a,b,c]Means in the same column with different superscripts for the same age are significantly different ($p<0.05$)

4.10.2 Effect of haplotype identified in exon 7 of IRF-5 gene on body weight and feed efficiency of Nigerian indigenous chickens

Effect of haplotype identified in exon 7 of IRF-5 gene on body weight and feed efficiency of Nigerian indigenous chicken is as shown in Table 35. Body weight was not significantly ($p>0.05$) affected by the haplotypes identified in exon 7 of IRF-5 gene except at week 12. Also, there was no significant ($p>0.05$) difference in the body weight of birds carrying haplotypes GGTCGG (1102.94±45.68 g) and haplotype GGTCTG (1118.13±56.94 g) at week 12.

Table 35: Effect of haplotype identified in exon 7 of IRF-5 gene on body weight and feed efficiency of Nigerian indigenous chickens

Age in weeks	Haplotype	N	Body weight (g)	Feed efficiency (%)
1	GGTCGG	27	78.00±5.76	55.12±4.82
	GGCCTG	9	81.02±7.77	56.19±6.42
	GGTCTG	13	81.33±6.68	66.54±5.73
	GGCTAA	18	92.96±7.07	56.06±5.39
4	GGTCGG	27	419.62±39.15	43.75±7.88^{b}
	GGCCTG	9	412.94±51.07	62.81±9.01^{a}
	GGTCTG	13	429.20±44.41	52.05±7.55^{ba}
	GGCTAA	18	379.13±46.24	62.54±8.07^{a}
8	GGTCGG	27	937.84±37.39	27.27±2.26
	GGCCTG	9	959.00±65.53	29.53±2.59
	GGTCTG	13	857.16±51.56	31.48±2.05
	GGCTAA	18	827.23±56.82	27.92±2.29
12	GGTCGG	27	1102.94±45.68^{b}	23.81±1.64
	GGCCTG	9	1274.55±70.07^{a}	25.72±1.90
	GGTCTG	13	1118.13±56.94^{b}	27.31±1.49
	GGCTAA	18	1277.60±60.83^{a}	24.29±1.67
16	GGTCGG	27	1242.57±95.87	6.31±0.89
	GGCCTG	9	1359.39±109.23	4.13±0.57
	GGTCTG	13	1293.67±101.66	3.08±0.61
	GGCTAA	18	1351.68±102.86	5.58±0.61
20	GGTCGG	27	1497.27±106.41	6.62±0.64
	GGCCTG	9	1401.72±125.97	7.04±0.74
	GGTCTG	13	1478.69±114.95	7.99±0.58
	GGCTAA	18	1475.05±116.72	6.49±0.64

N: number of observation

^{a,b}Means in the same column with different superscripts for the same age are significantly different ($p<0.05$)

4.11 Effects of haplotype identified in exons 3 and 7 of IRF-5 gene on linear body measurements of Nigerian indigenous chickens

4.11.1 Effect of haplotype identified in exon 3 of IRF-5 gene on linear body measurements of Nigerian indigenous chickens

Effect of haplotype identified in exon 3 of IRF-5 gene on linear body measurements of Nigerian indigenous chickens is as shown in Table 36. All the linear body measurements were not significantly ($p>0.05$) affected by haplotypes identified in exon 3 of IRF-5 gene.

4.11.2 Effect of haplotype identified in exon 7 of IRF-5 gene on linear body measurements of Nigerian indigenous chickens

Effect of haplotype identified in exon 7 of IRF-5 gene on linear body measurements of Nigerian indigenous chickens is presented in Table 37. All the linear body measurements were not significantly ($p>0.05$) affected by haplotypes identified in exon 7 of IRF-5 gene in Nigerian indigenous chickens except breast girth at week 12, wing length at week 8 and wing span at week 1.

Table 36: Effect of haplotype identified in exon 3 of IRF-5 gene on linear body measurements of Nigerian indigenous chickens

Age in weeks	Haplotype	N	BG (cm)	TL (cm)	SL (cm)	KL (cm)	WL (cm)	WS (cm)
1	GGCC	33	10.32±0.26	5.28±0.19	3.25±0.13	4.05±0.23	7.61±0.28	17.21±0.33
	AGTT	39	10.58±0.28	5.01±0.21	3.06±0.13	3.69±0.24	7.55±0.31	17.45±0.37
	AATC	15	10.36±0.36	4.74±0.27	3.09±0.16	3.50±0.28	7.37±0.39	18.18±0.48
4	GGCC	33	17.88±0.48	10.07±0.73	5.61±0.32	7.59±0.62	13.62±0.77	30.22±2.97
	AGTT	39	18.08±0.53	9.40±0.74	5.27±0.33	7.28±0.64	12.91±0.79	31.29±3.03
	AATC	15	18.06±0.68	9.45±0.81	5.53±0.37	6.89±0.69	12.40±0.87	29.32±3.26
8	GGCC	33	22.41±0.49	15.74±0.34	7.56±0.20	10.10±0.37	17.24±0.36	41.41±0.57
	AGTT	39	22.41±0.55	16.09±0.37	7.49±0.21	10.36±0.40	16.52±0.40	41.34±0.64
	AATC	15	21.72±0.74	15.58±0.49	7.29±0.24	9.70±0.50	16.75±0.53	41.59±0.85
12	GGCC	33	25.35±0.48	16.39±0.41	8.95±0.27	10.67±0.29	22.80±1.19	47.03±1.85
	AGTT	39	25.03±0.51	16.59±0.42	9.00±0.28	11.19±0.32	22.37±1.20	47.86±1.88
	AATC	15	25.37±0.64	16.24±0.46	9.10±0.32	10.89±0.43	22.86±1.26	47.70±2.03
16	GGCC	33	25.69±0.73	17.28±0.25	9.06±0.25	12.26±0.42	24.96±0.20	50.29±0.79
	AGTT	39	25.35±0.75	17.30±0.45	9.31±0.26	12.82±0.43	24.97±0.23	50.70±0.83
	AATC	15	25.73±0.84	17.01±0.35	9.28±0.30	12.79±0.48	24.72±0.30	50.26±1.00
20	GGCC	33	27.89±0.50	18.21±0.43	10.52±0.21	14.87±0.24	29.21±0.50	55.25±0.78
	AGTT	39	27.15±0.54	18.22±0.45	10.38±0.23	14.37±0.27	28.65±0.54	53.35±0.81
	AATC	15	26.95±0.69	17.88±0.51	10.35±0.29	14.29±0.36	30.16±0.69	54.43±0.95

N: number of observation

Table 37: Effect of haplotype identified in exon 7 of IRF-5 gene on linear body measurement of Nigerian indigenous chickens

Age in weeks	Haplotype	N	BG (cm)	TL (cm)	SL (cm)	KL (cm)	WL (cm)	WS (cm)
1	GGTCGG	27	10.37±0.26	4.97±0.19	3.15±0.12	3.57±0.22	7.89±0.28	18.02±0.34[a]
	GGCCTG	9	10.32±0.38	4.79±0.30	3.03±0.18	3.64±0.32	7.51±0.44	17.87±0.52[ab]
	GGTCTG	13	10.12±0.32	5.20±0.24	3.18±0.15	3.79±0.26	6.83±0.36	16.74±0.42[b]
	GGCTAA	18	10.87±0.34	5.08±0.26	3.16±0.16	3.99±0.28	7.81±0.39	17.81±0.46[ab]
4	GGTCGG	27	18.07±0.46	10.08±0.72	5.52±0.32	7.28±0.62	13.36±0.75	31.59±2.93
	GGCCTG	9	17.83±0.79	9.43±0.88	5.25±0.41	7.16±0.74	12.09±0.95	30.20±3.48
	GGTCTG	13	18.21±0.62	10.02±0.79	5.69±0.36	7.68±0.67	13.82±0.84	30.62±3.17
	GGCTAA	18	17.91±0.67	9.04±0.81	5.42±0.37	6.90±0.69	12.64±0.87	28.69±3.25
8	GGTCGG	27	22.36±0.48	15.38±0.32	7.59±0.20	10.34±0.35	17.37±0.35[a]	42.52±0.56
	GGCCTG	9	22.18±0.84	16.03±0.56	7.44±0.26	10.00±0.57	17.25±0.60[ab]	41.42±0.97
	GGTCTG	13	21.81±0.66	15.66±0.44	7.09±0.23	9.51±0.46	15.76±0.48[b]	40.41±0.77
	GGCTAA	18	22.36±0.73	16.14±0.48	7.68±0.24	10.38±0.49	16.95±0.52[ab]	41.45±0.84
12	GGTCGG	27	24.71±0.46[b]	16.51±0.41	8.85±0.27	10.96±0.28	22.64±1.18	46.77±1.83
	GGCCTG	9	24.38±0.73[b]	17.15±0.49	9.09±0.35	11.01±0.49	22.66±1.31	47.22±2.16
	GGTCTG	13	25.42±0.59[ba]	16.47±0.44	9.12±0.31	10.76±0.38	23.40±1.23	47.30±1.97
	GGCTAA	18	26.50±0.63[a]	16.81±0.45	9.15±0.31	11.64±0.42	22.02±1.26	48.84±2.02
16	GGTCGG	27	25.78±0.72	17.28±0.25	8.87±0.24	12.35±0.42	25.01±0.20	50.56±0.77
	GGCCTG	9	26.16±0.93	17.30±0.45	9.87±0.34	13.39±0.52	24.97±0.35	51.65±1.14
	GGTCTG	13	25.54±0.81	17.01±0.35	9.39±0.29	12.36±0.46	24.68±0.27	49.66±0.94
	GGCTAA	18	26.88±0.83	17.26±0.39	9.54±0.30	12.39±0.48	24.87±0.30	49.81±0.99
20	GGTCGG	27	28.05±0.49	18.08±0.43	10.27±0.21	14.48±0.24	29.69±0.48	54.77±0.76
	GGCCTG	9	26.89±0.78	17.57±0.54	10.36±0.32	14.45±0.41	29.63±0.79	53.80±1.06
	GGTCTG	13	26.60±0.63	18.31±0.48	10.51±0.27	14.38±0.33	29.23±0.63	53.40±0.89
	GGCTAA	18	27.77±0.68	18.45±0.50	10.52±0.28	14.73±0.36	28.81±0.68	55.41±0.94

N: number of observation

[a,b]Means in the same column with different superscripts for the same age are significantly different ($p<0.05$)

4.12 Effects of haplotype identified in exons 3 and 7 of IRF-5 gene on heat tolerance traits of Nigerian indigenous chickens

4.12.1 Effect of haplotype identified in exon 3 of IRF-5 gene on heat tolerance traits of Nigerian indigenous chickens

Effect of haplotype identified in exon 3 of IRF-5 gene on heat tolerance traits of Nigerian indigenous chicken is presented in Table 38. All the heat tolerance traits were not significantly ($p>0.05$) affected by the haplotypes except rectal temperature at week 4 and pulse rate at week 8. Significant difference ($p<0.05$) was recorded in rectal temperature of birds carrying GGCC (41.46±0.12 ^{0}C) and AGTT (41.05±0.13 ^{0}C) haplotypes at week 4.

4.12.2 Effect of haplotype identified in exon 7 of IRF-5 gene on heat tolerance traits of Nigerian indigenous chickens

Effect of haplotype identified in exon 7 of IRF-5 gene on heat tolerance traits of Nigerian indigenous chicken is as presented in Table 39. All the heat tolerance traits were not significantly ($p>0.05$) affected by the haplotypes identified in exon 7 of IRF-5 gene in Nigerian indigenous chickens.

Table 38: Effect of haplotype identified in exon 3 of IRF-5 gene on heat tolerance traits of Nigerian indigenous chickens

Age in weeks	Haplotype	N	Rectal temperature (^{0}C)	Respiratory rate (breaths/min)	Pulse rate (bears/min)	Heat stress index
4	GGCC	33	41.46±0.12^{a}	59.61±9.58	201.19±9.44	2.88±0.51
	AGTT	39	41.05±0.13^{b}	65.86±9.65	185.67±10.29	3.22±0.52
	AATC	15	41.38±0.17^{ab}	62.68±9.94	204.91±13.13	2.93±0.56
8	GGCC	33	41.26±0.13	36.14±1.95	196.66±14.69^{b}	1.73±0.17
	AGTT	39	41.34±0.14	37.29±2.04	216.62±14.85^{a}	1.57±0.17
	AATC	15	41.08±0.18	32.75±2.38	205.65±15.53^{ba}	1.47±0.18
12	GGCC	33	41.47±0.16	34.49±1.56	193.67±9.63	1.67±0.15
	AGTT	39	41.46±0.17	33.56±1.75	181.47±10.00	1.70±0.15
	AATC	15	41.38±0.18	32.02±2.33	198.24±11.49	1.48±0.19
16	GGCC	33	41.76±0.08	35.20±3.35	222.14±9.22	1.44±0.10
	AGTT	39	41.73±0.08	32.21±3.47	220.42±9.62	1.34±0.11
	AATC	15	41.72±0.11	30.20±3.92	218.18±11.19	1.29±0.14
20	GGCC	33	41.33±0.08	32.16±1.92	265.18±5.85	1.10±0.07
	AGTT	39	41.42±0.09	32.64±2.01	262.37±6.01	1.13±0.07
	AATC	15	41.23±0.11	31.60±2.35	261.07±6.67	1.12±0.08

N: number of observation

^{a,b}Means in the same column with different superscripts for the same age are significantly different ($p<0.05$)

Table 39: Effect of haplotype identified in exon 7 of IRF-5 gene on heat tolerance traits of Nigerian indigenous chickens

Age in weeks	Haplotype	N	Rectal temperature (^{0}C)	Respiratory rate (breaths/min)	Pulse rate (bears/min)	Heat stress index
4	GGTCGG	27	41.51±0.12	67.38±9.53	202.05±9.22	3.17±0.51
	GGCCTG	9	41.26±0.19	63.12±10.24	192.50±14.96	3.06±0.59
	GGTCTG	13	41.16±0.16	61.63±9.82	196.76±11.99	3.00±0.54
	GGCTAA	18	41.26±0.17	58.72±9.93	197.72±12.91	2.81±0.56
8	GGTCGG	27	41.42±0.12	34.73±1.92	196.31±14.58	1.65±0.16
	GGCCTG	9	41.13±0.20	36.63±2.67	207.11±16.20	1.63±0.20
	GGTCTG	13	41.05±0.16	33.53±2.26	207.66±15.26	1.50±0.18
	GGCTAA	18	41.31±0.18	36.68±2.35	214.16±15.50	1.58±0.18
12	GGTCGG	27	41.47±0.16	33.23±1.51	195.76±9.38	1.58±0.14
	GGCCTG	9	41.39±0.20	33.37±2.65	205.63±12.77	1.52±0.21
	GGTCTG	13	41.41±0.18	33.73±2.09	180.77±10.89	1.73±0.17
	GGCTAA	18	41.47±0.18	32.74±2.30	182.34±11.42	1.64±0.18
16	GGTCGG	27	41.79±0.08	34.57±3.28	222.29±8.96	1.43±0.10
	GGCCTG	9	41.72±0.12	32.87±4.33	217.61±12.52	1.42±0.16
	GGTCTG	13	41.62±0.10	31.50±3.74	220.62±10.56	1.28±0.12
	GGCTAA	18	41.80±0.11	31.21±3.90	220.47±11.11	1.31±0.13
20	GGTCGG	27	41.37±0.08	33.55±1.87	265.01±5.81	1.15±0.07
	GGCCTG	9	41.19±0.13	28.91±2.63	258.50±7.25	1.01±0.09
	GGTCTG	13	41.40±0.10	33.48±2.21	266.59±6.45	1.15±0.08
	GGCTAA	18	41.34±0.11	32.60±2.33	261.38±6.60	1.14±0.08

N: number of observation

4.13 Effects of haplotypes identified in exons 3 and 7 of IRF-5 gene on antibody response to Newcastle disease before and after vaccination in Nigerian indigenous chickens

The effect of haplotypes identified in exons 3 and 7 of IRF-5 gene on antibody response to Newcastle disease before and after vaccination in Nigerian indigenous chickens is shown in Table 40. Antibody response to Newcastle disease before and after vaccination was not significantly ($p>0.05$) affected by haplotypes identified in both exons 3 and 7 of IRF-5 gene in Nigerian indigenous chickens.

Table 40: Effect of haplotype identified in exons 3 and 7 of IRF-5 gene on antibody response to Newcastle disease before and after vaccination in Nigerian indigenous chickens

Region	Haplotype	N	Geometric mean titre before vaccination	Geometric mean titre after vaccination
Exon 3	GGCC	33	14.03±5.42	59.45±12.64
	AGTT	39	1.08±0.04	54.56±4.98
	AATC	15	1.00±0.00	86.93±21.80
Exon 7	GGTCGG	27	3.22±1.26	46.11±10.28
	GGCCTG	9	23.67±13.77	101.33±31.10
	GGTCTG	13	1.00±0.00	67.69±8.53
	GGCTAA	18	10.22±7.14	53.89±15.12

N: number of observation

CHAPTER FIVE

5.0 DISCUSSION

Genetic variation existed in the body weight, breast girth, shank length, wing length and wing span of Nigerian indigenous chickens. Adebambo *et al*. (2011) also reported large variation among the growth traits of Nigerian indigenous chickens and this suggested that improvement can be made on these birds through selection and complementary gene action.

The mean body weight and other linear body measurements recorded in this study were higher than the ones reported by Fadare (2014) and this may be due to the fact that the birds used in this study have undergone many years of genetic improvement compared to the level of genetic improvement on the birds used by Fadare (2014).

Although the lowest body weight was observed in frizzle feather chickens at week 1, the highest body weight and breast girth observed in this genotype at week 4 and 12 suggested compensatory growth in this genotype between week 2 and week 12. The highest body weight observed in frizzle feather chickens between week 4 and 8 suggested that this genotype could be used in the development of broiler lines. Also, the highest breast girth recorded in frizzle feather chickens between week 4 and 8 also corroborated its usefulness in the development of broiler lines. There was no significant difference in the body weight of all the three genotypes at week 20 and this indicated that the three genotypes performed alike at week 20.

Body weight, linear body measurements and feed efficiency measurements in all the three genotypes showed a gradual increase from 1 to 12 weeks of age. After the 12^{th} week, the body weight, linear body measurements and feed efficiency declined, a trend that was maintained until 20 weeks of age. The 12^{th} week could therefore be

regarded as the point of inflection for these birds. Asuquo (1984) and Momoh *et al*. (2010) had reported a similar observation that the point of inflection of growth in Nigerian indigenous chickens is the 12 week of age.

The significant differences in the feed efficiency observed among the three genotypes may indicate differences in their maintenance requirements and genetic make-up (Momoh *et al*., 2010).

Males had higher values of body weight and feed efficiency than females from week 8, although differences with respect to body weight was not significant until week 16 and feed efficiency was only significant at week 16. This difference indicated sexual dimorphism in favour of males and this has earlier been reported by Adedeji *et al*. (2008). Superiority of male chickens over females could be due to hormonal profile, social dominance and aggressiveness. The male hormone, testosterone, stimulates and maintain secondary sexual development and it also affects growth and development of body parts (Adeleke *et al*., 2011). Aggressiveness of males over females especially when reared together also put the females at a disadvantage for feed and water. Presence of sexual dimorphism in Nigerian indigenous chickens indicated their potential for development of sire and dam lines in breed development (Momoh *et al*., 2010).

Differences were observed among all the three chicken genotypes for the heat tolerance traits indicating that thermo-responses were genotype dependent in Nigerian indigenous chickens. Genetic factors clearly influenced the bird's physiological response to heat. Different lines of chickens have been reported by Lu *et al*. (2007) to exhibit different heritable responses to heat stress.

A high rectal temperature range of 41.16 to 41.86 ^{0}C was observed in this study. Chickens are warm blooded animals that usually maintain a high body temperature (Schmidt-Nielsen, 1997). They gain heat from the environment and through their metabolism. Birds regulate the balance between heat production and heat loss to maintain their body temperature. They transport the generated heat to the body surface to allow sensible heat loss from surfaces such as combs, shanks, wattles and other unfeathered areas to the surrounding environment because they don't have sweet gland (Anderson and Carter, 1993). The body temperature of chickens must remain within the narrow limit of 41^{0}C if its welfare is to be safeguarded and production maintained at acceptable levels (Justin, 2004).

The rectal temperature observed in the Nigerian indigenous chickens was in the range of 41.2 to 42.2 ^{0}C reported by Isidahomen *et al*. (2012). The highest rectal temperature observed for naked neck chickens in this study negated the potential of naked neck over other genotypes in response to thermotolerance as reported by Zerjal *et al*. (2013). The naked neck gene has been related to heat tolerance. The naked neck birds tend to gain more weight at high ambient temperature and had better capacity to maintain body temperature at high ambient temperatures compared to their fully-feathered counterparts (Yahav *et al*., 1998). Cahaner *et al*. (2008) reported that heat resistance correlates with reduced feathering density. The results obtained in this study for thermotolerance revealed that it is still uncertain which specific genes or epigenetic effects play a direct role in the chicken's ability to tolerate or succumb to heat stress.

Highest rectal temperature observed in naked neck chickens at 16 and 20 weeks of age might be due to high metabolic rate in this genotype leading to increase in rate of exchange of gases. The thermotolerance observed in frizzle feather chickens at 16 and 20 weeks of age might be due to their feather structure. The highest respiratory rate

observed in naked neck chickens at 16 and 20 weeks of age might be due to the size of these birds at these ages. Size of animals has been shown to affect respiratory rate. Heavier birds have higher respiratory rate which could be attributed to higher metabolism since they possess larger surface area which could lead to increase in rate of exchange of gases. Heat production is also affected by species, breed, level of production, feed quality, activities and exercise (Justin, 2004).

The pulse rate observed in this study was higher than the one reported by Adedeji *et al*. (2015) and this may be due to different rearing environment and different season of rearing. There existed a fluctuation in pulse rate at different ages and these fluctuations might have resulted from ability to cope with prevailing environmental condition because of natural selection which may have aided them in the accumulation or expression of genes for adaptation.

Heat stress index is defined as a function of the deviation of actual from targeted environmental temperature and bird's age. The higher the heat stress index, the higher the severeness of the heat stress. The heat stress index obtained in this study implied that the three chicken genotypes were stressed at 4 weeks of age which may be due to residual effect of brooding. Frizzle feather chickens were less heat-stressed at 4 and 20 weeks of age while naked neck chickens were more heat-stressed at 12, 16 and 20 weeks of age.

The result of sex effect on thermotolerance revealing that female birds had higher rectal temperature at 16 weeks of week and higher heat stress index at 20 weeks of age suggested hormonal differences and possibility of egg laying processes (Adedeji *et al*., 2015).

High mortality recorded in frizzle feather chickens may be due to cold shock as most of the frizzle feather chickens died when it rained. It is widely accepted that cold exposure can influence the function of endocrine system, antioxidation system and immune system (Helmreich *et al*., 2005). Hangalapura *et al*. (2006) demonstrated that cold stress could significantly influence the immune status of chickens. Rybakina *et al*. (1997) reported that cold stress suppressed humoral immunity in rats while Regnier and Kelley (1981) reported that cold stress decrease cell-mediated immunity in chickens.

Disease resistance in young chicks is crucial for the survival and productivity of chickens (Yonash *et al*., 1996). The variation in the immune response of young Nigerian indigenous chicks to Newcastle disease virus could reflect incomplete development of their immune system, partial expression or regulation of its genetic control (Sacco *et al*., 1994) and possible variation among genotypes in the maternal effect (Leitner *et al*., 1994). Naked neck chicks generated the highest level of immune response to Newcastle disease before vaccination. This high immune response at young age showed by naked neck may be due to high levels of maternally derived antibody (MDA) which are transmitted from hens to chicks and protect them at early age. Also chicks from immunized parents possess high level of maternally derived antibody which protect them against virulent and vaccine viruses (Rahman *et al*., 2002). Hamal *et al*. (2006) reported approximately 30% maternally derived antibody transfer from breeder hen to their progenies. High level of maternally derived antibody also protects and neutralizes vaccine viruses if the chicks are vaccinated (Awang *et al*., 1994). The lowest MDA against Newcastle disease in frizzle feather chickens observed in this study was in agreement with the findings of Adeleke *et al*. (2015) who

also reported lowest MDA to Newcastle disease in frizzle feather chicken using ELISA.

Significant effect of sex on MDA to Newcastle disease observed in this study was not surprising as Osei-Amponsah *et al*. (2013) also reported sex differences in immunocompetence in Ghanaian local, Sasso T-44 and broiler chickens. Significant effect of sex on MDA to Newcastle disease observed in this study was in disagreement with the findings of Eid and Iraqi (2014) who reported no significant effect of sex on response to Newcastle disease in broiler chickens. This dissimilarity may be due to different genotypes used, Nigerian indigenous chickens were used in this study while Hubbard broilers were used by Eid and Iraqi (2014) in their study.

Antibody response to Newcastle disease virus after vaccination differed in Nigerian indigenous chickens with naked neck chickens having the highest immune response. Antibody response to the same virus differs among chicken genotypes (Pitcovski *et al*., 2001) and selection for an antibody response may improve disease resistance in chickens (Gross *et al*., 1980). Highest immune response observed in naked neck chickens have also been reported by Fathi *et al*. (2005), Fathi *et al*. (2008) and Galal (2008). Rajkumar *et al*. (2010) also reported that naked neck allele in homozygous and heterozygous condition increased cell mediated immunity to phytohaemoagglutin-P and antibody titres to sheep red blood cell antigens compared to other genotypes.

The presence of SNPs in exons 3 and 7 of IRF-5 gene in Nigerian indigenous chickens was an indication that these regions are polymorphic. Absence of SNPs in exon 4 of IRF-5 gene in Nigerian indigenous chickens and exon 5 of naked neck and frizzle feather chickens was an indication that these regions are totally conserved. Conserved genomic regions are likely responsible for gene functions as sequence conservation

points to an important biological role (Bejerano *et al*., 2004). Bejerano *et al*. (2004) also reported that proteins that are coded for by conserved sequences provide some vital functions for the organism. The conserved amino acid sequence of exon 4 of IRF-5 gene of Nigerian indigenous chickens as well as exon 5 of naked neck and frizzle feather chickens will probably correspond to the active site of their proteins (Lesk, 2002). Several other genomic regions that are related to immunity have been found to be conserved in different livestock species. Cluster of differentiations 4 and 8 have been found to be conserved and they play active role in T-cell recognition and activation by binding to their respective class I and II major histocompatibility ligands on their antigen presenting cells acting as co-receptor for polymorphic T-cell receptor (Miceli *et al*., 1990)**.**

Equal C↔T and G↔A transition mutations observed in exon 3 of IRF-5 gene in Nigerian indigenous chickens implied that there was no substitution bias in transition mutations in this region. Presence of 67% transitions in exons 3, 5 and 7 of IRF-5 gene in Nigerian indigenous chickens was in agreement with the finding of Lesk (2002) who reported that transition mutations are more common than transversions. Comparison of DNA sequences of metazoan by Keller *et al*. (2007) showed an excess of transitional over transversional substitutions and this is due to the relatively high mutation of methylated cytosine to thymine. Transition bias observed in metazoans could be caused by a mutational bias due to intrinsic properties of DNA. Also, in coding regions, the bias could be explained by selection on nonsynonymous transversions. Transition and transversion can change the amino acid composition of the resultant protein but the biochemical difference in the protein product tends to be greater for transversion (Zhang, 2000). There is likely to be greater purifying selection against transversions and selection could therefore favour DNA repair mechanisms

that are efficient in preventing transversions (Keller *et al*., 2007). Natural selection favours amino acid replacements via transitions and transitions are less severe with respect to the chemical properties of the original and mutant amino acids (Wakeley, 1996). Presence of more transversions in exon 5 of IRF-5 gene in normal feather chickens was in agreement with the findings of Hale *et al*. (2009) who reported higher transversion sites in the regulatory genes such as Endonuclease reverse transcriptase and TC1-like transposase. Exon 5 of IRF-5 gene in normal feather chickens might be responsible for encoding transcription factors with multiple biological functions observed in the gene.

Diversity at immune gene loci has been linked to fitness (Lenz *et al*., 2013). Heterozygosity quantifies within individual genetic diversity and is also related to inbreeding. Low heterozygosity of SNPs observed in exon 5 of IRF-5 gene in normal feather chickens and exon 7 of IRF-5 gene in naked neck chickens may have deleterious effects on their fitness (Wright, 1992). Low heterozygosity can affect fitness in natural settings (Walling *et al*., 2011) as reduced genetic diversity may interact with extrinsic stressors, such as disease, to influence population dynamics (Forcada and Hoffman, 2014). There is also a positive correlation between heterozygosity and immunity (Brock *et al*., 2013). Heterozygosity-fitness correlation (HFC) have been studied in many populations and the explanation of HFC to inbreeding depression is that of linkage between one or few neutral markers and functional genes under balancing selection which could give rise to the frequently observed pattern of heterosis (Balloux *et al*., 2004). Heterozygosity is generally beneficial for individuals, mainly because high heterozygosity levels decrease the risk of expressing recessive deleterious alleles (Keller and Waller, 2002). HFC arises by genome wide effects of heterozygosity, which is usually called general effect

hypothesis (Hansson and Westerberg, 2002). However, it is strongly debated if heterozygosity measured across a set of genetic markers could reflect genome-wide heterozygosity and also inbreeding level. Alternatively, marker heterozygosity might reflect heterozygosity states at closely linked loci only (Balloux *et al*., 2004). Hence, the local effect hypothesis that HFC occurs due to LD between genetic markers and loci under selection.

Single nucleotide polymorphisms which are biallelic markers have a maximum PIC of 0.38 (Hildebrand *et al*., 1992) and this was obtained for mutation 347G>A in exon 7 of normal feather chickens. The implication of this highest PIC was that the marker is highly informative for linkage studies and any normal feather chicken chosen at random at this locus is likely to be heterozygous for that marker. The higher PIC of SNPs generally observed in exon 7 of normal feather chickens was an indication of genetic variation and a selective potentially using this exon for marker assisted selection in this genotype (Andersson, 2001). Various PICs values observed for different exons and different genotypes may be influenced by many factors. Factors such as breeding behaviour of species, genetic diversity, sample size, sensitivity of genotyping method and genomic location of the markers affect PIC of SNPs (Singh *et al*., 2013).

All the SNPs identified in exon 3 of IRF-5 gene in Nigerian indigenous chickens were synonymous mutations which are not expected to cause any amino acid variation. Although these SNPs are not expected to have effect on protein function, Sauna and Kimchi-Sarfaty (2011) reported that synonymous mutations have effect on disease. Silent mutations are now widely acknowledged to be able to cause changes in protein expression, conformation and function. There is also codon usage bias vis-à-vis synonymous codon, suggesting that synonymous codons were under evolutionary

pressure (Chamary *et al*., 2006). Synonymous mutations can also result in aberrant mRNA splicing that can cause disease (Cartegni *et al*., 2002). Evidence by Nackley (2006) also suggested that synonymous SNPs could affect mRNA stabillty and thus protein expression and enzymatic activity. Diseases like pulmonary sarcoidosis, haemophilia, cervical cancer, vulvar cancer and non-small-cell lung carcinoma have been associated with synonymous mutations through codon usage bias (Sauna and Kimchi-Sarfaty, 2011). Chen *et al*. (2010) conducted a survey of some associations between diseases and SNPs curated from 2,113 reports studying human genetic association and they concluded that non-synonymous and synonymous SNPs shared similar likelihood and effect size for disease association. There is also a range of mechanisms by which synonymous mutations can affect the yields of active, correctly folded protein and thus have an impact on physiological activity (Chamary *et al*., 2006).

Linkage disequilibrium is a property of SNPs on a contiguous stretch of genomic sequence that describes the degree to which an allele of one SNP is inherited or correlated with an allele of another SNP within a population (Bush and Moore, 2012). Linkage disequilibrium of 0.001 between 6G>A and 113G>C in exon 7 of IRF-5 gene in naked neck chickens implied that there was almost linkage equilibrium between the two SNPs and there was almost non-joint evolution of the two SNPs. Linkage disequilibrium between two SNPs reflects the history of natural selection, gene conversion and other forces that cause gene-frequency evolution (Slatkin, 2008). The very low LD between 6G>A and 113G>C in exon 7 of IRF-5 gene in naked neck chickens also implied statistical independence and random association. Genetic drift balanced by mutation and/or recombination could also have caused this very low LD as this has been reported by Ohta and Kimura (1969) to cause low level of LD.

LD of 1 obtained for 33A>G and 57T>C in exon 3 of IRF-5 gene in normal feather and frizzle feather chickens as well as among all the SNPs in exon 5 of normal feather chickens implied that all these SNPs can affect the same immune traits. These SNPs convey similar information and can be used as tag SNPs because their alleles tag the surrounding stretch of LD (Bush and Moore, 2012). The response to selection of any one of these SNPs might be accelerated or impeded by selection affecting the other. Selection alone can increase LD and this occurs when fitness are more than multiplicative, meaning that the average fitness of an individual carrying *AB* haplotype exceeds the product of the average fitness of individuals carry *A* or *B* alone (Felsentein, 1965). Also, LD of 1 observed in exon 5 of IRF-5 gene in normal feather chickens might have resulted from very high proximity of the SNPs. Closely linked polymorphic SNPs tend to be in strong LD with one another (International HapMap Consortium, 2007). High level of LD among SNPs is also assumed to be true for alleles that are involved in immunity (Slatkin, 2008). LD of 1 in exon 5 of IRF-5 gene in normal feather chickens might have also resulted from strong positive selection occurring in this region as positive selection quickly increases the frequency of advantageous alleles, with the result that linked loci remain unusually in strong LD with that allele which results in genetic hitch-hiking (Maynard and Haigh, 1974). LD of 1 observed in exon 5 of IRF-5 gene in normal feather chickens might also imply that the alleles of SNPs observed in this region are young or that much time has not passed since the allele arouse by mutation (Slatkin, 2008). Formation of new allele by immune genes such as IRF-5 might have resulted from host-parasite/pathogen coevolution (Croze *et al*., 2016).

Presence of six singleton variable sites out of 31 base pairs in exon 5 of IRF-5 gene in normal feather chickens was an indication of pathogen/transcription signal pressure

effect on the region. Rare allele variants may rapidly increase in frequency when pathogen pressure is strong and resistance enhances animal fitness but later may become selectively neutral due to pathogen counter-adaptation or a low frequency of attack called novel allele advantage (Lazzaro and Clark, 2003).

Historical recombination is important in understanding the role of recombination in the creation of patterns of variability observed in IRF-5 gene in Nigerian indigenous chickens. Recombination is an important evolutionary factor in many organisms. Lower bound (minimum number) were calculated for exons 3, 4, 5 and 7 of IRF-5 gene in Nigerian indigenous chickens because many recombination events in the history are typically undetectable, so the actual number of historical events in the gene may be unobtainable (Myers and Griffiths, 2003). Recombination might have created 170C>T identified in exon 7 of IRF-5 gene in Nigerian indigenous chickens as this SNP has not been previously reported in Literature. Recombination influences genetic diversity in many livestock species. All genetic variations are ultimately created through mutation but recombination can create new variants by combining types already present in the population. The presence of recombination event between 33A>G and 48G>A in exon 3 of IRF-5 gene in naked neck chickens implied that the two loci have mutated at most once since the ancestor of the chickens (Hudson and Kaplan, 1985). Absence of recombination in exon 3 of IRF-5 gene in normal feather and frizzle feather chickens as well as exons 5 in normal feather chickens was an indication that all the SNPs identified in these genotypes were ancient mutations. These ancient mutations might be responsible for functions of the gene which include immune defence against virus, stress response, cell differentiation, reproduction, growth and development (Chen and Royer Jr, 2010). Also, absence of recombination in exon 3 of IRF-5 gene in normal feather and frizzle feather chickens as well as exon

5 in normal feather chickens was an indication that each mutation site has the same family tree and that the history of these regions in these chicken genotypes consisted of just one tree. Recombination generates the history of a sample and the history of a sample is a collection of correlated family tree, one for each site (each nucleotide sequence). The family tree for a site traces the genealogy of a site back to its most common ancestor indicating which sampled variations are most closely related and when the most recent common ancestors occurred (Hudson and Kaplan, 1985). Presence of recombination among the SNPs in exon 7 of IRF-5 gene in all the three genotypes was an indication that these mutations are new variants that were not present in ancient chickens. The new mutations might be responsible for newly discovered functions, such as macrophage polarization and regulation of B-cell differentiation (Lien *et al*., 2010), of the gene.

Phylogenetic analysis revealed that exon 3 of IRF-5 gene in both naked neck and frizzle feather chickens were both related. Close relationship between exon 3 of naked neck and frizzle feather chickens implied high comparability and evolution from a most common ancestor. Evolutionary findings also demonstrated a clear distinction between exon 7 of red jungle fowl and other chicken genotypes.

There was an early duplication event in the evolution of all the exons examined which was displayed by two clades formed by all the exons analysed. The phylogenetic tree obtained for exon 7 in this study revealed that red jungle fowl formed ancestral lineage for other chicken genotypes. Formation of ancestral lineage by exon 7 of IRF-5 gene in red jungle fowl supported the hypothesis that domestic chicken originated from the tropical jungle fowl of the genus *Gallus* (Crawford, 1990).

Positive Tajima's D values observed in exon 3 and 7 of IRF-5 gene in Nigerian indigenous chickens was an indication of presence of low levels of low and high frequency mutations which can be linked to balancing selection and decrease in population size (Hahn *et al*., 2002). Since purifying selection was observed in exon 3, balancing selection can be excluded as the cause of these low levels of low and high frequency mutations and one can safely assume decrease in population size to be the cause. Positive Tajima's D values observed in exon 7 of IRF-5 gene of Nigerian indigenous chickens might have resulted from many haplotypes present in the region as positive Tajima's D values have been shown to be caused by presence of many haplotypes (Simonsen *et al*., 1995).

Significant negative Tajima's D value obtained for exon 5 of IRF-5 gene in normal feather chickens signified an excess of low frequency SNPs relative to expectation and this was an indication of population size expansion which can result from purifying selection, bottleneck or selective sweep (Suharyanto, 2011). Since balancing selection was observed in exon 5 of IRF-5 gene in normal feather chickens, purifying selection can be excluded as a cause of population size expansion in the region. Negative Tajima's D value also indicated an excess of rare alleles. All the SNPs identified in exon 5 of IRF-5 gene in normal feather chickens were singletons which are more or less rare alleles. Purifying selection, population expansion and selective sweeps can all produce rare alleles (Hahn *et al*., 2002).

Negative selective pressures were acting on exon 3 of IRF-5 gene in Nigerian indigenous chickens while positive selective pressures were acting on exon 5 of IRF-5 gene in normal feather chickens as well as exon 7 of the three chicken genotypes. Antagonistic co-evolution between host and pathogens is one of the major driving forces of molecular evolution of species (Paterson *et al*., 2010). Due to this, host

species are thought to maintain high standing allelic variation at immune and disease resistance loci, to counter a rapidly evolving and diverse pathogen fauna (Sommer, 2005). Immune genes are subject to acute selective pressures in order to resist pathogenic attacks (Downing *et al*., 2009). Presence of positive and negative selective pressures in IRF-5 gene in Nigerian indigenous chickens reflected the diverse functional roles that the gene plays in immunity (McTaggart *et al*., 2012). An important explanation for presence of both positive and negative selections in IRF-5 gene of Nigerian indigenous chickens was that molecules interacting directly with pathogens, either in recognition or destruction undergo more adaptive changes than molecules that do not (signal transducers). Presence of positive and negative selection in IRF-5 gene in Nigerian indigenous chickens may also be due to disease dynamics.

More positive selection was observed in IRF-5 gene in Nigerian indigenous chickens and this was in agreement with the study of international chicken genome sequencing consortium (2004) who reported that chicken host defence genes evolve under stronger positive selection than other functional categories of genes. It has been hypothesised that immune genes involved in the recognition of pathogens will show stronger signatures of positive selection than signal transduction molecules, which are more likely to be the subjects of purifying selection (Sackton *et al*., 2007). Consistent with the results obtained from other chicken genotypes by Downing *et al*. (2009) and fruit fly by Obbard *et al*. (2009), more positive selection observed in IRF-5 gene of Nigerian indigenous chickens supported the hypothesis that an average immune system gene has experienced more adaptive selection.

Exons 3 and 7 were classed as mixed folding structures which contained alpha helices, extended strands and random coils. Exon 4 was classified as single folding while exon 5 was classified as double folding. The secondary structure indicated whether a given

amino acid lies in a helix, strand or coil (Banerjee *et al*., 2009). Random coil dominated among the secondary structure elements of exons 3 and 7. These three structural elements observed in exons 3 and 7 were responsible for folding, stability and overall functions of the protein. Random coils were responsible for folding, stability and overall functions of exon 4 while extended strands and random coils were responsible for folding, stability and overall functions of exon 5.

Presence of IRF tryptophan pentad repeat DNA-binding domain profile in exon 3 of IRF-5 in NICs was not surprising as all interferon regulatory factors share significant homology in their N-terminal 115 amino acids, which contain the DNA-binding domain and it is characterized by five tryptophan repeats. The DNA-binding domain forms a helix-turn-helix structure responsible for the binding to the interferon stimulated response elements or interferon regulatory factor binding elements consensus in the target promoters. Three of the tryptophan repeats are reported to be involved in the binding to the DNA to orientate and stabilize amino acid contacts in the IRF molecule with the "GAAA"core sequences (Escalante *et al*., 1998).

Presence of casein kinase II phosphorylation sites in exons 4 and 7 of IRF-5 in Nigerian indigenous chickens might be responsible for the phosphorylation activities of IRF-5. Casein kinase II is a highly conserved serine-threonine kinase that uses ATP and GTP as phosphate donors. It is usually present as tetrameric holoenzyme complex of two catalytic subunits (alpha and/or alpha') and two regulatory beta subunits. Phosphorylation and dephosphorylation of proteins play a key role in cellular signalling in immune response. Upon RNA virus infection, the downstream molecules of IRFs could be activated by phosphorylation, which activates the transcription of IFN-B through either the TLR3 pathway or RIG-I pathway (Alexopoulon *et al*., 2001). Presence of casein kinase II phosphorylation sites in exons 4 and 7 of IRF-5 in

Nigerian indigenous chickens might also be responsible for the regulation of cell cycle function of the gene as previous findings by Singh and Ramji (2008) has shown that casein kinase II phosphorylates more than 300 substrates and controls a wide range of processes including regulation of cell cycle, apoptosis or programmed cell death (PCD) and transformation. Although casein kinase II is involved in apoptosis, it is also involved in cell growth and this might be responsible for cell growth functions of IRF-5 gene. Casein kinase II phosphorylation site derived its cell growth function and continuation of cell cycle from G_1 to S phase and G_2 to M phase checkpoints and it does this by protecting proteins from caspase-mediated apoptosis through phosphorylation of sites adjacent to caspase cleavage site, blocking the activity of caspase protein (Litchfield, 2003). This motif might also be responsible for inflammatory response of the gene as previous findings by Li *et al*. (2005) implicated casein kinase II in inflammation.

Presence of amidation site in exon 7 might be responsible for immune functions of the gene as previous findings by Sravanthi and Kumar (2017) revealed that amides have beneficial effects on B cells and also aid their growth and differentiation in mice. Amides as also revealed by Sravanthi and Kumar (2017) elicit a protective action on islet cells from inflammatory cytokines and prevents destruction of B cells.

Presence of cAMP- and cGMP- independent protein kinase phosphorylation site in exon 7 of IRF-5 gene in Nigerian indigenous chickens might be responsible for growth and differentiation functions of the gene. Cyclic AMP-independent protein kinase has been reported by Thevelein *et al*. (2000) to play a key role in regulation of basic cellular processes connected with metabolism, growth and differentiation. Cyclic AMP-independent protein kinase has also been found by Thevelein *et al*. (2000) to be

involved in the control of cell cycle progression, induction of thermotolerance and survival during starvation periods in yeast.

Presence of myristoylation site in exon 7 of IRF-5 gene in Nigerian indigenous chickens will have implication on the lipophilicity of its protein as previous findings by Farazi *et al*. (2001) has linked myristoylation with lipophilicity of protein molecules. Presence of myristoylation site in exon 7 of IRF-5 gene in Nigerian indigenous chickens may also be responsible for cell growth and development of the gene as previous findings by Toraskar *et al*. (2008) has shown that myristoylation is essential for growth, development and rapid cellular proliferation in variety of organisms.

The absence of N-glycosylation sites in IRF-5 of Nigerian indigenous chickens was surprising as it is a conserved domain in eukaryotes and it is one of the most abundant post-translational modification reactions in nearly half of all known proteins in eukaryotes. The implication of its absence in IRF-5 gene of Nigerian indigenous chickens is that this gene is not glycosylated.

Haplotypes GGCC, AGTT, AATC in exon 3; GTCCTG in exon 5 and GGTCGG, GGCCTG, GGTCTG, GGCTAA, GGCCTA, GGCCGG in exon 7 shared by the three chicken genotypes were the ancestral haplotypes and can be used in disease mapping and association studies. Haplotypes that are shared among individuals in a population is potentially powerful alternate method for mapping disease genes (de la Chapelle and Wright, 1998). Shared haplotypes are conserved, so they can be used in disease mapping as knowledge of their mode of inheritance is not needed (Houwen *et al*., 1994). These shared haplotypes may be due to translocation history. The specific haplotypes identified in this study highlighted the strong differentiation among sites.

Also, differences among haplotypes have been shown to support immune responses that affect disease susceptibility. Haplotype diversity arises from single nucleotide mutations, indels, meiotic recombination and gene conversion, while isolated SNPs provide evidence of diversity that is likely derived from mutational events (Hosomichi *et al*., 2008).

The significant effect of the haplotypes identified in exons 3 and 7 of IRF-5 gene on feed efficiency, body weight and some heat tolerance traits of Nigerian indigenous chickens implied that this gene has significant role in feed utilization, growth and response to stress. This was not surprising as the interferon regulatory factor (IRF) gene family encodes transcription factors with multiple biological functions which include immune defence against virus, stress response, cell differentiation, reproduction, growth and development (Chen and Royer, 2010).

5.1 CONCLUSIONS AND RECOMMENDATIONS

5.1.1 Conclusions

From the results obtained in this study, it can be concluded that:

- Genotypic differences accounted for variation in growth traits, feed efficiency, thermotolerance, survivability and antibody response to Newcastle disease in Nigerian indigenous chickens. Also, sexual dimorphism favoured male birds in term of body weight and feed efficiency from 8^{th} week of age.
- Exons 3 and 7 of IRF-5 gene in Nigerian indigenous chickens were polymorphic while exon 4 was conserved.
- Very low to moderate to high linkage disequilibrium existed among the single nucleotide polymorphisms identified in exons 3, 5 and 7 of IRF-5 gene in Nigerian indigenous chickens.
- Six haplotypes are present in exon 3, two in exon 5 and thirteen in exon 7 of IRF-5 gene in Nigerian indigenous chickens.
- Nigerian indigenous chickens have undergone recombination events in the exon 7 of their IRF-5 gene.
- Phylogenetic relationship between exon 7 of IRF-5 gene of Nigerian indigenous chickens and other chicken genotypes revealed that Red Jungle Fowl formed the ancestral lineage of Nigerian indigenous chickens.
- Prediction of selective events revealed that both adaptive and purifying selective forces were acting on IRF-5 gene of Nigerian indigenous chickens.
- Exons 3 and 7 of IRF-5 protein in Nigerian indigenous chickens were classified as mixed folding structures; exon 4 was classified as single folding while exon 5 was classified as double folding structure.

- Prediction of motifs present in IRF-5 gene of Nigerian indigenous chickens revealed the presence of IRF tryptophan pentad repeat DNA-binding domain profile in exon 3.
- Haplotypic differences in exon 3 of IRF-5 gene accounted for feed utilization and some heat tolerance traits while haplotypic differences in exon 7 of IRF-5 gene accounted for body weight and feed utilization in NICs

5.1.2 Recommendations

Based on the findings of this study, the following are thereby recommended:

- The highest body weight and breast girth observed in frizzle feather chickens between week 4 and 8 suggested that this genotype can be used in the development of broiler lines.
- Nigerian indigenous chickens can be used in development of sire and dam lines in breed development due to presence of sexual dimorphism in them.
- Naked neck chickens can be used to facilitate genetic improvement against Newcastle disease.
- Linkage disequilibrium among SNPs in IRF-5 gene of Nigerian indigenous chickens can be exploited to optimize genetic studies, preventing genotyping of SNPs that provide redundant information.
- Only the haplotypes present in the exons 3, 5 and 7 of IRF-5 gene in NICs can be used for association studies as most of the SNPs present in these regions were synonymous and the nonsynonymous ones were singletons. Haplotypes identified in exons 3 and 7 of IRF-5 gene can be used in haplotype-assisted selection when improving feed utilization and heat tolerance traits in Nigerian indigenous chickens.

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